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The Kidney in Plasma Cell Dyscrasias

Editor
G.A. Herrera

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The Kidney in Plasma Cell Dyscrasias

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The Kidney in Plasma Cell Dyscrasias

Volume Editor

Guillermo A. Herrera *St. Louis, Mo.*

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The Kidney in Plasma Cell Dyscrasias: A Current View and a Look at the Future

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A book with an identical title to the one now compiled: The kidney in plasma cell dyscrasias was edited by Minetti, D'Amico and Ponticelli and published in 1988 by Kluwer Academic Publishers [1]. The book is a collection of manuscripts compiling the presentations at a meeting by the same name held in Milan in 1987 in which the leading researchers and clinicians in the field participated. The book provides a rather comprehensive state of the art of the subject summarizing knowledge available two decades ago.

In the last chapter of this book titled Conclusions, Dr. J.S. Cameron makes a number of interesting comments worth reflecting on 18 years later. When he discusses mechanisms of renal damage, there is ample information provided regarding proximal and distal tubular nephrotoxicity. Mechanisms involved in these are summarized and; although the refined molecular understanding of the pathological processes that we command today did not exist then, the overall conceptual mechanistic views as to how damage occurs is quite similar to our current perception. What is remarkable is that he clearly stated that there was no idea as to how glomerular damage occurred in these monoclonal light chain-related disorders. The readers will observe as they peruse and read this book, that our understanding of how glomerular damage occurs has remarkably improved in the last two decades. The present book devotes several chapters to various glomerulopathies associated with deposition of immunoglobulin light and heavy chains, including those associated with amyloidosis, highlighting sequential events that take place and delineating crucial steps and key molecules involved amenable to modulation or control. Another notable difference is that there has been significant improvement in the therapy of these conditions using innovative

means. Likewise, the increased sophistication in therapy is highlighted in the book chapters dealing with this subject. Although the emphasis in these chapters addressing the therapy is placed on the management of cases with renal involvement, a distinct focus in addressing the diseases as a whole as they impact the patients' general health and prognosis has been maintained in the discussions.

The chapters in this book address the entire pathological spectrum of monoclonal light chain-related renal diseases and provide a comprehensive up to date compendium of information that should be valuable to a variety of disciplines. Drs. Steeusma and Kyle provide a historical account of how throughout the years we have increased our understanding of these diseases highlighting the key developments that have taken place in the field. This chapter provides a wonderful introduction to the entire book and clearly provides a historical account dealing with the sequence of events that have taken place throughout the years culminating in our current knowledge. Cotelingam and Veillon address the diagnosis of plasma cell dyscrasias in the anatomic and pathology laboratories. Significant advances in this area now permit a rather sophisticated and accurate evaluation of paraproteins in the serum and urine, not only for diagnostic purposes, but also to follow these patients and to assess pertinent prognostic and therapeutic issues. Mayo and Johns address in their chapter the use of serum free light chains in the diagnosis and monitoring of patients with plasma cell dyscrasias. They summarize the current knowledge regarding the applications of this relatively new test in clinical practice. Merlini summarizes in a succinct yet lucid fashion the main mechanisms involved in the pathogenesis of the various renal manifestations of plasma cell dyscrasias. The type of renal damage in these conditions is quite broad and heterogeneous. Understanding mechanisms involved not only clarifies how it happens mechanistically, but it also delineates basic science considerations that serve to explain why renal alterations can be so diverse, a good example of how research can translate from the bench to the bedside to enhance patients' management. Dr. Batuman has spent a significant amount of this career in deciphering how proximal tubular damage occurs in some patients with monoclonal light chain-associated diseases. His chapter provides a translational approach to the understanding of this subject. Likewise, Dr. Sanders has conducted sophisticated and elegant research in the area of distal nephron obstruction associated with myeloma. In his chapter, he outlines how crucial is for particular structurally abnormal light chains to interact with Tamm-Horsfall protein as they engage in creating distal tubular casts. My laboratory has been engaged in the study of the pathogenesis of glomerular damage in monoclonal light chain related renal diseases for the last 15 years. Dr. Keeling has been involved in defining how crucial are interactions between some light chains and mesangial cells resulting in the pathological alterations that we observe in these conditions and how functional alterations of mesangial cells inevitably affect the surrounding mesangial matrix. Dr. Picken's chapter provides an in-depth

excursion into light and heavy chain associated amyloidosis with emphasis on diagnostic aspects and pathogenesis. Dr. Steven's group has conducted seminal research dealing with the characterization of abnormal light chains and the impact of this biochemical characterization on the pathogenicity (including nephrotoxicity) of these monoclonal light chains. They provide us with a detailed summary of their research and how this information fits into the understanding of renal damage in plasma cell dyscrasias. Finally, the chapters by Roman-Pineda and Tricot, as well as Comenzo, leaders in the clinical management of these patients, detail current therapeutic protocols used, particularly in those patients with renal involvement. These two chapters clearly show the great advances that have taken place in the last 20 years in the treatment of these conditions.

It should be noted that heavy chain-associated renal diseases were not known until the 1990s, so it is in this book (not in the previously published) that conditions such as heavy chain deposition disease and heavy chain-related amyloidosis are discussed as specific entities. While our understanding of the pathogenesis of heavy chain-associated disorders is still primitive, we are becoming proficient at diagnosing them accurately.

Much has been done in the biochemical characterization of pathogenic light chains. This work has clearly shown that these pathological light chains exhibit peculiar amino acid alterations in the variable portion of the light chain molecule. While those alterations in some instances are quite characteristic (i.e. the amino acid substitution in position 30 of the variable portion of the κ 1 light chain molecule on acquired renal Fanconi's syndrome), in other monoclonal light chains-associated conditions the structural changes noted are significantly more variable and complex. The abnormality in the light chains associated with the Fanconi's syndrome has been shown to render the altered light chain resistant to catabolism, thus crystalline structures are formed in the cytoplasm of the proximal tubular cells.

Animal models of these diseases have not been easy to create. Recent developments have resulted in the creation of an animal model of acquired renal Fanconi's syndrome [2]. This exciting discovery should lead the way for other models of these diseases to be developed. Animal models are most useful to test new therapeutic interventions and provide a solid platform to test the clinical importance what has been observed in vitro. Undoubtedly, animal models bridge the gap between experimental work and reality. As demonstrated by Sirac et al. in their seminal paper, design of therapeutic interventions leading to reversibility of these disorders is possible once their pathogenesis is clearly elucidated [2, 3].

In the era of molecular understanding of diseases, monoclonal light chain-related renal disorders have not been left behind. These diseases are understood with much better details at the present time. While advances in molecular-targeted pharmacotherapy for renal disorders have taken place relatively slowly, it is anticipated that new therapeutic interventions will be designed at a much

faster pace in the near future. Obviously designing such therapeutic avenues is only possible because we have acquired a sound and comprehensive molecular understanding of the pathogenesis of these disorders.

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A History of the Kidney in Plasma Cell Disorders

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Abstract

Background: The kidneys are commonly injured in plasma cell dyscrasias. **Methods:** We reviewed the pertinent medical literature related to the historical development of clinical nephrology and diagnostic renal pathology; early case reports of patients with plasma cell disorders; and historical descriptions of multiple myeloma, amyloidosis, and the renal disorders that are associated with these conditions. **Results:** Medieval uroscopists recognized proteinuria, and in 1827 Richard Bright first linked proteinuria to both dropsy (edema) and the autopsy finding of chronically diseased, scarred kidneys. In the 1840s, Henry Bence Jones and William Macintyre described a peculiar form of proteinuria in a middle-aged English grocer with fragile, tumor-riddled bones; this proteinuria became known as 'Bence Jones type'. It was initially believed that Bence Jones proteins were harmless to the kidney, but after 1899 (when myeloma cast nephropathy was recognized), investigators observed numerous renal injury patterns associated with plasma cell dyscrasias. Gross observations of 'waxy degeneration' or 'lardaceous change' in organs including the kidney yielded to the misnomer 'amyloid' in 1854, when iodine staining suggested to Rudolf Virchow that the strange material present in these conditions was a form of starch or cellulose. During the 20th century, biochemists and physicians carefully studied patients with myeloma, in order to better define the nature and structure of normal and pathological immunoglobulins. **Conclusion:** Historical understanding of the kidney in plasma cell disorders reflects developments in understanding of the anatomy and physiology of the kidneys in health and in disease.

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General Considerations: Evolution of Clinical Nephrology and Diagnostic Renal Pathology

Introduction

Physicians have been interested in the role of the kidney in human disease since antiquity, yet clinical nephrology and diagnostic renal pathology are

relatively new biomedical disciplines, formalized only in the second half of the 20th century [1, 2]. In the early 19th century, abnormalities in the gross appearance of the urine and the kidney were first reported in association with the clinical conditions now grouped together as plasma cell disorders. In contrast, microscopic descriptions of specific renal disease patterns in the monoclonal gammopathies only began with the recognition of myeloma cast nephropathy in 1899, and most reports belong to the late 20th century [3, 4]. Advances in our collective understanding of the various renal injuries complicating plasma cell dyscrasias are best understood within the context of more general insights into the anatomy and physiology of the kidney, both in health and disease.

Early History

Many ancient texts contain observations of the kidneys and theories about their afflictions, but the first major treatise devoted solely to diseases of the kidneys and urinary tract was probably that of Rufus of Ephesus [5]. Rufus was a leading Greek physician who flourished during the reign of the Roman Emperor Trajan (98–117 AD) [6]. Later writers referenced Rufus extensively, including Claudius Galen (129–199 AD), and Rufus was especially influential among the medieval Islamic physicians, who translated more than 50 of his works into Arabic [7]. Rufus of Ephesus was appreciated for the richness of his clinical descriptions, and some credit him with being the first to notice the hardened, shrunken kidneys of what is now referred to as 'end stage' renal disease. Little else is known of Rufus, and only fragments of his original works have survived [6]. In the centuries after Rufus, many major authorities addressed urolithiasis and hematuria, but the kidneys themselves remained mysterious and received relatively little attention.

Uroscopy

Uroscopy, the practice of carefully examining the appearance of patients' urine – known colloquially as 'water gazing' performed by 'piss-prophets' (fig. 1) – was advocated by many historical medical authorities, including both Hippocrates (cf. 460–377 BCE) and Galen. In his famous *Canon of Medicine*, the Persian physician and philosopher Avicenna (Ibn Sina; 980–1037 AD) insisted that physicians should routinely examine their patients' urine. The practice of uroscopy reached its zenith during the time of French physician and humanist Pierre Gilles de Corbeil (Aegidius Corboliensis; 1165–1223) (fig. 2), whose influential treatise *De urinis, de pulsibus, de virtutibus, et laudibus compositorum medicamentorum* includes observations on dozens of subtly distinct physical states of urine. Frequently, uroscopy substituted for a physical examination, which many academic doctors in this era considered improper or unpleasant.

Fig. 1. A classic image of uroscopy: 'The Physician' by Gerrit Dou (1613–1675), a Dutch painter who was a pupil of Rembrandt van Rijn. Dated 1653, oil on oak, 49.3 × 37 cm, Kunsthistorisches Museum, Vienna. In this case, the reddish color of the liquid in the flask and the imagery suggest that the physician was performing a pregnancy test. In the 17th century, urine was sometimes mixed with red wine for this purpose, a procedure that altered the appearance of urine proteins observed in many pregnant women.

Medical practitioners in the Middle Ages practiced uroscopy so often that the icon of the glass urine flask became identified with physicians as closely as the image of the stethoscope would in the 20th century (fig. 1). Geoffrey Chaucer's physician-pilgrim – who esteemed Rufus of Ephesus as one of the many authorities supporting his opinions – is depicted gazing at a urine flask in the illuminated Ellesmere manuscript of the 14th-century *Canterbury Tales*, in the rather unlikely position of practicing uroscopy while riding on horseback (fig. 3). Chaucer's Host praises the physician for his storytelling by blessing his urine examinations and his flasks:

Fig. 2. A 1967 semipostal stamp from Belgium illustrating Pierre Gilles de Corbeil (1140–1224), also known as Aegidius Corboliensis, who distinguished more than 19 substances in urine, separating them by consistency, sedimentation, quantity, and quality. Author's collection.

I pray to God so save thy gentil cors,
And eek thyne urynals and thy jurdones,
Thyn ypocras, and eek thy galiones,
And every boyste ful of thy letuarie,
God blesse hem, and oure lady Seinte Marie!
(Introduction to The Pardoner's Tale, lines 304–308) [8]

Urinalysis

Gross observation of urine has been practiced for millennia, but chemical analysis of the urine was first systematized in the early 1800s. During this period, the rise of 'animal chemistry' (i.e., what is now called clinical chemistry) in British, French and German hospitals facilitated detailed analyses of all body excreta, especially the urine. Richard Bright (1789–1858), an energetic and enormously popular physician at Guy's Hospital in London, was not the first to recognize albumin in the urine, but he did develop a simple test for proteinuria in 1827: holding a small quantity of urine in a spoon over a candle [9]. Bright was the first to connect urine that curdled when treated in this way with the clinical condition of dropsy (edema) and the autopsy finding of shriveled, scarred kidneys (fig. 4). For more than a century after his work, all chronic kidney diseases, especially progressive parenchymal renal disorders, were grouped together and called 'Bright's disease'. Bright also clearly distinguished renal edema from the forms of edema associated with heart and liver disease, although he was not the first to do so [10]. The same milieu of exhaustive chemical analysis that Bright worked in also made possible the description of the Bence Jones protein in the 1840s [11], the landmark event in the history of the plasma cell dyscrasias (described in more detail below).

Renal Histology

Marcello Malpighi (1628–1694) of Bologna, one of the founders of microscopical anatomy and a contemporary of the Dutchman who invented the

Fig. 3. Fourteenth-century version of point-of-care urinalysis: Geoffrey Chaucer's physician water-gazing. Detail from the Ellesmere Chaucer in the Huntington Library.

microscope, Anton van Leeuwenhoek (1632–1723), was the first to describe the renal glomeruli ('Malpighian corpuscles') in his text *De renibus* in 1666 [12–14]. Malpighi identified these structures by perfusing renal arteries with a solution of India ink and alcohol. The eponym 'Malpighi' is rarely used today to describe glomeruli, because of potential confusion with the Malpighian corpuscles of the spleen (i.e., the while pulp of the spleen, lymphoid follicles). Coincidentally, in both the kidney and the spleen, Malpighian corpuscles are the preferential places for amyloid proteins to deposit.

Almost two centuries after Malpighi, William Bowman (1816–1892) in London identified a small capsule surrounding the glomerulus (in 1841), a structure which now carries his name [15, 16]. The delicate interface between the glomerulus and Bowman's capsule is frequently disrupted in plasma cell dyscrasias. In 1862, another major portion of the renal filtration apparatus – the U-shaped loop of the renal tubule – was noted by Friedrich Gustav Jacob Henle (1809–1885), who worked at the University of Göttingen in Germany and benefited from newly invented achromatic microscope lenses [17, 18]. Axel Key (1832–1901), a Swede, is credited with discovery of mesangial cells in 1860 – but Key is much better known for his diplomatic skills, which he used while

Fig. 4. A portrait of Richard Bright, who first linked dropsy (edema), proteinuria, and kidney disease. From Thomas Joseph Pettigrew's *Medical Portrait Gallery*. London, Fisher, Son, & Co. 1840.

serving as the Rector of Karolinska Institute in Stockholm to persuade Alfred Nobel and the executors of Nobel's will to sponsor the Nobel prize in Physiology or Medicine [19].

Despite these anatomical advances, theories of renal physiology remained somewhat simplistic until the early 20th century, when a series of painstaking animal and human experiments by many investigators – above all Homer Smith (1895–1962) in New York – led to the present paradigm [20, 21]. The work of Smith and his contemporaries revealed that the kidney is the essential organ for maintaining physiological salt, water, and acid-base balance; these studies also clarified the precise role of the kidney in excreting various metabolic by-products, toxins, and other substances from the body. Even though the mechanisms of these complex processes are now well understood, the fact that the kidney is able to ensure homeostasis over such a wide range of diets and under the influence of diverse hormonal signals and vascular states still seems remarkable.

Renal Pathology

Detailed understanding of microscopic renal pathology awaited the advent of surgical kidney biopsy, and later percutaneous needle biopsy. Surgical biopsy in the modern sense only became possible after 1837, when Gabriel Gustav Valentin (1810–1883) in Bern invented the first crude microtome [22]. A tissue fixative was also essential; mercuric chloride and later formaldehyde were introduced in the mid-19th century for this purpose, and both still enjoy widespread use.

Clinical reports and autopsy data from the middle of the 19th century frequently included rudimentary renal histology on unstained specimens. Later in the 19th century, various tissue stains were introduced, allowing more detailed microscopic studies [23]. The decades after 1856, when aniline dyes first came into use, saw the introduction of most of the stains that are still used by pathologists today, including the classic hematoxylin (derived from Central American logwood trees) and eosin combination, which debuted in 1875 [24]. With these new tools, pathologists – principally those working in the influential German universities – were able to describe a wide range of abnormal morphological patterns, including several in the kidney. The 1914 renal pathology textbook of Franz Volhard and Karl Fahr of Giessen, Germany [25] is widely considered to be the culmination of the early surgical pathology era [2].

Percutaneous Biopsy

Despite this body of work, systematic observations of renal histopathology during life remained uncommon until the mid-20th century, and most reported pathological patterns represented end-stage disease. Surgical biopsies were rarely undertaken in clinical practice, and until the 1940s there were few attempts at percutaneous needle biopsy. Notable among the successful early biopsies was that of Stockholm orthopedist Johann Henning Waldenström (1877–1972) in 1928, during the evaluation of a patient with amyloidosis [26]. Waldenström was the father of Jan Gosta Waldenström (1906–1996) [27], who described his eponymous macroglobulinemia in 1944 [28].

A new era in renal pathology arrived when Poul Iversen and Claus Brun in Copenhagen developed a reproducible technique of percutaneous biopsy of the kidney in early 1949, publishing their results (with an initial 50% success rate) to global interest in 1951 [29]. Biopsy technique was refined in 1954 by Robert C. Muehrcke (1921–) and Robert M. Kark (1911–2002) in Chicago, who used a style of needle (Vim-Silverman) that is still common today (fig. 5). Kark and Muehrcke also obtained biopsies with patients in the prone position, resulting in a much greater degree of success [30]. Much later, ultrasound guidance of the biopsy needle made this procedure even safer.

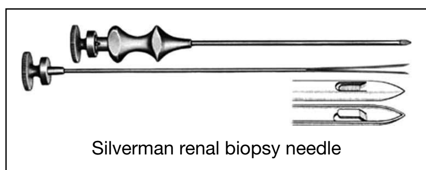

Fig. 5. Diagram of a Silverman needle for percutaneous renal biopsy.

Evolution of Nephrology

The advent of hemodialysis as a 'renal replacement therapy' is chiefly the legacy of Willem Kolff (1911–), a true medical pioneer who used scavenged parts to build the first rudimentary dialysis machines in the Netherlands at the end of the Second World War, working under extremely difficult conditions [31]. Percutaneous biopsy coupled with hemodialysis led directly to the development of the medical specialty of clinical nephrology, since by the 1950s both a technically challenging treatment (dialysis) as well as a new specialized diagnostic procedure (biopsy) were available. In 1965, the American College of Physicians recognized the discipline of nephrology as a subspecialty of internal medicine [1]. The American Society of Nephrology was founded in 1966, and the nephrology subspecialty board offered its first certifying examination in 1972 [1].

Electron microscopy was added to the armamentarium of renal pathologists in the late 1950s [32], contemporary with immunofluorescence [33] and silver staining of ultrathin tissue sections [34]. Together, these valuable tools led to more refined characterization of the diverse pathologies that had once been grouped together simply as 'Bright's disease', as well as recognition of several specific patterns associated with plasma cell dyscrasias [3, 35].

Multiple Myeloma and the Kidney

The first clear recognition of a plasma cell disorder as a unique clinicopathological entity resulted from detailed observations of a single patient, the English grocer Mr. Thomas McBean, from the time his initial symptoms in 1843 until his death at the age of 45 on January 1, 1846 [36, 37]. Dr. William Macintyre (1791–1857), a Harley Street practitioner, examined a urine specimen from Mr. McBean, who had complained to Macintyre that his body linen was stiffened by his urine. Macintyre found no evidence of sugar in the urine, but observed a strange precipitate [38]. Dr. Thomas Watson, another leading physician in London, saw the patient in consultation at Macintyre's request. Watson sent the following note with some urine samples to Henry Bence Jones (1813–1873) (fig. 6) [39], then a 31-year-old physician with a growing reputation as a chemical pathologist, who was working at St. George's Hospital at Hyde Park Corner:

Fig. 6. A portrait of Henry Bence Jones in the late 19th century, several decades after his description of what became known as Bence Jones protein.

Saturday, November 1, 1845
Dear Dr. Jones,
This tube contains urine of a very high specific gravity. When boiled, it becomes highly opake. On the addition of nitric acid it effervesces, assumes a reddish hue, and becomes quite clear, but as it cools, assumes the consistence and appearance which you see. Heat re-liquefies it. What is it? [11, 40].

Bence Jones corroborated Watson's findings that the addition of nitric acid produced a strange precipitate in the urine that was re-dissolved by heat, and that this precipitate formed again upon cooling. He credited Macintyre with first observing this peculiar property of the urine [11]. An exhaustive series of chemical analyses including evaporation, precipitation, filtration, and various solubility tests suggested to Bence Jones that this substance was probably a new form of protein, which he believed to be a 'hydrated deutoxide of albumen' [11]. Ordinary albumin did not react with nitric acid the way this new substance

did. While the substance was somewhat akin to 'hydrated tritoxide of protein' that had been reported in the 'inflammatory crust of the blood' by the Dutch chemist Gerardus Mulder (1802–1880) in 1838 [41], Mulder's substance contained no sulfur or phosphorous, and did not precipitate with 'ferro-prussiate of potash' (potassium ferricyanide).

Despite Mr. McBean's heavy proteinuria, at autopsy his kidneys appeared normal, both grossly and microscopically. Amyloidosis was well-recognized by that time (as 'lardaceous change' – see more below), and thus it is unlikely that Mr. McBean's kidneys were involved by amyloidosis. Macintyre wrote that in this case, the kidneys had 'proved equal to the novel office assigned them', and had 'discharged the task without sustaining, on their part, the slightest danger' [38].

Mr. McBean was considered by his physicians to have suffered from an unusual variant of 'mollities and fragilitas ossium', terms that were used in the 19th century to refer to any condition of bone fragility, including osteogenesis imperfecta and various other congenital or acquired osteopenias. John Dalrymple (1803–1852), surgeon to the Royal Ophthalmic Hospital in Moorfields, England, recognized at autopsy that McBean's peculiar form of mollities ossium was due to replacement of bone by a 'gelatin-form substance of a blood red color and unctuous feel' [42]. Dalrymple also noted that the disease had apparently begun in the cancellous bone, and then grew and produced regularly-sized, round, dark red projections that were visible through the periosteum. Microscopic examination revealed strange new cells; illustrations from his report are recognizable as plasma cells.

Some earlier cases of mollities ossium are also consistent with potential multiple myeloma, notably that of 39-year-old Sarah Newbury, the second patient described by Samuel Solly, a distinguished London surgeon, in a case series published in 1844 [43]. Mrs. Newbury suffered excruciating bone pain with fractures in her thighs, clavicles, humerus, radius and ulna. Autopsy findings revealed that the cancellous portion of her sternum had been replaced by a red substance, which Macintyre reported to be similar to the red substance seen in the bones of Mr. McBean [38].

In 1867, Herman Weber (1823–1918), a German physician working in London (and the father of the more famous Frederick Parkes Weber, who gave his name to many clinical conditions, including Sturge-Weber, Osler-Weber-Rendu, and Klippel-Trenaunay-Weber syndromes), described a 40-year-old man with mollities ossium and amyloidosis who had suffered severe sternal and lumbar pain [44]. This was the first time a connection was made between myeloma and amyloidosis. In this case, the amyloid deposits were found in the kidneys and spleen.

The term 'multiple myeloma' was introduced in 1873 by J. von Rustizky in Kiev, of whom little is known other than that he had once worked in Friedrich von

Recklinghausen's laboratory in Strasbourg. During an autopsy, von Rustizky noted eight separate tumors of the bone marrow in the patient, which he called 'multiple myelomas' [45]. The report did not comment on whether Bence Jones proteinuria was found during life.

In 1889, Otto Kahler (1849–1893), an internist from Prague working in Vienna, described the case of a 46-year-old physician named Dr. Loos, who developed pain in his right upper thoracic area aggravated by taking a deep breath, followed by intermittent but severe pains in multiple areas of the body [46]. Albuminuria was first noted in Dr. Loos in 1881, 2 years after the initial bone pain and anemia. The patient lived for 8 years after his first symptoms. Autopsy findings included large round cells in the ribs and thoracic vertebrae. Kahler recognized that the urinary protein excreted by Dr. Loos had the same characteristics as that described by Bence Jones.

The first recognized case of multiple myeloma in the United States followed shortly thereafter, reported in 1894 by James Bryan Herrick (1861–1954) and Ludvig Hektoen at Rush Medical College in Chicago [47]. Remarkably, Herrick is also credited with the first description of sickle cell anemia, as well as being the first to link angina with acute coronary syndromes [48, 49].

The nature and origin of the protein described by Bence Jones were completely mysterious until the middle of the 20th century. Proteinuria had been recognized centuries before Bright and Bence Jones arrived on the scene, but had long been thought to be identical with albuminuria [50, 51]. For instance, Frederick Dekkers (1648–1720) in Leiden noted in 1694 that the urine of consumptives became milky when placed over heat [52]. However, it is not clear whether Dekkers was truly describing albuminuria, or whether he was just reflecting contemporary ideas of transformation of food in the stomach, followed by further changes in the liver and then the kidneys – theories popularized by the brilliant but eccentric alchemist Theophrastus Bombastus von Hohenheim (Paracelsus) (1493–1541) in a 1527 essay [53]. Domenico Cotugno (1736–1822) of Bari, Italy – physician to the King of the Two Sicilies – in 1765 described a case of what is quite clearly nephrotic syndrome following a quotidian fever, probably malaria, which was treated with quinine [54]. Cotugno refers to 'urine, which is well known not to be coaguable', and goes on to describe the case of an edematous soldier whose urine, when exposed to fire, turned into 'a white mass, like egg albumin' – the same observation exploited by Bright 60 years later [51] William Cruickshank (? – approximately 1811), a Scotsman who was an ordinance chemist working at the Woolwich Arsenal in southeast London, noted that 'in some diseases; however, particularly general dropsy or anasarca, nitrous acid...produces a milkiness and in some cases a coagulation similar to...the serum of the blood...in morbid states of the urine, is detected...even by heat'. Three decades before Bright, Cruickshank had

noted that in the forms of dropsy associated with diseases of the liver or heart, the urine did not demonstrate this feature [55]. The clinical specimens were provided to Cruickshank by the Surgeon General of the Royal Artillery, John Rollo; the two also collaborated on the discovery of the element strontium [51].

Although initially myeloma proteins were felt to be harmless to the kidney, it was soon apparent that this sanguine view was false. In 1909, Alfred von Decastello (1872–?) in Vienna described an association between myeloma and tubular plugging by an amorphous substance. This syndrome became known as cast nephropathy, or 'myeloma kidney' [56]. (Decastello, a colleague of Karl Landsteiner, also described the AB blood group). By the early 1920s, most medical experts accepted the concept that Bence Jones proteins could be nephrotoxic [35, 57], but the origin of the protein was still unclear. In 1899, A. Ellinger in Germany suggested that there might be an abnormal protein in the blood in patients with myeloma that was similar to the Bence Jones protein, but the evidence he provided was not conclusive [4]. A Bence Jones-like protein was more convincingly detected in the blood in 1929 [58]. Arne Tiselius (1902–1971) in Uppsala, Sweden, a 1948 Nobel Laureate in Chemistry, reported an improved method of serum electrophoresis in 1937, which allowed separation of serum globulins into three components: alpha, beta, and gamma [59]. In 1939, Tiselius isolated antibody activity in the gamma fraction [60], while Lewis Longsworth (1904–1981) and his colleagues at the Rockefeller Institute used Tiselius' electrophoretic techniques and first noted the classic myeloma 'M-spike' in that same year [61].

Beginning in the 1930s, there was considerable debate between two schools of thought on the origin of Bence Jones protein. The first theory, championed by Adolf Magnus-Levy (1865–1955) in Berlin [62], held that proteinaceous materials found in the distal nephron were simply the result of overproduction of normal serum proteins by the bone marrow. The contrary view was held by Maxwell Wintrobe (1901–1986) and M.V. Buell at Johns Hopkins in Baltimore. In a description of the phenomenon of cryoprecipitation in 1933, Wintrobe and Buell championed the belief that the pathologic proteins in plasma cell disorders were distinct from all normal serum components [63]. The clinical syndrome of cryoglobulinemia was named by A.B. Lerner and C.J. Watson in 1947 [64].

In the early 1950s, protein chemist Frank W. Putnam at the University of Chicago performed a series of experiments in myeloma patients with radioisotopes, which helped clarify the origin of Bence Jones proteins. Putnam first showed that the Bence Jones proteins from 18 different patients with myeloma were all unique, though they clustered into two antigenic groups. In 1955, Putnam and Hardy [65] showed that Bence Jones proteins derived directly from the body's metabolic pool of nitrogen, rather than being a breakdown product of some sort of plasma precursor. The following year, biochemist Leonhard

Korngold and his assistant Rosa Lipari at Sloan Kettering and Cornell in New York City distinguished two different classes of Bence Jones proteins, and they showed a relationship between these and the serum proteins of multiple myeloma as well as normal globulins [66]. The immunoglobulin light chains are now called kappa and lambda, after Korngold and Lipari's surnames. Stanhope Bayne-Jones (1888–1970) and D.W. Wilson at Johns Hopkins had recognized two or three distinct groups of Bence Jones proteins as early as 1922, but had not shown their relationship to serum proteins [67].

In 1962, Gerald M. Edelman (1929–) and Joseph A. Gally at the Rockefeller Institute in New York conclusively demonstrated that light chains prepared from serum immunoglobulin, myeloma proteins, and Bence Jones proteins from the same patient's urine were identical in all respects: in molecular weight, amino acid sequence, chromatographic appearance, and thermal solubility [68]. The immunoglobulin light chains precipitated at temperatures between 40°C and 60°C, and dissolved upon boiling, just as Bence Jones had described more than a century earlier. Edelman shared the 1972 Nobel Prize for his 'discoveries concerning the chemical structure of antibodies'.

By the early 1960s, the harmful nature of myeloma proteins to the proximal tubules of the kidney was well established, and investigators began to recognize additional injury patterns beyond cast nephropathy. The first description of an association between myeloma and acquired Fanconi syndrome (i.e., defective proximal tubular reabsorption) came in 1963 [69]. Tubular cast formation in myeloma was correlated with the degree of renal failure in the late 1970s. Non-amyloid glomerulopathies were also first described in this era. In 1957, Sidney Kobernick and J.H. Whiteside at McGill University in Montreal first documented glomerular abnormalities in myeloma patients, including patterns of nodular glomerulosclerosis that resembled lesions seen in diabetics [70]. Other groups quickly confirmed this observation [71]. R.E. Randall [72] at the University of St. Andrews in Scotland reported light chain deposition disease in 1976. Non-amyloidogenic light chains in myeloma are sometimes called 'Randall-type'. A much rarer condition, heavy chain deposition disease, was recognized in the early 1990s by Pierre Aucouturier and his colleagues in Poitiers, France [73]. In 1984, myeloma was linked to cases of rapidly progressive ('crescentic') glomerulopathy [74], and Mayo Clinic investigators reported a case series of monoclonal gammopathy-associated focal and segmental glomerulosclerosis in 2005 [75].

The Kidney in Systemic Amyloidosis

The word 'amyloid' (starch-like) was coined in the late 1830s by the German botanist Matthias Schleiden (1804–1881) of the University of Jena, in

his seminal formulation of the cellular theory of life [76], a theory that was extended to animal tissues by his colleague and compatriot Theodore Schwann (1810–1882). The word amyloid derives from the Latin word *amylum*, and is a transliteration of the Greek *αμυλ-ον*, a term that meant 'not ground at the mill' and that referred to fine grains, especially starch.

Amidon and *amydon* were used as cooking terms for starch in the Medieval period. Starch – a complex carbohydrate that is insoluble in water – is a combination of two polymeric carbohydrates: amylose and amylopectin. Starch is distinct from *cellulose*, a word once used as a synonym for 'cellular' in the sense of compartmentalized, but which after the 1830s saw more specific use to refer to a long-chain polymer of beta glucose that forms the primary structural component of plants, and is indigestible by humans. Cellulose was first noted to be common in plant cell walls in 1838 [76].

Antoine Portal (1742–1832), physician to Charles X and Louis XVIII of France, was probably the first to describe a substance similar to lard in the liver of an elderly woman in 1787, and later in the liver of an 8-year-old boy; Portal also noted kidneys 'three times fattier than normal' [77, 78]. Because the material from lardaceous liver hardened like albumin when exposed to heat, Portal thought that his patients had some sort of albuminous obstruction. In 1842, the Bohemian physician Carl Freiherr von Rokitansky (1804–1882), working at the University of Vienna, described in his widely-read pathological handbook [79] the condition of lardaceous liver enlargement as a result of infiltration by an albuminous, gelatinous, grayish material. He noted that there was an association between the presence of this material and scrofula, rickets, syphilis, or mercury use. Rokitansky also stated that the finding of lardaceous liver was, '*nicht gar zu selten die Bright'sche Nierenkrankenheit und deiser verwandte Züstande der Nieren Combinirt*' (page 312, volume 3 of the *Handbuch*) – i.e., that not uncommonly, fatty degeneration of the liver was associated with Bright's disease of the kidney or various other renal conditions.

In 1854, Rudolf Virchow (1821–1902) in Berlin noted that the corpora amylacea – tiny degenerative bodies in the nervous system that had been described by Czech anatomist Jan Evangelista Purkyně (1787–1869) – stained a peculiar violet-brown color with iodine and dilute sulfuric acid [80, 81]. Virchow used the term 'amyloid' for the material that comprised these bodies, because he was convinced that these were identical or at least very similar to cellulose, although the staining pattern was a slightly different color than that seen with starch [82]. Virchow much preferred 'amyloid' to the commonly used terms 'lardaceous' or 'waxy', because the staining pattern suggested to him that the material was carbohydrate and not fat; he often commented that those who used the term 'lardaceous' were not good connoisseurs of bacon. The French school continued to prefer the term 'lardaceous' as described by Rokitansky,

whereas English and Scottish physicians used the term ‘waxy’, in part because noted Edinburgh physician John Abercrombie (1780–1844) in 1828 described the liver in these conditions as a uniform dull yellow that closely resembled the color of impure beeswax [83, 84].

Virchow’s ideas were influential, and he held tenaciously to his original beliefs about cellulose despite growing contradictory evidence, probably because he was convinced he had found a fundamental and previously unrecognized connection between animals and plants, unifying these two great kingdoms of life [82]. It is a consequence of Virchow’s considerable prestige that the somewhat inaccurate term ‘amyloid’ persists, even though it was subsequently demonstrated even in his lifetime that the degenerative substance was not a typical carbohydrate, nor was it likely to be a fat. For instance, German organic chemists Friedrich August Kekulé (1829–1896) and Nikolaus Friedreich (1825–1882) noted that the ‘lardaceous’ material was actually nitrogenous [85]. (Kekulé later became famous for his discovery of the cyclic nature of the benzene molecule, an idea that he claimed came to him after a dream in which a snake was seen eating its own tail) George Budd (1808–1882) of King’s College in London performed a similar analysis, using a sample from a patient of his brother William in Bristol – a patient with an enormous, pale, lardaceous liver that turned out to have 3 times as much albumin as fat [86]. Budd also recognized similar changes in the kidney [84, 86]. In 1854, W.T. Gairdner of London came to similar conclusions about the lack of genuine fat in lardaceous degeneration [87].

Primary amyloidosis was probably first reported in 1856, when Samuel Wilks (1824–1911), then at Guy’s Hospital in London [88], described a 52-year-old man with lardaceous viscera, including the kidney, in whom the changes were unrelated to known associated conditions such as syphilis, osteomyelitis, other osseous disease, or tuberculosis. All previously described patients had secondary amyloidosis consequent to chronic inflammation. Wilks’ patient had albuminuria as well as dropsy. Wilks also reported involvement of the adrenal glands with lardaceous disease in 1860, in a patient who had syphilis and lardaceous changes that also affected the liver, kidneys, and spleen [89]. In 1869, William Howship Dickinson (1832–1913) in London described a case of lardaceous disease involving only the kidney, which was attributed to an ovarian abscess [90, 91].

During this era, the ‘sago spleen’ was first noted. Sago is the pith found inside of the stems of some cycad plants of the genesis *Cycas*, the most notable being *Cycas revoluta*. *Segu* is a Malay word; Europeans first encountered this substance in the 1550s during explorations of New Guinea and the Moluccas in the South Pacific. Today, sago continues to be used in steamed puddings and as a thickener for other dishes. It has similar consistency to tapioca, the pith of the cassava plant.

The iodine-sulfuric acid staining method was the initial test for amyloid, most notably used (after Virchow) by Johann Heinrich Meckel von Hemsbach (1822–1856) [92], whom Virchow succeeded as prosector at the Charité Hospital in Berlin following Meckel's untimely death from tuberculosis. Meckel annoyed Virchow by holding the view that the lardaceous deposits consisted of 'cholesterine' (an older term for cholesterol), and that they were not the result of cellulose degeneration.

Metachromatic stains for amyloidosis, an improvement over less specific iodine techniques, were introduced in 1875 [82, 93]. The aniline dyes methyl violet and crystal violet produce characteristic color changes in amyloid tissues [94]. Congo red, another aniline compound (an azo dye), was first introduced in the 1880s as a textile dye [95]. Hans-Hermann Bennhold (1893–1976) in Tübingen noted its relative specificity for amyloid in 1922, and intravenous Congo red infusions became an early diagnostic test for amyloidosis [96]. In 1927, psychiatrist Paul Divry (1889–1967) and biochemist Marcel Florkin (1900–1979) in Liége, Belgium, recognized the specific apple green birefringence of amyloid fibrils when stained with Congo red and viewed under polarized light [97]. The fibrillary structure of both primary and secondary amyloid proteins were reported in 1959 by Alan S. Cohen and Evan Calkins (both fl. 2006) in Boston, and resulted from detailed electron microscopic observations [98, 99].

As with most conditions, it is possible to find early case reports potentially consistent with amyloidosis [92]. For instance, in 1639, the Belgian physician Nicolaus Fontanus (Fonteyn; Nicolao Fontano) in his monograph *Responsionum & Curationum Medicinalium Liber Unus* described a patient with ascites, jaundice, epistaxis with an abscess in the liver and a large spleen filled with strange white stones [100]. There is no mention of kidney anomalies. In 1654, Thomas Bartholin (1616–1680) of Copenhagen, the discoverer of the lymphatic system, described the autopsy of a woman whose spleen resembled indurated flesh in his *Historiarum Anatomicarum Rariorum Centuria* [101]. Bartholin reported that her spleen was so hard that it could scarcely be cut with a knife, and incision of the spleen produced a sound like that of cutting spongy timbers. However, it is not possible to be certain about either of these patients, because of the brief description and lack of special stains at the time.

In 1929, the Soviet physician Mikhail Innokentevich Arinkin (1876–1949) in Leningrad reported aspiration of sternal bone marrow in life. An earlier report of marrow aspiration in 1908 by Giovanni Ghedini in Italy had gone largely unheeded, in part because Ghedini chose to aspirate the tibia, which was less representative of the overall marrow architecture than marrow from the axial skeleton [102–104]. Bone marrow examination subsequently became routine in the evaluation of blood disorders, and it is now common to stain bone marrow with Bennhold's Congo red to determine the presence of amyloid

within the marrow, including in the blood vessels. Even though the diagnosis of amyloidosis can usually be made by examining other tissues, renal biopsy is still often necessary to determine the specific pathological pattern in the kidney.

Conclusion

Injury to the kidney is one of the cardinal features of plasma cell disorders, and an unusual case of proteinuria led to the first clear description of one of these hematological malignancies in the 1840s. Myeloma cast nephropathy and amyloid deposition are the most common renal damage patterns, but several other types of renal dysfunction exist. The history of evolving understanding of this group of conditions illustrates the importance of careful bedside observation and clinical reasoning, supported by developments in basic sciences.

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Pathologic Studies Useful for the Diagnosis and Monitoring of Plasma Cell Dyscrasias

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Abstract

The pathologic diagnosis of multiple myeloma and other plasma cell dyscrasias includes histopathologic examination of a bone marrow aspirate and biopsy and clinical laboratory tests to assess end organ damage and other prognostic information. Plasma cell dyscrasias are characteristically classified and staged based on the results of these pathologic studies in conjunction with other clinical and radiologic parameters. New staging systems such as the International Staging System (ISS) use readily available laboratory tests to stratify prognostic subgroups. The continued introduction of new laboratory assays will help improve our understanding of plasma cell dyscrasias and the therapeutic management of these patients.

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The pathologic diagnosis of multiple myeloma and other plasma cell dyscrasias includes a bone marrow aspirate and biopsy to assess the percentage of plasma cells and clinical laboratory tests to assess end organ damage and other prognostic information. Plasma cell dyscrasias result from a clonal expansion of neoplastic plasma cells that usually secrete a monoclonal protein or M-component. These diseases are characteristically classified and staged based on the results of pathologic studies and other clinical and radiologic parameters. The World Health Organization classification of 'plasma cell neoplasms' is shown in table 1 [1].

The diagnosis of multiple myeloma requires evaluation of a Wright-Giemsa stained bone marrow aspirate and a hematoxylin and eosin stained core biopsy section. Current diagnostic criteria, shown in table 2, include a minimum of one major and one minor criteria [1]. Alternatively, three minor criteria

Table 1. WHO classification of plasma cell neoplasms [1]

Plasma cell myeloma
- Indolent myeloma
- Smoldering myeloma
- Non-secretory myeloma
- Plasma cell leukemia

Osteosclerotic Myeloma (POEMS syndrome)

Immunoglobulin deposition diseases
- Primary amyloidosis
- Systemic light and heavy chain deposition diseases

Heavy chain diseases (HCD)
- γ-HCD
- μ-HCD
- α-HCD

Plasmacytoma
- Solitary plasmacytoma of bone
- Extramedullary plasmacytoma

Table 2. Diagnostic criteria for multiple myeloma

Major criteria[1]
- Marrow plasmacytosis >30%
- Plasmacytoma
- M-component
  - Serum IgG >3.5 g/dl or IgA >2 g/dl
- Urine Bence-Jones protein >1 g/24 h

Minor criteria[1]
- Marrow plasmacytosis of 10–30%
- M-component present
- Lytic bone lesions
- Immunoglobulin levels reduced to less than 50% of normal

[1]The diagnosis of multiple myeloma requires one major and one minor criteria or three minor criteria (must include marrow plasmacytosis and a M-component).

which must include marrow plasmacytosis and presence of a M-component are sufficient for a diagnosis of multiple myeloma. On bone marrow aspirate (fig. 1) and biopsy (fig. 2), an increase in plasma cells is usually evident. The morphology of these cells is variable. The neoplastic cells may resemble benign mature

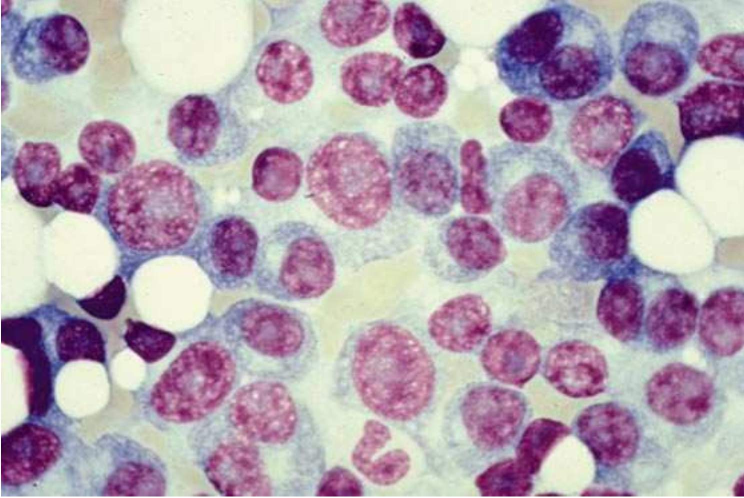

Fig. 1. Bone marrow aspirate smear with marked plasmacytosis and some proplasmacytic forms (Wright-Giemsa, original magnification ×400).

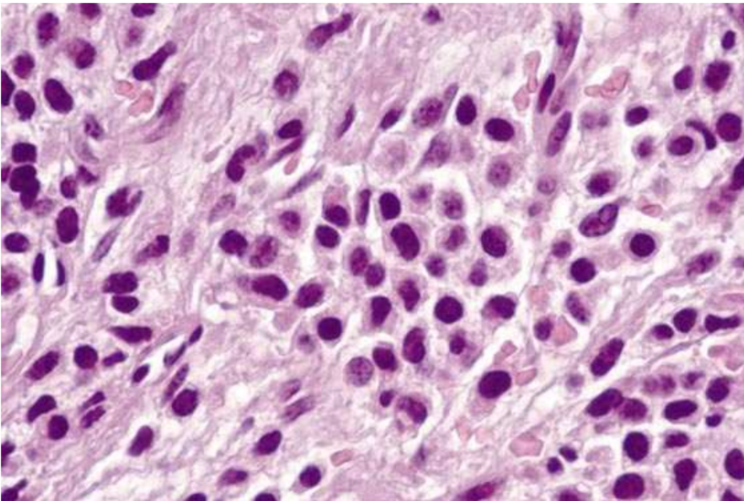

Fig. 2. Bone marrow core biopsy replaced by myeloma cells with associated myelofibrosis (hematoxylin and eosin, original magnification ×400).

plasma cells. Atypical forms may include immature plasma cells with prominent nucleoli, pleomorphic plasma cells including multinucleated and polylobated forms, and even anaplastic cells (fig. 3) not easily identified as plasma cells. Some patients have immature plasma cells with blastic features

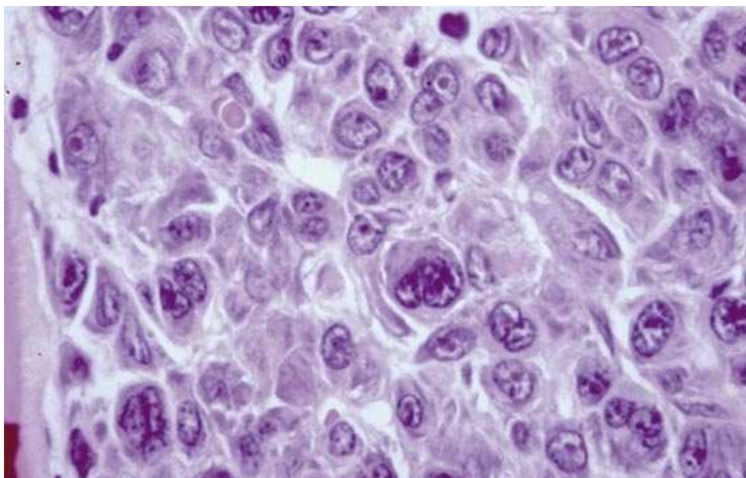

Fig. 3. Bone marrow core biopsy with anaplastic multiple myeloma (hematoxylin and eosin, original magnification ×400).

(plasmablasts). Cytoplasmic immunoglobulin in the plasma cells may result in Mott, Russell, and flame cell formation. An accompanying proliferation of histiocytes resembling Gaucher cells and crystalline structures may be observed. The plasma cells may be distributed in the bone marrow in small clusters, but sheets of plasma cells may also be evident. Early studies demonstrated that plasmablastic morphology and a diffuse pattern of marrow infiltration are usually associated with a poor prognosis [2, 3].

The percentage of bone marrow plasma cells is determined from morphologic examination with or without the use of immunohistochemistry. A bone marrow aspirate differential may underestimate the degree of bone marrow plasmacytosis, depending on the site of aspiration. Immunoperoxidase stains of the core biopsy for CD38 and CD138 (fig. 4) allow easy quantitation of the percentage of plasma cells. Immunohistochemistry is a good method for estimating tumor burden, and is particularly helpful in cases with only a mild plasmacytosis. When greater than 30% of the marrow cellularity is replaced by plasma cells, a diagnosis of multiple myeloma is expected. Although reactive plasmacytosis can rarely be associated with plasma cell infiltrates that comprise over 30% of the marrow cellularity, detection of large aggregates comprised of more than 10 plasma cells, cytologic atypia, and documentation of clonality are morphologic features that indicate a neoplastic process. Clonality may be confirmed on core biopsy recuts utilizing immunoperoxidase stains (fig. 5) or in situ hybridization

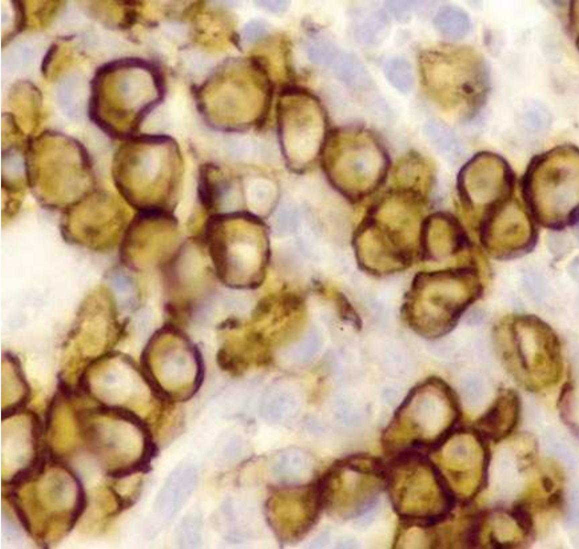

Fig. 4. CD38 positive myeloma cells comprise greater than 75% of the marrow cellularity on the immunoperoxidase stain (original magnification ×400).

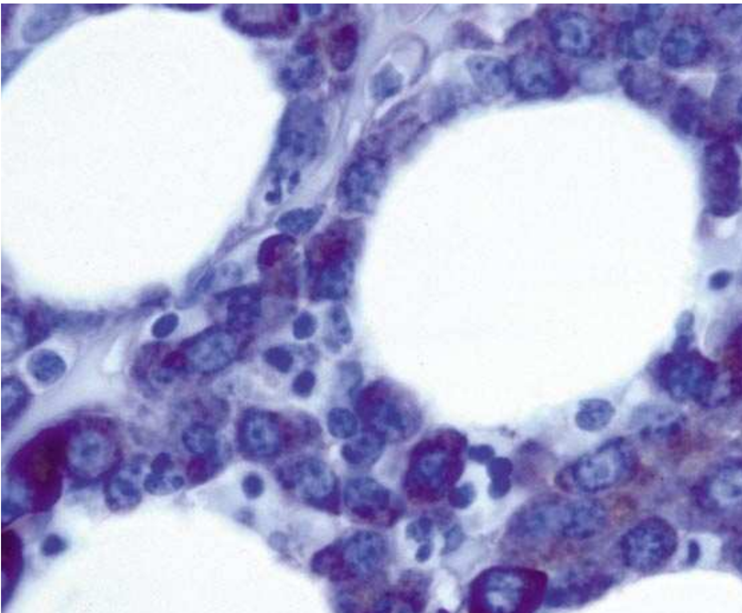

Fig. 5. Immunoperoxidase stain demonstrating κ light chain restriction in the myeloma cells (original magnification ×400).

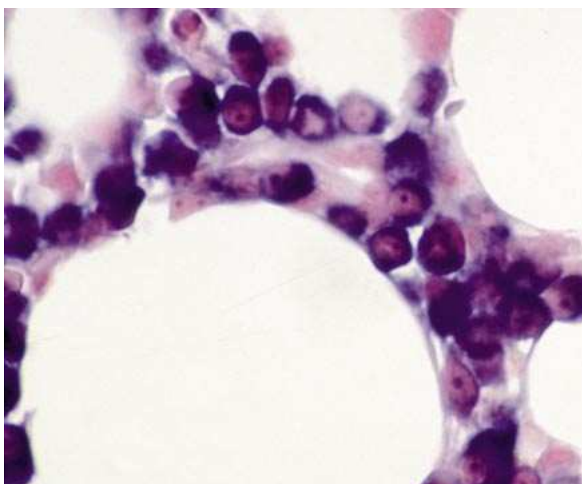

Fig. 6. In situ hybridization probe demonstrating κ light chain restriction in the myeloma cells (original magnification ×400).

probes (fig. 6) for κ and λ. Light chain restriction or clonality is often defined as a κ:λ ratio of greater than 5:1 or less than 1:2. In situ hybridization probes are characteristically easier to read and have less background staining. In rare cases, the neoplastic plasma cells are negative on in situ hybridization staining but have documented clonality on immunoperoxidase staining. Rarely, clonality cannot be assessed by either staining method. In these cases, clonality must be evaluated on flow cytometric or molecular genetic studies.

The plasma cell neoplasms are typically characterized by the secretion of a monoclonal immunoglobulin, often referred to as a M-component. A monoclonal immunoglobulin is found in the serum and/or urine of approximately 99% of patients. Serum protein electrophoresis (fig. 7) performed on agarose or cellulose acetate characteristically reveals a peak or restriction band in the γ region in these patients. The quantity of the monoclonal protein is determined by densitometry. On immunofixation (fig. 8) or immunoelectrophoresis, the monoclonal immunoglobulin can be characterized by identification of the heavy and light chain isotypes. The monoclonal protein is IgG in approximately 50% of cases. Monoclonal IgA accounts for approximately 20% of cases, followed by monoclonal light chains in 15% of cases, and IgD in 2% of cases. In rare patients, two immunoglobulin clones are identified (diclonal gammopathy). The type of monoclonal protein produced has prognostic significance with light chain only patients having the worst median survival and IgA patients having a

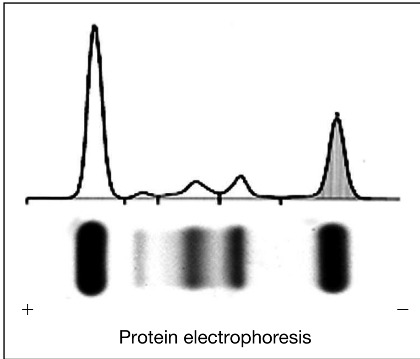

Fig. 7. Serum protein electrophoresis demonstrating a major restriction band in the γ region. Densitometric scan reveals that this M-component comprises approximately 27% of the total protein (Courtesy of Angela Grantham).

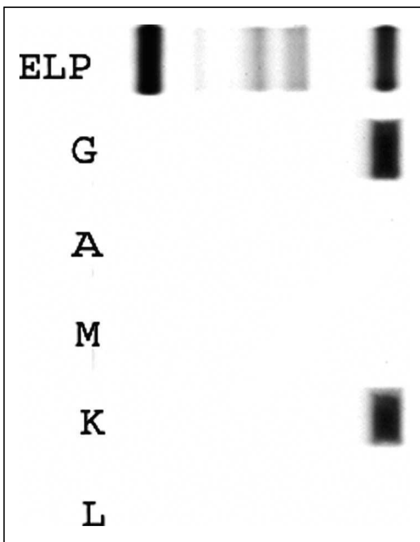

Fig. 8. Immunofixation electrophoresis reveals that the monoclonal protein is IgG κ (Courtesy of Angela Grantham).

worse median survival than patients with a monoclonal IgG [4]. In approximately 1% of patients, the neoplastic plasma cells do not secrete immunoglobulin. This may be due to plasma cells that synthesize, but do not secrete immunoglobulin or plasma cells that do not synthesize immunoglobulin. Quantitation of immunoglobulin levels, preferably by nephelometry, is useful for evaluating the amount of monoclonal protein, the levels of residual polyclonal immunoglobulins, and monitoring response to treatment. Production of normal immunoglobulins is usually decreased in multiple myeloma due to displacement of normal cells by neoplastic plasma cells. A greater than 50%

reduction in normal serum immunoglobulin levels is often evident, resulting in recurrent infections. Rarely, immunoglobulin levels are normal. When evaluating monoclonal proteins in urine, a 24-hour urine collection is essential. The total amount of protein excreted per day is calculated. An aliquot of the sample is utilized for protein electrophoresis and immunofixation. The amount of the monoclonal protein in urine can be estimated from the percentage of monoclonal protein in the aliquot and the total 24-hour urine protein.

An immunoassay for serum free light chains allows quantitation of κ and λ light chains that are in circulation, but are not bound to immunoglobulin heavy chains. Quantitation of κ and λ free light chains and determination of a κ:λ ratio is a sensitive and specific method for detecting free light chain diseases [5] such as primary systemic amyloidosis, light chain deposition disease, non-secretory multiple myeloma, and light chain multiple myeloma. The combination of serum protein electrophoresis and immunofixation with the quantitative free light chain assay improves detection of some patients with plasma cell dyscrasias. The quantitative free light chain assay is also useful for monitoring treated patients.

Assessment of hematologic parameters in patients with plasma cell dyscrasias typically includes a complete blood count with differential and peripheral blood smear examination. Anemia often occurs, a result of marrow effacement and decreased erythropoietin production by damaged kidneys. The anemia is usually normocytic and normochromic and often moderate in severity. Peripheral blood leukocyte counts are usually normal and thrombocytopenia is uncommon. Increasing numbers of plasma cells in the peripheral blood are usually associated with advanced stages of disease, and leukemic myelomatosis (fig. 9) is uncommon. Hypergammaglobulinemia is often associated with rouleaux (fig. 10) formation on the peripheral blood smear as well as an increased erythrocyte sedimentation rate. Rarely, excess immunoglobulin produced results in an increased serum viscosity and hyperviscosity syndrome. Monoclonal proteins can interfere with the function of coagulation factors and result in a prolongation of the prothrombin time and activated partial thromboplastin time that may be associated with bleeding complications.

Numerous biochemical laboratory studies are essential for the diagnosis and staging of patients with plasma cell dyscrasias and the monitoring of their response to treatment. Monoclonal light chains in the urine (Bence-Jones protein) cause tubular damage that leads to renal failure. Renal function has a major impact on prognosis [6]. Approximately 25% of patients have creatinine levels >2 mg/dl at diagnosis and many more develop renal insufficiency as their disease progresses. The skeletal destruction that occurs in multiple myeloma frequently results in hypercalcemia. Hyperuricemia is also noted in up to one-third of patients. Increased serum lactate dehydrogenase (LDH) level, a result of cell turnover, is characteristically seen in aggressive forms of the

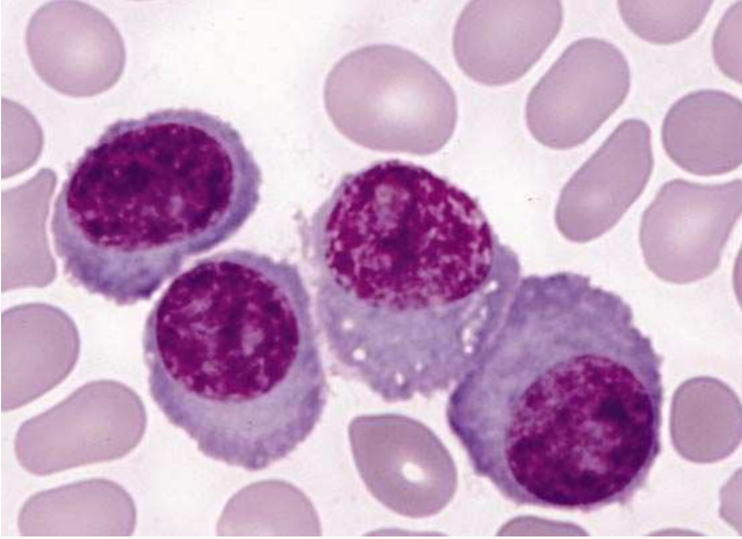

Fig. 9. Peripheral blood smear with secondary plasma cell leukemia or 'leukemic myelomatosis' (Wright-Giemsa, original magnification ×400).

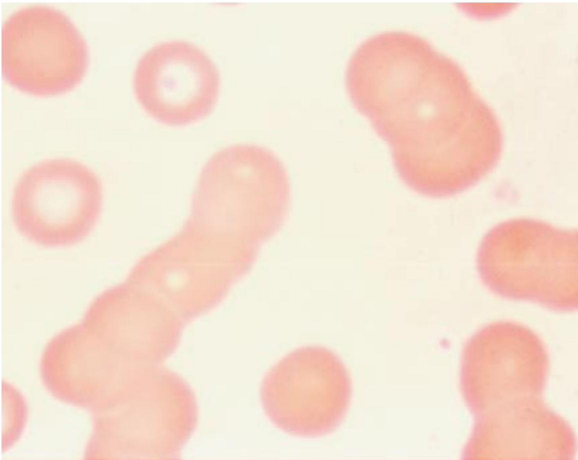

Fig. 10. Peripheral blood smear with rouleaux formation (Wright-Giemsa, original magnification ×400).

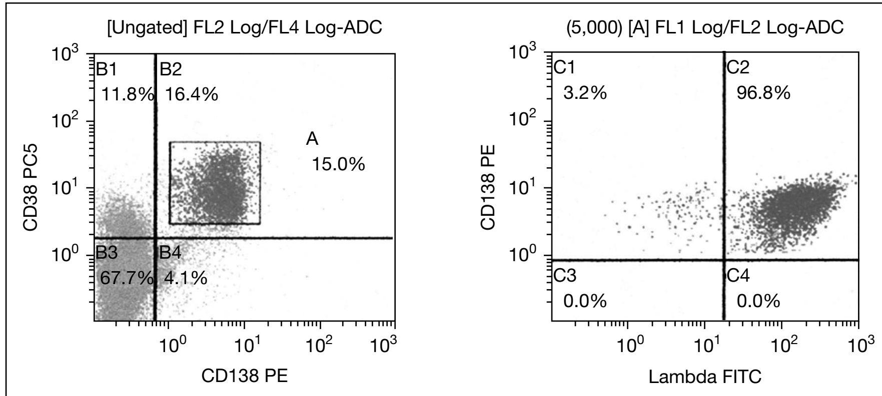

Fig. 11. Flow cytometry studies reveal that CD38 and CD138 positive plasma cells comprise 15% of the marrow cellularity. Lambda light chain restriction is evident (Courtesy of Angela Grantham).

disease. β_2-Microglobulin is an independent prognostic variable in multiple myeloma. Increased β_2-microglobulin is associated with advanced myeloma stage and a poor prognosis. Previous studies have shown however that β_2-microglobulin levels are not helpful for monitoring the course of the disease [7]. Serum albumin is another important prognostic variable. Low serum albumin is associated with rapid disease progression and a poor performance status. The new International Staging System [8] and the previously proposed Southwest Oncology Group stages [9] indicated that β_2-microglobulin and serum albumin are the best markers to stratify prognostic subgroups.

Flow cytometric studies are performed on cell suspensions from bone marrow aspirates collected in EDTA. These studies may also be performed on unfixed core biopsies subjected to vortex disaggregation. Fine needle aspiration is useful for sampling soft tissue lesions. Flow cytometric studies for evaluation of a plasma cell dyscrasia characteristically include (at a minimum) monoclonal antibodies for CD45, CD38, CD138, κ, and λ. Plasma cells characteristically express CD38 and CD138 but do not express CD45. They also express CD19, but do not typically express other mature B cell markers, including CD20 and CD22. Evaluation of light chain expression is performed utilizing cytoplasmic κ and λ and selective gating on CD38 and CD138 positive cells (fig. 11). The majority of neoplastic plasma cells exhibit light chain restriction on cytoplasmic κ and λ studies (a marker of clonality) and two or more aberrant immunophenotypic markers [10]. Other common phenotypic abnormalities include expression of CD56, decreased expression of CD38, and loss of CD19 expression. Less frequently reported abnormalities include expression of CD10, expression of CD20, expression of CD22, or expression

of CD28. Because of these phenotypic abnormalities, coexistence of residual normal plasma cells and neoplastic plasma cells, a common finding in patients with monoclonal gammopathy of undetermined significance (MGUS), can be readily detected. The prognostic significance of the myeloma cell phenotype has been evaluated in multiple studies. An immature phenotype with expression of CD20 and surface immunoglobulin, lack of CD56, or overexpression of CD19, CD28, or CD44 have been associated with a poor prognosis. Adhesion molecules such as CD56 may have an important role in the development of extramedullary disease and plasma cell leukemia [11, 12]. Expression of antigens associated with multidrug resistance, may be associated with a poor response to traditional therapeutic agents. While flow cytometric studies are useful in the identification of plasma cell dyscrasias and monitoring response to treatment [13], these studies often underestimate the percentage of tumor cells and occasionally yield false negative results (unpublished observations). Negative flow cytometric studies should be followed by immunohistochemical analysis of core biopsy recuts in cases of suspected disease. Immunohistochemical evaluation on a core biopsy is the preferred method for estimating tumor burden [13]. Flow cytometric studies can be utilized to identify circulating myeloma cells, which may have prognostic significance in some treatment groups.

Flow cytometry is a useful method for the evaluation of DNA content and plasma cell proliferation. A double staining procedure for plasma cell surface antigens (such as CD38 and/or CD138) and nuclear DNA (utilizing propidium iodide) is used for the specific analysis of DNA content and S-phase in marrow plasma cells. DNA ploidy obtained by flow cytometric studies is expressed as a DNA index. This index is the ratio of the modal fluorescence channel of the G_0/G_1 peak of the myeloma cells and the modal fluorescence channel of normal G_0/G_1 cells present in the sample. A DNA index of 1.0 is considered to be diploid. If the DNA index is above or below 1.0, the tumor cells are said to be aneuploid (hypodiploid – DNA index <0.95 and hyperdiploid – DNA index >1.05). The reported incidence of DNA aneuploidy varies, but is probably seen in approximately 50% of cases with multiple myeloma. Most aneuploid tumors are hyperdiploid and studies indicate that these patients have a better outcome [14]. Hypodiploid tumors, although less common, have a reportedly worse prognosis [15]. The percentage of bone marrow plasma cells in S-phase has been shown to be an independent prognostic factor, with a high number of plasma cells in S-phase ($>2.5\%$) predicting a poor prognosis [16]. Studies have indicated that this measure of proliferative activity is the most important prognostic factor in multiple myeloma patients greater than 65 years of age [17]. Alternatively, proliferative status can be assessed utilizing Ki-67 staining of plasma cells on sections obtained from the paraffin embedded core biopsy. Increased Ki-67 expression is associated with advanced disease.

Conventional cytogenetic studies are performed on bone marrow aspirate collected ideally in sodium heparin. Metaphase cells are analyzed on unstimulated cell cultures utilizing GTG banding. Conventional cytogenetic analysis is difficult due to the low proliferative rate of most neoplastic plasma cells, and metaphases evaluated are often from non-neoplastic bone marrow cells. The utilization of cytokine stimulated bone marrow cultures and the addition of molecular cytogenetic techniques such as fluorescent in situ hybridization (FISH) that eliminate the need for metaphase cells, have increased the availability and clinical utility of genetic information. Structural and numerical chromosomal abnormalities are described in the majority of patients with multiple myeloma, but the prognostic significance of most of these abnormalities is unknown. Complex karyotypes with multiple chromosomal gains and losses are most frequently identified. Gains in chromosomes 3, 5, 7, 9, 11, 15, 19, and 21 are commonly reported. Losses are often reported in chromosomes 13, 17, X, and Y [18]. Translocations, deletions, and mutations are also frequently reported. Translocations involving the immunoglobulin heavy-chain gene (IgH) and various oncogenes are common. Translocations involving the IgH locus on 14q32 are reported in approximately half of the patients with multiple myeloma [18]. Monosomy or partial deletion of chromosome 13 (13q14) is reported in up to 40% of new cases of multiple myeloma, and is associated with a poor prognosis [18, 19]. Translocations such as t(11;14)(q13;q32) involving the *BCL-1* gene; t(4;14)(p16.3;q32) resulting in activation of *FGFR3* and *MMSET* genes; t(14;16)(q32;q23) resulting in activation of *c-MAF*; and t(14;20)(q32;q11) resulting in *MAFB* activation are also associated with a poor prognosis [20]. Deletion of *p53*, located on the short arm of chromosome 17 (17p13.1), is associated with aggressive disease and has been reported with increased frequency in plasma cell leukemia [21]. Deletion of 7q is associated with drug resistance and a poor response to therapy. Amplification of chromosome band 1q21, associated with overexpression of the cell cycle regulator gene CKS1B, is associated with disease progression and a poor prognosis [22, 23]. Trisomies of chromosomes 9 or 19 are associated with a more favorable prognosis. While some abnormalities are detected on conventional cytogenetic studies, many abnormalities may only be detected by molecular genetic studies utilizing techniques such as FISH on interphase nuclei. These studies can be performed on aspirate smears and even paraffin embedded core biopsies. Molecular genetic studies currently performed on cases of suspected or known plasma cell dyscrasias vary, but may includes assays for the following: rearrangements of the (IgH) locus IgH on 14q32; 13q14 deletion; 17p13.1 deletion associated with deletion of *p53*; t(11;14)(q13;q32) involving the *BCL-1* gene; 7q deletion, and assessment of aneuploidy for chromosomes 3, 5, 7, 9, 11, 15, 19, and 21.

FISH studies (fig. 12) have become the preferred method for detecting non-random cytogenetic changes at the time of diagnosis. Other molecular

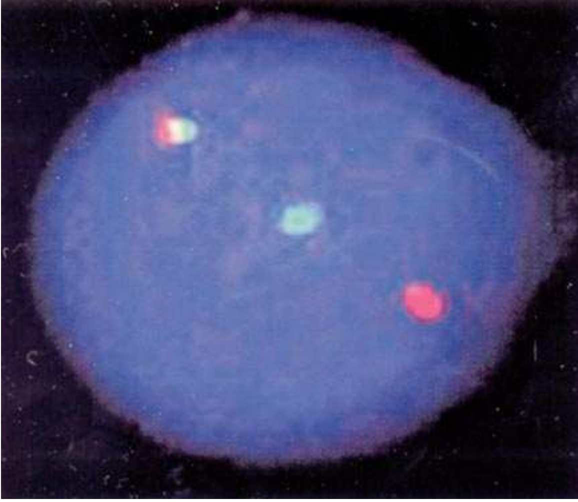

Fig. 12. Fluorescence in-situ hybridization (FISH) demonstrating a translocation involving the immunoglobulin heavy chain gene (IgH) on chromosome 14 at 14q32. The intact gene is indicated by a yellow signal and the rearranged gene by an orange and a green signal.

genetic techniques such as polymerase chain reaction procedures for evaluation of heavy chain and light chain gene rearrangement can be used to document clonality. This technique may be helpful in cases where clonality cannot be demonstrated by other techniques. It can also be useful for documenting residual clonality when evaluating response to treatment.

Evaluation of the results of these pathologic studies in conjunction with other clinical and radiologic findings is essential for the diagnosis of multiple myeloma and other plasma cell dyscrasias. Clinical variants of plasma cell dyscrasias shown in table 3 are classified based on the quantity of the monoclonal immunoglobulin, the presence or absence of lytic bone lesions, and the degree of bone marrow plasmacytosis. Plasma cell leukemia, defined as circulating peripheral blood plasma cells greater than 2.0×10^9/l or 20% of peripheral blood leukocytes, may occur at the time of diagnosis (primary plasma cell leukemia) or may arise late in the course of the disease (secondary plasma cell leukemia, referred to also as 'leukemic myelomatosis'). Plasma cell leukemia is more commonly observed in light chain only disease or in cases of multiple myeloma that secrete IgD or IgE. MGUS is considered to be a precursor lesion to multiple myeloma. These patients have a monoclonal protein but no evidence of multiple myeloma or other lymphoplasmacytic disorders associated with monoclonal protein production. These patients are often asymptomatic and the monoclonal protein is often incidentally discovered. The disorder is most commonly seen in older individuals

Table 3. Comparison of MGUS and multiple myeloma variants

	MGUS	Smoldering myeloma	Indolent myeloma	Multiple myeloma
Bone marrow plasmacytosis	<10%	10–30%	>30%	>30%
Monoclonal protein	IgG < 3.5 g/dl; IgA < 2 g/dl	IgG > 3.5 g/dl; IgA > 2 g/dl	IgG = 3.5–7 g/dl; IgA = 2–5 g/dl	IgG > 3.5 g/dl; IgA > 2 g/dl
Laboratory studies	Normal hemoglobin, serum calcium, and creatinine	Normal hemoglobin, serum calcium, and creatinine	Normal hemoglobin, serum calcium, and creatinine	Anemia, hypercalcemia, and increased creatinine common; Reduced normal immunoglobulins; Bence-Jones protein in urine
Lytic bone lesions	Absent	Absent	≤3 lytic lesions; no compression fractures	Present
Symptoms	None	None	None	Present

and is reported in approximately 3% of patients over 70 years of age. Approximately 25% of the patients diagnosed with MGUS have developed multiple myeloma or other lymphoplasmacytic diseases during a 20-year follow-up [1]. Evolution of the disease typically occurs after approximately 10 years.

Laboratory studies essential for the diagnosis and management of patients with multiple myeloma and other plasma cell neoplasms are outlined in table 4. Cytogenetics and molecular genetic studies such as FISH are clinically useful in the evaluation of prognostic factors that may determine treatment subgroups. The clinical staging of multiple myeloma is typically based on the level and type of the M-component, the presence or absence of lytic bone lesions, and the results of laboratory studies including hemoglobin, serum calcium, and serum creatinine levels. The Durie-Salmon stage introduced in 1975 is shown in table 5 [24]. This system was the standard staging tool used for many years for patient stratification and treatment decisions. Creatinine levels provided the basis for substaging into lower versus higher risk subgroups. Newer staging systems including the Southwest Oncology Group staging system and the International Staging System (table 6) are based on the results of a serum β_2-microglobulin and a serum albumin, two easily obtainable assays [8].

Table 4. Laboratory studies useful in the evaluation of plasma cell dyscrasias

Laboratory test	Clinical significance
Complete blood count	Anemia with hemoglobin <8.5 g/dl is associated with advanced stage of disease
Basic metabolic panel	Increased serum calcium is associated with advanced stage of disease; increased creatinine is a result of renal insufficiency and is associated with a worse prognosis
Serum protein electrophoresis	A monoclonal protein or M-component is found in the serum or urine of the majority of patients
Immunofixation or immunoelectrophoresis	M-component identification in serum or urine including Bence-Jones protein in urine
Immunoglobulin levels	The amount of M-component is useful in the diagnosis of multiple myeloma and its clinical variants; reduced normal immunoglobulins is a minor criteria useful in the diagnosis of multiple myeloma
β_2-microglobulin	Increased β_2-microglobulin is associated with a poor prognosis
Cytogenetics	Complex karyotypes with multiple chromosomal gains and losses are most frequently identified
Molecular genetic studies	Deletions and gene rearrangements are common and often associated with a worse prognosis
Bone marrow aspirate and biopsy	Classification of plasma cell dyscrasias is based on the percentage of plasma cells in the bone marrow
Immunohistochemistry	Stains performed on the core biopsy are useful for quantitation of plasma cells; evaluation of plasma cell proliferation, angiogenesis, and other prognostic factors can also be performed
Flow cytometry	Documentation of clonality and quantitation of plasma cells may be performed, ploidy and S-phase have prognostic significance

Table 5. Durie-Salmon Staging System

Stage	Clinical and laboratory findings
I	• M-component levels: IgG <5 g/dl, IgA <3 g/dl, or urine Bence-Jones protein <4 g/24 h • Bone lesions absent or a solitary lesion evident • Normal laboratory parameters including hemoglobin, serum calcium, and immunoglobulin levels
II	• M-component levels: IgG = 5–7 g/dl, IgA = 3–5 g/dl, or urine Bence-Jones protein 4–12 g/24 h • Lytic bone lesions present • Abnormal laboratory studies including anemia, increased serum calcium, and reduced immunoglobulin levels
III	• M-component levels: IgG > 7 g/dl, IgA > 5 g/dl, or urine Bence-Jones protein >12 g/24 h • Advanced bone disease with multiple lytic lesions • Hemoglobin <8.5 g/dl, serum calcium >12 mg/dl
Subclasses	Based on renal function
• A	• Serum creatinine <2 mg/dl
• B	• Serum creatinine >2 mg/dl

Laboratory studies used in the evaluation of residual disease following treatment are similar to those used at the time of diagnosis. Microscopic examination of a bone marrow aspirate and biopsy for detection of residual disease is often difficult because of the presence of normal plasma cells. Flow cytometry and immunohistochemistry are the most useful methods for identification of residual clonal plasma cells [13].

New laboratory tests and techniques will continue to be evaluated and integrated into routine testing. Serum levels of interleukin (IL)-6, soluble IL-6 receptor, soluble IL-2 receptor, and expression of IL-2 receptors on bone marrow plasma cells or peripheral blood mononuclear cells correlates with disease activity and disease stage. Serum IL-6 and soluble IL-6 receptor levels are increased in up to 50% of patients with multiple myeloma, and high levels of

Table 6. International Staging System (ISS) for multiple myeloma

Stage	Criteria
I	Serum β_2-microglobulin <3.5 mg/l Serum albumin ≥3.5 g/dl
II[1]	• Serum β_2-microglobulin <3.5 mg/l but serum albumin <3.5 g/dl • Serum β_2-microglobulin ≥3.5 mg/l but <5.5 mg/l (irrespective of serum albumin level)
III	Serum β_2-microglobulin ≥5.5 mg/l

[1]Stage II has 2 categories.

serum IL-6 are associated with a poor prognosis [25, 26]. These studies may have potential use in cases where other prognostic markers such as β_2-microglobulin and serum lactate dehydrogenase levels reveal conflicting or borderline results. C-reactive protein, an acute phase reactant, is a reflection of IL-6 activity. C-reactive protein is increased in approximately 40% of patients with multiple myeloma. Evaluation of C-reactive protein at diagnosis provides useful prognostic information. It is an inexpensive and simple assay that can be used instead of serum IL-6 levels [27]. The evaluation of biochemical markers of bone disease such as serum ICTP (carboxy-terminal telopeptide of type-I collage) and urinary Ntx (amino-terminal collagen type-I telopeptide) may lead to new treatment options [28].

Immunohistochemical studies for detection of multi-drug resistance, angiogenesis, or anti-apoptotic factors, although not currently used in routine clinical practice, will provide important prognostic information for determining treatment protocols in the future [29, 30]. New molecular genetic techniques, such as target FISH (cell mapping on a stained slide followed by destaining and FISH analyses of plasma cells) [31] and clone-specific cytoplasmic immunoglobulin staining method coupled with FISH (cIg-FISH) [31] offer new opportunities for monitoring of residual disease. Gene expression profiles improve our understanding of the pathogenesis of these diseases. These molecular insights will lead to new classification schemes, more targeted therapies, and improved outcomes in these patients. The continued introduction of new laboratory and other diagnostic technologies will help to improve our understanding of plasma cell neoplasms and our approach to the management of these patients.

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Serum Free Light Chains in the Diagnosis and Monitoring of Patients with Plasma Cell Dyscrasias

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Abstract

Serum free light chain assay is a recently available test for diagnosis and monitoring of patients with plasma cell dyscrasias. In particular, this test is especially useful in patients that were previously difficult to follow with traditional laboratory methods. These patients include non-secretory multiple myeloma, amyloidosis and light chain deposition disease. In addition other uses for the test include monitoring response to treatment and earlier detection of relapse in all patients with plasma cell dyscrasias. Potential uses include assessing progression of patients with monoclonal gammopathy of undetermined significance, smoldering multiple myeloma, solitary bone plasmacytoma and extramedullary plasmacytoma to multiple meyloma. Analytical considerations for the assay are also discussed.

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Traditional Methodology for Diagnosis and Monitoring of Patients with Plasma Cell Dyscrasias

The first step in the laboratory evaluation of a patient with a suspected plasma cell dyscrasia has traditionally been electrophoresis of serum and urine. Electrophoresis should be considered for any patient that presents with one or more of the following symptoms or laboratory findings: unexplained anemia, elevated erythrocyte sedimentation rate, back pain, osteoporosis or lytic bone lesions, renal insufficiency, hypercalcemia, deficiency of polyclonal immunoglobulins, recurrent infections or fatigue [1]. The majority of plasma cell dyscrasias are associated with a monoclonal immunoglobulin detectable by electrophoresis. The monoclonal protein or 'M' protein is an immunologically homogeneous protein produced by a single proliferating clone of plasma cells. The 'M' protein is

composed of two identical heavy chains of the same class and subclass and two light chains of the same type. The isotypes of the heavy chains are designated by Greek letters, with the corresponding intact immunoglobulin designated by the appropriate Roman capital letter. The isotypes are gamma (γ) in IgG, mu (μ) in IgM, alpha (α) in IgA, delta (δ) in IgD, and epsilon (ε) in IgE. The two types of light chains are lambda (λ) and kappa (κ). High resolution electrophoresis should be performed due to its ability to detect small M-proteins in the α2, β_1, β_2 or γ region. The 'M' protein migrates as a localized band on agarose gel electrophoresis and appears as a localized spike on the densitometry tracing. If an 'M' protein is identified, immunofixation is required to confirm the presence and to determine the type [1]. Additionally, if clinical suspicion is high, immunofixation with κ and λ antisera may be useful to detect small M-proteins even if high-resolution electrophoresis is negative [2].

The quantity of the 'M' protein should be determined and followed by densitometry. Changes in the level of the previously identified monoclonal protein in serum or urine should be assayed by high-resolution electrophoresis at regular intervals that vary from every 1–2 months for patients being treated for multiple myeloma (MM), Waldenstrom's macroglobulinemia, or amyloidosis (AL) to every year for patients with monoclonal gammopathy of undetermined significance (MGUS). Immunofixation does not need to be repeated unless there is a change in the electrophoretic migration, an additional band, or to confirm complete remission after treatment [2].

To define the level of uninvolved immunoglobulins, direct measurement by rate nephelometry is indicated at diagnosis for all patients with a plasma cell dyscrasia [2]. This method should not be used as a sole screening method for an M protein, as it cannot distinguish between polyclonal and monoclonal immunoglobulins.

Multiple Myeloma

When a patient has an identified serum M-protein by electrophoresis and immunofixation, an aliquot of a 24-hour urine specimen should be tested by electrophoresis and immunofixation to determine the amount of monoclonal immunoglobulin and/or light chain excreted in the urine [3]. The amount of monoclonal protein is determined by multiplying the percent of the M-protein times the total protein in the 24-hour specimen. In a review of 1,027 patients with newly diagnosed MM, Kyle et al. [4] determined that almost 80% of patients had a monoclonal light chain in the urine, and that 20% of the patients had light chain only myeloma. Ninety-seven percent of patients had an M-protein detectable in either the serum or the urine at diagnosis. Diagnostic criteria established by the International Myeloma Foundation for MM require all three of the following: (1) monoclonal plasma cells in the bone marrow

>10% and/or presence of a biopsy-proven plasmacytoma, (2) monoclonal protein in the serum and/or urine, (3) one or more of the following: (a) (C) Hypercalcemia (>10.5 mg/l or upper limit of normal), (b) (R) renal insufficiency (plasma creatinine >2 mg/dl), (c) (A) anemia (hemoglobin <10 g/dl or 2 g < normal), (d) (B) lytic bone lesions or osteoporosis [5]. Changes in the level of the previously identified monoclonal protein in the serum and/or urine should be monitored every 1–2 months for patients being treated for MM. High-resolution protein electrophoresis is the method of choice for monitoring. Repeat immunofixation is not indicated unless the M-protein is not detectable or a new band is observed [2]. In the cases where the protein is no longer detectable by protein electrophoresis, even if still observed by immunofixation, quantitation is not possible by conventional means.

AL Amyloidosis

AL amyloidosis (primary systemic amyloidosis) is characterized by accumulation of monoclonal free light chains (FLCs) as fibrillar protein deposition in multiple organs. The monoclonal plasma cells secrete light chains of the λ type with approximately twice the frequency of κ type. The disease is rare before the age of 40 and males represent approximately 60–65% of patients. Approximately 20% of patients with amyloidosis have associated Waldenstrom's disease or MM, but 80% of patients do not have co-existing disease [6]. In the absence of myeloma or Waldenstrom's disease, the marrow contains a low percentage of monoclonal plasma cells. Typically, the patients present with cardiac or renal failure, but other organs as well as the skin and peripheral nerves may be involved. Laboratory diagnosis of amyloidosis includes electrophoretic studies; however in a significant number of patients (5–20%) a serum or urine monoclonal protein is undetectable, depending on the electrophoresis sensitivity [7]. Even when detectable, the monoclonal band is often too small to be quantified by densitometry, which has limits of sensitivity of 500–2,000 mg/l [8]. In the presence of typical clinical symptoms and a high index of suspicion, a tissue biopsy is necessary to confirm the diagnosis of amyloidosis. Monitoring patients with amyloidosis by conventional electrophoretic means is limited by assay sensitivity.

Light Chain Deposition Disease

Light chain deposition disease (LCDD) is a rare condition that also involves the precipitation of monoclonal FLCs in various tissues. The disease differs from amyloidosis by being more prevalent in younger women (30–50 years) and having a majority of κ (85%) vs. λ chains deposited without amyloid P component. Renal failure is a common presenting symptom [7]. As many as 50% of patients have no evidence of neoplastic plasma cell proliferation. Some

of the patients have detectable monoclonal protein in the serum and/or urine demonstrable by electrophoresis or immunofixation. Approximately 25% of patients have no monoclonal protein detected by these conventional laboratory methods. In these patients, these tests are not useful for diagnosis and/or monitoring. Other laboratory tests that are supportive are routine chemistries to evaluate renal and hepatic function and complete blood count to evaluate anemia.

Non-Secretory Myeloma

Non-secretory myeloma is characterized by the absence of monoclonal immunoglobulins in serum and urine using electrophoresis testing. Monoclonal plasma cells are usually demonstrated in the bone marrow by immunohistochemical staining. Depending on the level of sensitivity of the laboratory testing, non-secretory myeloma accounts for approximately 1–5% of myeloma patients [9]. Some patients have detectable monoclonal serum proteins using more sensitive tests such as isoelectric focusing, other patients have monoclonal plasma cells that are able to synthesize but unable to secrete immunoglobulin into the blood and still others appear to be true 'non-producers' [9]. The patients have the clinical features of MM, but the plasma tumor cells have no detectable immunoglobulins. In the study from the Mayo Clinic by Kyle et al. [4], 3% of patients were designated as having non-secretory myeloma. Traditional laboratory testing in the form of serum and urine electrophoresis and immunofixation is of limited or no value in the diagnosis and follow-up of non-secretory myeloma patients. Clearly, a more sensitive test is required.

The History of Bence Jones Proteins and the Development of Free Light Chain Assays

In November of 1845, Dr. Henry Bence Jones was sent a specimen of urine from a wealthy London grocer named Thomas Alexander McBean by Drs. Macintyre and Watson [10]. Mr. McBean had been under the care of his general practitioner, Dr. Thomas Watson for several months for bone pain and fractures. Dr. Macintyre was summoned to see the patient and noted a peculiarity in his urine. The urine was of very high specific gravity, became slightly opaque upon boiling, assumed a reddish hue upon the addition of nitric acid with clearing, but upon cooling, the precipitate re-appeared. Dr. Bence Jones received a note from Dr. Watson, which described the phenomenon and asked for Dr. Jones' opinion on what the substance might be. Dr. Jones, upon further testing of the urine, and seeing the patient, concluded that the substance was an oxide of albumin that should be looked for in other cases of 'mollities ossium'. Today, this unusual substance is

known as FLC immunoglobulin, or Bence-Jones protein (BJP), the hyphen added only after Dr. Jones' death [11]. It was not until 1956 that Korngold and Lapri, using the Ouchterlony technique [12], showed that the antisera raised against groups I and II types of BJPs also reacted with myeloma proteins [13]. The two types of BJPs were designated κ and λ in honor of their observations.

In normal individuals, plasma cells produce a slight excess of light chains that spill over into the urine and are catabolized by the proximal tubule cells. κ light chains are produced in approximately twice the amount as λ light chains in humans. λ chains have a tendency to form dimers, which results in slower renal clearance. The end result is that normal serum contains a lower concentration of κ FLCs than λ FLCs. In myeloma patients, the amount of light chains produced can overwhelm the absorptive capacity of the tubules and are excreted in the urine. This generally occurs when FLC production exceeds 10–30 g/day [14]. Synthesis by the tumor, therefore, can be significant before FLC is detected in the urine. The amount of FLC excreted in the urine is also dependent on renal function. Serum assays for BJP, or FLCs were unsatisfactory for many years due to the fact that the antibodies against κ and λ light chains could not distinguish between the free and bound forms of the molecules. Sensitivity is an important consideration as well. The concentration of FLCs in serum is several orders of magnitude less than the concentration bound to intact immunoglobulins. In 1975, size-separation chromatography prior to radioimmunoassay was used successfully to measure FLCs in serum [15]. Cole et al. [16] developed a method for immunoquantitation of FLCs in the serum of myeloma patients in 1978. The method involved ultrafiltration of the patient's serum to separate free from bound light chains followed by radial immunodiffusion utilizing polyclonal antisera raised against several patients' purified κ or λ light chains. The radial immunodiffusion system was not sensitive enough, however, to detect FLCs in the serum of normal patients. Both methods were too laborious for routine clinical laboratory use and efforts were made to develop antibodies against the 'hidden' epitopes on κ and λ light chains; i.e., the epitopes that are not exposed when the light chains are bound to the heavy chains. Presumably, if such antibodies were developed with a high enough specificity and sensitivity, the necessity for physical separation of the bound and FLCs would be eliminated. In 2001, immunoassays were developed using polyclonal antibodies that were able to measure FLCs at normal serum concentrations [17]. The assays were demonstrated to have minimal cross-reactivity with whole immunoglobulins and no reactivity with the alternative light chains, both critical aspects. These assays are available on a number of automated instruments and are based on the principle of latex particle enhanced immunoturbidimetry or immunonephelometry. These assays have detection limits that are >50-fold lower than electrophoresis and approximately 20-fold lower than immunofixation.

Serum Free Light Chains in the Diagnosis and Monitoring of Plasma Cell Dyscrasias

Multiple Myeloma

In general, it is agreed that the utility of serum free light chain (sFLC) assays in the diagnosis and monitoring of intact immunoglobulin MM (IIMM) is not clearly established, and further studies are required [18, 19]. In a study by Mead et al. [20], 96% of intact MM patients (included heavy chains of all types) had abnormal sFLC ratios and/or concentrations. All of the patients had an intact monoclonal immunoglobulin detectable by electrophoresis, and this is the traditional method for following response to treatment in these patients. In another study by Kang et al. [21], 59 out of 61 patients with IIMM had elevated κ or λ FLC concentrations according to their specific light chain type and had concurrent abnormal κ/λ ratios. Serial measurements may be useful in IIMM, however. The half-life of sFLCs is on the order of hours compared to days or weeks for intact immunoglobulins and may give a more rapid indication of the efficacy of treatment. In addition, FLC concentrations may be useful when the concentration of intact monoclonal immunoglobulin is too low to reliably measure by densitometry.

New response criteria in myeloma [22] are the following for complete response (CR): (a) serum and urine immunofixation negative, (b) bone marrow biopsy $< 5\%$ plasma cells and (c) disappearance of soft tissue plasmacytoma. Stringent CR includes all of the above *plus* normal FLC ratio and absence of clonal plasma cells in the bone marrow by IHC or IF.

Approximately 20% of patients with MM have light chain MM (LCMM). LCMM patients may have no monoclonal spike, or a minimal one in serum, but have a large FLC spike in urine. Quantitation of the 24-hour urine M spike is used to monitor disease activity. A 2003 study by Bradwell et al. [23] found that in 224 patients with LCMM, all had an abnormal serum κ/λ ratio. Abraham et al. [24] monitored serial changes in sFLCs and urine M-protein values and found excellent correlation. The use of sFLCs may replace the need for 24-hour urine collection to monitor LCMM patients.

Amyloidosis

As mentioned previously, some patients with amyloidosis do not have FLCs detectable in serum or urine by immunofixation or traditional electrophoresis. Follow up of these patients is difficult, because the response to therapy is monitoring reduction of the monoclonal immunoglobulin protein. Abraham et al. [25] compared the sensitivity of sFLC assays with serum or urine immunofixation for detecting monoclonal light chains in 95 patients with amyloidosis. The sFLC results showed a 95–100% sensitivity in patients whose serum was negative for monoclonal κ or λ by immunofixation, and a sensitivity of 86% for

those patients whose serum and urine were both immunofixation negative. Katzmann et al. [26] in a study of 110 untreated amyloidosis patients found an abnormal κ/λ ratio in 91% of the patients as compared to 69% positivity by serum immunofixation or 83% by urine immunofixation. All three tests combined had a 99% diagnostic sensitivity. Although the current gold standard for detection of monoclonal FLC is immunofixation, the assays are qualitative. Response criteria in amyloidosis now includes the use of sFLCs, with a normal κ/λ ratio and serum and urine immunofixation negative being required for CR [27]. In April 2006, Dispenzieri et al. [28] published a retrospective analysis of 93 patients with amyloidosis who underwent peripheral blood stem cell transplantation. They made three important observations. Firstly, the absolute levels of the immunoglobulin FLCs appeared to be more important than the FLC ratio in these patients. Secondly, the attainment of a low absolute value of involved immunoglobulin rather than percent reduction was the best predictor of hematologic response and overall survival after transplant. Thirdly, pretransplantation FLC values had prognostic value for overall long-term survival.

Light Chain Deposition Disease

LCDD is a rare condition and only small studies [8, 26] have been carried out to date. One hundred percent and 89% of the LCDD patients, respectively in these studies were found to have abnormal FLC ratios. In a recent case report, Brockhurst et al. [29] reported the diagnosis and monitoring of a patient with LCDD with sFLCs. The patient's serum electrophoresis, immunoglobulin and urinary BJP assays were all normal. This is the first case to demonstrate a direct relationship between the measurement of the precursor protein in serum and renal function in LCDD. These results indicate a potential role for sFLC assays in the diagnosis and monitoring of other LCDD patients. Further studies are needed.

Non-Secretory Myeloma

Approximately 1–5% of myeloma patients are non-secretory [9]. In a study of 28 patients with non-secretory myeloma, Drayson et al. [30] found that 19 patients had abnormal κ/λ ratios, 4 had suppression of either κ or λ or both FLCs and 5 out of 28 had normal ratios with normal to borderline κ and λ values. In 6 patients, they assessed the role of sFLCs in disease monitoring. The changes in the concentration of the FLCs in these patients were in accordance with their clinical progress. In cases where there is a high clinical index of suspicion, and no monoclonal immunoglobulins by conventional electrophoretic/immunofixation methodology, FLCs offer a means to diagnose and monitor a significant proportion of patients with non-secretory myeloma.

Future Applications of Serum Free Light Chain Assays and Possible Studies

Serum Free Light Chains and Renal Disease

There is limited data about FLCs in renal disease. Increased FLC concentrations can occur not only when an M-protein is present, but when polyclonal immunoglobulin synthesis is increased, as in autoimmune diseases such as systemic lupus erythematosus or when FLCs are retained in the blood circulation for a longer period of time due to renal failure. In patients with renal failure, the half life of sFLCs lengthens from an average of 2 h for κ and 4–6 h for λ to a maximum of 2–3 days [31]. As renal function deteriorates in patients, the relative amounts of κ and λ light chains changes slightly, resulting in an increase in the κ/λ ratio [32]. In a study of 13 patients with acute or chronic renal failure of various etiologies, it was found that the FLC concentrations were elevated in all patients, but the κ/λ ratio was within the reference interval [33]. The presence of renal failure in patients with plasma cell dyscrasias often makes it difficult to quantify urinary loss of monoclonal light chain to assess disease progression or response to treatment. In these patients, it is important to incorporate the calculated κ/λ ratio with the actual concentrations of FLCs when interpreting results. Some of these patients may have increased levels of both FLCs, with a concurrent abnormal FLC ratio. Further studies are needed to compare results of sFLC concentrations and ratios and renal function in patients with plasma cell dyscrasias.

Serum Free Light Chain Analysis in Assessing the Progression of Individuals with Monoclonal Gammopathy of Undetermined Significance, Smoldering Multiple Myeloma and Solitary Bone Plasmacytoma or Extramedullary Plasmacytoma

MGUS is described as an asymptomatic, premalignant lesion with a bone marrow population of monoclonal plasma cells, a low monoclonal protein in the serum and/or urine and no evidence of the end-organ damage, including hypercalcemia, renal failure, anemia or osteolytic bone lesions seen in MM or other plasma cell dyscrasias [34]. MGUS is the most common of the plasma cell dyscrasias, representing 56% (578) of 1,026 patients diagnosed with a monoclonal protein and is 20 times more common than MM [35].

Smoldering MM (SMM) is a category between MGUS and MM and is defined as stage IA myeloma by the Durie/Salmon staging system. While mild anemia (hemoglobin >10 g/dl) or slightly elevated serum creatinine (<2.0 mg/dl) may be seen, end-organ damage such as abnormal renal function

is considered an indicator of progression of disease [5]. Both MGUS and SMM may progress to MM throughout the lifetime of the patient, resulting in long-term monitoring and follow-up. This creates anxiety for the patients and their families and diagnostic dilemmas for physicians as to selection and frequency of testing.

Solitary bone plasmacytoma (SBP) and extramedullary plasmacytoma (EMP) represent 3–5% of plasma cell neoplasms. Approximately half will progress to MM within 3–5 years [36]. To be classified as SBP or EMP, these patients, like MGUS patients, must be free of evidence of end-organ damage or tissue impairment except for the biopsy-proven plasmacytoma. Once again, the patients need long-term follow-up and monitoring for progression of disease.

Monoclonal Gammopathy of Undetermined Significance and Smoldering Multiple Myeloma

Diagnostic Criteria

The diagnosis of MGUS requires a low serum and/or urine monoclonal protein level (<3.0 g/dl for serum IgG, <2.0 g/dl for serum IgA and urine κ or λ monoclonal protein <1.0 g/24 h), bone marrow monoclonal plasma cells <10% and no evidence of end-organ damage (normal calcium, hemoglobin, creatinine, skeletal X-ray survey or other imaging for bony lesions and no clinical or laboratory features of amyloidosis or light chain deposition disease) [5]. These values are monitored for the lifetime of the patient and increased levels are considered indicative of progression. Some studies use the cut-off <3.0 g/dl for any type of immunoglobulin to meet the criterion of low serum monoclonal proteins for inclusion of patients in the MGUS category [37]. Revised criteria for response to treatment for the plasma cell dyscrasias came out in 2006 and include sFLC analysis and ratios [22].

SMM also has three criteria which must all be met and is considered an intermediate category between MGUS and MM. These criteria include a monoclonal protein present in the serum and/or urine, monoclonal plasma cells in the bone marrow and/or a tissue biopsy and not meeting the criteria for MGUS, MM or solitary plasmacytoma of bone or soft tissue [5]. Studies from the Mayo Clinic use ≥3.0 g/dl for serum monoclonal protein or ≥10% bone marrow plasma cells with no evidence of end-organ damage attributable to plasma cell proliferative disorder for categorization as SMM [38].

Prevalence and Epidemiology of MGUS and SMM

Sera from 21,463 residents over the age of 50 in Olmsted County, Minnesota were tested by agarose-gel electrophoresis (routine serum protein

electrophoresis). This represented most of the population over 50 years of age (76.6%) in that county. Any serum with a discrete band was further tested by immunofixation. MGUS was identified in 694 persons (3.2%), with higher age-adjusted rates in men than in women (4.0 vs. 2.7%, $p < 0.001$). The prevalence of MGUS in persons 70 years old or older was 5.3% and in the group 85 years of age or older was 7.5% [39]. These findings correspond to the association with age and sex seen in MM [4]. A study in France found similar percentages of MGUS in an adult population [40]. A NEJM study summarizing multiple studies found similar rates if age was used to separate prevalence rates [39].

When compared with MGUS, SMM has an increased risk of progression to malignancy and a shorter time to progression. While MGUS has a 1% per year risk of progression to MM (higher risks of progression to MM from MGUS have been cited which did not take into account death from other causes in MGUS patients), SMM is felt to progress at a rate of 10–20% per year [39, 41]. Cumulative time to disease progression in MGUS is 12% in 10 years, while SMM is typically 12–32 months [31, 35]. Follow-up for SMM, therefore, is typically more frequent than for MGUS because of the acuity of SMM, with serum testing every 3 or 4 months and survey for bone lesions yearly.

The incidence and mortality of MM are approximately 2-fold greater among African-Americans than European-Americans and represent the highest worldwide incidence rates [42]. MM is rare among Asian-Americans, with an incidence of 1–2 cases per 100,000. This appears to be related to racial influences, rather than environmental, as the low incidence of MM among Chinese and Japanese immigrants persisted in their descendents in California and Hawaii [43]. A study of veterans at 142 VA hospitals in the US showed approximately 3-fold higher age-adjusted prevalence rate of MGUS in African-American veterans vs. veterans identified as white [44]. This suggests that the increased risk of MM among African-Americans results from an increased risk of MGUS, rather than an increased risk of progression from MGUS to MM for African-Americans. The fact that the HLA-Cw2 antigen is associated with an increased risk of developing MM and that familial cases of MM and MGUS do occur, suggest strong genetic influences as well [43, 45].

Socioeconomic status, however, has also been shown to account for some of the increased risk among African-Americans and most likely is a surrogate for negative environmental characteristics, such as exposure to carcinogens or infectious agents, lack of access to medical care and poor nutrition [45]. Occupations involving sheet metal work or wood dust exposure are known to increase the risk of MM [46]. Employment in the nuclear industry and agriculture has been investigated, and suggests a weak role for radiation and benzene [43, 46]. Rheumatoid arthritis and chronic antigen stimulation show an association

with MM, while an association with human herpes virus 8 (known to be involved in Kaposi sarcoma) is less clear [43, 47–49].

Pathogenesis and Cytogenetics

MGUS continues to carry a risk of progression to MM every year after the diagnosis is made. The cumulative risk of progression for MGUS at 10 years is 12%, at 20 years is 25% and at 25 years is 30%. It is also very unusual for a serum monoclonal protein to disappear during long-term follow-up without treatment for disease (0.4% in one long-term study) [35]. These facts suggest that at least a portion of the MGUS population has sustained an initial 'hit' and requires a second trigger to progress to MM. This group of MGUS patients are said to be non-evolving and transformation to malignancy will not take place without the occurrence of a second, currently undefined event. The non-evolving form of MGUS is more common, patients are more likely to have an IgG monoclonal protein and have less risk of progression [50, 51].

A second group of MGUS patients are considered to be evolving if there is an unequivocal increase in the amount of the monoclonal protein in the first 3 years of follow-up. The evolving patients are more likely to have an IgA or IgM monoclonal protein (55% of the evolving patients) than an IgG monoclonal protein, although IgG is more common in MGUS as it is in MM [51]. In a large study, IgG comprised the monoclonal component of MGUS in 68.9% of patients, while IgM was found in 17.2%, IgA in 10.2% and biclonal in 3.0% and the light chain expressed was κ in 62.1% of patients and λ in 37.9% [39]. The evolving MGUS patients have a higher progression rate than the non-evolving patients, with 55% progression at 10 years and 80% at 20 years, while the non-evolving patients' progression rates were 10% at 10 years and 13% at 20 years. Forty-eight percent of evolving patients developed MM, but only 5% of the non-evolving patients developed MM [51].

SMM also possesses both evolving and non-evolving patterns. The evolving patients frequently have a previously identified evolving MGUS (59%), while only 4% of the non-evolving SMM patients had any MGUS identified prior to diagnosis of the SMM [50]. The evolving patients had a progressive increase in monoclonal protein, while the non-evolving patients had a stable level, which increased abruptly if they developed MM [52]. For the evolving pattern of SMM at least, this supports the idea of SMM as an intermediate step towards MM transformation [50, 52].

Overall, conventional cytogenetics is not as successful in the evaluation of MGUS as in other malignancies with more metaphase events. Fluorescence in situ hybridization, however is performed on interphase cells and has identified many chromosomal abnormalities shared by MGUS and MM. MGUS patients acquire more chromosomal changes slowly over time and efforts are being

made to determine which of these (if any) hold the key to malignant transformation to MM [53]. Flow cytometry is useful in detecting chromosomal aneuploidy.

MM cytogenetic studies show two subgroups of genetic abnormalities. The hyperdiploid group with 48–74 chromosomes is associated with recurrent trisomies, especially chromosomes 3, 5, 7, 9, 11, 15 and 19. MGUS and SMM patients showed a similar percentage (40%) of hyperdiploid abnormalities as MM patients, suggesting that the hyperdiploid changes may originate early in the course of the disease. These have a better prognosis than the second subgroup, known as the non-hyperdiploid group [54–57].

The non-hyperdiploid group involves primary translocations, of which the immunoglobulin heavy chain locus at 14q32 and 13q deletions are most commonly involved [54–57]. Non-hyperdiploid abnormalities are also thought to occur early, especially those involving 14q32 and chromosome 13 deletions. Rearrangements of 14q32 occurred in almost 50% of 186 patients with MGUS or SMM and the translocation partner may affect the progression of disease. Translocations involving 11q13 with 14q32 are seen in approximately 15% of MGUS, SMM and MM patients, while 4p16.3 is less common in MGUS or SMM, but may be seen in up to 10% of MM patients [53]. Deletion of 13 is observed in all stages, but results have varied as to whether the percentage is the same in MGUS or SMM as in MM. Deletion 13q is, however, common in MGUS and SMM and is therefore unlikely to play a role in progression to MM. Non-hyperdiploid patients have a worse prognosis [53, 56, 57].

Risk of Progression to Multiple Myeloma

The discovery of reliable, inexpensive, readily available, relatively non-invasive testing to predict high risk of progression from MGUS or SMM to MM would provide reassurance to patients with lower risk disease and decrease unnecessary testing. This would also allow for consideration of prophylactic intervention or therapeutic trials for high risk patients in whom the risk of adverse effects might then be considered justifiable, which is not true for the general population of MGUS patients. Many studies have shown differences between neoplastic plasma cells and normal plasma cells, including differential chemokine receptor expression, CD markers, such as CD126 (interleukin-6 receptor α-chain), dysfunctional T regulatory cells, DNA methylation changes and expression of VEGF receptors on plasma cell [58–62]. Valid testing to predict progression of disease has been difficult to find.

In a long-term study of MGUS patients, the level of monoclonal immunoglobulin was found to be predictive of progression as was the immunoglobulin class of the monoclonal protein, with IgM and IgA more likely to progress to malignancy [35]. MGUS patients with an IgM monoclonal protein

showed progression rates of 6.8% at 5 years, 23.2% at 10 years and 45.4% at 15 years, with 5.5% developing Waldenström's macroglobulinemia and 3% developing non-Hodgkin lymphoma [63]. Increased BM plasmacytosis (≥5% for MGUS or ≥10% for SMM) and presence of urinary BJP were risk factor for progression in an Italian study of 1,231 MGUS patients [64]. High erythrocyte sedimentation rate and polyclonal immunoglobulin reduction were predictive in some studies, but not others [35, 64].

The sFLC assay is a recent test for the quantification of the unbound κ and λ chains in the serum. It is readily available, relatively non-invasive and not affected by renal catabolism, unlike urine measurements of monoclonal proteins. Serum FLCs are usually low in normal and MGUS patients, but an abnormal ratio of free κ to free λ can distinguish monoclonal FLCs from polyclonal FLCs and can be used as a marker of clonal evolution and progression in MGUS patients [34]. The risk of progression in 379 patients with an abnormal sFLC ratio was significantly higher compared with 769 patients with a normal ratio (hazard ratio = 3.5; CI = 2.3–5.5; $p < 0.001$). This was independent of the amount and type of the intact serum monoclonal protein. The cumulative annual rate of progression was 0.8% per year in the patients with an abnormal sFLC ratio as compared to 0.3% per year in the patients with a normal sFLC ratio (after adjusting for competing risk of death since MGUS patients have a higher risk of dying from diseases other than progression of their plasma cell dyscrasias) [65].

A German study to determine the serum concentrations of monoclonal FLC required to produce renal overflow and BJPs in urine detectable by immunofixation electrophoresis (IFE) analyzed paired sera and 24-hour urine samples from MM patients. Of 98 sera with abnormal sFLC ratios in which the monoclonal light chain was κ, 51% had a positive urine IFE. The median value for the κ sFLC in these patients was almost 3-fold higher than the median κ sFLC value in the patients with negative urine IFE and was approximately six times the upper limit of the reference range of 19.4 mg/l. Of the 107 sera with abnormal sFLC ratios and a λ monoclonal protein, only 35% had a positive urine IFE. There was an even greater discrepancy between the median value for the λ monoclonal sFLC in patients with positive urine IFE and the median value of the λ sFLC in those with a negative urine IFE (almost a 7-fold difference, with the median value approximately 11 times the upper limit of the reference range of 26.3 mg/l). While a few patients with low sFLC levels have positive urine IFE, they concluded that relatively high concentrations of monoclonal sFLCs are required to produce renal overflow and positive urine IFE tests for BJP. They also noted that while serum levels of monoclonal FLCs are the primary factor in determining urinary excretion of monoclonal FLCs, that renal function will also affect the urinary excretion, especially for κ monoclonal proteins [66].

Table 1. Risk stratification model to predict progression of monoclonal gammopathy of undetermined significance to myeloma or related disorders, incorporating all three predictive factors (serum M-protein <1.5 g/dl, normal sFLC ratio (0.26–1.65) and IgG subtype monoclonal protein as indicators of lower risk) [65]

	Number of patients	Relative risk, 95% CI	Absolute risk of progression at 20 years, %	Absolute risk of progression at 20 years, accounting for death as a competing risk, %
Low risk (no factors abnormal)	449	1.0	5	2
Low-intermediate risk (any one factor abnormal)	420	5.4	21	10
High-intermediate risk (any two factors abnormal)	226	10.1	37	18
High risk (all three factors abnormal)	53	20.8	58	27

A risk-stratification model for MGUS utilizes the sFLC ratio, the immunoglobulin subtype and the serum monoclonal protein level to stratify patients into low, intermediate or high risk of progression to MM. In this model, sFLC ratio between 0.26 and 1.65, IgG subtype and a monoclonal protein level of less than 1.5 g/dl are defined as lower risk. Using all three predictive factors, low risk is defined as having all three parameters normal, low-intermediate risk involves one abnormal factor, high-intermediate risk has two abnormal factors and high risk has all three factors abnormal. Low-risk MGUS patients had an absolute risk of progression of 2% at 20 years, while low-intermediate was 10%, high-intermediate was 18% and high risk was 27% at 20 years when death from other causes is accounted for (see table 1) [65].

SMM is less well-studied than MGUS, but is currently being reviewed by the Mayo Clinic group for risk of progression due to age, gender, hepatosplenomegaly, hemoglobin, calcium, creatinine, albumin, presence of bone lesions, type and amount of monoclonal protein, number of BM plasma cells, reduction of polyclonal immunoglobulins and the presence, type and amount of monoclonal light chain in the urine [67]. The MD Anderson group has a stratification model for progression of SMM to MM based on two factors – monoclonal protein ≥3 g/dl and IgA monoclonal protein, to make low (52 mos to progression), intermediate (25 mos to progression) and high risk (9 mos to progression) categories ($p < 0.01$). BJP of >50 mg/day, BM plasmacytosis >25%, β_2-microglobulin >2.5 mg/l, decrease of polyclonal IgM to ≤30 mg/dl and abnormal MRI were all indicators of shortened time to progression and abnormal MRI was shown to be beneficial in

predicting earlier progression in a subpopulation of the intermediate category [41]. As with MGUS, SMM patients who have abnormal sFLC ratios progress to MM at a much shorter median time (713 days) as compared to a median of 1,323 days in SMM patients with normal sFLC ratios [68]. Serum FLC ratios are included in the new uniform response criteria for MM and are becoming important for following any patient with a plasma cell dyscrasia [22].

Future models for risk stratification will most likely also include cytogenetics. Already, bone lesion development can be predicted based on translocations, while deletions of the p53 locus are associated with plasma cell leukemia [69]. Circulating plasma cells detected by immunofluorescence in peripheral blood predict increased risk of progression in MGUS and SMM and may be incorporated into risk stratification schemes as well [70]. Microarray expression analysis has detected 74 genes differentially expressed between MGUS and MM and further studies should indicate which of these are necessary for progression [71]. BM angiogenesis increases progressively from MGUS to SMM to MM and may be related to disease progression or prediction of response to therapeutic agents [72]. Flow cytometry is a sensitive monitor for the presences of neoplastic plasma cells after bone marrow transplantation [73]. Flow cytometry could also be useful to predict progression and monitor response to therapy, if CD markers can be found that elucidate the difference between MGUS and MM.

Solitary Bone Plasmacytoma and Extramedullary Plasmacytoma

Diagnostic Criteria

Three criteria must all be met, which include a biopsy-proven monoclonal plasmacytoma of bone (SBP) in a single site only, BM with $<$10% monoclonal plasma cells and no other myeloma-related organ dysfunction, including hypercalcemia, renal failure, anemia or osteolytic bone lesions. X-rays, MRI and/or FDG PET imaging (if performed) must be negative outside the primary site. The primary lesion may be associated with a low serum and/or urine monoclonal protein. EMP can be a manifestation of MM, where it confers a poor risk. Therefore it is important to rule-out MM before making a diagnosis of EMP [5].

Prevalence and Epidemiology of SBP and EMP

SBPs represent 3–5% of plasma cell neoplasms and may be more common in women than in men. Most present with a painful lesion, but some asymptomatic patients are diagnosed when X-rayed for another condition. Approximately 50% progress to MM in 3–4 years; 30–50% survive for 10 years [36]. Intraperitoneal injection of mineral oil in certain strains of mice induces

the formation of plasmacytomas, but environmental influences in man are not well-studied in this relatively rare neoplasm [43].

EMP can occur in any organ, but the upper respiratory tract is the most common location (85%). The gastrointestinal tract is the next most common site of involvement, but they have been reported in lung, bladder, thyroid, testis, ovary and tonsil [74]. Only 15% progress to MM [36]. Both SBP and EMP are treated with radiotherapy.

Risk of Progression to Multiple Myeloma

SBPs demonstrate a small monoclonal protein in approximately 50% of patients. Persistence is associated with a worse outcome. While this is useful in predicting prolonged disease stability, a year or two after successful completion of therapy may be required for the monoclonal protein to clear [75].

In a study of 116 SBP patients, the sFLC ratio at diagnosis was found to be abnormal in 47% and these patients had a higher risk of progression to MM than those with a normal sFLC ratio. The patients with abnormal sFLC ratio had a risk of progression of 44% at 5 years and 51% at both 10 and 15 years. The patients with normal sFLC ratio had a risk of progression of 26% at 5 years, 32% at 10 years and 36% at 15 years.

A risk stratification model uses sFLC ratio abnormality at diagnosis and persistent elevated serum monoclonal protein (≥0.5 g/dl, 1–2 years following diagnosis and treatment). It defines groups with a low risk of progression to MM (13% risk), an intermediate risk (26%) and a high risk (62%) [76].

EMPs have low or no detectable monoclonal immunoglobulin in three-fourths of patients' serum or urine [77]. When present, the monoclonal protein is typically IgA [36]. Polyclonal immunoglobulins must be normal and CT or MRI should confirm the solitary nature of the lesion. PET scan and sFLC analysis should help with diagnosis and identification of high risk patients in this rare plasma cell neoplasm.

Relationship between Serum Free Light Chains and Patient Outcome in Plasma Cell Dyscrasias

In their review of 1,027 patients with newly diagnosed MM, Kyle et al. [4] determined that age, plasma cell labeling index, platelet count, serum albumin value and the log of the creatinine value were the most important prognostic factors. The size of the monoclonal M-protein was not helpful in excluding the diagnosis of MM. There is limited data about sFLCs and overall survival in patients with plasma cell dyscrasias. As mentioned previously, Dispenzieri and colleagues [27] determined that the absolute values of FLCs before and after treatment in 93 patients with amyloidosis were more prognostic than the ratio of

FLCs. Further studies in patients with other plasma cell dyscrasias need to be done to evaluate the relationship between sFLCs and patient outcome.

Analytical Considerations in the Use of Serum Free Light Chain Assays

The use of sFLCs offers great promise for the investigation of plasma cell dyscrasias; particularly in non-secretory myeloma and amyloidosis. There are analytical considerations that must be addressed as with any new test in the clinical laboratory. Tate et al. [33] studied analytical issues with The Binding Site assay extensively. The reagents are available for use on a number of assay systems to include the Roche Modular P™, Beckman Immage™, ProSpec™ and The Binding Site Minipeh™. Even though the reagent source is the same, there is not necessarily harmonization between assay systems (some are immunonephelometric, some are immunoturbidimetric), or between lots of the polyclonal antisera. Clinicians need to bear this in mind if they are monitoring serial values of FLCs in patients. It is important to stay with the same assay system and to contact the laboratory director if there appears to be a value inconsistent with clinical picture.

Some other analytical issues that were addressed were over- or underestimation of FLC concentrations. This is a potential problem whenever an M-protein is measured by immunonephelometric or immunoturbidimetric assays. In general, the monoclonal M-protein may react differently with the reagent antisera than do the polyclonal calibrators. This can potentially occur with any intact monoclonal immunoglobulin or FLC upon dilution [24], yielding a falsely elevated value. Antigen excess can cause a falsely low value and the specimen should be diluted if this is suspected. Some instruments (e.g. Beckmann Immage™) have parameters built in to check for antigen excess.

Analytical imprecision of the assay systems tested by Tate et al. yielded CV's of up to 10–11%, which is higher than the intra-individual variation. They recommended that borderline high or low specimens be repeated due to the assay imprecision. It is apparent that attention to tight quality control and appropriate calibration is important.

Reference intervals and diagnostic ranges were determined in 282 normal serum samples by Katzmann et al. [8]. The reference intervals were calculated as the 95% central tendency, and the diagnostic range included all specimens tested. Their results are summarized in table 2. They also calculated the sensitivity, specificity, positive and negative predictive values and accuracy for the test using the reference interval and the diagnostic range for the 282 reference patients, and 25 polyclonal hypergammaglobulinemia patients as well as sera from 66 patients with amyloidosis, LCDD and MM. The results are summarized in table 3.

Table 2. Free light chain (FLC) reference intervals and diagnostic ranges [8]

	95% reference interval	Diagnostic range
κ FLC	3.3–19.4 mg/l	–
λ FLC	5.7–26.3 mg/l	–
κ/λ FLC	0.3–1.2	0.26–1.65

Table 3. κ/λ FLC comparison diagnostic ranges and reference intervals [8]

	Reference interval		Diagnostic range	
	Estimate	95% CI	Estimate	95% CI
Sensitivity, %	98	91–100	97	89–100
Specificity, %	95	92–98	100	98–100
PPV, %	78	65–89	100	91–100
NPV, %	100	98–100	99	97–100
Accuracy, %	96	93–98	99	98–100

In general, sensitivity assays to measure sFLCs has greatly improved the ability to diagnose and monitor patients with plasma cell dyscrasias. In particular, patients with non-secretory myeloma or amyloidosis are difficult to diagnose and follow due to inability to detect monoclonal proteins in serum or urine by conventional electrophoretic means. Serum FLC assays are extremely beneficial in these cases. There is a need for further studies to determine the utility of sFLC assays in diagnosing and monitoring intact immunoglobulin myeloma and monoclonal gammopathy of undetermined significance.

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Mechanisms of Renal Damage in Plasma Cell Dyscrasias: An Overview

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Abstract

The kidney is a target organ in plasma cell dyscrasias. Usually the offending molecules are the monoclonal light chains (LCs), but the complete immunoglobulins can participate in the pathogenesis of organ damage. The primary structure of the monoclonal proteins is at the basis of the ultrastructural organization of their aggregates which translates into characteristic kidney injuries. The kidney targeting is due to the concurrence of several factors such as the local catabolism of monoclonal LCs, specific interactions of the monoclonal proteins with tissue and cellular components, and local environmental conditions. Glomerulopathic LCs interact with mesangial cells producing, through two distinct pathways, LC amyloidosis or monoclonal immunoglobulin deposition disease. Tubulopathic LCs damage the proximal tubule causing Fanconi's syndrome or precipitate in the distal tubule determining the cast nephropathy. The use of electronmicroscopy combined with immuno-labeling has allowed the identification of other rare patterns of kidney damage. In patients with known plasma cell dyscrasia, the recognition of these patterns of renal injury should lead to appropriate therapeutic intervention.

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Renal function is frequently disturbed in plasma cell dyscrasias (PCD) [1]. The kidney is the major catabolic site of circulating monoclonal light chains (LCs), which are filtered through the glomerulus and presented to the proximal tubule [2]. The uptake of LCs is constant, not influenced by the isoelectric point of the protein, and occurs through binding to the megalin–cubilin complex [3]. Endosomes containing the low-molecular-weight proteins acidify and lysosomal enzymes are added to form lysosomes, which degrade the proteins and return their amino acid components to the circulation across the basolateral membrane of proximal tubule epithelial cells. When the endocytotic heteromeric

Table 1. Spectrum of conditions associated with a monoclonal immunoglobulin

B-cell derived malignancies	MGUS
multiple myeloma and related conditions:	Chronic immune stimulation
plasma cell leukemia, POEMS	chronic infections (e.g. *H. pylori*)
plasmacytoma:	autoimmune diseases (e.g. rheumatoid arthritis)
solitary of bone, extramedullary	Immunodeficiencies
lymphoproliferative diseases:	primary (e.g. X-linked, combined, etc.)
Waldenström's macroglobulinemia	secondary (e.g. AIDS)
non-Hodgkin lymphomas	Transient
chronic lymphocytic leukemia	passive neonatal from mother
Monoclonal Ig-related disorders	pediatric infections
cryoglobulinemia types I and II	viral infections
AL amyloidosis	during bone marrow reconstitution after autologous or allogeneic stem cell transplant
LCDD, LHCDD	drug hypersensitivity
chronic cold agglutinins	
Ig-related polyneuropathies	

complex of the proximal tubule becomes saturated, LCs appear in the tubular fluid of distal nephron segments and finally in the urine; if the urinary LCs are monoclonal they are also referred to as Bence Jones proteins.

The PCD include a wide spectrum of clinical-pathologic diseases (table 1). In many of these disorders the clinical presentation is dominated by the proliferative activity of the B-cell clone. Other conditions are dominated by the effects of the tissue deposition of monoclonal proteins: intact immunoglobulin molecules, LCs, heavy chains, or truncated portions of these proteins. They may involve the four components of the kidney parenchyma: glomeruli, tubules, interstitium and blood vessels (table 2). It appears that the amino acid sequence of the monoclonal LC is the primary determinant of the pattern of renal parenchymal deposition and clinical disease. This is supported by the finding that the same pattern of renal disease observed in patients can be reproduced by injecting mice with the corresponding monoclonal protein [4].

Light Chain Cast Nephropathy (Myeloma Kidney)

Pathogenesis

The most common form of renal disease associated with PCD is LC cast nephropathy (LCCN). LCCN is a purely tubulointerstitial lesion and is strongly associated with multiple myeloma, although it may be rarely observed also in

Table 2. Patterns of renal diseases associated with plasma cell dyscrasias and main biochemical and structural features

Renal injury	Pattern of renal disease	Ultrastructural organization	Composition of deposits
Glomerulopathies	Amyloidosis*	β-pleated sheet fibrils (8–12 nm diameter), congophilic and apple-green birefringent under polarized light	AL: κ:λ 1:3 AH: γ or μ
	LCDD*	Granular, electron-dense	κ:λ 3:1
	LHCDD	Granular, electron-dense	γ + κ or λ
	HCDD	Granular, electron-dense	mainly γ
	GOMMID	Microtubules (20–60 nm diameter) in parallel arrangement	IgG κ or λ
	Waldenström's macroglobulinemia glomerulonephritis	Granular, massive intracapillary deposits	IgM κ or λ
	Proliferative glomerulonephritis with monoclonal Ig deposits	Granular, rarely with substructure by EM	IgG κ or λ
	Cryoglobulinemic glomerulonephritis	Granular, with substructure by EM	Type I: IgG κ or λ Type II: IgMk and polyclonal IgG
Tubulopathies			
proximal	LC Fanconi syndrome	Intracellular crystals	κ:λ 9:1
distal	Myeloma cast nephropathy	Electron-dense casts ± crystals	THP + LC

*All compartments of the kidney may be involved by amyloid and LC amorphous deposits.

AL = LC amyloidosis; AH = heavy chain amyloidosis; GOMMID = glomerulonephritis with organized microtubular monoclonal immunoglobulin deposits; HCDD = heavy chain deposition disease; LC = light chain; LCDD = LC deposition disease; LHCDD = light–heavy chain deposition disease; THP = Tamm-Horsfall protein.

Waldenström's macroglobulinemia [5]. As a result, this entity is more commonly referred to as myeloma cast nephropathy or 'myeloma kidney'. This condition is characterized by distinctive tubular casts composed of monoclonal LCs and Tamm-Horsfall protein (THP, uromodulin) causing tubulointerstitial injuries. THP is a heavily glycosylated protein synthesized in the medullary thick ascending limb of the loop of Henle. The relative affinity of monoclonal LCs for THP determines their propensity for the development of LCCN [6, 7]. Monoclonal LCs interact through their complementary determining region (CDR) [8] with a specific binding site on the THP [9]. The heterotypic aggregation of

the LC and THP determines the cast formation and subsequent intratubular obstruction of distal tubule and the thick ascending loop of Henle [10]. Cofactors frequently encountered in myeloma patients, such as hypercalcemia with increased calciuria, dehydration, acidosis, and use of furosemide are known to enhance the binding between THP and monoclonal LCs [10]. Indeed, dehydration and poor renal perfusion, in the presence of LCs proteinuria, represent a common pathogenetic mechanism for precipitating acute renal failure. Therefore, it is of primary importance to avoid the occurrence of these aggravating cofactors through preventing dehydration and prompt treatment of hypercalcemia. The initial binding rate between LC and THP and the following aggregation rate increase when the amount of LC increases [10]. This finding was confirmed in an animal model [7], and underlines the importance of the rapid reduction of the urinary monoclonal LCs. The casts produce distal tubule obstruction with tubular–glomerular feedback.

Renal Involvement

Clinical Features

Renal failure is found in about 20–40% of patients at diagnosis, and in more than 50% during the course of myeloma. The major cause of renal failure is LCCN, which accounts for about 90% of the cases [11]. Autopsy of a series of patients with PCD found LCCN in 30% of them [12]. Other causes of renal failure are amyloidosis, plasma cells infiltration, renal deposition of calcium or uric acid, infections, and nephrotoxic drugs. Over 50% of patients with kidney failure regain good renal function. When renal insufficiency persists, amyloidosis, LC deposition disease, plasma cells infiltration or an irreversible form of myeloma kidney can be suspected. LCCN is the most common cause of acute renal failure in patients with multiple myeloma [13]. Recently, an uncommon cause of acute renal failure was reported to be mediated by acute tubular interstitial nephritis associated with monotypic LC accumulation at tubular basement membranes [14]. The prognosis for survival depends on the stage of the disease at the time of diagnosis and the response to treatment [15–17]. Recovery of renal function is an important favorable prognostic indicator. Markers of a poor prognosis are hypercalcemia, early infections, and interstitial fibrosis detected on renal biopsy [18].

Pathologic Findings

The characteristic pathologic picture is the presence of large, waxy, fractured casts in the lumina of distal and collecting tubules, commonly surrounded by giant cells. The casts are mainly composed by monoclonal LCs, THP and occasional rhomboid or needle-shape crystals. These casts are markedly eosinophilic, polychromatophilic, and are stained by periodic acid-Schiff. A cellular

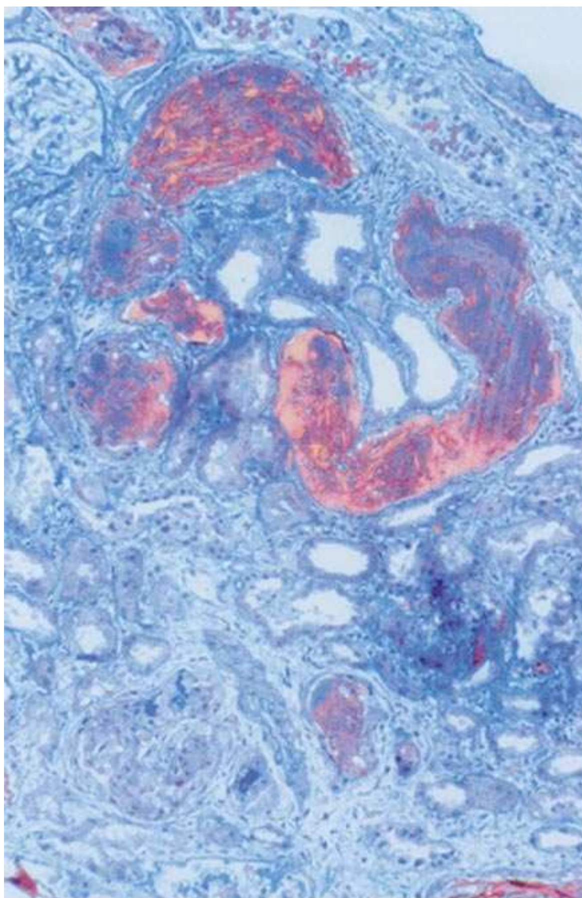

Fig. 1. Myeloma cast nephropathy: normal glomeruli contrasting with marked tubulointerstitial lesions (AFOG×150).

reaction occurs around the casts, involving both mononuclear and multinucleated giant cells, which probably originate from infiltrated monocytes. Both the proximal and distal tubules are diffusely damaged: epithelial cells appear flattened or may show varying degrees of degeneration, necrosis, atrophy and denudation of the basement membrane. Interstitial lesions are irregularly distributed: edema, fibrosis and infiltrates of plasma cells may be moderate or severe [19]. Sometimes there are areas of pyelonephritis or calcium deposits. Glomeruli usually have a normal appearance, which contrasts with the severity of the tubulointerstitial lesions (fig. 1).

Light Chain Amyloidosis

Pathogenesis

Amyloidosis is a protein misfolding disorder in which soluble proteins aggregate as insoluble extracellular amyloid fibrils causing functional and structural organ damage [20]. At least 24 different proteins can form amyloid deposits that may be localized or systemic, complicating the differential diagnosis of this disease. The fibrils are formed by the N-terminal fragment, comprising the variable region and a portion of the constant region, of a monoclonal immunoglobulin LC, produced by a bone marrow plasma cell clone. There are anecdotal reports of amyloidosis caused by the deposition of γ or μ heavy chain variable region fragment, possibly associated to the third constant domain (CH3), constituting heavy chain amyloidosis (AH) [21–23]. Typically, the amyloid plasma cell clone infiltrates the bone marrow to a modest extent (median bone marrow plasma cell percentage = 7%), often requiring anti-LC immunostaining to be identified. Only a small proportion of monoclonal LCs form amyloid fibrils in vivo. Thus, the ability to form amyloid is related to peculiar structural characteristics of the LC. Unlike in other PCD, the λ LC isotype is prevalent in AL (λ/κ ratio, 3:1), suggesting the existence of amyloid-associated Vλ germline genes. Substantial over expression of Vλ*6a* and Vλ*3r* gene segments accounts for this phenomenon [24, 25]. For amyloid κ LCs, the gene families VκI and VκIV appear to be more frequently rearranged in AL [26]. The central event to amyloid formation involves the conversion of soluble LCs into insoluble fibrils, a process requiring major conformational changes. It is possible that the amyloid-associated germline genes identified so far, such as Vλ*6a*, Vλ*3r* and possibly Vκ*B3* (VkIV) might have enhanced propensity to aggregate. Since amyloid LCs are mutated, attention has been focused on amino acid substitutions. Comparative analyses have differentiated amyloid from non-amyloid proteins based on certain amino acid substitutions in key positions within the VL [27], and amino acid changes were shown to be responsible for lesser thermodynamic stability, a feature that is linked to propensity for fibril formation [28, 29]. However, mutations could also influence LC amyloid formation through other mechanisms, such as hydrophobic and electrostatic perturbations, as shown by site-directed mutagenesis of a recombinant Vλ6a domain [30]. Proteolytic remodeling is also important since the major protein constituent of the amyloid deposits is represented by LC fragments of 12–18 kDa comprising the amino terminus [31]. The molecular mechanisms underlying the tissue targeting responsible for the prevalent involvement of a particular organ are under close scrutiny. LCs derived from the *λ6a* germline gene are preferentially associated with kidney involvement [24–26]. Following the interactions with a yet uncharacterized cell surface receptor and internalization in mesangial cells, the

amyloidogenic LCs are transported to mature lysosomes for processing, leading to the formation of fibrils which are then extruded into the extracellular matrix [32, 33]. The amyloid deposits progressively replace the extracellular matrix through the combined effects of decreased synthesis of mesangial matrix, probably due to diminished production of transforming growth factor-β [34] and its increased degradation mediated by up-regulated expression of matrix metalloproteinases (MMP) without concomitant up-regulation of tissue inhibitors of metalloproteinases [35].

Specific interactions with cell surface receptors, such as the multiligand receptor for advanced glycation end products (RAGE) [36] and recognition of specific epitopes by LC dimers retaining antibody binding activity [37] can also play a role in tissue specificity.

Renal Involvement

Clinical Features

The clinical manifestations of amyloidosis are protean and depend on the organs involved. Patients most commonly present with vague symptoms such as fatigue, anorexia, weight loss, postural hypotension, shortness of breath, peripheral edema, and less frequently with macroglossia and periorbital purpura. Kidney involvement is observed in about 60–74% of cases [38, 39]. The majority of patients with renal disease present with massive proteinuria, which in about one third of cases is a typical nephrotic syndrome. Renal failure at onset is seen in 20% of patients [39], almost always with high proteinuria. In the 5–10% of patients there is renal failure without proteinuria, and in these cases the amyloid deposition is predominantly vascular rather than glomerular. End-stage renal disease occurs in more than 20% of patients by 13–14 months from diagnosis. The presence of nephrotic syndrome and elevated serum creatinine values are the most important poor prognostic factors [40]. Median survival in patients with renal damage is 69 months, while it is 21 months in patients with cardiac involvement [39]. The diagnosis of amyloidosis must be made by a tissue biopsy, preferably an abdominal fat aspirate: the diagnostic sensitivity of abdominal fat aspirates in 705 AL patients referred to our center was 87% [39]. Although the risk of bleeding is increased in amyloidosis, a kidney biopsy may be necessary to rule out other renal disease and to evaluate the extent and the pattern of renal involvement.

Pathologic Findings

All compartments of the kidney may be involved by amyloid deposits, though such deposits seem to predominate within the glomeruli (fig. 2). Identification of amyloid is best achieved using Congo red stain, viewed with polarized light to give the typical apple green birefringence. At light microscopy amyloid appears as an

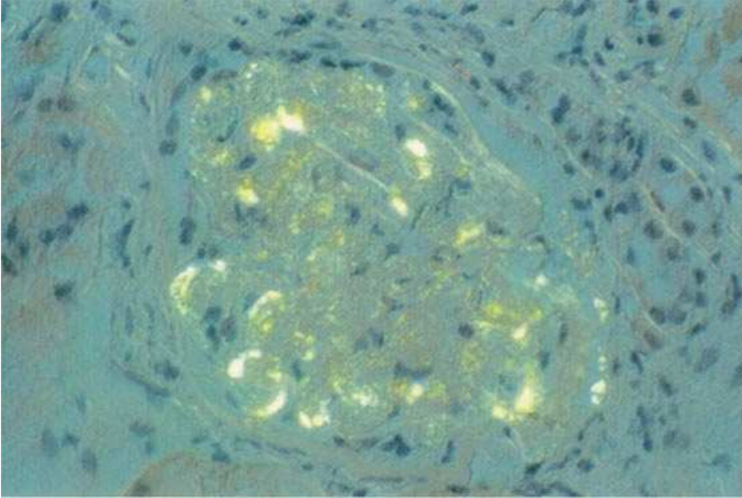

Fig. 2. Amyloidosis: a glomerulus under polarized light shows many pale green or yellow deposits (Congo red stain × 260).

amorphous, weakly eosinophilic substance, which stains rose-pink to orange to Congo red. In the glomeruli amyloid is found in the mesangium, where it forms nodules of various sizes, in the intercapillary space, and in the capillary walls. It has been postulated that deposits begin near the vascular pole and progress through the glomerular tuft [41–43]. In severe cases capillary wall deposits form large spicules on the epithelial side. The presence of many spicules seems to be associated with a more rapidly progressive course [44]. Amyloid deposits may also involve tubular basement membranes, arterioles, arteries and interstitial tissue. At electron microscopy amyloid deposits contain fine fibrils, measuring approximately 8–10 nm in width and 30–1,000 nm in length. This pattern of distribution gives rise to the spike-like features observed by light microscopy.

Monoclonal Immunoglobulin Deposition Disease

Pathogenesis

The rare non-amyloidotic monoclonal immunoglobulin deposition diseases (MIDD) can be classified into three entities: LC deposition disease (LCDD), light- and heavy-chain deposition disease (LHCDD), and heavy-chain deposition disease (HCDD). The clinical features, immunopathology and molecular mechanisms of these diseases have been reviewed [45].

LCDD was originally described by Randall et al. in two patients with end stage renal failure [46]. In most patients with this disease the LC is κ, and the deposits, predominantly localized to kidney basal membranes, are amorphous and electrondense. LCDD is usually associated with κI and κIV LCs. As expected, the primary structure of the LCs is the main determinant of their deposition in tissues also in LCDD, as suggested by data obtained in animal models. Analysis of the primary structures of LCDD-related κ LCs showed unusual hydrophobic residues exposed to solvent in CDR1 or CDR3 regions. Sequence alignment of VκI and VκIV revealed the presence of hydrophobic residues, leucine and isoleucine, or tyrosine at positions 27 and/or 31 in all known cases of LCDD [47]. Both fibrillar and non-fibrillar monoclonal LC deposits may coexist in the same patient, and the identity of the amino acid sequence of the deposited protein has been reported [48]. The molecular mechanisms underlying the possibility that the same protein can adopt dramatically different structural organizations have been investigated. The data obtained from the analysis of a recombinant amyloidogenic VκIV indicate that a given protein might have more than one critical intermediate conformations during the aggregation pathway, and that such different conformations may lead to different types of deposits [49, 50]. The experimental data indicate that sequence-specific intrinsic properties of the protein determine whether it will remain soluble or aggregate; and that fibrillar and granular aggregates form along different pathways which are governed by the environmental conditions.

Herrera and colleagues have contributed greatly to the elucidation of the molecular mechanisms of glomerular damage observed in LCDD. The glomerulopathic LCs interact with a surface receptor and are internalized in mesangial cells where they are degraded in the early endosomes [32]. The mesangial cells transform to a myofibroblastic phenotype with active production of extracellular matrix mediated by the increased production of transforming growth factor-β [51]. In particular there is de novo expression of mesangial matrix components, which are usually absent or expressed in very small amounts in normal mesangium, such as fibrillary collagens and tenascin [33]. The LCDD-LCs induce a marked reduction of matrix MMP-7 and -3 in mesangial cells with no significant changes in the expression of the tissue inhibitors of metalloproteinases [35]. Tenascin is catabolized predominantly by MMP-7, and it is known to accumulate in the center of mesangial nodules in patients with LCDD and nodular glomerulosclerosis [52]. In LCDD the initial inhibition of MMP-7 promotes the accumulation of tenascin in the mesangial matrix, potentiating further matrix increase. These findings highlight the crucial role of mesangial cells in the generation of glomerulosclerosis [53, 54].

Renal Involvement

Clinical Features

AL amyloidosis and LCDD share several features, being both a light chain deposition disease, but also have peculiar aspects. While LCDD is frequently associated with multiple myeloma (in 65% of the cases in a recently reported large LCDD population) [55], only one-tenth to one-fifth of AL are associated with clinically overt myeloma. The kidney is the organ most frequently involved in both conditions: in practically all patients (96%) with LCDD and in approximately three quarters of AL patients. Renal function is more severely and rapidly compromised in LCDD than in AL, with almost all LCDD cases having renal insufficiency at presentation, while urinary protein excretion is substantial in both conditions, exceeding 3.5 g/day in 53 and 40% of AL and LCDD patients, respectively [39, 55]. Extrarenal symptomatic deposition in LCDD patients can occur in the heart (21%), liver (19%), and peripheral nervous system (8%). At 1 year renal survival is 54%, and the variables associated with a worse renal survival are age and degree of renal insufficiency at presentation. At 1 year patient survival is 66%, and the variables independently associated with a worse prognosis are age, associated multiple myeloma and extrarenal LC deposition [55]. HCDD is less frequent than LCDD, indeed, only 20 cases have been described so far. HCDD is more frequently caused by deposition of γ heavy chains with rare α and μ reported [56, 57]. Although these heavy chains have a deletion of the CH1 domain, this deletion seems necessary but not sufficient for deposition, since VH probably also contributes to tissue deposition [57]. The most common clinical presentation is nephrotic syndrome, almost always with renal insufficiency. The clinical course of HCDD is similar to LCDD. Cases of combined light and heavy chain deposition have also been reported [57].

Pathologic Findings

LCDD is diagnosed by renal biopsy using fluorescent anti-LC antibodies and electron microscopy [55], while light microscopy patterns are only suggestive. At light microscopy the typical lesions are prominent nodules within the mesangial areas, which are stained by periodic acid-Schiff but not by Congo red. Another lesion is thickening of the peripheral basement membrane, which may resemble type II membranoproliferative glomerulonephritis. Tubules show deposits of ribbon-like material in the outer part of the basement membrane. Deposits may also be observed in the arteries, arterioles, capillaries, veins and interstitium. It is worth stressing that immunofluorescence may be negative in about 10% of cases and electron microscopy in about 20% [55]. Therefore, an incomplete morphological examination could lead to a wrong diagnosis. On immunofluorescence, linear peritubular deposits of monotypic LCs can be detected in 90–95% of patients [55, 58]. Deposits are also found along

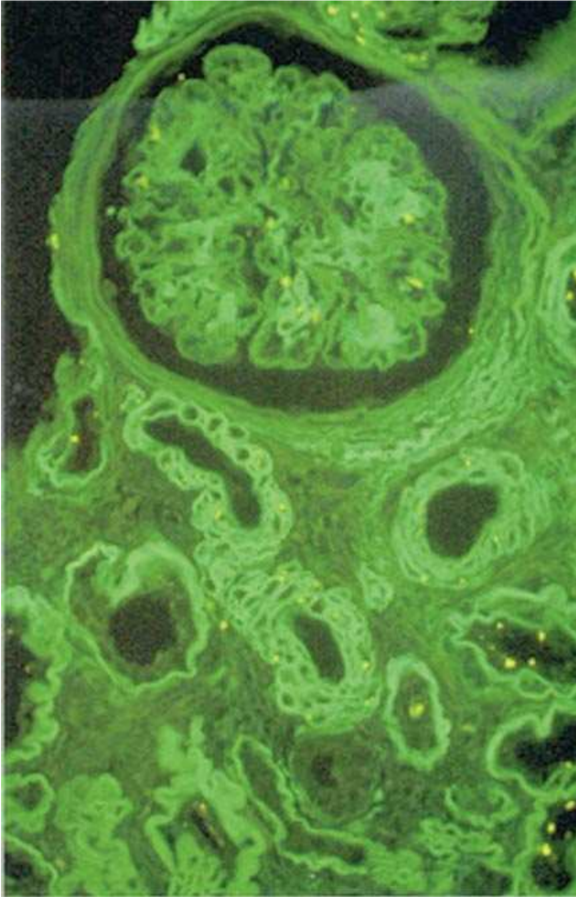

Fig. 3. LC deposition disease: peritubular, vascular and glomerular deposits of κ LCs (Immunofluorescence ×150).

glomerular basement membranes, in mesangial nodules, along Bowman's capsule, along vascular structures and in the interstitium (fig. 3). Electron microscopy shows finely granular, electron-dense deposits, in the same locations of immunofluorescence. Myeloma cast nephropathy can be associated in 16–32% of the cases and AL amyloidosis in 3–33% of LCDD patients [55]. Monoclonal protein is detected by immunofixation in serum or urine in 94% of LCDD patients with a κ/λ ratio of 68/32. The deposits may be formed by heavy chain instead of LC in HCDD. In ~10% of LCDD cases monotypic HC is associated with monotypic LC in deposits (LHCDD).

Crystal-Storing Histiocytosis: Adult Fanconi's Syndrome

Pathogenesis

Crystal-storing histiocytosis is an intralysosomal accumulation of monoclonal LCs which aggregate in crystals, observed in association with both plasma cell and lymphoid disorders [59, 60]. LC type κ have been almost exclusively involved without a consistent association with a particular heavy chain. Crystals can form in either histiocytes in soft tissues or parenchymal cells in bone marrow, lymph nodes, spleen, liver, stomach, adrenals, renal proximal tubules, thyroid follicule, or gallbladder surface epithelial cells [61]. The initial clinical presentation depends on the site of crystal formation and is, therefore, varied. Some patients present with soft tissue masses in which predominantly histiocytes, but also fibroblastic cells, contain crystals. The crystal formation in renal proximal tubules is at the basis of the adult Fanconi's syndrome (FS). This is a generalized disorder of proximal tubule function, leading to the urinary leak of phosphate, uric acid, glucose, amino acids, and low molecular-weight proteins (β_2-microglobulin, retinol-binding protein, and α_1-microglobulin), often associated with proximal (type II) renal tubular acidosis. In adults, FS is usually acquired following the administration of certain drugs, heavy metal poisoning, or, more frequently, urinary secretion of a monoclonal LC, almost always of the κ isotype. Progress has been made in understanding the mechanisms by which monoclonal LCs accumulate and form crystals within proximal tubules. Almost all κ LCs in patients with FS belong to the Vκ1 subgroup and derive from two germline genes, O2/O12 or O8/O18 [62, 63]. Unlike other monoclonal LCs, which, after endocytosis, are degraded in the lysosomal compartment of proximal tubular cells, LCs from patients with FS usually are resistant to proteolysis [64]. Particular modifications in the primary structure of the Vκ domain account for this resistance to enzymatic degradation and also promote self-reactivity, leading to interactions between monomers and the formation of organized crystals in the endocytic compartment of proximal tubule cells. However, the mechanisms involved in proximal tubular dysfunction have yet to be elucidated, as highlighted by a recent report of a patient with indolent Waldenström's macroglobulinemia and full-blown FS with accumulation of a monoclonal κ LC within proximal tubules, without crystals. This LC belonged to the Vκ3 subgroup and derived from the L2/L16 germline gene, showed no common substitution with previous FS κ LC and was sensitive to proteolysis. This report indicates that the molecular characteristics of κ LC in FS and the pathogenesis are more heterogeneous than initially believed [65]. FS frequently evolves into renal failure. High concentrations of monocytes chemoattractant protein-1 were found in urine collected from patients with idiopathic FS. The inflammatory effects of high concentrations of monocytes chemoattractant protein-1 may contribute to the progressive interstitial and tubular damage leading to renal failure [66].

Renal Involvement

Clinical Features

Clinical and pathological features of LC-associated FS are heterogeneous. Typically, but with notable exceptions, FS occurs in patients with smoldering myeloma, or precedes the development of overt myeloma [67]. Osteomalacia and chronic renal failure are the most common manifestations leading to the diagnosis of LC-associated FS. Less frequently, FS may be observed in the setting of high-mass myeloma with typical cast nephropathy and may be associated or not with crystals in proximal tubules [62, 64, 68]. Principal laboratory abnormalities include aminoaciduria, renal glycosuria, hypophosphatemia, hyperchloremic metabolic acidosis, hypokalemia, proteinuria of tubular origin, and hypouricemia. The major manifestations include osteomalacia, polyuria, chronic acidosis, and episodes of dehydration.

Pathologic Findings

No morphologic features are specific to FS; however, the presence of extensive tubular atrophy combined with crystals within the proximal tubular cells are considered characteristic of this anomaly [68]. The monoclonal LC inclusions are highly organized into crystals within the lysosomal compartment of proximal tubular cells. Crystalline inclusions also are detected commonly in the cytoplasm of macrophages and malignant plasma cells and may be involved in the slow progression of the lymphoproliferative disease.

Cryoglobulinemic Glomerulonephritis

Pathogenesis

Cryoglobulins are circulating immunoglobulins that possess the unique characteristic of precipitating reversibly when cooled below 37°C. There are three types of serum cryoglobulins. Type I cryoglobulins are composed of a monoclonal population of immunoglobulins, mainly IgG, and occasionally IgM or IgA, and are strongly associated with underlying B-cell neoplasms and PCD. Type II cryoglobulins are composed of monoclonal immunoglobulins (most commonly monoclonal IgMκ) with specificity for the Fc portion of polyclonal IgG. Type II cryoglobulins are the most common and are associated with HCV infection, autoimmune disease, and lymphoproliferative diseases. Type III cryoglobulins, like type II, are of mixed composition, including a polyclonal population of immunoglobulins with specificity for the Fc portion of immunoglobulins. Type III cryoglobulins are not associated with lymphoproliferative diseases.

Renal Involvement

Clinical Features

Renal disease in cryoglobulinemia is observed in about 20% of patients at diagnosis, and in more than 50% of patients during the course of the disease. Renal involvement is manifested by isolated proteinuria and microscopic hematuria (30% of cases), nephrotic syndrome (20% of cases), chronic renal failure (20% of cases), and acute renal failure (10% of cases). In most patients, the renal course is indolent and protracted, and is characterized by proteinuria, hypertension and hematuria. Approximately 30% of patients experience a partial or complete remission of renal symptoms. End-stage renal disease is observed in about 10–15% of patients [69]. Extrarenal clinical features include palpable purpura, fatigue, Raynaud's syndrome, arthralgias, lymphadenopathy, hepatosplenomegaly, and peripheral neuropathy. Reduced serum concentrations of C4 are often prominent. The principal causes of death are infections, liver failure, neoplasms, and cardiovascular or cerebrovascular complications favored by severe arterial hypertension [69].

Pathologic Findings

Cryoglobulinemic glomerulonephritis is characterized by thickening of the glomerular basement membrane, cellular proliferation, and occlusive eosinophilic deposits [70]. Glomerular intracapillary proliferation is marked and involves resident glomerular cells and infiltrating blood-borne cells. The more specific morphological findings are: (1) intraluminal thrombi composed of precipitated cryoglobulins (on light microscopy); (2) diffuse IgM deposition in the capillary loops (on immunofluorescence microscopy); and (3) subendothelial fibrillar deposits (on electron microscopy) (fig. 4).

Waldenström's Macroglobulinemia

The kidneys are not often involved in Waldenström's macroglobulinemia, and glomerular lesions are seldom of clinical significance. The most important etiologic factors of renal dysfunction are infiltration of the renal parenchyma with lymphoplasmacytic cells, amyloidosis, hyperviscosity, immune complex deposition, and LC deposition disease. Nephrotic syndrome is rare, and is usually due to amyloidosis. Acute renal failure may occur in association with capillary hyaline thrombi. Renal insufficiency is rare [71].

Pathologic Findings

The characteristic findings are deposits of an eosinophilic material within the capillary lumen. The deposits are sometimes so voluminous to occlude the

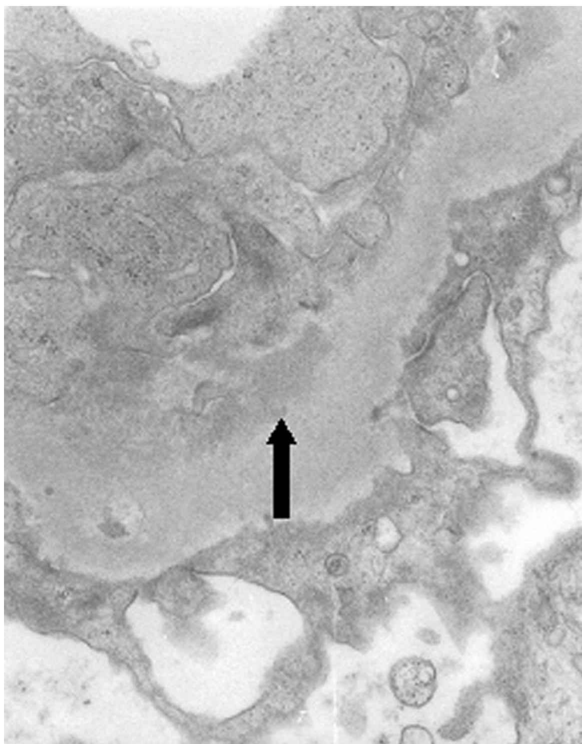

Fig. 4. Membrano-proliferative glomerulonephritis in cryoglobulinemia: osmiophilic deposits with light crystalloid structure in the subendothelium (E.M. × 15,000).

capillary lumen. Stained with anti-serum against IgM, the deposits can be seen on immunofluorescence microscopy (fig. 5). Some patients have been reported to have nodular glomerulosclerosis, as observed in LCDD. Most of the patients show interstitial infiltrates of lymphoplasmacityc cells [72].

Glomerulonephritis with Organized Microtubular Monoclonal Immunoglobulin Deposits

A singular form of organized monoclonal immunoglobulin deposits, defining cryocrystalglobulinemia, is characterized by highly organized crystalline substructures affecting various organs, especially the kidneys and the synovium [73]. This condition is associated with various B cell-derived immunoproliferative disorders, most frequently chronic lymphocytic leukemia

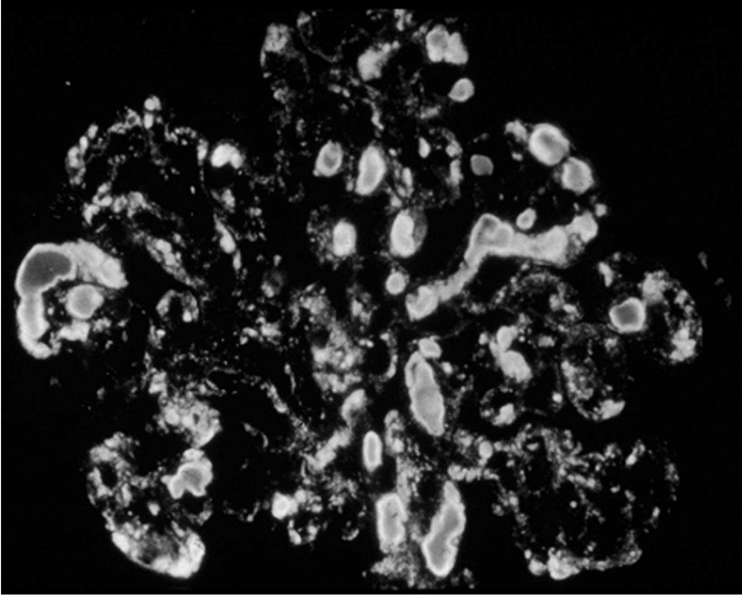

Fig. 5. Glomerular IgM deposits in Waldenström's macroglobulinemia: massive endoluminal deposits of IgM (Immunofluorescence ×200).

[74, 75] and is rarely observed in multiple myeloma [76]. Glomerulonephritis with organized microtubular monoclonal immunoglobulin deposits (GOMMID) has been reported in a number of patients with chronic lymphocytic leukemia, most of whom did not have cryoglobulins and had low concentrations of circulating monoclonal immunoglobulins, detectable only with sensitive techniques, or undetectable. The microtubules seen in GOMMID differ from the fibrils seen in non-amyloid fibrillary glomerulonephritis not only by their mean diameter (10–60 vs. 10–20 nm, respectively), but mostly by their protein content (monoclonal immunoglobulin vs. polyclonal IgG) and their respective parallel vs. random arrangement [77]. In addition to signs of kidney involvement, patients may have systemic vasculopathy and widespread erosive arthropathy, mimicking rheumatoid arthritis [73].

Proliferative Glomerulonephritis with Monoclonal Immunoglobulin Deposits

Recently, 10 cases with proliferative glomerulonephritis caused by glomerular deposits of monoclonal IgG that do not conform to any previously reported

pattern of glomerular involvement by a monoclonal gammopathy have been reported [78]. A serum monoclonal IgG was detectable in 50% of the cases. None of the patients had overt multiple myeloma or lymphoma at presentation or over the course of follow-up nor did they have clinical or laboratory features consistent with type I cryoglobulinemia. All patients had proteinuria and nephrotic syndrome was present in 44%. Eighty percent of patients had renal insufficiency.

The main histological feature on light microscopy was diffuse endocapillary proliferative or membranoproliferative glomerulonephritis, characterized by marked endocapillary hypercellularity and leukocyte infiltration. The monoclonal immunoglobulins formed granular electron-dense deposits seen primarily in mesangial and subendothelial sites by means of electron microscopy. Using immunofluorescence techniques, the deposits were found to be monoclonal in origin, staining for a single LC isotype and a single γ subclass. There was no evidence of deleted immunoglobulin molecule. Complement deposition was present in 90% of cases, and 40% of patients had low serum complement concentrations. A case with monoclonal IgA deposits and proliferative glomerulonephritis associated with paracrystalline deposits was recently reported [79].

Conclusions

Monoclonal proteins cause a wide array of kidney lesions through molecular mechanisms which are being progressively elucidated. Specific interactions of monoclonal proteins with the extracellular matrix and with mesangial, endothelial and tubular cells are involved in distinct patterns of renal damage. Proinflammatory, proapoptotic, and profibrotic mediators play important roles in determining the progressive interstitial and tubular damage leading to renal failure. These findings represent the basis for the development of new therapeutic agents to be used in a comprehensive treatment strategy, including chemotherapy to suppress the synthesis of the monoclonal protein, and the prevention of aggravating cofactors, such as hypercalcemia, acidosis and nephrotoxic drugs. The nephropathies caused by monoclonal proteins are formidable models for understanding the mechanisms of kidney damage. The lessons learned from these models may contribute to improving the care of kidney diseases.

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Proximal Tubular Injury in Myeloma

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Abstract

Renal involvement is common in multiple myeloma and implies much worse prognosis. Most of the kidney disorders associated with myeloma are caused by the excess production of monoclonal light chains, and renal involvement is almost always accompanied by light chain proteinuria. Light chains have variable effects on the kidney; some are more toxic than others and different light chains affect different structures in the kidney. In normal quantities light chains are filtered relatively unhindered in the glomerulus and endocytosed by the proximal tubule cells through the tandem endocytic receptors megalin/cubilin and targeted to degradative sites. Proximal tubule injury is the most common mode of renal involvement and it can manifest in a variety of ways. When light chains are overproduced the proximal tubular endocytic process is overloaded and cell stress responses that include phosphorylation of MAPKs, prominently, p38 MAPK, and nuclear transcription factors NF-κB, AP-1 are activated resulting in production of inflammatory and proinflammatory cytokines, TNF-α, interleukin-6, 8, and monocyte chemo-attractant protein-1. In early stages of myeloma, light chain nephrotoxicity often presents with proximal tubular functional abnormalities, such as Fanconi syndrome. These proximal tubule alterations often progress to a severe tubulointerstitial kidney disease, the most common type of kidney involvement responsible for end-stage renal failure seen in myeloma patients.

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Introduction: Renal Involvement in Myeloma

Multiple myeloma is characterized by neoplastic proliferation of a single clone of plasma cells producing an M-protein. The clone of plasma cells proliferates in the bone marrow and frequently invades the adjacent bone causing skeletal destruction that results in bone pain and fractures. Anemia, hypercalcemia and renal involvement are among main features of multiple myeloma. Renal involvement is present in approximately 20–50% of the cases at diagnosis

and includes a variety of renal disorders [1–5]. Depending on the type of the monoclonal immunoglobulin and its associated light chain produced by the neoplastic B-cell clone, there may be preferential involvement of the proximal tubule, the distal tubule, or the glomerulus. Proximal tubule involvement is more common and initially manifests with subtle proximal tubular disorders, such as Fanconi syndrome, which is associated with glycosuria, amino-aciduria, hyperuricosuria, and renal tubular acidosis [6]. These functional abnormalities often progress to end-stage kidney disease and portends a worse prognosis complicating the management of this already difficult-to-treat cancer.

A recent review from the United States Renal Data System (USRDS) shows that renal morbidity from multiple myeloma is a considerable burden [7]. Of the 375,152 patients in the registry initiated on ESRD therapy between January 1, 1992 and June 30, 1997, 3,298 (0.88%) had myeloma associated kidney disease. These patients were disproportionately male (59.5 vs. 53.2%) and Caucasian (76.2 vs. 64.1%) and older (68 $\pm$ 11.78 vs. 60.69 $\pm$ 16.55 years. Patients with myeloma associated kidney disease had lower serum hemoglobin, higher creatinine, and were more likely to have been started on hemodialysis than peritoneal dialysis. The 2-year all-cause mortality of patients with myeloma kidney involvement during the study period was significantly greater, 58 vs. 31% in all other patients. Furthermore, myeloma kidney disease was independently associated with 2.5-fold increase in all-cause mortality [7].

The monoclonal light chains produced by a neoplastic clone of B cells cause almost all of the renal disorders with the exception of hypercalcemic nephropathy, rarely, heavy chain or intact immunoglobulin deposition and even more rarely, plasma cell infiltration. Thus it can be argued that perhaps the renal disorders that accompany myeloma are unique to myeloma or to light chain overproduction states and that these lesions are not encountered in other diseases, although there is emerging evidence that renal involvement in myeloma may involve common pathophysiologic pathways with other proteinuric diseases. Nevertheless, proximal tubule changes seen in myeloma are always associated with the monoclonal light chain excess. There is now considerable body of work that has improved our understanding of the mechanisms of kidney involvement in multiple myeloma and on nephrotoxicity of light chains. In this chapter, we will focus on the recent insight into mechanisms of proximal tubular injury in myeloma.

Renal Handling of Light Chains

For a more complete understanding of renal disorders associated with light chain proteinuria a brief overview of the renal handling and metabolism of light chains would be helpful. Free immunoglobulin light chain fragments, mw

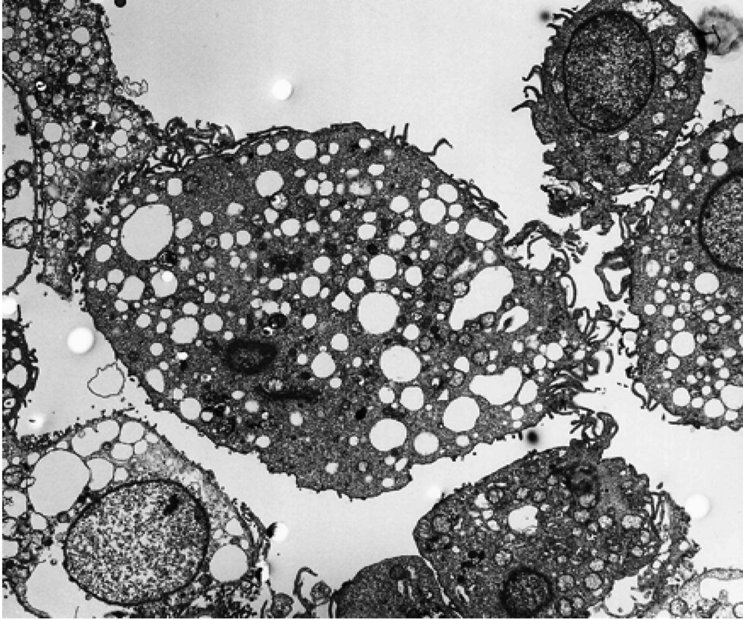

Fig. 1. Light chain exposed human proximal tubule cells by EM examination demonstrate apoptosis and extensive vacuolization.

approximately 22–25 kDa in monomeric form, are present in plasma. In normal humans aged 21–90 using a new method Katzmann et al. estimated the reference range for free κ light chains at 3.3–19.4 mg/l, and for free λ light chains at 5.7–26.3 mg/l. Although the precise filterability of free light chains in the glomerulus is unknown, because of their size and relative to albumin cationic net charge, light chains have been assumed to be relatively freely filtered in the glomerulus [8, 9]. In one review, the glomerular sieving coefficient for free κ light chains is given as 0.09 [9]. Using this value for the total free light chain (κ + λ), an average filtered load of 100–600 mg/24 h light chain can be estimated to be presented to the kidney tubule. That the urine is virtually free of light chains – normal humans excrete less than 3–5 mg/24 h light chain [9–11], clearly implies a huge capacity by the renal tubule to absorb filtered light chains. Avid internalization of light chain can be easily demonstrated by immunocytochemistry by exposing human proximal tubule cells to FITC tagged light chains, in vitro. Within 20–30 min, intense uptake of light chains into cells can be visualized in a vacuolar distribution within the cytosol (fig. 1). In earlier studies, using immunocytochemistry, immunofluorescence and immunoelectron microscopy techniques in light chain perfused rat kidney tubules Sanders et al. [12] demonstrated the presence of light-chain protein in endosomes and lysosomes.

Originally, light chains were believed to be internalized by the proximal tubule cells by a passive process through pinocytosis or fluid phase endocytosis [9, 13]. Later studies have shown that filtered light chains are endocytosed by a receptor mediated process [14]. Free light chains are now characterized as one of many ligands that bind to the tandem scavenger receptor system cubilin/megalin and are endocytosed via the clathrin dependent endosomal/lysosomal pathway [15–17]. The megalin/cubilin system, related to the LDL receptor superfamily [18–20], functions as a low-affinity, high capacity receptor as was predicted from the earlier classical receptor studies [14]. This endocytic receptor mechanism is interesting in that cubilin has no cytoplasmic domain, but its partner receptor, megalin, which functions as its chaperone, has a single transmembrane domain which contains NPXY motifs that allow phosphorylation when a ligand binds to either component, and endocytosis of the bound ligand is initiated. It appears that some light chains bind preferentially to cubilin, while others to megalin. Either way, receptor binding results in endocytosis [14, 21]. Maneuvers that interfere with clathrin lattice formation, such as hypertonicity, block light chain endocytosis indicating a significant role of clathrin-coated pit pathway [14]. Endocytosis of light chains is then followed by vesicular trafficking and requires vesicular acidification. Inhibitors of vesicular acidification by using the H-ATPase inhibitor bafilomycin, and other agents that interfere with lysosomal acidification such as, chloroquine, inhibit light chain endocytosis and degradation confirming that light chain endocytosis occurs through the endosomal–lysosomal pathway [14, 22, 23]. Degradation studies showed that all light chain digestion is not confined within the lysosomes, and there maybe significant degradation of light chains bound to the proximal tubule cell membrane, probably through the action of protelytic enzymes within the brush border membrane [14].

It is known that receptor associated protein (RAP), a ~40 kDa high-affinity, chaperone-like ligand for megalin is required for functioning of cubilin/megalin. RAP is found primarily in the endoplasmic reticulum and it plays a necessary role in the folding and exocytic trafficking of the members of the LDL receptor gene family including megalin. RAP deficiency is associated with the appearance in the urine of cubilin/megalin ligands, such as, light chains, retinol binding protein, etc., confirming that it is essential for proper endocytic function of the proximal tubule [18].

Light Chain Proteinuria and Toxic Effects of Light Chains

The presence of light chains in the urine, light chain proteinuria, often termed Bence-Jones proteinuria, may occur as a result of overproduction,

increased filtration, or decreased proximal tubular reabsorption. Appearance of light chains in the urine is most often a consequence of overproduction, as seen in multiple myeloma and Waldenstrom's macroglobulinemia, but may also occur in lymphomas, leukemias and monoclonal gammopathy of unknown significance (MGUS) [24–34]. Increased filtration and presence of light chain in the urine presumably without overproduction may occur in glomerular disorders that may be associated with overt proteinuria as a result of increased perm-selectivity of the glomerular basement membrane [35–39]. Increased light chain in the urine can be found in early diabetics [35, 38, 40] and in patients with active lupus nephritis [25]. Some kidney diseases that spare the glomeruli and involve predominantly the tubulointerstitium exhibit a characteristic proteinuria that consists of proteins smaller than albumin, also termed, low-molecular-weight proteinuira, because of the diseased tubules inability to efficiently reabsorb the filtered proteins [9]. Light chains are almost always present among the low-molecular weight proteins excreted in the urines of patients with tubular proteinuria that may occur as a result of decreased proximal tubular reabsorption in tubulointerstitial kidney disease [15, 18, 41–45].

Light chain proteinuria is associated with a spectrum of proximal tubular disorders, and most of these disorders can be attributed to the toxic effects of light chains on the proximal tubule cells [46–50]. Furthermore, when overproduction exceeds the endocytic capacity of the proximal tubule, increased concentrations of light chains in the renal tubule may be available for interaction with Tamm-Horsfall proteins and formation of the typical myeloma casts [51]. There is considerable variability among the light chains that are associated with kidney disorders in myeloma [29, 50, 52–54]. In some patients, small amounts of light chain proteinuria may be associated with severe nephropathy while in others light chain proteinuria up to 8–9 g/day or greater maybe associated with minimal if any renal dysfunction. This has been taken as evidence of variability among light chains' nephrotoxicity. In general, the variability in light chain nephrotoxicity has been found determined by the variable region, V_L, of light chain molecule [29, 52, 53, 55–57]. For example, among the light chain subtypes, λVI light chains have been found most frequently but not exclusively in amyloid light chain (AL) amyloidosis [58]. In an in vitro model λVI light chains formed amyloid rapidly while others did not. The precise determinants of variable toxicity, however have not been fully identified. It is now widely accepted that both κ and λ light chains can be equally nephrotoxic, although λ light chains are more frequently associated with amyloidosis while κ chains are more frequent than λ in Fanconi syndrome [29, 59–62]. The earlier reports that the net electrical charge, pI, of the light chain molecule correlated with toxicity has not been confirmed in later studies [63–66].

Tubular Functional Abnormalities

These changes are often subtle and frequently missed in early stages. Tubular functional alterations, like other myeloma associated renal disorders, are associated with light chain proteinuria. Proximal tubular events prevail and full Fanconi syndrome, characterized by aminoaciduria, renal-proximal tubular acidosis, glycosuria, kaliuresis, phosphaturia, hyperuricosuria may occur [29, 47, 48, 56, 67]. Increased urinary excretion of potassium, uric acid and phosphate is often associated with hypokalemia, hypouricemia and hypophosphatemia and may be clues to a proximal tubular dysfunction that may initiate clinical investigations identifying the underlying myeloma [59, 68]. Multiple myeloma is the most common cause of proximal renal tubule acidosis in the adult.

The pathophysiology of Fanconi syndrome appears to be mediated through direct toxic effects of myeloma light chains on proximal tubule cells [69–71]. Although κ light chains are more frequently associated with Fanconi syndrome both subtypes have been implicated [29, 53, 56, 61, 62, 72–74]. This is consistent with studies that have demonstrated that both κ and λ light chains can inhibit transport of glucose, amino acids and phosphate, in vitro, at concentrations that can be found in the tubule fluid of patients with multiple myeloma and light chain proteinuria. Furthermore, light chains directly inhibit the renal isoform of Na-K-ATPase on proximal tubule cells at both protein and gene level [71]. This may interfere with cells' energy metabolism, disrupt the actin cytoskeletal organization [75], and may be a contributing mechanism for the proximal tubular functional abnormalities [71].

Light chain exposure has been reported to result in a variety of alterations in the proximal tubule cells in rat kidneys infused with light chains, and in kidney biopsies from humans with light chain related renal disease. These changes include cellular desquamation, cytoplasmic vacuolization, and focal loss of microvilli [12, 76]. In many cases Fanconi syndrome is associated with crystalline deposits in the kidney, and accumulation of proteolysis resistant light chain sequences in the endocytic compartment of proximal tubule cells has also been suggested as a possible mechanism [77]. Prolonged exposure of proximal tubule cells to light chains lead to apoptosis, and cell drop-out might also conceivably be a mechanism for decreased transport functions in the proximal tubule (fig. 2) [75]. These morphological alterations may well be contributing to the functional abnormalities associated with light chain proteinuria.

Clinical studies have shown that many multiple myeloma patients with light chain proteinuria also suffer from a concentrating defect that may result in polyuria and polydypsia, in the absence of hypercalcemia, suggesting again a direct toxic effect by light chains [6]. The precise cellular mechanisms for this

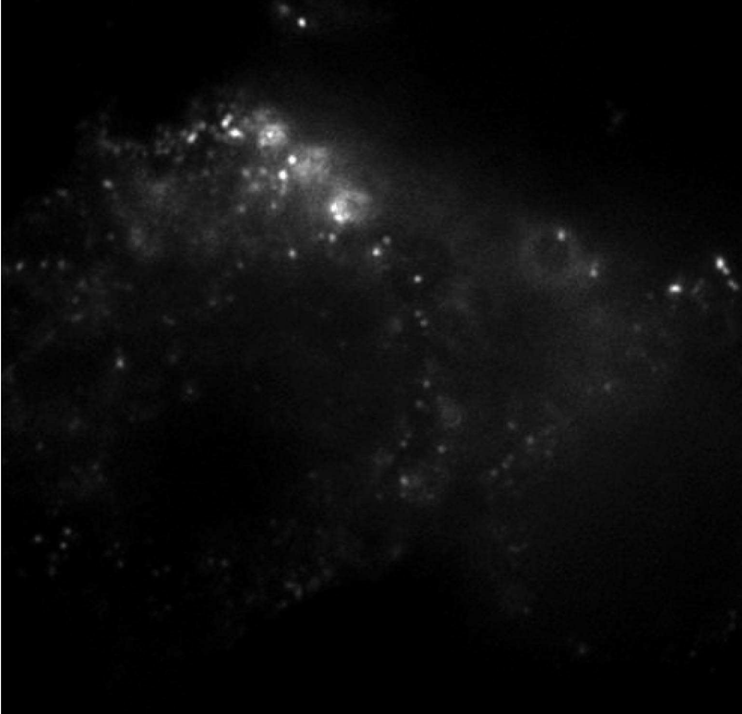

Fig. 2. FITC-light chain is seen in the vacuoles (presumably endosomes) within the cytosol of two adjacent human proximal tubule cells in culture.

disorder in myeloma have not been identified, but are attributed to the tubulointerstitial changes and tubule unresponsiveness to vasopressin, i.e., a nephrogenic diabetes insipidus. Distal tubular changes are often associated with distal renal tubular acidosis, and rarely a mixed picture involving both proximal and distal tubular dysfunction can occur [68, 78, 79].

Myeloma Kidney

The most common type of renal involvement in multiple myeloma is a chronic tubulointerstitial nephropathy characterized by tubular atrophy and tubulointerstitial fibrosis (fig. 3) often associated with casts, and also referred to as 'cast nephropathy' [2, 48, 66, 80–87]. There is much research on pathophysiologic mechanisms and considerable new insight on the role of light chains in the genesis of chronic kidney injury. Sanders et al. have demonstrated

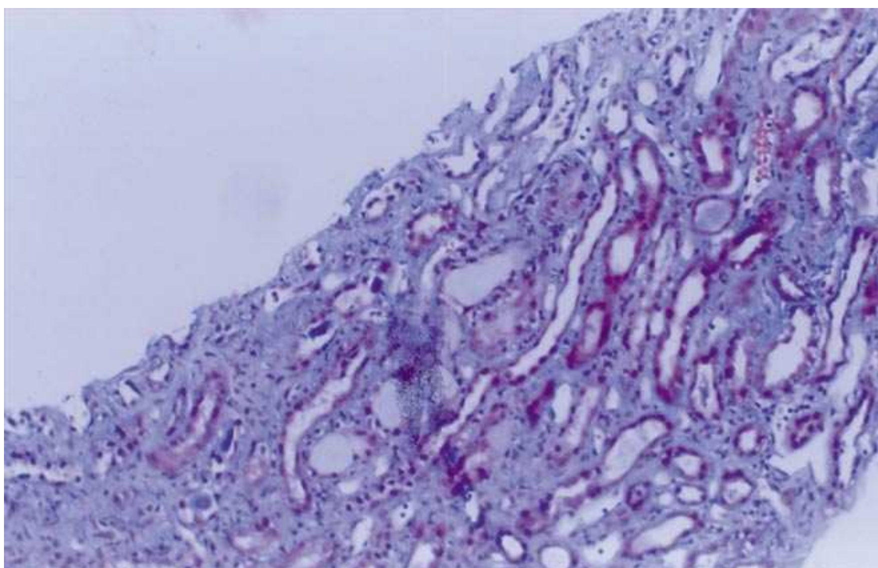

Fig. 3. Kidney biopsy from a patient with multiple myeloma and myeloma kidney shows extensive tubulointerstitial fibrosis and tubule atrophy. Masson trichrome, 150 ×. (Photograph is courtesy of Dr. Suzanne Meleg-Smith, Professor of Pathology, Tulane University Medical School.)

that certain types of light chains, which have a propensity to form casts behave as ligands binding to defined sites on Tamm-Horsfall proteins and that these light chains are responsible for 'cast nephropathy' [46, 48, 85, 88–91]. Mice injected with Bence-Jones proteins from humans with various types of kidney lesions develop similar renal injury patterns including cast formation [54]. However, these casts, except in the case of acute cast nephropathy, are seldom extensive. Furthermore, how occasional casts would lead to a pervasive sequence of events that result in tubulointerstitial inflammation, scarring and fibrosis has not been fully addressed.

Recent studies with cultured proximal tubule cells have revealed intriguing clues to a novel pathophysiology of the chronic tubulointerstitial nephropathy that accompanies myeloma [22, 23, 71, 75]. These studies probed the role of the most abundant cell type in the kidney, the proximal tubule epithelium, which is also the cell type responsible for endocytosis and catabolism of filtered light chains, and demonstrate a significant role of proximal tubule cells in the pathogenesis of myeloma kidney. A series of myeloma light chains collected and purified from the urine of multiple myeloma patients with mild to moderate renal insufficiency and without albuminuria, i.e., without evidence of glomerulopathy, were shown in

these studies to induce apoptosis and increased DNA degradation in cultured proximal tubule cells [75]. Prolonged exposures were also shown to lead to necrosis. More interestingly, these tubulopathic light chains were also shown to induce inflammatory/proinflammatory cytokine production [22, 23]. Detailed investigations suggest that these cytokine responses are due to activation of cell stress responses due to increased endocytosis and overloading by myeloma light chains. In these studies, light chains are shown to induce production of interleukins (IL)-6, -8, TNF-α, and monocyte chemotactic protein. These cytokine responses were mediated by activation of NF-κB and AP-1 and dependent on light chain endocytosis. Maneuvers that either inhibit the activation of these transcription factors or block light chain endocytosis, both abrogated cytokine responses. Light chain activation of nuclear transcription factors appears to be signaled through the MAPKs ERK 1/2, JNK and p38. Pharmacologic inhibitors of these MAPKs blocked these cytokine responses in vitro in proximal tubule cells exposed to light chains. The totality of these findings strongly argue for a major role for proximal tubule cells and cytokines produced by these cells in the pathogenesis of chronic tubulointerstitial injury that is the hall-mark of myeloma kidney. These observation, in vitro, provide evidence that excessive light chain endocytosis, i.e., light chain protein overloading induces transcription and secretion of inflammatory and proinflammatory cytokines in the renal proximal tubular cells, that in turn may initiate renal interstitial fibrosis and tubular destruction. Ongoing studies also demonstrate that light chains induce TGF-β1, epithelial mesenchymal transition in cultured human proximal tubule cells (unpublished observations). Fibroblastic transformation of renal epithelial cells maybe an important mechanism of renal fibrosis seen in myeloma kidney.

Furthermore, the relatively non-toxic pharmacologic inhibitors of both NF-κB and MAPKs may provide potential therapeutic options for patients with myeloma kidney [22, 23]. A recent study showed that pituitary adenylate activating polypeptide (PACAP)-38, a naturally occurring peptide with well-known immune modulatory and anti-inflammatory effects [92] can protect proximal tubule cells from toxic effects of light chains in cultured cells, in vitro, and in rat kidneys infused with light chain, in vivo [93]. This peptide’s renoprotective effects, both in vivo and in vitro, were associated with the inhibition of activation of NF-κB, and p38 MAPK, further confirming the pathophysiologic role of these transcription factors and the cytokines in myeloma kidney.

At present, there is no specific treatment for myeloma kidney, and therapy is focused on controlling the tumor burden. Early intervention and institution of one of the several chemotherapy protocols or bone marrow transplantation before significant renal injury appears will often spare the patient from kidney disease. However, reversal of established kidney disease is often not possible. It is therefore reasonable to implement renoprotective measures early during the

course of the disease, and prevent or promptly treat events such as volume depletion, hypercalcemia, which can expose the kidneys to renal injury. Based on the recent insight into the role of inflammatory and pro-inflammatory cytokines in the pathogenesis of myeloma kidney, anti-inflammatory measures, such as, using steroids should be a reasonable approach. Indeed, steroids are frequently used as part of various therapeutic protocols in myeloma, however, there are no studies evaluating whether steroids are uniquely beneficial in ameliorating or reversing kidney involvement in myeloma. Recent studies using PACAP38, in vitro, and in animals, suggest that this agent might be helpful, however, there are no human studies with this agent.

Acute Cast Nephropathy

Some myeloma patients present with severe acute oliguric renal failure often associated with significant dehydration and with massive cast deposition both in distal but more prominently in proximal tubules [4, 94–96]. Occasionally, this presentation is seen concomitantly with contrast administration and additive nephrotoxicity of contrast dye as well as the accompanying dehydration have been implicated as contributing factors [4, 97, 98]. Acid pH in tubular fluid, reduced renal plasma flow and hypercalcemia are among other factors implicated in cast formation. Precipitates of Tamm-Horsfall glycoprotein in the presence of contrast media have also been demonstrated [99–101]. Hypercalcemia, Bence-Jones proteinuria, and hypovolemia have been present in most patients who developed this syndrome. Contrary to the widely held contention that contrast dye use is contraindicated in patients with myeloma, it has now been shown that contrast procedures can safely be performed provided that the patients are adequately hydrated using normal saline prior to, during and after the procedure [102].

Acute cast nephropathy is generally associated with the worst outcome among all types of kidney involvement in myeloma and warrants aggressive treatment, which some investigators believe should include plasmapheresis. There are no good large controlled trials demonstrating convincingly the effectiveness of plasmapheresis [103]. However, in one small controlled trial in a total of 29 patients, Zucchelli et al. [104] found plasmapheresis was associated with superior renal outcomes and better overall survival.

Light Chain Deposition Disease and Amyloidosis

Glomerular involvement in myeloma may manifest as either light chain deposition disease (LCDD) or as AL amyloidosis [2, 26, 105, 106]. Both disorders

are associated with monoclonal light chains often in the setting of multiple myeloma or occasionally with MGUS, a condition in which there may be overproduction of modest quantities of a monoclonal immunoglobulin but without overt myeloma. Rarely, LCDD presents without any detectable gammopathy and in kidney biopsy the nodular glomerular deposits can be mistaken for diabetic nephropathy if immunohistochemical studies are not carried out [107]. Although there may be peritubular deposition of light chains and amyloid, resulting in tubulointerstitial manifestation, both of these disorders clinically present predominantly as glomerular kidney diseases and are discussed in greater detail elsewhere in this book.

Vascular Lesions

Although isolated involvement of the renal vasculature is unusual, glomeruli and other vessels are concomitantly affected by light chain deposition. Amyloid or granular light chain deposition can be found. Light chain deposits are generally localized to the walls of arterioles and small and medium arteries. Both proliferative and non-proliferative vasculopathies can occur and contribute to progressive loss of renal function and tubulointerstitial destruction in some patients [2].

Neoplastic Cell Infiltration

Actual renal parenchymal infiltration by neoplastic plasma cells is a very rare entity as either a solitary plasmacytoma or a manifestation of multiple myeloma [108–110]. On some occasions, especially when associated with amyloidosis, the kidneys may attain a huge size and become palpable by abdominal examination. Plasmacytoid cells have been recovered from urine sediment in some patients with massive cell infiltration.

Hypercalcemia

Mild hypercalcemia (11–13 mg/dl) occurs in more than 25% of patients; marked hypercalcemia (>13 mg/dl) may also be encountered. The hypercalcemia is secondary to enhanced bone resorption mediated by osteoclast activating factors, a family of cytokines including lymphotoxins, interleukin-1β, a parathyroid related protein, and interleukin-6 produced by the neoplastic cells or by marrow stromal cells [111–114]. Hypercalcemia can interfere with renal

concentrating ability and simultaneously may have vasoconstrictive actions on renal vasculature causing decreased glomerular filtration rate, hypovolemia and pre-renal azotemia. Decreased urine formation and tubule flow rate enhance cast formation and sometimes may precipitate acute cast nephropathy. Saline administration usually reverses hypercalcemia, provided that renal function is not severely impaired. Loop diuretics also increase calcium excretion, but furosemide should not be given until the patient is clinically euvolemic, because it may facilitate nephrotoxicity from light chains. Generally most patients respond to volume repletion and chemotherapy. Rarely more aggressive management is necessary. Both gallium nitrate and bisphosphonates are nephrotoxic and should be administered only after adequate hydration. Response to biphosphonates occurs within the first several days of treatment; interrupting therapy is indicated as the calcium level normalizes. The effect of these agents is transient but often allows time for chemotherapy and hydration to prevent recurrence of hypercalcemia [115, 116]. In some patients with markedly elevated serum levels of abnormal immunoglobulins, the total concentration of serum calcium is elevated because of binding to globulins, but the ionized fraction remains normal [117]. This spurious hypercalcemia does not require treatment. Similarly, spurious hyperphosphatemia due to binding of inorganic phosphorus to the elevated immunoglobulins has also been observed [118, 119].

Acute Uric Acid Nephropathy

In patients with multiple myeloma hyperuricemia can occur from the increased nucleic acid turnover, either spontaneously or as a result of chemotherapy. Although patients with other lymphoproliferative disorders may have marked hyperuricemia (uric acid $>$20–25 mg/dl) and acute uric acid nephropathy, this is rare in patients with multiple myeloma. Judicious use of hydration with normal saline together with alkalinization of the urine and pre-treatment with allopurinol has all but eliminated this complication [120].

Obstructive Nephropathy

Obstructive nephropathy directly related to myelomatosis can occur secondary to ureteral amyloidosis, nephrolithiasis, papillary necrosis, huge proteinaceous renal pelvic cast formation, and neurogenic bladder due to spinal cord or nerve injury resulting from vertebral collapse [121, 122].

Hyperviscosity Syndrome

Although hyperviscosity occurs frequently in Waldenstrom's macroglobulinemia, rarely it can also be seen in multiple myeloma when the serum concentration of the monoclonal gamma globulin reaches very high levels [31, 117, 123]. It is usually manifested by impaired urine concentration, azotemia, and occasionally hematuria. Very rarely, it can cause acute renal failure or permanent renal damage. Under emergency situations, plasmapheresis with the removal of large amounts of macroglobulins can be accomplished. Exchange transfusions may also be done.

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Paraproteinemic Renal Diseases that Involve the Tubulo-Interstitium

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Abstract

The renal response to deposition of monoclonal light chains represents a spectrum of pathologic changes that can be divided into glomerular or tubulo-interstitial processes. Involvement of the tubulo-interstitium can include activation of the proximal tubule, proximal tubule injury/cell death, and cast nephropathy. In these diseases, the culprit is not the intact immunoglobulin protein but instead the immunoglobulin light chain. Recent non-invasive tests, including immunofixation electrophoresis or quantification of serum free light chains, have increased the sensitivity for detection of an abnormality in circulating free light chains and are invaluable ancillary tools, but short of renal biopsy, the diagnosis of these diseases can prove challenging. A description of the pathobiology and overview of the approach to management of these light chain-mediated renal lesions is provided.

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The spectrum of paraproteinemic renal diseases continues to expand, but can be divided into those diseases that manifest primarily as glomerular or tubulo-interstitial injury [1]. Patterns of tubular injury include a proximal tubulopathy and cast nephropathy (also known as 'myeloma kidney'). Immunoglobulin light chain deposition is directly responsible for the pathology in paraproteinemic renal diseases that produce tubulo-interstitial injury. Unlike most endogenous proteins, immunoglobulin fragments, particularly light chains, have a propensity to produce tubular damage. One reason for this association is related to the renal handling of these low-molecular-weight proteins. Light chains, once termed Bence Jones proteins [2], appear in the circulation as monomers ($M_r \sim 22$ kDa) and dimers ($M_r \sim 44$ kDa). Metabolism of these proteins is directly

related to glomerular filtration [3] and reduction in glomerular filtration rate increases circulating concentrations of light chains [4]. As will be discussed, nephron loss increases the filtered load of light chains in the remaining nephrons, providing an increased potential for nephrotoxicity.

Once filtered, light chains are reabsorbed into the proximal tubule by binding initially to a receptor that consists of megalin and cubilin [5–8]. After endocytosis, vesicles containing the ligand/receptor complex acidify to release the light chain and other bound ligands and permit recycling of the receptor back to the apical membrane to load more cargo; the subsequent addition of lysosomal enzymes permits hydrolysis of the light chain protein and return of the amino acid components to the circulation [9–11]. Although saturation of the multiligand endocytotic receptor complex of the proximal tubule permits light chains to appear in the tubule fluid of distal nephron segments and finally in the urine, overproduction of monoclonal light chain can result in significant concentration of these proteins in the proximal tubule.

The predominant clinical and laboratory evidence suggests that each light chain has unique toxicity that must therefore be ascribed to the individual physicochemical properties of these proteins. Light chains are modular proteins composed of two domains: a variable (V_L) and a constant (C_L) region [12]. Within the globular V_L domain are four framework regions that consist of β sheets that develop a hydrophobic core [13–17]. Interspersed among the framework regions are three separate hypervariable segments known as complementarity determining regions (CDR1, CDR2, and CDR3). The CDRs form loops structures and represent those regions of sequence variability in the light chain [13–15]. Diversity among the CDR regions occurs because the V_L domain is synthesized by combining V and J gene segments. Because the number of human Vκ and Vλ gene segments are estimated to be 30–50 and 20–30, respectively, and there are probably 5 Jκ and 20–30 Jλ gene segments [18], a large number of combinatorial possibilities exist. Thus, while possessing similar structures, no two light chains are identical. Mian et al. [19] suggested that amphipathic amino acids, especially tryptophan and tyrosine, are frequently present in CDR regions, because they allow flexibility to interact with a wide range of proteins. Thus, while required to promote antigen binding, these residues potentially allow cross-reactivity with other proteins, such as Tamm-Horsfall protein.

Involvement of the Proximal Tubule

The highly efficient protein reclamation system of the proximal tubule provides an important mechanism of conservation of amino acids and other essential molecules that are carried by proteins, such as folate [10, 11, 20].

However, this process can produce detrimental results in the setting of overproduction of monoclonal light chain proteins. Endocytosis of monoclonal light chain into the proximal tubule epithelial cell initiates a cascade of events that range from 'activation' of the cell to necrosis.

Activation of the Epithelium of the Proximal Tubule

Much attention in recent years has turned to the possibility that the process of protein reabsorption by the proximal tubule results in chemokine and cytokine release, which accelerates interstitial fibrosis and progressive renal disease. For light chains, this process has been characterized in some detail. Proximal tubular reabsorption of light chains sparks the activation of NF-κB [21] and mitogen-activated protein kinases [22]; these signaling pathways cooperate to promote chemokine and cytokine production by these cells [21–23]. Intrarenal production of these agents facilitates the infiltration of inflammatory cells that in turn increase intrarenal TGF-β production, resulting in matrix protein deposition and renal fibrosis. While many investigators have focused on the effects of albumin, which was recently reviewed [24], compared to human albumin, the effect of light chains on cells in culture was much more dramatic [22]. As glomerular filtration rate falls, the serum concentrations of monoclonal (and polyclonal) κ and λ light chains increase [4, 25]; thus the tubular concentration will increase in the remaining viable nephrons and proximal tubular epithelium. Activation of the proximal tubule with elaboration of chemokines and cytokines might also play a role in facilitating renal fibrosis in cast nephropathy [23]. Although it is not clear if all light chains produce this effect, it is interesting to speculate that, regardless of the underlying cause, renal failure per se increases the load of light chains (even polyclonal) presented to the proximal tubule, resulting in persistent activation of the epithelium and perpetuation of a pro-fibrotic environment that accelerates renal disease progression.

Proximal Tubule Necrosis

Endocytosis of light chains can produce more severe injury to the proximal tubule epithelium (fig. 1), with associated clinical manifestations of renal failure [26–28]. Proximal tubular epithelial injury and necrosis was associated with endocytosis of the light chain into proximal tubule cells with subsequent distention of the endolysosomal system. Functional and morphologic alterations (fig. 1a) of these cells followed [26, 27]. The physicochemical property(s) of the monoclonal light chains responsible for the more severe form of epithelial cell injury is unknown at present, but may relate to aggregation of light chain as it is concentrated in the epithelial cell after endocytosis and subsequent impairment of hydrolysis of the protein.

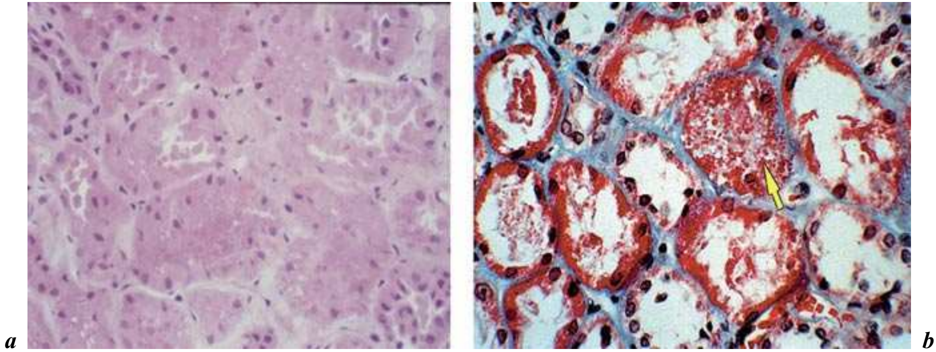

Fig. 1. Proximal tubule lesions associated with monoclonal light chains. ***a*** Hematoxylin and eosin, ×350. Proximal tubule cell injury with desquamation and fragmentation of tubular cells. Note cellular fragments in the tubule lumen. ***b*** Trichrome stain, ×350. Injured proximal tubule cells with intracytoplasmic crystalline-like structures are depicted (yellow arrow).

Fanconi Syndrome

A classic renal presentation of multiple myeloma is Fanconi syndrome, which is characterized by a renal tubular acidosis type II and defective sodium-coupled co-transport processes, producing aminoaciduria, glycosuria and phosphaturia. Renal biopsy typically shows crystalline or fibrillary-like inclusions of light chain protein in the epithelium of the proximal tubule (figs. 1b, 2 and 3). Fanconi syndrome may herald the onset of multiple myeloma, although the myeloma is generally low-grade, perhaps because of formation of crystals of light chain in the plasma cell also. The light chains that produce this syndrome are almost always members of the κ1 subfamily. Unusual non-polar residues in the CDR1 region and absence of accessible side chains in the CDR3 loop of the variable domain of κ1 light chains result in homotypic crystallization of the light chain [29, 30]. Presumably, if the monoclonal light chain was not toxic to the plasma cells and increased amounts of the light chain was released into the circulation and presented to the proximal tubule, a more severe type of renal injury would result.

Cast Nephropathy

Another more common mechanism of light chain-mediated tubule damage relates to intratubular obstruction from precipitation of light chains in the distal nephron (fig. 4) [27]. The mechanism of cast formation by light chains has been clarified and centers on Tamm-Horsfall protein [27, 28, 31–35]. Tamm-Horsfall

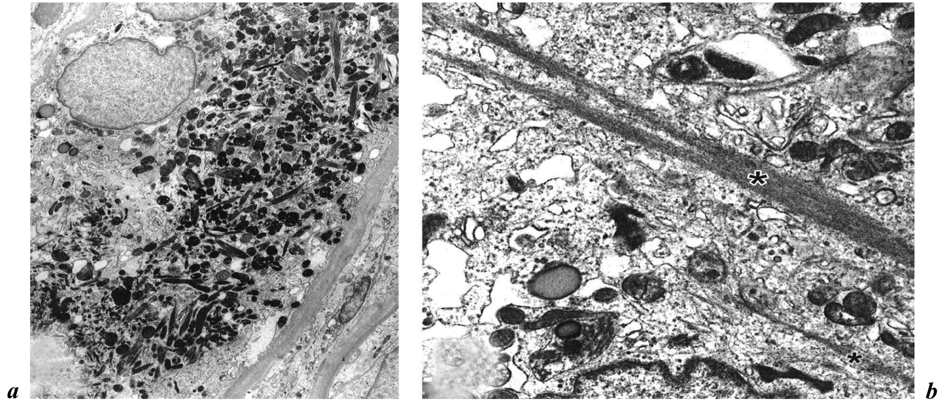

Fig. 2. Proximal tubular lesion with intracytoplasmic crystalline-like inclusions. ***a, b*** Transmission electron microscopy. Uranyl acetate and lead citrate. ***a*** × 7,500. ***b*** × 17,500. ***a, b*** Intracellular fibrillary-like inclusions in proximal tubular cell are shown with ***b*** exhibiting details of inclusions.

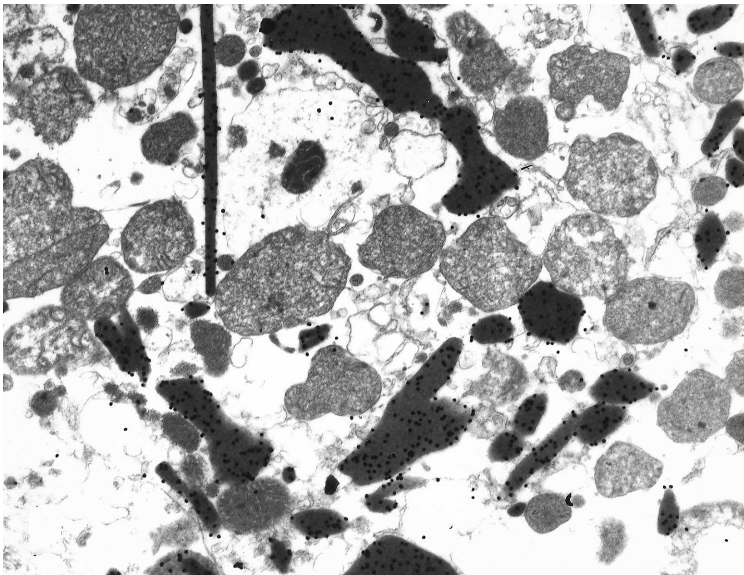

Fig. 3. Proximal tubule crystalline-like cytoplasmic inclusions. Transmission electron microscopy. Uranyl acetate and lead citrate. Immunogold labeling for kappa light chains. × 12,500 Crystalline-like inclusion are strongly labeled for kappa light chains, as depicted by gold particles (black dots) on top of them.

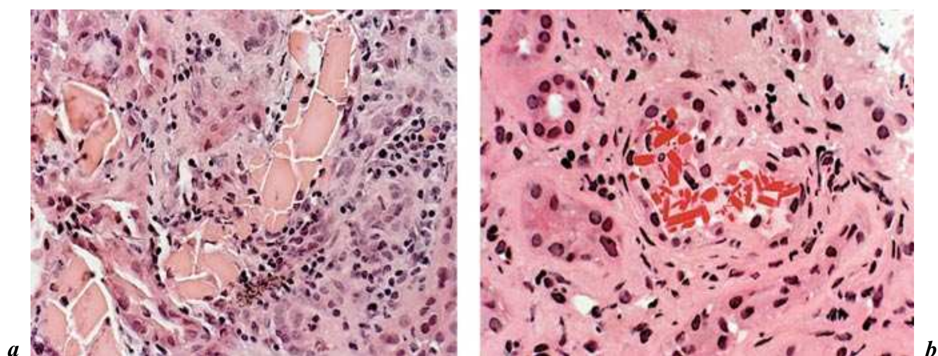

Fig. 4. Light chain cast nephropathy (myeloma kidney). ***a*** × 160. ***b*** × 350. Photomicrographs depicting some of the types of casts observed in cast nephropathy. ***a*** Prominent interstitial inflammatory infiltrate is present surrounding a distal nephron cast with fracture planes, typical of this condition. ***b*** Distal nephron cast composed of crystals.

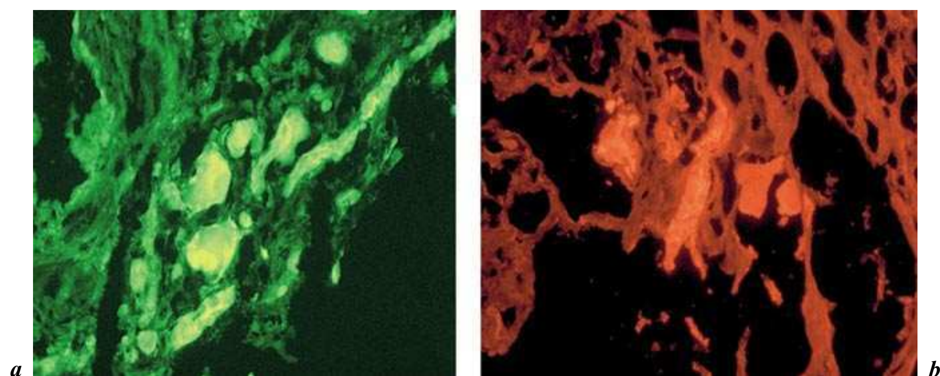

Fig. 5. Light chain cast nephropathy (myeloma kidney). Direct immunofluorescence for Tamm-Horsfall protein (Texas red) – **(*a*)** ×160, and monoclonal lambda light chains (fluorescein) – **(*b*)** ×160. Distal nephron casts contain both Tamm-Horsfall protein and monoclonal light chains.

protein is typically found in the casts and forms the matrix upon which the cast is formed (fig. 5). Only cells of the thick ascending limb of the loop of Henle synthesize Tamm-Horsfall protein, the major protein constituent of normal urine [36, 37]. Tamm-Horsfall protein belongs to the class of glycophospholipid-anchored protein that permits extracellular localization on the apical surface of the cell [38]. A soluble form of THP also appears in tubular fluid of the distal nephron and urine. Although constitutively expressed, an increase in dietary salt increases expression of THP in rats [39].

Intravenous infusion of nephrotoxic human light chain in rats acutely elevates proximal tubule pressure and simultaneously decreases single nephron glomerular filtration rate; intraluminal protein casts were identified in these kidneys [40]. Isolated mechanical obstruction of single nephrons in vivo reduces glomerular blood flow and produces atrophy of the nephron proximal to the obstruction [41, 42]. Using an in vivo isolated microperfusion model of cast nephropathy in rats, casts were observed to form exclusively in the distal portion of the nephron, beginning with the thick ascending limb of Henle's loop; removal of THP prevented cast formation in vivo [32]. Human light chains that precipitate in vivo in the rat co-aggregate in vitro with human THP [30]. Light chains bind THP with differing affinities. Enzymatic removal of the carbohydrate moiety of THP did not alter binding by light chain [33]. A single 9-amino acid binding domain for light chain (residues 225–233) was mapped on THP [35]. Using a yeast two-hybrid assay [43] with a segment of human THP known to contain the light chain binding domain as the 'bait' protein, the interacting domain on both κ and λ light chains was found to be the CDR3 region [44].

Diagnosis

Diagnosis of a paraproteinemic renal lesion is hampered by the general lack of sensitivity and specificity of currently available non-invasive tests. Because of the insensitivity of assays based upon routine protein electrophoresis of serum and urine, these tests have been replaced by more sensitive antibody-based assays that include immunoelectrophoresis and immunofixation electrophoresis. More recently, a quantitative assay that detects free light chains in the circulation has been developed and provides the required sensitivity to identify patients who have circulating monoclonal free κ or λ light chains. This test is perhaps more convenient since it obviates the need to obtain urine for analysis. While these tests do not diagnose a paraproteinemic renal lesion, they can increase suspicion that co-existent renal dysfunction is related to monoclonal light chain deposition, particularly if free light chains are detected. The gold standard for diagnosis remains renal biopsy with demonstrated evidence of deposition of monotypical light chains in the area of injury.

Treatment

Most tubulo-interstitial renal lesions occur in the setting of multiple myeloma and complicate therapy because of the attendant renal dysfunction. These patients should be managed with cytoreduction therapies available in the

medical community. Increasingly, the approach taken has been high-dose chemotherapy with autologous peripheral stem-cell transplantation (HDT/SCT) [45, 46]. The presence of renal failure is not considered a contraindication for HDT/SCT, although procedure-related mortality is increased and this therapy should be performed at institutions that specialize in the management of these patients [47, 48]. Recovery of renal function can occur following HDT/SCT even in myeloma patients already receiving renal replacement therapy [47, 49].

Regardless of the form of cytoreduction therapy undertaken, adjunct therapy designed to limit the interaction of light chain with THP should also be pursued. Because this interaction is affected by tubular fluid flow rates, luminal concentrations of calcium and NaCl, presence of furosemide, and concentrations of both light chain and Tamm-Horsfall protein [28, 32], hypercalcemia and hyperuricemia should be anticipated and corrected. Patients should be encouraged to drink water up to 2–3 l/day as tolerated and adhere to a low-salt diet. Radiocontrast agents, non-steroidal anti-inflammatory drugs, and diuretics should be avoided.

While an earlier study [50] supported a role for plasmapheresis specifically in the setting of acute renal failure related to cast nephropathy, a recent randomized controlled trial suggested no benefit from plasma exchange therapy [51]. Although this trial included 104 patients, a wide 95% confidence interval around the differences between the groups suggested that some benefit or harm in specific subgroups might have been missed. In addition, renal biopsy was not performed, the duration of the study was extended due to difficulty in admitting patients, and it appeared that some participants (14 out of 58 in the treatment group; 4 of 39 in control group) did not have monoclonal light chain detected in either the serum or urine. Tests to confirm reductions in light chain concentrations with treatment were also not performed. Despite these limitations, this study demonstrated that there was no benefit in plasma exchange in acute renal failure in multiple myeloma. However, while plasma exchange might not be a satisfactory approach to the management of these patients, the findings do not disprove the concept that efforts to rapidly decrease serum light chain concentrations should be included in the therapeutic strategies to improve renal function, particularly in the setting of acute renal failure.

Conclusion

The diversity of tubulo-interstitial renal lesions associated with paraproteinemias can be explained by the physicochemical properties of the light chains, which, when pathologic, are responsible for these lesions. Monitoring circulating light chains during treatment may provide the nephrologist prognos-

tic insight into the potential for improvement in renal function, since continued presentation of the offending light chain to the nephron will perpetuate deposition and injury.

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The Mesangium as a Target for Glomerulopathic Light and Heavy Chains: Pathogenic Considerations in Light and Heavy Chain-Mediated Glomerular Damage

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Abstract

Certain structurally abnormal light and heavy chains are known to be nephrotoxic and alter mesangial homeostasis producing pathological alterations. Many of the mechanisms involved in light chain–mesangial interactions have been deciphered using an in vitro model, providing a framework for understanding the sequence of events that leads to irreversible glomerular changes and eventually renal failure. The molecular events involved in the pathogenesis of these disorders are now for the most part well-established. These studies have delineated the sequence of steps involved and crucial events have been determined. An animal model is being developed which will undoubtedly contribute significantly to validate the information that has been obtained from the in vitro models. The present chapter will address the pathogenesis of these disorders with an emphasis on highlighting crucial steps possibly amenable to therapeutic intervention to stop or ameliorate adverse consequences leading to irreversible changes.

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Glomerulopathic Light and Heavy Chains: Factors Involved in Their Pathologic Effects

Glomerular Structure: Interactions of Light and Heavy Chains with Glomerulus

The glomerulus (fig. 1) consists of an anastamosing network of capillaries and two layers of epithelia: the visceral epithelium (podocytes), and the parietal

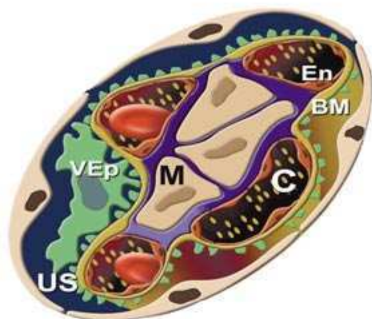


Fig. 1. Schematic representation of the renal glomerulus highlighting mesangial area (M) and endothelium (En) separating it from the circulating blood through the capillary spaces (C) with usual gaps which permit light and heavy chains to gain access to the mesangium. Non-glomerulopathic LCs due to their low-molecular-weight traverse the glomerular basement membrane (BM) through filtration slits in the visceral epithelial cells (VEp) and pass into the urinary space (US) to the proximal and distal tubules.

epithelium separated by a basement membrane. Its central stalk of mesangial cells and a network of fibrillary matrix comprise the mesangium which is invested by the glomerular basement membrane. Blood supply to the glomerulus is maintained by the anastamosing capillaries whose walls acts as a selective filter. The renal glomerulus, therefore, is a complex structure performing specialized functions culminating in the filtration of plasma and the production of urine. The endothelium and subendothelial zones are the first to interact with circulating abnormal light and heavy chains in patients with plasma cell dyscrasias. Because most light chains (LCs) present in the circulation have a low-molecular-weight, they normally traverse the glomerular wall and are delivered to the proximal tubules. Normally, 90% of the circulating LCs are metabolized by an endosomal process in the proximal tubules and their amino acids returned to the circulation. The fate of structurally abnormal light and heavy chains is certainly less predictable. Some may interact with the glomerular basement membranes and are trapped in the peripheral capillary walls while others find their way into the mesangium [1]. Purported receptors for glomerulopathic LCs on the surface of mesangial cells are most likely the driving force for these LCs to engage in interactions with mesangial cells. Glomerulopathic LCs center their pathologic actions on the mesangium.

The glomerular mesangial cell is one of the major cell types and accounts for 30–40% of the total population of the glomerulus [2]. Mesangial cells are of mesenchymal origin and are most akin to vascular smooth muscle cells. These cells generally have an indented nucleus and a small amount of cytoplasm containing organelles such as mitochondria, lysosomes, ribosomes, endoplasmic reticulum and stacked Golgi cysternae [3]. Numerous bundles of small intracellular filaments with spindle densities or dense bodies and associated attachment plaques are present in the normal mesangial cells (fig. 2). They are contractile, phagocytic and capable of proliferation. Ultrastructurally, the mesangial cell

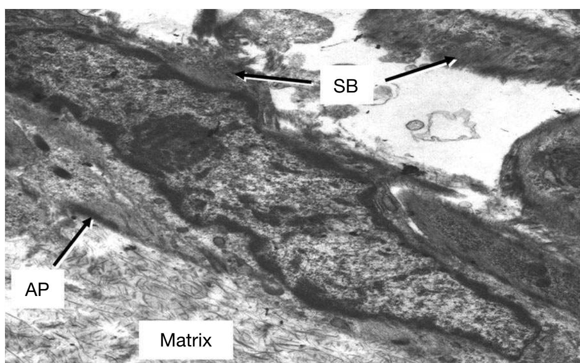


Fig. 2. Normal mesangial cell grown on Matrigel (matrix). Numerous bundles of small intracellular filaments with spindle densities or dense bodies (SB) (seen also in portion of adjacent mesangial cell) and associated attachment plaques (AP) are clearly identifiable in normal mesangial cells. Transmission electron microscopy, uranyl acetate and lead citrate ×12,500.

appears to be remarkably irregular in shape with numerous processes of varying lengths projecting into the surrounding extracellular matrix and connecting with the glomerular basement membrane. Their main functions are to secrete and regulate the extracellular matrix, produce a number of biologically active mediators and enzymes, and to provide structural support for the glomerular capillary loops [4].

Mesangial cells manipulate macromolecules such as lipids, immune complexes and advanced glycation end products (AGEs). These macromolecules, trafficking through the mesangium via mesangial channels based on their size, concentration, charge, or affinity for either matrix components or mesangial cells surface receptors, are either taken up by these cells or remain trapped in the matrix [5]. Receptor-mediated uptake processes have been demonstrated for lipoproteins, AGE, immunoglobulin A (IgA) and immunoglobulin G (IgG) complexes. Clearance by the mesangial cells following degradation of these complexes, or mesangial cell injury leading to inflammation may be a consequence of the modification and concentration of said complexes. Likewise, mesangial cells may engage in interactions with certain structurally abnormal LCs resulting in pathological consequences.

Light and Heavy Chains: Structural Considerations

LCs are units of the immunoglobulin molecule produced by B lymphocytes and plasma cells. They vary in molecular weight from approximately 12,000–30,000 Da and may aggregate into polymers. Both kappa (κ) and

lambda (λ) LCs have a constant polypeptide region of 105–107 amino acids. The identity of the LC is attributed to its variable region, which contains 110–120 amino acids. Structural variability in the variable region of the LC accounts for their organ pathogenicity and, more specifically, nephrotoxicity [6–9]. Amino acid substitutions in the variable portion of the abnormal LCs result in alterations in the primary, and in some cases tertiary, structure and conformation of the LCs associated with LC deposition disease (LCDD) and AL-amyloidosis [8–11]. These amino acid substitutions introduce hydrophobic residues in the exposed portions of the variable regions of the LCs. Post-translational modifications such as glycosylation may also be responsible for the glomerulopathic properties of some LCs. Glycosylated LCs are four times more frequently associated with amyloidosis than non-glycosylated LCs [9, 12]. More than 85% of the physico-chemically abnormal LCs produced by patients with plasma cell dyscrasias have been reported to be nephrotoxic but only approximately 30% are glomerulopathic.

The abnormal circulating heavy chains in related disorders (heavy chain deposition disease [HCDD] and AH-amyloidosis) exhibit deletions of the CH1 domain of the heavy chain molecule in most cases [13]. Deletions in the CH2 domain and hinge region have also been described but they are much less common, especially the latter which is quite rare [14, 15]. Lack of the CH1, CH2 or hinge domains in the mutant heavy chain molecules precludes association with the immunoglobulin binding protein and corresponding LCs; therefore, the heavy chains are secreted and circulate as free units being delivered to the kidney [16].

An entire chapter is dedicated to a detailed description of the structural abnormalities that can be found in light and heavy chains and how these impact on their pathogenicity.

Pathogenetic Considerations

Glomerulopathies

Within the glomeruli, pathologic light and heavy chains engage in two distinct characteristic patterns of injury. Fibrillary monoclonal light/heavy chain-related renal disease seen in AL and AH-amyloidosis, respectively, and conditions characterized by the granular deposition of these immunoglobulin components (light and heavy chain deposition diseases), either in combination or by themselves, constitute these patterns. Although all types of light and possibly heavy chains can cause amyloidosis, it is usually more commonly associated with λ LC than κ (approximately 2–3 times) and rarely seen in association with heavy chains (AH-amyloidosis) [6, 7]. It has also been shown by Solomon et al. [17] that λVI LCs are more specifically associated with AL-amyloidosis. Comenzo

et al. [18] have provided corroborating evidence by demonstrating a striking tropism of the λVI LCs for glomerular AL-amyloidosis. Only a few AH-amyloidosis cases have been reported, the majority gamma (IgG)-related. Only gamma and mu (IgM) AH-amyloidosis have been documented in the literature. LCDD is usually associated with κLCs (predominantly κI and IV) while HCDD is most often associated with gamma heavy chains.

In amyloidosis, amyloid deposition is first seen focally in the mesangium with the normal mesangial matrix being replaced by amyloid fibrils [19–21] (fig. 3a). Progression of the disease results in further amyloid deposition within the glomerulus eventually involving peripheral capillary walls and totally obliterating its normal appearance and structure (fig. 3b, c) and may also affect interstitium and vessel walls. Ultrastructural examination of amyloid reveals randomly distributed, non-branching fibrils which are 7–12 nm in diameter (fig. 3d). Deposits exhibits a salmon-pink appearance on Congo Red staining (fig. 4a), while polarized light microscopy of these deposits show an apple-green birefringence when stained with Congo Red [22, 23] (fig. 4b). If the Congo Red stain is viewed under fluorescence using the fluorescein gate it shows a deep red color (fig. 4c). The accumulation of these fibrils in the kidney and other organs of the body might be at least partially attributed to the failure of the immune system in recognizing amyloid as foreign in nature [24]. The overall incidence of AL-amyloidosis in patients with myeloma is approximately 7–10% [25].

The mesangium is also the glomerular compartment where pathological alterations occur in LCDD. The classic glomerular pattern of nodular glomerulosclerosis seen in LCDD (fig. 5a) is usually preceded by other morphological manifestations such as mesangial or membranoproliferative patterns [26, 27], processes centered in the mesangium. The prominent mesangial nodularity within the glomerulus, characteristic of LCDD in its mature stage, results from the deposition of extracellular matrix proteins admixed with monotypic LC deposits (fig. 5b). Thickening of the glomerular peripheral capillary walls and tubular basement membranes are also noted resulting from the deposition of LCs. Ultrastructurally, the deposits are seen as punctate, powdery, granular, electron-dense material (fig. 5c). The incidence of LCDD in patients with plasma cell dyscrasia is approximately 5% [28]. Only the nodular glomerulosclerotic pattern has been described in HCDD, a possible reflection of the limited experience with this disease at this time.

LC-mediated glomerular injury, while initially affecting mesangial cell function [4], causes several downstream alterations within the mesangium. These include matrix expansion due to increased extracellular matrix (ECM) protein synthesis and deposition [20, 26, 27, 29–31], phenotypic transformation of mesangial cells [30, 32–37] and the formation and deposition of amyloid within the mesangium [20, 26]. Unfortunately, knowledge regarding the pathogenesis

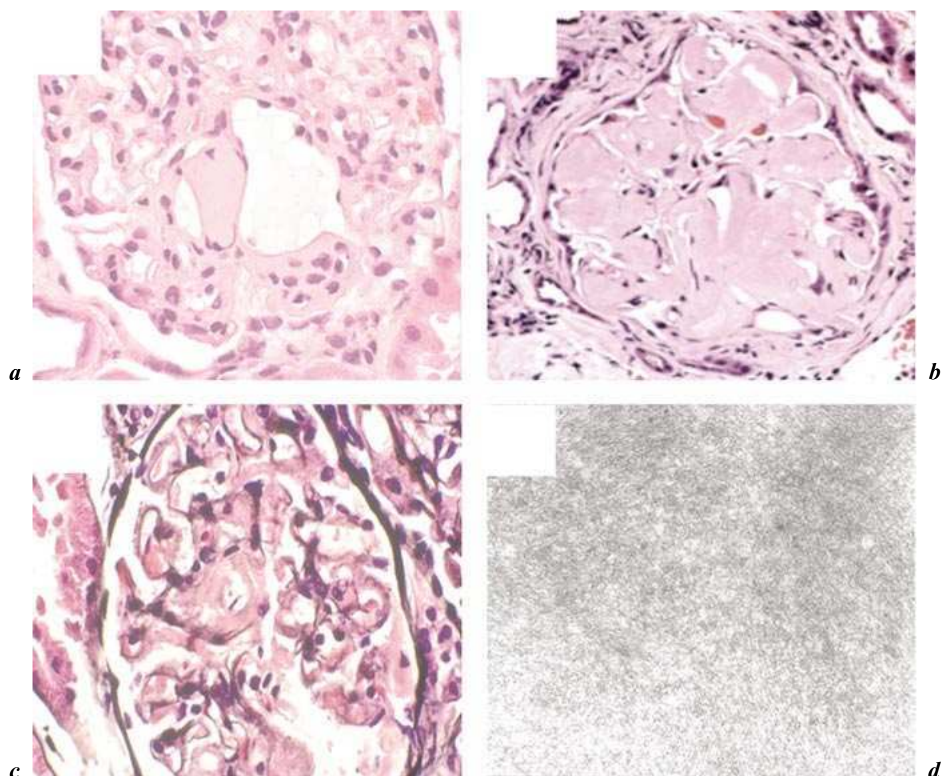


Fig. 3. Glomerulus with AL-amyloidosis. ***a***, ***b*** Hematoxylin and eosin stain ×400, ***c*** Jones silver methanamine stain ×400. ***d*** Transmission electron microscopy, uranyl acetate and lead citrate ×25,500. ***a*** Illustrates early mesangial amyloid deposition while in (***b***) the process is more advanced and the normal glomerular structure has been replaced by abundant amyloid deposition. ***c*** highlights the replacement of normal mesangial matrix which would normally by argyrophilic (black) with the silver stain. ***d*** Shows typical randomly distributed 8–10 nm non-branching filaments indicative of amyloid which have replaced the mesangial matrix entirely.

of HCDD is extremely primitive but the end result – nodular glomerulosclerosis, is the same as in LCDD, suggesting that there should be close similarities in pathogeneses between these two disorders.

Phenotypic transformations of the normal smooth muscle type mesangial cells to either a macrophage phenotype (fig. 6) or a myofibroblastic phenotype is considered to be a vital step in the pathogenesis of glomerular AL-amyloidosis and LCDD, respectively [35, 37]. The former transformation involves the acquisition of numerous mature lysosomes which are involved in the active

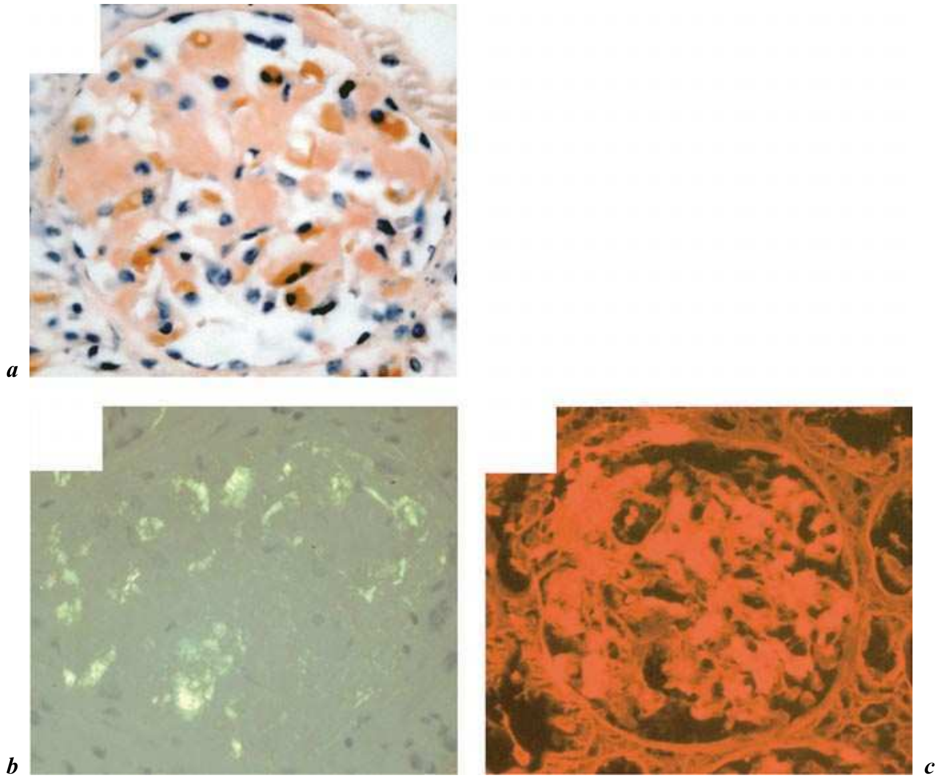


Fig. 4. Glomerulus with AL-amyloidosis. ***a*** Congo Red stain ×400, (***b***) polarization of (***a***) ×400, ***c*** Congo Red stain specimen viewed under fluorescent light using fluorescent gate. ***a*** Illustrates classical salmon-pink appearance of amyloid which when polarized, ***b*** reveals apple-green birefringence. In (***c***), intense red color is associated with areas where amyloid is deposited when a Congo Red stained specimen is examined under fluorescence microscope.

degradation and remodeling of the abnormal LCs. This phenotype is associated with AL-amyloidosis and the cells express high levels of CD68 and cathepsin D (fig. 7). These cells also express decreased levels of actin; smooth muscle actin and muscle specific actin. In contrast, the transformed myofibroblast is shown to possess numerous rough endoplasmic reticulum cisternae and Golgi stacks identifying the cells ability to produce excessive amounts of proteins. They do not express appreciable amounts of CD68 and cathepsin D, but increased amounts of smooth muscle actin, muscle specific actin and early endosomal antigen (EEA). This phenotype is noted in LCDD (fig. 7). The occurrence of these two distinct transformed cells provides evidence for two divergent

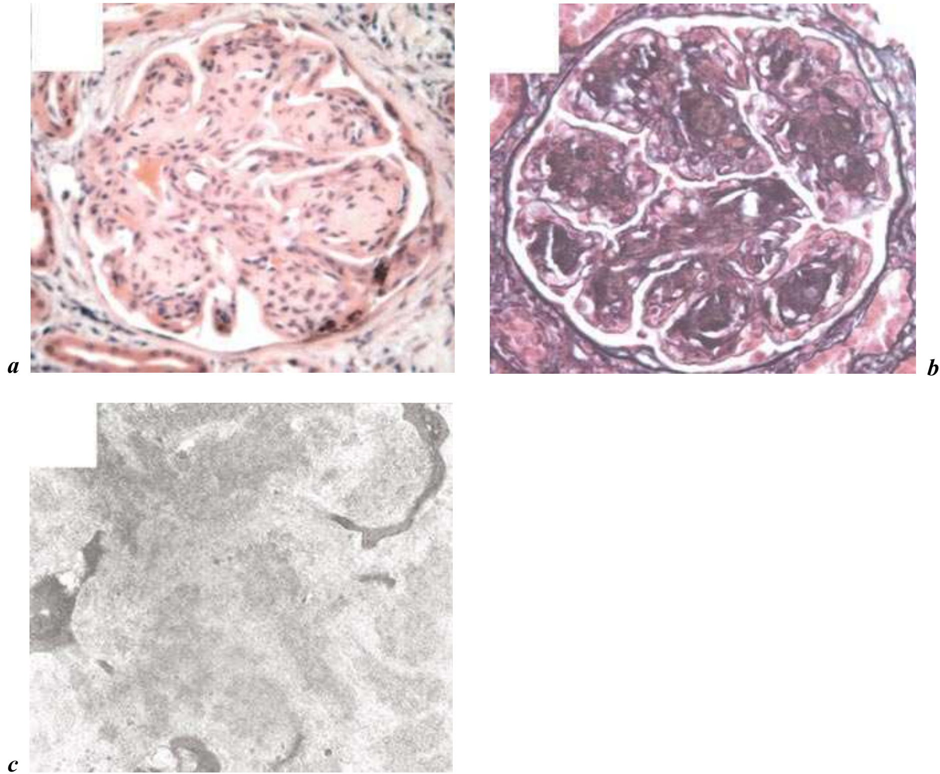


Fig. 5. Light chain deposition disease. ***a*** Hematoxylin and eosin stain ×400, ***b*** silver methenamine stain ×400, ***c*** transmission electron microscopy, uranyl acetate and lead citrate. Prominent mesangial nodularity is noted in this advanced stage of LCDD (***a***). ***b*** Demonstrates intense silver staining in mesangial areas as a result of increased extracellular matrix and LC deposits. ***c*** Punctate electron dense material characteristic of LC deposits in the mesangium.

pathways in the pathogeneses of AL-amyloidosis and LCDD; related to the type of LC to which the normal mesangial cell is exposed.

The production and accumulation of numerous lysosomes by the macrophage phenotype in the mesangial cell is essential in the processing of amyloidogenic LCs with the subsequent formation of amyloid [35]. Acquisition of the macrophage phenotype by human mesangial cells has been shown to be associated with macrophage-like functions, expression of Fc receptors, pinocytosis of immune complexes and insoluble mediator and release of a number of cytokines and mediators [34]. Amyloid fibrils formed by these cells are later deposited into the mesangium where they accumulate (fig. 6). Amyloid accumulation is aided by the destruction the normal matrix by matrix metalloproteinases

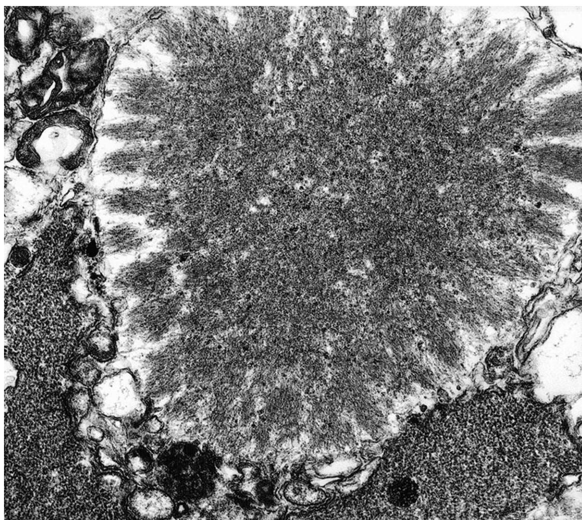


Fig. 6. AL-amyloidosis. Transmission electron microscopy, uranyl acetate and lead citrate ×30,000. Abundant amyloid deposition in the mesangium. Adjacent mesangial cell shows a macrophage phenotype and mature lysosomes at the cell periphery intimately associated with extracellular amyloid fibrils.

(MMPs) whose activation is stimulated by the extracellular amyloid deposits. Ultrastructural labeling studies have shown amyloidogenic LCs to be present in mature lysosomal structures prior to amyloid formation. Lysosomal enzymes have been reported to play a crucial role in the processing of LCs and in the formation of amyloid fibrils in vivo [38, 39], while in vitro studies [40] have shown a similar association.

How do Glomerulopathic Light and Heavy Chain Engage in Interactions with Mesangial Cells?

Non-pathologic LCs are freely filtered through glomerular capillary walls and do not interact with the mesangium at all. Experimental work has been done exclusively on the interactions of mesangial cells with glomerulopathic LCs [41]. There has not been any work published addressing how abnormal heavy chains engage in pathologic mesangial alterations. Therefore, only LC–mesangial cell interactions will be addressed.

Glomerulopathic, but not tubulopathic LCs engage with mesangial cells and alter mesangial homeostasis. AL and LCDD LCs compete with purported receptor(s) present on mesangial cells [41]. The structurally abnormal LCs avidly engage in interactions with mesangial cells through these receptors. Non-pathologic and tubulopathic LCs do not participate in interactions with

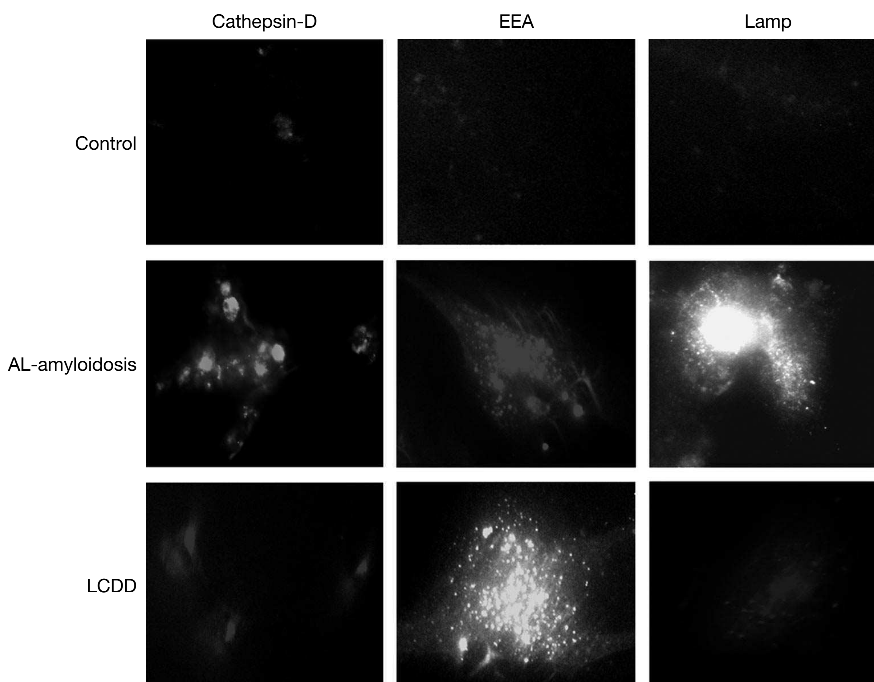


Fig. 7. Fluorescence microscopy. Mesangial cells incubated with LCDD-LCs reveal abundant expression of EEA and no LAMP and cathepsin-D, while those incubated with amyloidogenic LCs show abundant LAMP and cathepsin-D, but minimal amounts of EEA ×750.

mesangial cells, presumably because the receptors present on mesangial cells are not 'compatible' with the structural/conformational characteristics of these LCs and, as a result, receptor–ligand interactions do not occur and mesangial homeostasis is preserved.

While all glomerulopathic LCs compete for the same mesangial receptors, non-glomerulopathic ones do not [41]. The specific nature and characteristics of the receptor for LCs on mesangial cells remains elusive. There is, however, a receptor for LCs in the brush border of proximal tubules which has been characterized (cubulin/megalin) but the receptor on mesangial cells is understood to be different. Further work is currently being performed to refine our understanding of the LC receptor on mesangial cells.

Interactions between Glomerulopathic Light Chains and Mesangial Cells: Initial Events

Signaling molecules are activated as pathologic LCs engage in interactions with receptors on the mesangial cells surface. Two signaling molecules are of

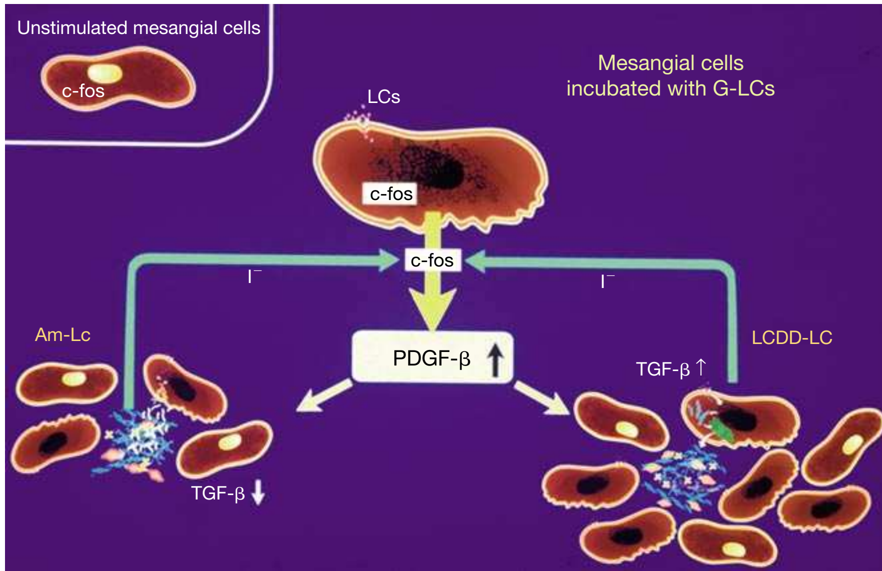
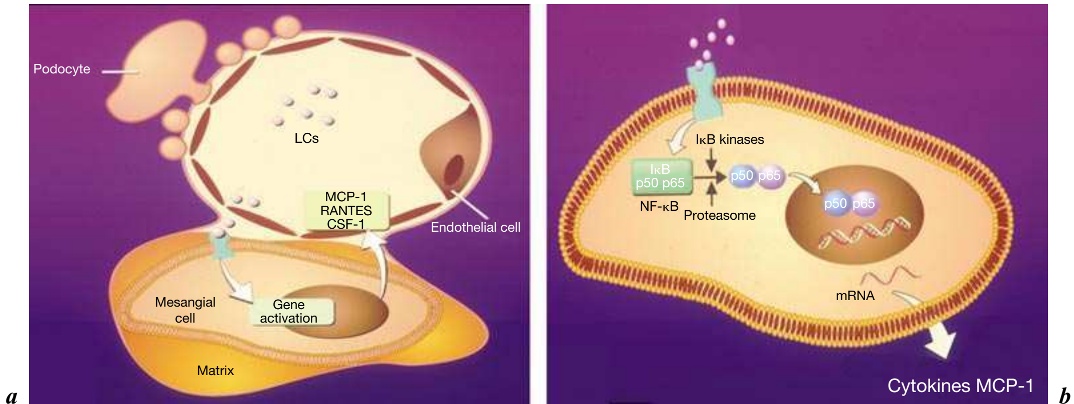


Fig. 8. Schematic representation of early events that occur when mesangial cells are exposed to different types of glomerulopathic LCs. Upon incubation of mesangial cells with glomerulopathic LCs, c-fos changes its normal cytoplasmic to a nuclear location. c-fos, through the action of PDGF-β controls cell proliferation and is also responsible for cell surface changes (ruffling) and rounding-up of mesangial cells. PBGF-β is activated in both conditions (AL-amyloidosis and LCDD). TGF-β is increased in LCDD and decreased in AL-amyloidosis.

unique importance in this regard: c-fos and NF-κB [42, 43] (figs. 8, 9). These molecules migrate from their normal cytoplasmic locale to the nucleus within minutes of exposure of mesangial cells in culture to glomerulopathic LCs obtained from the urine of patients with LCDD and AL-amyloidosis. c-fos participates in altering mesangial cell shape resulting in rounding-up and surface ruffling, as glomerulopathic LCs interact with mesangial cells. Surface ruffling results in the amplification of surface area to enhance interactions with LCs. Activation of NF-κB results in stimulation of cytokine production such as MCP-1 by mesangial cells via activation of genes involved in this process [42–44] (fig. 9a, b).

Early events also include autocrine production of PDGF-β by mesangial cells resulting in cellular proliferation; an effect also controlled by c-fos [43] and discussed more extensively in the following section. Incubation of mesangial cells with tubulopathic LCs do not alter c-fos or NF-κB, thus homeostasis is preserved.

The fact that amyloidogenic LCs are avidly phagocytosed by mesangial cells via a clathrin-mediated process is important from a pathogenesis standpoint.



a *b*

Fig. 9. Schematic representation of NF-κB activation. Upon exposure to glomerulopathic LCs NF-κB changes its normal cytoplasmic to a nuclear localization. Gene activation leads to the production of cytokines such as MCP-1, among others.

Phagocytosis of LCs can be observed within minutes of incubating amyloidogenic LCs with mesangial cells [41]. The phagocytosed LCs are then transported to the mature lysosomal system where they are processed. Rab proteins are involved in the intracellular transport of LCs in mesangial cells [41]. In contrast, LCDD-LCs are only minimally phagocytosed with most remaining at the mesangial cell surface where, by interacting with their corresponding receptors they engage in active stimulation and transformation of mesangial cells. The phagocytosed LCDD-LCs are catabolized in the endosomal compartment and are not delivered to mature lysosomes [41].

PDGF-β and TGF-β: Growth Factors Responsible for Alterations in Mesangial Homeostasis

Production and activation of PDGF-β by mesangial cells occurs in both AL-amyloidosis and LCDD and is responsible for early mesangial proliferation in these diseases by increasing the number of mesangial cells available to contribute to alter mesangial homeostasis. The production of PDGF-β is more pronounced when LCDD-LCs interact with human mesangial cells in culture than when AL-amyloidosis LCs interact.

During the early phases of AL-amyloidosis and much more noticeable in those of LCDD, cell proliferation under the influence of PDGF-β occurs and dominates the pathologic manifestations. The mesangio and membranoproliferative variants of LCDD are a result of predominant PDGF-β production and activation (without significant TGF-β contribution at this time). In more advanced

phases, matrix deposition predominates in LCDD under the influence of TGF-β, as detailed below, and cell deletion occurs. In early amyloidosis some degree of mesangial cell proliferation is virtually always present, also a result of the action of PDGF-β. As time progresses and amyloid deposition occurs in the mesangial matrix, other molecules acquire a more significant role and cell proliferation ceases. Eventually amyloid deposition takes over the mesangium, as mesangial matrix is replaced. Apoptosis is enhanced when mesangial cells are exposed to glomerulopathic LCs in vitro [45]. Apoptosis of mesangial cells is likely the predominant mechanism responsible for cell deletion in these diseases [46].

The other crucial growth factor that participates in the progression of these disorders is TGF-β [47–49]. TGF-β production is potentiated by LCDD-LCs while TGF-β is inhibited in AL-amyloidosis (fig. 8). TGF-β plays a fundamental function in the mesangium; it regulates matrix production by mesangial cells [48, 49]. The fact that TGF-β is produced and activated clearly explains the matrix expansion that occurs in the advanced phases of LCDD (fig. 5a, b). The in vitro model of LCDD has been used to delineate the intricacies of the pathogenesis of this disorder [43]. Likewise, the fact that TGF-β is inhibited in AL-amyloidosis (presumably as a result of amyloid deposition), provides a clear explanation for the inability of the damaged matrix actively destroyed by the activated MMPs to be restored. This results in the typical picture seen in advanced amyloidosis where the normal mesangial matrix is destroyed and replaced by amyloid fibrils (fig. 3c).

Matrix Metalloproteinases in Mesangial Interactions with Glomerulonephritis Light and Heavy Chains

MMPs are structurally and functionally related zinc-dependent endopeptidases [50–55], and are mostly ubiquitous enzymes with a high degree of sequence homology. All MMPs are synthesized as zymogens and are secreted extracellularly either as free entities or transmembranely attached [56]. They are arranged into eight distinct structural classes; five classes include the secreted MMPs, while the other three classes account for the membrane-tethered MMPs [57]. MMPs function in the modulation of cell–matrix through ECM turn-over where they influence cell signaling by tightly regulating the structure and composition of the ECM, thereby impacting on cell proliferation, differentiation and apoptosis [58]. Their roles are evident in several biological processes; some normal, others pathological.

Several structural domains have been described in MMPs; some of which exists commonly among them, while others are activity-specific domains [59]. These domains along with substrate affinities have contributed to the classification of MMPs [60]. These domains (fig. 10) include the signal peptide or

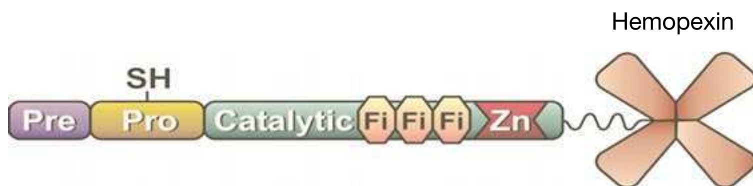


Fig. 10. Schematic representation of an MMP structure. Different structural components of a typical MMP are shown in this scheme. Catalytic = Catalytic domain; Fi = fibronectin repeats; Hemopexin = hemopexin-like domain; Pre = pre-domain; Pro = pro-domain; Zn = zinc.

predomain, the propeptide domain, the catalytic domain, the C-terminal hemopexin-like domain, the furin-cleavage site insert, and the fibronectin-like repeats.

Although MMPs are described as having some substrate specificity, it has been shown that individual enzymes degrade several different proteins. In fact, the spectrum of substrates among the enzymes is quite similar and there is much cross-activity in protein degradation among the MMPs [52, 61]. Evidence indicates that some matrix substrates such as denatured and native non-fibrillary collagens, elastin, basement membrane components, and fibronectin can be degraded by MMPs-2, 3, 7 and 9. However, the collagenases consisting of MMPs-1, 8, and 13 have been described to cleave the interstitial collagens I, II, and III among other ECM proteins [62, 63]. Evidence has also shown MMP-1 to partially degrade these collagens by making single, site-specific cleavage within the triple helix of these matrix molecules allowing for further degradation by other MMPs.

Denatured collagen (gelatin) is digested primarily by the gelatinases (MMPs-2 and 9, which are also known as the gelatin-binding MMPs). MMP-2, has also been reported to be a key player in the modulation of the glomerular basement membrane [64] and although sharing substrate specificities with MMP-9, their patterns of expression are distinct [65]. MMPs-7 and 26, known as the matrilysins, are unique in that they possess only the essential domains. Although they have been described to degrade numerous ECM components including fibronectin and gelatin, MMP-7 have also been reported to play the primary role in the degradation of tenascin [66–68], and its expression is dramatically inhibited in certain glomerulopathies such as LCDD characterized by mesangial expansion and ECM accumulation [26, 31, 68].

Tenascin is identified as an abnormal protein present in abundance occupying the central areas of mesangial nodules in LCDD [5, 26], and in diabetic nephropathy [69]. Tenascin, a large oligomeric glycoprotein, has a peculiar ‘octopus’-like structure (hexabrachion configuration) and is not an easy ECM

protein to degrade. Tenascin accumulation has been reported in conditions such as pulmonary fibrosis and hyperplastic scars and keloids [70–72], where it plays a key role in providing tensile strength and appears to be responsible for permanency of fibrous connective tissue and inability to reverse the fibrotic process.

Six forms of membrane-type MMPs (MT-MMPs) have been described and they too are capable of degrading a number of ECM proteins including gelatin, fibronectin and laminin [73, 74]. These MT-MMPs share 30–50% sequence homology and possess common multi-domains [75, 76]. Four are anchored by their trans-membrane domain [MT1-MMP (MMP-14), MT2-MMP (MMP-15), MT3-MMP (MMP-16), and MT5-MMP (MMP-24)]; while MT4-MMP (MMP-17) and MT6-MMP (MMP-25) are glycosylphosphatidylinositol anchored to the cell [73]. The MT-MMPs have a furin/pro-hormone convertase cleavage site at the end of the propeptide domain. These MT-MMPs possess structural domains similar to the secreted MMPs, except for two distinguishing ones: the trans-membrane domain and a cytoplasmic tail.

MT-MMPs play a major role in the biology of other MMPs where they form complexes with tissue inhibitor of metalloproteinases (TIMP) and secreted MMPs, resulting in the activation of the MMPs (e.g. MMPs-2 and 9) [56, 77, 78]. MT1-MMP is reported to act as a surface receptor for pro-MMP-2 resulting in the cleavage of its pro-domain. Additionally, interactions of MT-MMPs with an increasing array of cellular proteins have been described. Such proteins include TIMPs, cytoplasmic and cytoskeletal proteins, as well as cell–cell and cell–matrix receptors. Their ability to initiate intracellular contact with the extracellular milieu is remarkable and alludes to their many undiscovered properties in the realm of proteolysis.

In addition, the activities of these MT-MMPs are also inhibited by TIMPs even though there are significant differences in their affinities for the various TIMP molecules compared to MMP–TIMP interaction. With the exception of MT4-MMP these MMPs are avid endopeptidases with an increasing repertoire of substrates among the ECM components such as collagen I, fibrinogen, fibrin, pro-TNF-α, CD44, and α_2-macroglobulin [73].

The Altered Mesangium in LCDD and AL-Amyloidosis

Another important pathogenic effect of glomerulopathic LCs relates to activation of genes in mesangial cells which are normally quiescent. For example, LCDD genes responsible for fibrillary collagen and tenascin production become activated under the influence of TGF-β. As a result, the composition of the mesangial matrix changes dramatically, which can then contribute to accelerate the process of glomerulosclerosis [79]. As previously discussed, the composition of the altered mesangium in LCDD is much different than that of the normal mesangium [80].

In AL-amyloidosis ECM proteins also change in the mesangium but the markedly accelerated activity of MMPs dominate the pathological scenario resulting in matrix destruction and replacement by amyloid fibrils, keeping the newly produced matrix proteins at the periphery of the destroyed mesangial areas [26]. The altered mesangium in turn modulates mesangial cell function creating a cyclic enhancement of disturbance of mesangial homeostasis [68, 81, 82].

Conclusions

Glomerulopathic LCs have been reported to play pivotal roles in the pathogenesis of LC-mediated glomerulopathies. Interaction of these LCs with mesangial cells initiates a receptor-mediated process which then controls downstream events. Based on the type and degree of structurally abnormal LC, processes such as endocytosis, activation of growth factors and cytokines, and mesangial matrix alterations are regulated. Two distinct glomerulopathies have been described with different pathogeneses. However, damage to the glomerulus is a sequel shared by both, which if left uncontrolled eventually results in renal failure.

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Immunoglobulin Light and Heavy Chain Amyloidosis AL/AH: Renal Pathology and Differential Diagnosis

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Abstract

Among the varied and biochemically diverse group of protein folding disorders that are collectively known as the amyloidoses, AL-amyloidosis where deposits are derived from the immunoglobulin light chain fragments, is the most prevalent systemic form of the disease found in the western world. In contrast, AH-amyloidosis, resulting from the deposition of immunoglobulin heavy chains, is a rare disease with very few cases thus far reported. Both diseases primarily affect older individuals and are always associated with some form of plasma cell/B cell lymphoproliferative process. The overwhelming majority of monoclonal light chains are nephrotoxic leading to frequent renal involvement, although a wide variety of other organ systems may be involved. The most common clinical presentation is proteinuria and the disease is often diagnosed by renal biopsy. The kidneys are the most frequent site of amyloid fibril deposition in AL and light microscopic examination of Congo red stained sections is the prime means of detection. Electron microscopy may be helpful in the detection of small deposits and in the differentiation of amyloid from other types of renal fibrillar deposits. Current treatment of systemic amyloidoses depends upon the type of amyloid deposits; thus, accurate typing, using a panel of antibodies, is of paramount importance. While the differential diagnosis of amyloidoses continues to expand with increased awareness of hereditary types, currently, the main challenge is diagnosis of AL/AH with confidence. Future goals include the development of more precise and sensitive diagnostic tools. This chapter presents the pathology of AL/AH, current standards of diagnosis and the differential diagnosis. Whenever possible, the most recent references, considered as being particularly useful to clinicians and pathologists serving patients with renal amyloidosis, have been selected.

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The amyloidoses comprise a group of protein folding disorders of diverse etiology in which deposits of abnormally folded proteins share unique staining

Table 1. Renal involvement in human systemic amyloidoses

Amyloid protein	Precursor	Syndrome
AL/AH	Immunoglobulin Light/heavy chain	Multiple myeloma/plasma cell dyscrasia-associated, aka 'primary'
AA	Serum AA protein	Sporadic: secondary Periodic fevers[1] Familial mediterranean fever, other
ATTR	Transthyretin	Hereditary[2–4]
AFib	Fibrinogen A α-chain	Hereditary[2,5]
AApoAI	Apolipoprotein AI	Hereditary[2,6]
AApoAII	Apolipoprotein AII	Hereditary[2,7]
ALys	Lysozyme	Hereditary[2,8]
AGel	Gelsolin	Hereditary[2,9]
ACys	Cystatin C	Hereditary[2,9]
$A\beta_2M$	β_2-Microglobulin	Dialysis-associated

[1]Genetic defect in proteins involved in the inflammatory response but not the amyloid precursor protein per se [15].
[2]Genetic defect involving the amyloid protein precursor [1, 21].
[3]polyneuropathy and cardiomyopathy, some mutants with severe renal involvement [16].
[4]Senile form derived from wild transthyretin with cardiomyopathy, renal involvement in medulla and vessels [24].
[5]Severe nephropathy with glomerular involvement [17].
[6]Hepatic, cardiomyopathy, nephropathy with involvement of renal medulla, some mutants with neuropathy [18].
[7]Nephropathy [19].
[8]Nephropathy, gastrointestinal [20].
[9]Clinically silent [23].

properties and a fibrillar ultrastructure [1]. These deposits ultimately lead to the destruction of tissues and progression of disease. While biochemically diverse, all types of amyloid have a β-pleated sheet secondary structure which, in turn, is responsible for their diagnostic staining characteristics and stability under physiologic conditions [1]. A biochemical approach is the foundation of the current classification of amyloid. This is based upon the amyloid fibril protein type, also referred to as the precursor protein (table 1) [1]. By convention, the amyloid type is designated A (for Amyloid), followed by an abbreviation derived from the name of the amyloid fibril precursor protein. There are more than 20 types of precursor proteins associated with various forms of amyloidosis and many more variants; additional types of amyloid are likely to be discovered in the future. Amyloidosis can be localized, systemic, or localized and systemic (table 1) [1–8].

In AL (also referred to as primary amyloidosis) the amyloid fibril is derived from the immunoglobulin light chain or, more frequently, its fragment [1–5, 7, 8]. AL is associated with various B cell lymphoproliferative disorders such as the multiple myeloma – plasma cell dyscrasia (PCD) spectrum; malignant lymphomas and macroglobulinemia may also be complicated by AL [1–5, 7–9]. While AL is typically a systemic disease, in some patients localized deposits of AL may be seen forming a tumor mass [6, 7]. The term AH designates amyloid derived from immunoglobulin heavy chain. Similar to AL, AH is also associated with some form of plasma cell/B cell lymphoproliferative process. AH is rare, with only a few cases derived either from truncated γ or μ-heavy chains thus far reported [10–12].

In the United States and the western world, AL-amyloidosis is the most prevalent type of systemic amyloidosis [2–5, 7, 8]. In contrast, in developing countries, secondary (AA) amyloidosis is far more common than AL [13, 14]. Worldwide, 45% of all systemic amyloidosis is estimated to be of the AA type [14]. Typically, AA amyloidosis is associated with an underlying chronic inflammatory process, which in the developed world is exemplified by chronic inflammatory arthritides. By contrast, in developing countries, long-standing infectious processes are the main culprits; the high prevalence of chronic infections is considered to be responsible for this higher incidence of AA amyloidosis [13, 14]. In certain geographic areas, i.e. the Mediterranean basin but increasingly also in other parts of Europe and the US, periodic fevers are being diagnosed in a subset of patients with AA [13, 15]. Periodic fevers are the result of genetic defects in the inflammatory response and not in the precursor protein per se. The best known example is Familial Mediterranean fever; recently, several others have also been identified [15]. In contrast, in the hereditary amyloidoses, mutations affect the amyloid precursor protein itself and thereby render it amyloidogenic [16–23]. These mutations are diagnosed with increasing frequency. In several recent series, 10% of new patients [7, 8, 21–23] were shown to have hereditary amyloidosis. Both in the US and worldwide, amyloid derived from numerous (>100) variants of transthyretin (ATTR) is the most common type of hereditary amyloidosis [16, 21–23]. Interestingly, in elderly patients, the wild-type transthyretin may also form systemic amyloid deposits, which, however, preferentially accumulate in the myocardium (Senile Cardiac Amyloidosis [24, 25]). In the UK and Northern Europe, amyloid derived from fribrinogen (AFib) appears to be the most common form of hereditary amyloidosis [17, 22, 23]. Other types of amyloidoses affecting the kidney are listed in table 1. Although most of the hereditary amyloidoses affect the kidney, the degree of renal damage varies. AFib leads to rapid renal failure, whereas slow progression to renal insufficiency is seen in AApo AI and II and ALys [17–20]. In ATTR, some mutations may also be associated with renal failure while other variants

provide only clinically silent deposits [16, 22, 23]. The latter can be seen in senile cardiac amyloidosis and other rare types of systemic amyloidosis such as ACys and AGel [22–24]. Importantly, due to differences in gene penetration, a family history may be missing and the clinical picture may mimic AL [7, 8, 22, 23]. For these reasons, it is perceived that hereditary amyloidoses may be underdiagnosed. Patients on long-term dialysis may develop amyloidosis derived from β_2-microglobulin (Aβ_2M) [2–5].

In contrast to Alzheimer's disease, a well-known example of a localized amyloidosis, systemic amyloidoses are relatively rare. With an estimated eight new cases of AL per million per year, it's incidence is similar to Hodgkin disease, chronic granulocytic leukemia, and polycythemia rubra vera [7]. The incidence of AL amyloidosis increases with age and autopsy studies suggest that the actual incidence may be higher [8]. In one study, among nephrotic adults older than 44 years 10% had AL amyloid nephropathy [26]. AL and AH-amyloidosis primarily affect older individuals and patients with this condition who are younger than 40 years of age are uncommon [2–5, 7, 8, 26].

The majority of AL patients have free monoclonal light chains with minimal, $< 10\%$, bone marrow plasmacytosis. However, approximately 10% of AL patients have frank myeloma. Conversely, AL develops only in 12–15% of patients with multiple myeloma and in $< 5\%$ of patients with 'benign' monoclonal gammopathy. In some cases, AL may be the only evidence of dyscrasia. These issues are addressed in depth in other chapters of this book.

Renal Involvement in Plasma Cell Dyscrasias

Renal involvement in dysproteinemias is frequent and heterogeneous [27–31] with approximately 85% of all monoclonal light chains being nephrotoxic. Apart from AL/AH amyloidosis, other pathologies include light chain (myeloma) cast nephropathy, acute tubulopathy, inflammatory tubulointerstitial nephritis and non-fibrillar deposition diseases (light- or heavy chain-related, LCDD/HCDD) [27–36], which are discussed elsewhere in this book. Combined patterns, such as AL-amyloidosis and LCDD/HCDD, LCDD and light chain cast nephropathy, LCDD and HCDD/AH or tubulopathy and AL are uncommon [30–36].

Light and Heavy Chain-Related Amyloidosis (AL and AH)

Clinical Presentation

Clinically, in addition to fatigue and weight loss, the symptoms depend on the organ system involved [2–5, 7, 8]. There may be edema, orthostatic hypotension,

peripheral neuropathy, cardiac arrhythmias and congestive heart failure, carpal tunnel syndrome, hepatomegaly without filling defects, early satiety, malabsorption, gastrointestinal bleeding or abnormalities of motility with atony, pseudo-obstruction, chronic diarrhea or constipation [2–5, 7, 8, 37–39]. Macroglossia, which is considered to be almost pathognomonic for AL, occurs in 20% of patients. Truncal purpura affects 15% of AL patients [7, 8]. However, similar skin lesions ('petechiae') have recently been reported in lysozyme amyloidosis [20]. Similarly, amyloid in bone marrow, formerly considered specific for AL, has also recently been reported in lysozyme amyloidosis [20]. Infiltration of submandibular glands by amyloid with corresponding swelling and involvement of the shoulder joint ('shoulder pad') are also typically associated with AL. In contrast, vitreous 'floaters' (opacities) are seen in ATTR [4, 5, 7, 8]. Clinical renal disease in AL is reported in more than 50% of patients [7, 8]. The most common clinical presentation is proteinuria with or without renal insufficiency. The proteinuria can be massive, and the accompanying edema can be resistant to diuretics. Initially, the glomerular filtration rate may be normal, but progression to renal failure typically follows unless the underlying lymphoproliferative process is treated successfully. Patients with extraglomerular deposits of amyloid may present with renal insufficiency without marked proteinuria.

Amyloidosis is often diagnosed by renal biopsy, and renal involvement may be the first manifestation of systemic disease, with subsequent studies demonstrating systemic involvement by amyloidosis. Extrarenal localized AL amyloid deposits may be seen in the genitourinary tract. Localized amyloid requires no systemic treatment [5–7].

Gross Pathology

In autopsy studies of patients with amyloidosis, kidneys were frequently enlarged. In a recent autopsy study, the average kidney weight in AL-amyloidosis was 200 g [40]. However, the overwhelming majority of patients with AL have normal-sized kidneys on pre-biopsy radiologic evaluation [41]. There is no correlation between the weight of the kidneys and renal function, or the compartment involved by amyloid deposition. In advanced cases, parenchymal atrophy contributes to and, in some cases, is primarily responsible for kidney size.

Light Microscopy

The kidneys may be involved in all types of amyloidosis and kidneys are the most frequent site of amyloid fibril deposition in AL and AA [2–5, 16–20, 22, 23]. Amyloid can be deposited in any of the renal compartments: glomeruli, interstitium, tubules and vessels. In hematoxylin and eosin (H&E) stained sections,

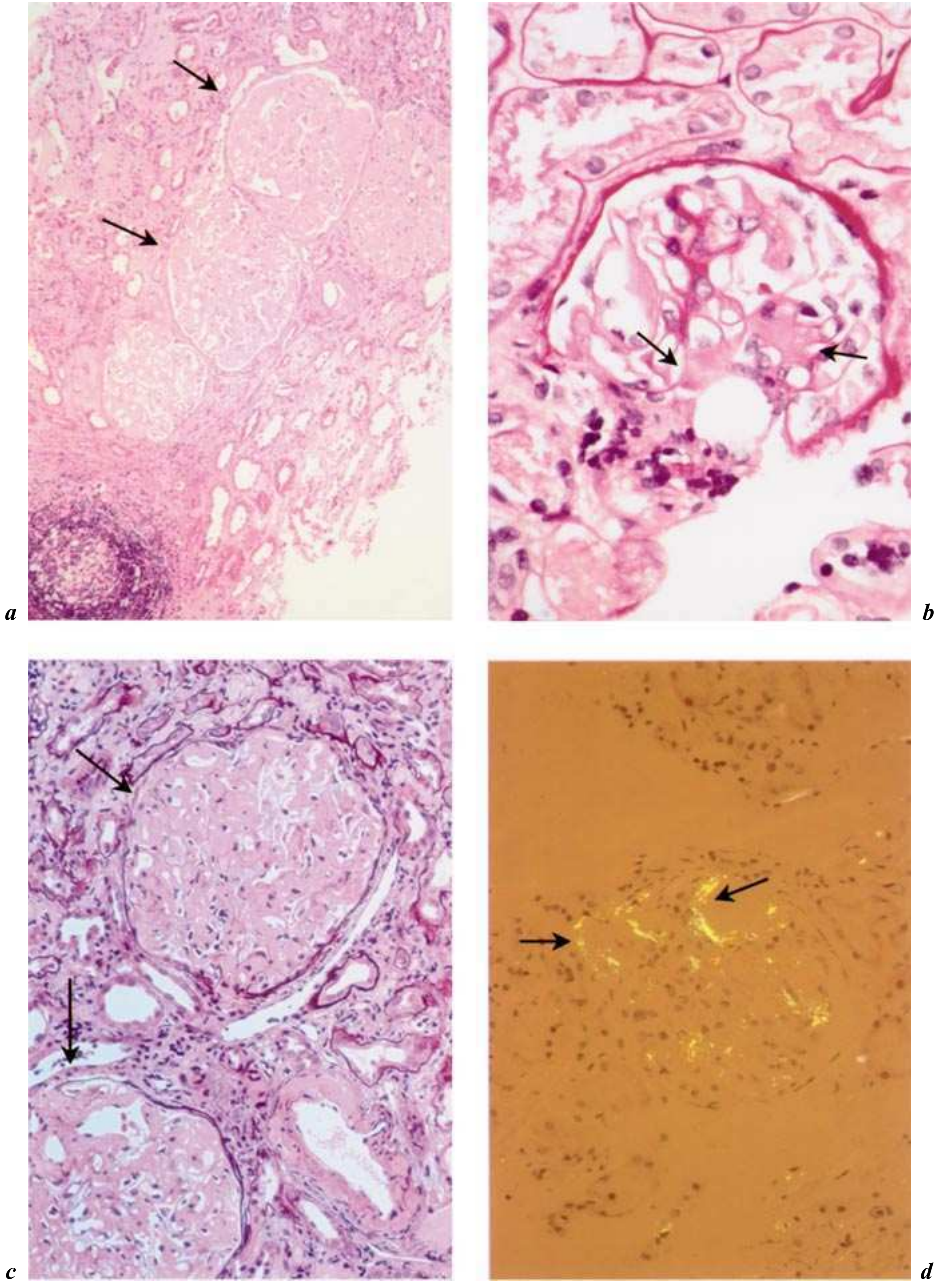

Fig. 1. AL-amyloidosis, paraffin sections. ***a*** Complete obliteration of the glomerular architecture by amyloid deposits in advanced stage disease (arrows), H&E ×100. ***b*** PAS stained section showing early segmental mesangial deposits (arrows) which are only weakly

amyloid deposits have an eosinophilic, amorphous, 'hyaline' appearance (fig. 1a). Amyloid deposits are weakly PAS positive (fig. 1b) and silver stain negative (fig. 1c). However, small deposits may not be apparent on H&E, PAS or silver stain; hence, Congo red stain should be used not only to confirm, but also to rule out, the presence of amyloid. By definition, amyloid has a high affinity for Congo red, and the gold standard for diagnosis of amyloid requires the demonstration of apple green birefringence under polarized light [1, 14, 42–44] (fig. 1d). Typically, in bright light, amyloid deposits stained with Congo red appear salmon pink or pale rose. However, in thinner sections (such as those that are typically cut for kidney biopsies) deposits may be inconspicuous in bright light. Thicker sections are frequently recommended for amyloid stains in order to increase the frequency of detection of small deposits, but this may not be practical for thin core biopsies. In the author's own experience, examination of additional sections of regular thickness (typically two or more) may yield equivalent results. The use of a proper polarizer, a strong light source, the removal of all color filters, and examination in the dark are critical for the detection of small deposits [4, 14]. Excessive retention of Congo red dye by tissue may lead to false positives [7]. Examination of Congo red stained slides by fluorescence light microscopy has been reported to be more sensitive than examination in bright field [44]. Thus, using the fluorescence gate, even small deposits of amyloid can be detected by virtue of their characteristic bright red appearance. Thioflavin T or S fluorescence has also been advocated as more sensitive than the Congo red stain in the detection of small amounts of amyloid [45, 46], but the efficiency of binding to amyloid may vary [45]. Congo red stain and immunohistochemistry have been tried together in a single slide, and it has been proposed that this combination may be more sensitive than the examination of the Congo red stain alone [44]. Other stains, such as crystal violet, methyl violet, sirius red and sulfated alcian blue, are less sensitive and less specific (and some also fade).

Glomerular amyloid deposition begins in the mesangium and, as was elegantly shown by Herrera and coworkers [46] using an in vitro model, the mesangial cell plays a critical role in the processing of amyloidogenic light chains (fig. 1b, 1d). Newly formed fibrils replace the normal mesangial matrix and subsequently extend into the peripheral capillary walls [27, 28, 47–49]. Glomerular amyloid deposits may have segmental, diffuse mesangial, nodular or pure basement membrane patterns [27, 28, 47–49]. Early segmental amyloid deposits may be small and inconspicuous on H&E stained sections. Larger

PAS positive, ×200. ***c*** Jones silver stained section demonstrates loss of argyrophylia within deposits of amyloid (arrows), same specimen as (***a***) ×170. ***d*** Congo red stained slide viewed under polarized light showing apple green mesangial deposits (arrows), same specimen as (***b***) ×170.

deposits form acellular nodules, which may asymmetrically expand the mesangium. The nodular amyloid deposits mimic diabetic nephropathy and other forms of nodular glomerulosclerosis. In end-stage amyloidosis, the entire glomerular architecture is altered by amyloid [47, 48]: the glomerular tuft is hypocellular, amyloid deposits compress the mesangial cells, and the capillary wall is thickened and frequently narrowed or obliterated (fig. 1a). Near complete glomerular obliteration by amyloid is typically seen in amyloid derived from fibrinogen (AFib) [17, 22, 23]. On H&E sections, obliteration of glomerular architecture by amyloid (either segmental or global) may be mistaken for sclerosis. A multinucleated giant cell reaction or crescents may accompany glomerular amyloid deposition associated with capillary wall rupture [50, 51]; however, crescent formation in the setting of amyloidosis is rare and is most commonly reported in association with AA amyloidosis.

Interstitial and peritubular deposits of amyloid are seen in approximately 50% of cases [47]. While such deposits frequently accompany glomerular involvement, in some patients (and certain amyloid types) the extraglomerular deposits are predominant or exclusive [18]. Occasional patients with AA-amyloidosis, and amyloidosis derived from the wild-type of transthyretin and apolipoprotein AI, may show amyloid deposition limited to the interstitium and medulla [18, 24]. In AL, scattered aggregates of lymphoplasmacytic cells may surround deposits of amyloid (fig. 1a). Tubular atrophy is seen most often in advanced stages of amyloidosis.

The involvement of extraglomerular vessels is frequent and may range from inconspicuous deposits to those that completely replace the vessel wall, with luminal occlusion. Vascular amyloid may mimic hyalinosis and even fibrinoid necrosis. Although vascular amyloid frequently coexists with glomerular deposits, in rare cases the vessels may be selectively involved by amyloid. Examples include certain cases of AA or AApo AII [52, 53]. It should be stressed, however, that morphology is not a substitute for the immunohistochemical typing of amyloid since a considerable overlap exists between various types of amyloid [48].

For screening purposes, a minimally invasive test, such as the examination of an abdominal fat aspiration biopsy, has been advocated. However, while the detection of amyloid in abdominal fat aspirates is highly specific (99%), the sensitivity has varied in different series, from 52 to 100% [4, 14].

Immunofluorescence

The routine performance of immunofluorescence studies in the evaluation of kidney biopsies has the additional advantage of securing the best source of material for amyloid typing, i.e. unfixed frozen tissue. Kidney biopsies are routinely studied using a panel of antibodies, which includes IgG, IgA, IgM, C3,

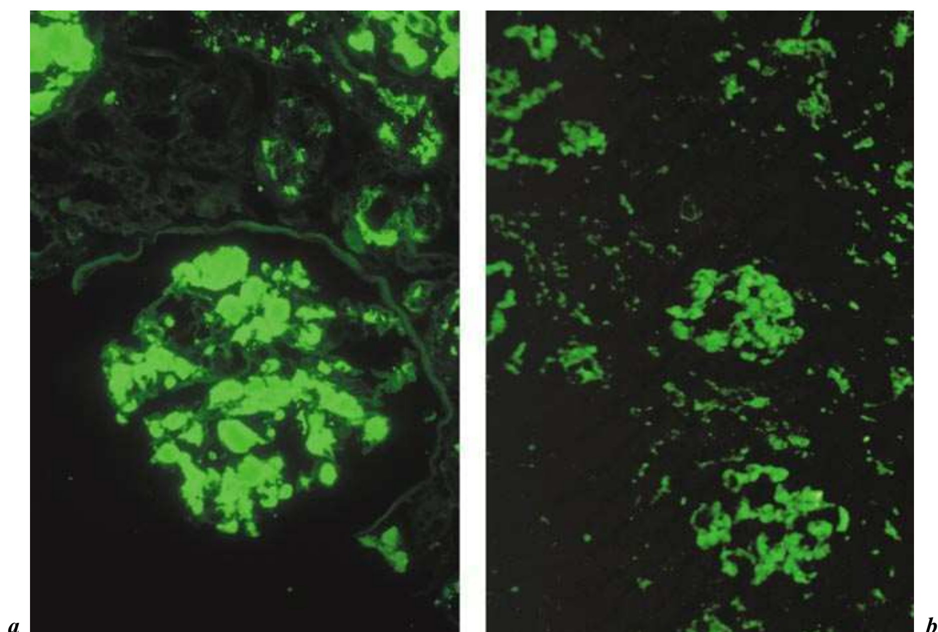

Fig. 2. AL-amyloidosis, frozen sections, direct immunofluorescence stain. ***a*** Bright fluorescence stain for λ light chain outlines deposits of amyloid within glomeruli and in the interstitium, ×200. In contrast, stain for κ light chain was completely negative (not shown). ***b*** Stain for amyloid P component, outlining deposits of amyloid in the glomeruli, interstitium and in the extraglomerular vessels, which correspond to Congo red positive areas (not shown), ×100.

C1q, fibrinogen and albumin. Most laboratories also include κ and λ light chains, at least for adult kidney biopsies. The inclusion of these two latter stains has been pivotal in the early detection of various pathologies associated with underlying PCD, including amyloid. The few cases of AH-amyloidosis thus far reported have been derived from either γ or μ-heavy chains [10–12]. In the diagnosis of this condition, specific antibodies to the heavy chains are applicable. Interestingly, the heavy chains in AH are also truncated and have been shown to lack C_H1 and C_H2. Therefore, antibodies against these deleted portions of the heavy chains can be used to support the diagnosis (by the absence of a reaction).

For the diagnosis of amyloid, interpretation of stains is made in conjunction with the presence of Congo red positive deposits and includes the demonstration of light chain (or heavy chain) restriction for AL (or AH) (fig. 2a) or a stain for fibrinogen in the case of amyloid derived from fibrinogen (AFib). All

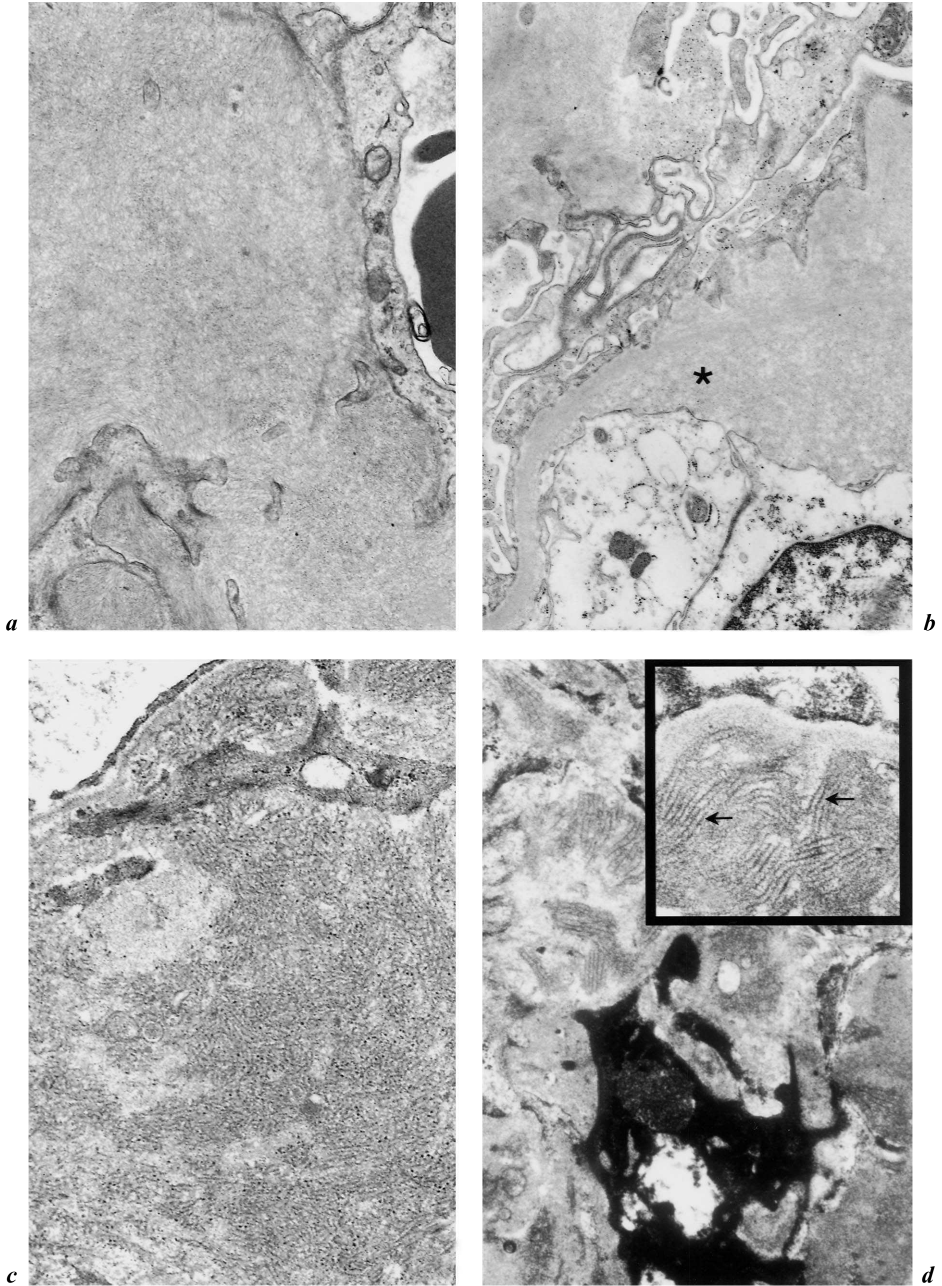

Fig. 3. Transmission electron microscopy, uranyl acetate and lead citrate. ***a*** Fibrillary ultrastructure of amyloid deposits, ×20,000. ***b*** Early deposits of amyloid are seen in the

deposits of amyloid are also positive for amyloid P component, regardless of the amyloid type (fig. 2b). In immunofluorescence studies on frozen sections, only a truly fluorescent appearance is diagnostic. A well-known pitfall is the over-diagnosis of a stain that, despite being relatively bright, is not truly fluorescent. Please see further discussion under amyloid typing.

Electron Microscopy

By definition, in the electron microscope, amyloid deposits are fibrillar. The fibrils are relatively straight, non-branched, measure 8–12 nm in width and have highly variable lengths, up to several μm (fig. 3a and b). Extracted fibrils, examined in negatively stained preparations, often exhibit a twisted shape consistent with a substructure of bundled protofilaments 2–3 nm in diameter [54–56]. Interestingly, amyloid fibrils in β_2-microglobulin-associated amyloidosis have been reported to exhibit short curvilinear bundles rather than rigid fibrils, at least focally [57].

In early glomerular involvement, fibrils replace the mesangial matrix (fig. 3b). In an experimental model of mesangial cells in culture, Herrerra and coworkers [58] showed that, following delivery of fibrils to the extracellular mesangial matrix, activation of metalloproteinases occurs, which ultimately leads to destruction of the mesangial matrix and its replacement by amyloid. From the mesangium, amyloid deposits may extend into the peripheral capillary walls, occupying the subendothelial or subepithelial spaces (fig. 3b) [56] or replacing the lamina densa. At times, massive subendothelial aggregates of amyloid may be seen [56]. With regression of amyloidosis following treatment, the deposits contain few fibrils which are surrounded by amorphous debris [56], similar to stage IV membranous nephropathy. Extraglomerular amyloid parallels the distribution of deposits seen by Congo red stain as discussed above. It should be noted that the examination of a Congo red stain is sufficient for the diagnosis of amyloid. While electron microscopy per se is not required for the diagnosis of amyloid, it may be helpful in the detection of small deposits; however, in this

mesangium and begin to extend into the paramesangial subendothelial space (asterisk), whereas the more peripheral segment of the glomerular basement membrane is not affected ×7,500. ***c*** Deposits in fibrillary glomerulopathy at the same magnification as deposits of amyloid in (***a***). Although morphologically similar, deposits in fibrillary glomerulopathy are approximately twice as thick as amyloid fibrils, ×20,000. ***d*** Immunotactoid deposits with fibrils which are almost four times thicker than amyloid and have hollow cores (arrows), ×7,500; insert: ×20,000.

context, it is important to differentiate between amyloid deposits and other fibrils such as deposits in fibrillary or immunotactoid glomerulopathy [59–62]. The former are very similar to amyloid fibrils, but are approximately twice as thick and Congo red stain and Thioflavin T and S are negative [59, 60, 62] (fig. 3c). In immunotactoid glomerulopathy, deposits may exhibit a hollow core and lattice structure [55, 59, 62] (fig. 3d). On rare occasions mixed deposits can be encountered, consisting of isotypic amyloid and LCDD, usually in different renal compartments [30, 31]. The author has also encountered a case of proximal tubulopathy with crystals and intestinal amyloid [36].

Ultrastructural immunogold labeling has been applied to amyloid typing in cases with negative (or equivocal) immunohistochemical/immunofluorescence results [56, 63, 64]. Please see under amyloid typing.

Amyloid Typing – The Current Standard of Care

Current treatment of systemic amyloidoses depends on the type of amyloid deposits. From a patient management point of view, the diagnosis of renal amyloid should be considered to comprise three broad categories: AL, AA and hereditary types. Available treatments are quite radical and, for AL, include aggressive chemotherapy with autologous peripheral stem-cell rescue to control the underlying PCD [7, 8]. In contrast, for hereditary amyloidoses, liver transplantation is offered to eliminate the source of abnormal protein; this treatment has been available since the early 1990s [22, 23, 65]. In patients with AA, control of the acute phase response is currently the standard of care. Other treatments targeting the process of amyloid formation in AA and ATTR are currently [8] at various stages of clinical trial. These latter treatments, although not as aggressive, require a long-term commitment. Moreover, hereditary diseases also involve testing of proband, family and counseling. Finally, prognosis is also type dependent. Therefore, there is a need for unequivocal typing of amyloid deposits following their detection by Congo red stain [7, 8, 22, 23, 64]. Given the above listed differences in patient management, systemic amyloidosis can be grouped into three main categories: AL, AA and hereditary amyloidoses [7, 8]. In the setting of long-term dialysis, amyloid derived from β_2-microglobulin (Aβ_2M) should also be considered [3–5, 57]. However, this consideration applies to non-renal biopsies, since kidney biopsies are hardly, if ever, performed under such circumstances due to the fact that renal involvement virtually never occurs in this condition. Importantly, diagnosis of the amyloid type cannot be based on history alone and histochemical typing is imperative [7, 8, 22, 23]. The author has recently diagnosed AL amyloid in two patients who, by history, were expected to have AA amyloid. One patient had a long history of ankylosing spondylitis and the other had long-standing Crohn's disease (Picken et al., in press). In both of these patients, the histochemical diagnosis of AL was subsequently corroborated by ancillary studies. Patients with

Waldenstrom's macroglobulinemia may have either AL or AA [9]. In some patients, more than one type of deposits may be encountered. Several methods have been tried for amyloid typing such as immunofluorescence on frozen tissue, immunoperoxidase on paraffin sections and immunoelectron microscopy. In the mid 1980s, Gallo reported encouraging results in amyloid typing using immunfluorescence studies on frozen tissues, and this approach remains preferable to this day [66]. In particular, immunofluorescence is the technique of choice for detection of amyloid derived from immunoglobulin light and heavy chains. In formalin fixed/paraffin embedded tissues the protein epitopes are denatured; also, fixation 'locks' plasma proteins in tissues. These factors are responsible for the higher backgrounds found in stains with antibodies against plasma proteins. In contrast, these issues are not of concern in unfixed frozen tissues and, indeed, immunofluorescence studies performed on unfixed samples (frozen tissue, air-dried fat aspirates, bone marrow smears) give much better results than paraffin sections [3, 4].

For the reasons listed above, immunohistochemistry on formalin fixed/paraffin embedded tissues generally results in higher background staining, which can make interpretation challenging. However, in such cases, the use of a panel of antibodies is helpful in that it provides the opportunity to identify the strongest antibody reaction (fig. 4a, b). Moreover, the inclusion of a stain for amyloid P component serves as a built-in positive control and is useful for comparison. Interestingly, all types of amyloid have been shown to contain amyloid P component by biochemical and immunohistochemical methods [3, 4]. In contrast, deposits of L/HCDD lack amyloid P component [67]. Amyloid P component may also be seen in the organized deposits of fibrillary glomerulopathy, and in normal glomerular basement membranes and blood vessels, but in significantly lesser amounts than in amyloid deposits [68].

As indicated above, AL amyloid fibril proteins are derived from the aminoterminal end of the light chain, which contains a variable region. These proteins are very heterogeneous and the outcome of the immunohistochemical stain largely depends on the extent to which the C-region is present. Thus, it is expected that not all AL amyloid deposits will be reactive with commercial antibodies for κ or λ light chains [3, 4, 5, 65]. In AH-amyloidosis, the amyloidogenic protein is also composed of fragments of the heavy chains, and the C_H1 and C_H2 constant domains have not been detected in any of the cases thus far reported [10–12]. For AL/AH diagnosis, restriction for the light chain (either κ or λ) or for heavy chain determinants (γ or μ) must be demonstrated in association with Congo red positive deposits. In contrast in L/HCDD, restriction of the light (or heavy) chain is not associated with Congo red positivity.

The percentage of renal biopsies with amyloid deposits that are not reactive in frozen sections with commercial antibodies against κ or λ light chain, but which

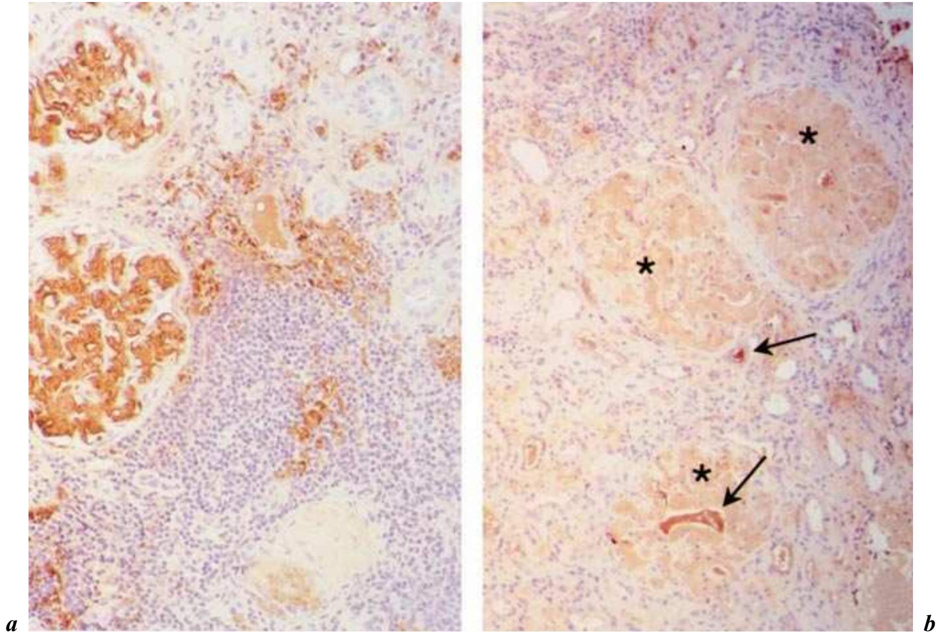

Fig. 4. AL-amyloidosis, paraffin sections, immunoperoxidase stain. ***a*** Strong stain for λ light chain in glomeruli and interstitium corresponding to deposits of amyloid, same specimen as fig. 2a, ×150. ***b*** In contrast, stain for κ light chain shows only focal positivity corresponding to serum proteins (arrow) and only a 'blush' stain in areas corresponding to amyloid (asterisks), same specimen as (***a***), ×150.

were subsequently shown to be AL, varies between 13.6% (author's own experience) and 35.3% [69]. Understandably, all equivocal or frankly negative cases must be studied further. In contrast, AA amyloidosis is relatively easy to diagnose by immunohistochemistry in both frozen tissues and paraffin sections [3, 5, 7, 8, 14], as is (generally) ATTR (amyloid derived from transthyretin). However, in some cases, difficulties have been reported with ATTR [5, 37]. Similar considerations apply to amyloidoses [22, 23]. Better results can be obtained following the pre-treatment of sections with formic acid or guanidine [23, 70].

For routine amyloid typing, a panel of antibodies limited to κ and λ immunoglobulin light chains, amyloid A protein, transthyretin, fibrinogen and amyloid P component, is recommended. A stain for β_2-microglobulin should also be added in the setting of dialysis. However, it should be noted that this latter consideration applies to non-renal specimens, since kidney biopsies are usually not performed in patients on long-term dialysis. In view of the limitations discussed above, it is recognized that some cases may be negative, and additional studies, best done in specialized centers, will be needed. In the

author's own experience this antibody panel allowed definitive typing of amyloid deposits in 90% of kidney biopsies. Overall, typing of amyloid deposits in other tissues using the same antibody panel was successful in 90 and 85% of cases respectively, in frozen and paraffin sections. Additional antibodies which may be needed include Apolipoprotein AI, AII and Lysozyme; however, these latter stains are best performed at laboratories that are specialized in their use (table 1). Since stains for κ and λ light chains and fibrinogen are typically included in the immunofluorescence antibody panel used for native kidney biopsies, the amyloid typing panel would require only the addition of stains for amyloid P component, AA and transthyretin.

In recent years, the importance of discrimination between AL and hereditary amyloidoses has emerged. Two recent studies have demonstrated that hereditary amyloidosis may be misdiagnosed as AL in some patients [22, 64]. In one study, 350 patients with systemic amyloidosis were evaluated, in whom the diagnosis of AL was suggested by clinical and laboratory findings and by the absence of a family history. However, 10% of patients were actually shown to have familial amyloidosis [22]. Interestingly, a low-grade monoclonal gammopathy was detected in 24% of these patients. In a study by Comenzo et al. [64], 6% of screened patients and 2% of asymptomatic patients had both monoclonal gammopathy and a hereditary variant amyloid. Thus, careful clinicopathological correlation, as well as exclusion of other types of amyloid, is important. Additional studies may involve screening for transthyretin variants by isoelectric focusing and mass spectrometric characterization, and analyses for genetic mutation by direct DNA sequencing and restriction fragment-length polymorphism [21–23, 64]. Variants of apolipoprotein AI, apolipoprotein AII, fibrinogen-α chain, lysozyme and gelsolin can be evaluated by referral centers.

Results superior to immunohistochemistry have also been reported with immunoelectron microscopy [5, 56, 63, 64]. Unfortunately, this latter technique, pioneered by Herrera and coworkers [56], is available only in selected laboratories, since its wider use is constrained by the expertise required to perform this technique. The biochemical typing of amyloid protein in formalin-fixed, paraffin-embedded specimens has also been reported [71, 72]. The most recent approach involves the use of proteomics techniques to detect and type amyloid deposits [73]. Again, these newer techniques are available only in specialized laboratories; thus, immunofluorescence and/or immunoperoxidase stains are the most widely used methods.

A single patient may suffer from different amyloid diseases simultaneously. Renal glomerular AL and vascular AA deposits were reported in a patient with ankylosing spondylitis [53]. A case of mixed cardiac deposits derived from wild type ATTR and AL and another case of ATTR with AApo AIV have also been reported [37]. In certain patients on dialysis, or with mutations of proteins

involved in hereditary amyloidosis, immunohistochemistry detected deposits of the amyloid protein that were Congo red negative. Such deposits are referred to as 'preamyloidotic'. Although such deposits may be harbingers of fibrillogenesis, their true significance is at present undetermined.

Detection of renal amyloid deposits is overwhelmingly associated with systemic disease. However, amyloid in other parts of the genitourinary system can be localized. This distinction is important because localized amyloid requires no systemic treatment and has a good prognosis [6, 7].

Etiology and Pathogenesis of AL

AL is unique among the amyloidoses since it represents both a protein folding disorder and a clonal PCD, where the amyloid fibrils are derived from the immunoglobulin light chain or fragments thereof. In dysproteinemias, there is uncontrolled production of antibodies with disturbance of the normal production and assembly of light and heavy chains. Thus, in AL there is an excess of light chains. It is postulated that certain amino acid sequences, as well as post-translational modifications of the light chains, such as glycosylation, may be responsible for the amyloidogenic potential of a given light chain [54, 74–76]. Consequently, only 10–15% of patients with multiple myeloma develop AL, λ light chains are more frequently involved than κ light chains, and certain subgroups, in particular λVI, are over represented in AL [77]. However, no common sequence motif has thus far emerged. In experimental studies in mice, only animals injected with light chains isolated from patients with AL developed amyloid. Similarly, as reported by Herrera et al. [78], in experimental studies with mesangial cells in culture, only light chains isolated from patients with AL were associated with fibril formation. The mechanisms involved in AH-amyloidosis remain unclear [10–12].

It is postulated that amino acid substitutions affect the stability and interactive properties of misfolded or partially folded light chains. Partially folded intermediate forms may be prone to self-assemble into protofilaments and fibrils and form a nidus, i.e. a small amount of abnormally folded protein. Such a nidus provides a template that facilitates fibrillogenesis and subsequent exponential progression of the process. Tissue extracts containing amyloid fibrils ('amyloid enhancing factor') have been shown to accelerate all forms of amyloidogenesis in experimental models, both in vivo and in cell culture, by the 'seeding' mechanism [37, 78]. These issues are addressed in depth in other chapters of this book.

The mechanisms by which amyloid damages tissue and organ function are poorly understood. Progression of organ dysfunction is more rapid in AL than in hereditary amyloidoses. Keeling and Herrera [58] have shown that activation of metalloproteinases occurs following delivery of fibrils to the extracellular mesangial environment, leading to destruction of the mesangial matrix and its replacement by amyloid. Cell culture studies of cardiac myocytes showed that

partially folded light chains are toxic [79]. Whether amyloid fibrils per se, or its protofibrils, are the main culprit is still not clear [54]. Thus, in ATTR, monomers of transthyretin rather than tetramers are thought to be pathogenic.

Both earlier, and more recent experimental studies have demonstrated that mesangial cells play a pivotal role in glomerular amyloidogenesis in AA and AL [58, 78, 80, 81]. As shown by Herrera and coworkers [81], using rat and human mesangial cells in culture, there is different trafficking of the light chains isolated from patients with AL versus those with LCDD, even though they both appear to share the same putative receptor. These differences result in divergent patterns of light chain deposition, i.e. fibrillar (in AL) versus non-fibrillar (in LCDD). It is, therefore, not a coincidence that deposits of amyloid and LCDD first form in the mesangium. Although AL is a systemic disease, tissue distribution of the deposits is not random and the role of local tissue factors in the distribution of amyloid deposits has been proposed. In addition, however, recent studies by Comenzo et al. [77] and Abraham et al. [82] have shown that some light chains are more likely to give one pattern of organ involvement than another. Thus, λVI shows nephropathic tropism [77, 82]. Interestingly, phenotypic differences between different mutants of transthyretin are also present, and a hereditary amyloidosis derived from a mutant of fibrinogen (AFib) also shows striking renal tropism [17, 22, 23]. These issues are discussed in depth in other chapters of this book.

In addition to the amyloid fibril protein, all types of amyloid deposits thus far studied have been shown to contain the normal plasma glycoprotein, serum amyloid P component (SAP), glycosaminoglycans (GAGs), apolipoprotein E and various basement membrane proteins [83, 84]. Their role in fibrillogenesis is under investigation. SAP, a member of the pentraxin protein family, which also includes C-reactive protein, exhibits calcium-dependent binding to amyloid fibrils. SAP scintigraphy is used in several European centers to assess the amyloid load, as well as disease progression or regression following treatment [85]. In vitro and in vivo experimental studies of AA amyloidosis have shown that certain low-molecular-weight GAG mimickers have anti-amyloidogenic properties that derive from their ability to block amyloid protein binding sites [84]. Currently, one such compound for patients with AA amyloidosis is being tested in clinical trials [8].

Treatment, renal transplantation and prognosis are discussed in depth in other chapters of this book.

Conclusion

In conclusion, as the treatment options expand, there is increasing need for early and accurate diagnoses. The biggest challenge for the future will be to

devise a non-invasive test, possibly based on body fluids (blood) rather than tissues. Since amyloid is essentially a protein disorder, proteomics methodologies may offer a solution, despite their inherent complexities. In the future, the focus may shift toward the detection of pre-amyloidotic deposits and in particular circulating monomers (protofibrils). Such an approach will open the quest for prevention. Finally, an association of cerebral amyloidosis with ageing has long been recognized; however, several extracerebral systemic amyloidoses are also age dependent. Thus, an increased understanding of the process of amyloidogenesis in general may offer some clues to understanding the process of aging per se. For now, however, the main focus is on typing AL amyloid deposits with confidence.

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Diversity and Diversification of Light Chains in Myeloma: The Specter of Amyloidogenesis by Proxy

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Abstract

Background/Aims: Primary amyloidosis and the cancer, multiple myeloma, are characterized by the overproduction of free antibody light chains. Approximately 10% of myeloma patients develop amyloidosis; primary amyloidosis may be thought of as the pathological analog of monoclonal gammopathy of undetermined significance. The kidney is a common site of accumulation of amyloid fibrils and is also the target of other light chain pathologies. Understanding the structural origin of these pathologies is complicated by the extreme primary structure heterogeneity of light chains. **Methods:** Patterns of light chain germline gene usage in myeloma patients were compared to those found in other immune system disorders: lymphoma, leukemia, systemic lupus erythematosus and rheumatoid arthritis. **Results:** Significant differences in apparent gene usage are found in the various diseases; several germline gene products have not been documented in myeloma patients to date. **Conclusion:** The plasma cell dyscrasias including myeloma, lymphoma, leukemia, and monoclonal gammopathy of undetermined significance are usually monoclonal diseases; however, the light chains produced are not homogeneous. Thus, the pathological risk for the patient may change during the course of the illness. Mutation rates in light chains observed during clonal diversification parallel mutations occurring in all genes in the malignant cells and could be a clinically useful biomarker.

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The overexpression of antibody light chains during multiple myeloma presents a pallet of several potential pathological outcomes. In some cases, these disease complications are the result of protein aggregation. Tubular cast nephropathy arises from amorphous precipitation in distal tubules [1–8] facilitated by interactions of the light chain [1, 5, 9–13] with Tamm-Horsfall glycoprotein

[14–16] produced in the Loop of Henle. Cast formation can generally be controlled [1, 7, 17] by maintaining adequate hydration of the patient. Chemotherapy is also used to reduce the production of protein. It is probable that cast formation is akin to the 'salting out' phenomena of proteins. As water is resorbed in the kidney, protein concentration is increased. If it reaches a saturation point, precipitation of the light chain or light chain-Tamm-Horsfall complex, necessarily occurs. The saturation point is determined by the amino acid composition of the protein produced by the patient and is modulated by extrinsic factors such as salt and ion composition of the urine, other metabolites in the urine, pH, and temperature.

Light chain deposition disease (LCDD) manifests itself as amorphous, punctuate deposits on basement membranes in kidneys and other organs [18–33]. Although the protein deposits found in LCDD are amorphous, three-dimensional crystals are often found in light chain-related Adult Acquired Fanconi's Syndrome (AAFS). It is not known if the crystals are the vector of kidney damage to the proximal tubule, whether the propensity to form crystals is a correlate of relevant physicochemical properties of the particular light chain, or if the crystal formation is simply a consequence of high protein concentrations that result in tissue damage for other reasons.

In contrast to the classic crystals found in AAFS, fibrils produced during light chain amyloidosis (AL) can be considered to be two-dimensional quasi crystals in which the inter-strand and intra-sheet spacings of a β-domain are present. No consistent repeat distance is found in the axial direction. Although it is typically accepted that β-strands are aligned perpendicularly to the fibril axis, there is in fact no definitive experimental evidence for this assertion. The structural properties of amyloid fibrils are consistent regardless of the source of the protein, and are also found in the fibrils generated by proteins that do not physiologically generate fibrils [34]. In addition, all amyloid fibrils bind Congo red resulting in a chromophoric shift in the dye [35–38]; ThioflavinT [39] and Thioflavin S are also bound by fibrils resulting in the induction of fluorescence. Note, however, that binding of Thioflavin with induction of fluorescence is not proof of amyloid fibrils. For instance, such interactions have been found with acetylcholinesterase [40] and some mutants of human κ4 light chains (Stevens et al., unpublished results).

Amyloid fibrils formed by light chains may be localized to the kidney [41–45] or other organs [46–60] or limited to a single site (amyloidoma) [53, 56, 60–70]. In other patients, fibril deposition is found in several organs or systemically throughout the body [14–16, 58, 71–75].

Does the existence of five types of light chain deposition phenomena (inclusive of non-deposition), in addition to direct toxicity of some light chains, coupled with the diversity of site deposition phenomena found within the categories

of LCDD and AL, preclude ever understanding the linkage with the amino acid sequence? We postulate that it is not impossible – but much work remains. In this chapter, we review sources of primary structural diversity exhibited by light chains; recent reviews have addressed structural aspects of light chain variable domains in relation to disease processes [76–81]. In addition to the several mechanisms of diversification that result from normal immunological processes during B-cell differentiation, we suggest the existence of a dynamic form of diversification that is the result of the neoplastic process. This process may lead to a phenomenon that might be informally termed amyloidogenesis by proxy.

Light Chain Diversity

What is the evidence that the primary structure of the light chain is a principle determinant of its pathological destiny? By analogy to the fact that variations in light and heavy chain sequence determine the interaction specificity of an antibody, it was not unreasonable to presume that differences in primary structure of light chains produced by different patients would control the pathological outcome. However, sequencing of light chains that formed amyloid or other pathological deposits never revealed the equivalent of the glutamic acid to valine substitution at position six in the β chain of hemoglobin that sufficiently alters the physicochemical properties of the normal protein to generate sickle cell hemoglobin. Nevertheless, a linkage of pathological properties to primary structure of the light chain, rather than a outcome of patient-specific factors, was provided by Solomon et al. [82, 83], who demonstrated that infusion of human Bence Jones proteins into mice revealed that the pathological character of the protein that was observed in the patient was reprised in the mouse. Thus, the pathologenic nature of a particular light chain was neither patient, nor species, dependent. Nevertheless, support for contributions by the patient was also demonstrated [84]. Chromatography methods were used to demonstrate that some Bence Jones proteins formed aggregates under one or more physiologically relevant buffer conditions and that the observation of aggregation distinguished pathological and non-pathological light chains [85]. More recently, Apstein's group demonstrated that light chains associated with cardiac deposition in human patients induced heart dysfunction in mice following infusion [86] providing further support for the determinative role of the light chain.

The role of the light chain gene can be parsed at several levels. The most crude division reflects the fact that humans produce two groups of light chain, κ and λ, which share as much as almost 50% sequence identity to as little as 15% identity depending upon which genes are compared. Each group has been divided into subgroups: four major subgroups are found among κ, six major subgroups in

λ. Each group may also be said to have small outlier subgroups. Within most subgroups, there are sub-subgroups, each generated by the expression of a particular germline gene. It is within each specific sub-subgroup that direct ties between amino acid variations and differences in pathological propensities are likely to be revealed. The division of light chains into groups, subgroups, and sub-subgroups is, at some level, artificial. Sub-subgroups are products of individual germline genes; subgroups are an amalgamation of germline genes that share, directly or iteratively, more than average identity. The two groups are most fundamentally distinguished by the fact that a highly conserved N-terminal cysteine is at position 22 in one group (λ) and at position 23 in the other group (κ). All light chain variable domains, as well as heavy chain variable domains, share a common evolutionary origin as do numerous other representatives of the immunoglobulin superfamily [87–90]. Effectively, assignment of sub-subgroups, subgroups, and groups sorts germline genes on the basis of the length of time that has passed since gene duplication and sequence dispersion occurred.

κ vs. λ

Although examples of both κ and λ light chains have been found for the five clinical options outlined above, there appears to be preferential tendencies. For instance, AAFS is very rarely attributed to λ light chains; the primary structure of the only such representative reported to date was originally described some 20 years ago [91, 92]. In contrast, the amino acid sequences of at least 10 examples of κ light chains associated with AAFS have been determined. The differences found in LCDD are less extreme, with 20 κ sequences determined compared to seven λ examples.

As part of a long-standing effort to relate light chain primary structures to pathogenesis, with emphasis on amyloidosis, we have compiled a database of more than 570 light chain sequences (currently 271 κ and 301 λ). Entries are restricted to sequences that are of full length or nearly so to maximize the structural information content. Figure 1 depicts the pathological distribution of light chain sequences in this database. Note that these data do not reflect the usage pattern of light chain germline genes by multiple myeloma patients, nor does it accurately reveal the relative occurrence frequencies of pathological complications. The first sequences that were determined and provided the initial insights into the composition of the human immunoglobulin repertoire probably more closely paralleled the underlying usage frequency; thus it is not unreasonable to suspect that either the κ1 group or κ2 group is the most frequently expressed. However, in recent years, sequencing efforts have been motivated by pathological correlation. Thus, the sequences of light chains associated with amyloidosis

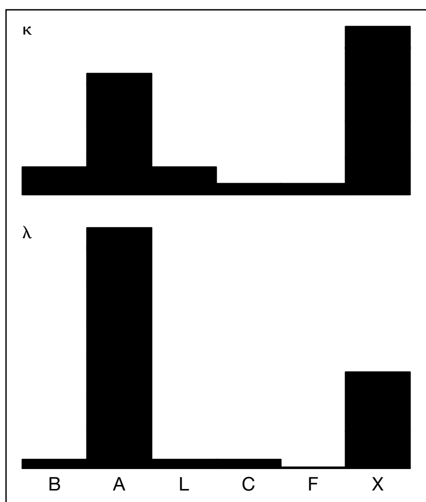

Fig. 1. Distribution of pathologies associated with light chain variable domains obtained from patients with plasma cell dyscrasias, almost all involving multiple myeloma and primary amyloidosis. The distribution of variable domains that have been selected for primary structure analysis does not parallel the usage of light chain germ line genes in myeloma. A = Amyloidosis; B = benign (non-pathological light chains); C = cast formation; F = adult acquired Fanconi's syndrome; L = light chain deposition disease; X = no clinical characterization.

constitute more than half the database, whereas a 10–15% representation would be more consistent with its appearance in myeloma combined with its frequency as primary amyloidosis.

Although the number of amyloid-associated λ chains in the database is about double that of amyloid-associated κ chains, this occurrence is due in large part to the large number of entries of the λ6 subgroup, which consists of only one germline gene. Interest in the proteins encoded by the λ6 germline gene (IGLV6S1) [93] accounts for a large part of this predominance due to the virtually 100% association of these molecules with fibril formation [94–97]. Nevertheless, more cases of amyloidosis involving λ light chains than κ light chains are found [98]. It remains unknown whether this reflects specific structural features of λ chains that render them more prone to fibril formation. Other potential rationalizations include the possibility that λ producing B-cells are for some reason more vulnerable to tumorigenesis; since relative germline gene usage in myeloma patients compared to the healthy population is not known, this possibility cannot be dismissed. A third possibility may also account for overrepresentation of λ light chains even if there is no specific structural feature(s) that enhances amyloidogenesis by λ light chains (except for λ6 light

chains) and if the frequency of λ chain usage in the myeloma population matches that found in the healthy population. The ratio of free κ and free λ light chains differs in the urine and serum [99, 100]. This is a consequence of a higher proportion of covalent dimers formed by λ light chains. Although proteins of size approximating that of serum albumin can be filtered through the glomerulus, the partitioning of proteins between serum and urine has a natural size dependence. Thus, free monomers, which predominate the κ population, are more efficiently removed from serum than are the covalent dimers that characterize λ production. Thus, λ light chains have a longer serum half-life and can attain higher serum concentrations. Both factors would increase the predominance of λ fibril formation on a simple probabilistic basis.

κ vs. κ; λ vs. λ

In addition to possibly different pathological tendencies that distinguish κ and λ light chains, different subgroups within the two groups also exhibit differential propensities. The extreme case, noted above, is that found for λ6 light chains, which are almost invariably linked to amyloidogenesis. Among κ light chains, products of the O18–O8 (κ1b) germline gene, appear to be particularly prone to amyloidognesis resulting in the accumulation of numerous primary structures for this sub-subgroup, which now account for more than 25% of the κ variable domain data. Proteins produced from the B3 gene (κ4) have received attention as being possibly over represented among LCDD-producing light chains [101, 102].

If different germline gene products are preferentially disposed towards particular pathological outcomes, then the frequency of usage of the approximately 25 human κ germline genes and 25 λ germline genes provides a low resolution picture of the risk distribution for myeloma patients as a population. This applies whether or not the myeloma usage pattern parallels or diverges from ‘normal’ usage. It is known that light chain usage patterns can be dramatically altered in various disease states, at least when the immune system itself is part of the disease. Whereas, the myeloma data do not reflect the actual gene usage among myeloma patients, it is probable that datasets linked to other diseases are reasonable approximations of actual usage. Little or none of the data in these analyses were ‘cherrypicked’ on the basis of sequence-derived novelty.

Table 1 compares the (apparent) gene usage in myeloma to that found in patients with the autoimmune disorders, systemic lupus erythematosus (SLE) and rheumatoid arthritis (ART), and another plasma cell dyscrasia, leukemia (LK). Although differences with the myeloma dataset are compromised by non-randomly selected light chains, there are clear differences between the other three sets. For instance, κ sequences outnumber λ sequences by approximately

Table 1. Comparative apparent germline gene usage in immunological disorders

Frequency × 1,000						Frequency × 1,000					
Gene	SLE	ART	LYM	LK	MYL	Gene	SLE	ART	LYM	LK	MYL
L12a	106	48	69	79	111	humlv114	53	18	67	39	66
O18–O8	39	14	63	72	269	humlv122	201	70	172	108	140
O12–O2	162	48	89	209	133	IGLV1S2	72	49	49	69	27
A30	17	5	17	19	18	VL2.1	170	44	117	138	136
L1	56	14	52	62	59	DPL12	85	21	117	133	86
L18	0	10	6	17	7	humlv318	25	64	37	153	33
L5	34	10	11	36	26	hsiggl150	16	165	61	74	30
O11–O1	0	0	3	7	11	VIII.1	38	106	123	108	159
A19–A3	67	14	32	36	48	humlv418	85	28	18	30	50
A2	11	10	17	7	7	IGLV6S1	50	54	37	10	176
L2–L16	95	183	121	58	63	V3–2	3	21	92	5	3
A27	223	370	181	156	52	humlv801	13	168	6	5	27
L6	45	207	92	55	18	DPL24	6	0	25	0	0
B3	73	58	190	101	155	IGLV8	63	15	12	5	0
B2	0	0	3	0	7	V2–19	0	5	0	0	10
L22	0	0	0	0	0	V2–15	0	0	0	0	0
A5	0	0	0	0	0	V5–4	19	41	6	10	0
L25	0	0	0	0	0	V1–11	6	0	0	10	10
L11	28	0	29	7	7	V2–14	31	85	37	64	43
L10	0	0	0	0	0	V2–8	3	3	0	5	3
A23	39	10	23	74	4	DPL–22	25	23	12	5	0
A11	6	0	3	2	4	V4–1	22	21	12	10	0
A7	0	0	0	0	0	V4–4	3	0	0	5	0
O14	0	0	0	0	0	V1–20	9	0	0	15	0
A10	0	0	0	2	0	V1–9	0	0	0	0	0
Entries	179	208	348	417	271	Entries	318	388	163	203	301

ART = Rheumatoid arthritis data; LK = leukemia data; LYM = lymphoma data; MYL = multiple myeloma data; SLE = system lupus erythematosus data.

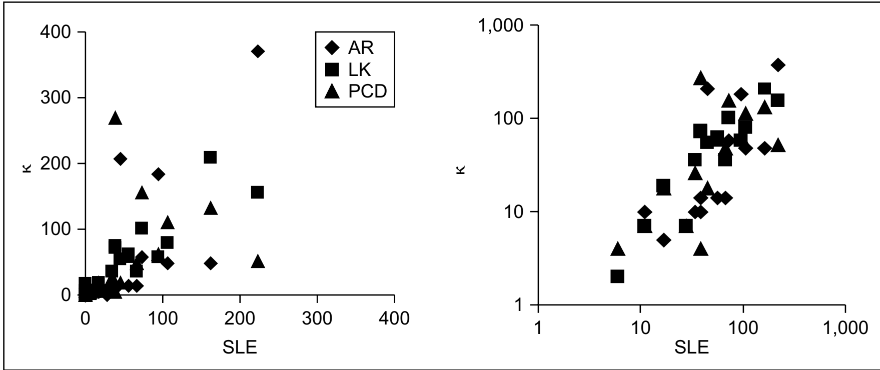

Fig. 2. Correlation plots of κ germline variable domain gene usage in SLE, ART, LK, and found in the plasma cell dyscrasia (PCD) sequence database. Because apparent gene usage spans two orders of magnitude, a logarithmic scale plot is presented on the right. Axes are enumerated as 1,000× the fractional representation in the respective data sets.

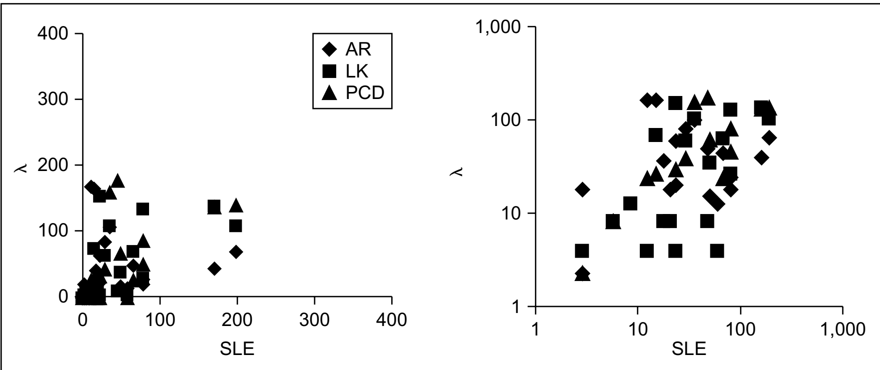

Fig. 3. Correlation plots of λ germline variable domain gene usage in SLE, ART, LK, and found in the plasma cell dyscrasia sequence database.

two to one in the LK data set. This relationship is approximately reversed in the two autoimmune datasets. The arthritis κ data are dominated by the three κ3 germline genes (L2–L16, A27, L6), which together account for three-quarters of the κ data. The arthritis λ response is dominated by λ3b (humlv150) and λ8 (humlv801), which cumulatively provide one-third of the λ sequences. This contribution is fifteen-fold greater than their representation among the myeloma-related data, perhaps suggesting that these germline genes encode proteins that have relatively little pathogenic potential or relative intrinsic expression in myeloma. Figures 2 and 3 display the correlation of κ and λ gene

usage among these data sets using both linear and logarithmic scales. The logarithmic plot is used to take into account the two orders of magnitude span of gene usage frequencies.

Products of several germline genes in myeloma have not (yet) been documented although they appear in the other data sets, which are of comparable size or smaller. For instance, no examples of IGLV8, V5–4, DPL22, DPL24, V4–1, V4–4, and V1–20 λ germline products have been sequenced from protein or cDNA obtained from myeloma patients. One consequence of this is that presumably no antisera specific for these variable domains have been developed, which limits the ability to identify the potential usage of these genes.

Figure 4a provides aligned sequences of the seven λ sequences noted above. Of particular interest are five: DPL24, V5–4, V4–1, V4–4, and DPL22. These genes are distantly related to humlv801 (λ8), which has been identified eight times among 301 λ sequences from myeloma patients. As indicated in table 1, this gene appears to be overexpressed in ART. In common with humlv801, these five genes encode an extended CDR2. A four-amino acid insert is found in all of these genes with the exception of DPL22, in which five amino acids are encoded. Additional novelty in this cohort of genes is found in V4–1 and V4–4 in which Asp and Ala are encoded at positions 66a and 66b, an insert otherwise occupied only in IGLV6S1 (λ6).

Figure 4b compares the sequence of the A7 germline gene [103] to other κ2 light chain genes. A7 is closely related to A23, its counterpart in the duplicated κ locus, differing at only three positions. Notably, threonine replaces methionine at position 89, adjacent to the conserved cysteine at position 88. More notably, phenylalanine replaces the other conserved cysteine at position 23, thus, voiding the intradomain disulfide bond that is a hallmark of these β domains largely throughout the immunoglobulin superfamily. Is this reported phenylalanine an error, or is the gene defective and unable to encode a functional antibody component? Apparently this replacement of Cys23 is not an error. Santos-Esteban and Curiel-Quesada [104] have described the isolation from a phage display library of a functional antibody that binds a blood group antigen [105]. The sequence is shown in figure 4b; the primary structure contains both Phe23 and Met89 in addition to two somatic mutations. The antibody is evidently functional as it was identified by binding the blood group antigen. To our knowledge, this is the only example of expression of the A7 gene that currently appears in the NCBI database.

κ_x vs. κ_y; λ_x vs. λ_y

The extent of sequence variation that occurs within light chain variable domain groups is comparable to that found between groups. Figures 5 and 6 provide

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O11-O1      (k2a) divmtqtplslpvtpgepasiscrssqslldsddgntyldwylqkpgqspqlliyt
O14               divmtqtplslpvtpgepasiscrssqslldsddgntyldwylqkpgqspqlliyt
A19-A3      (k2b) divmtqsplslpvtpgepasiscrssqsllhsng-ynyldwylqkpgqspqlliyl
A2          (k2c) divmtqtplslsvtpgqpasisckssqsllhsdg-ktylywylqkpgqppqlliye
A23               divmtqtplsspvtlgqpasiscrssqslvhsdg-ntylswlqqrpgqpprlliyk
A5                eivmtqtplslsitpgeqasiscrssqsllhsdg-ytylywflqkarpvstlliye
A7                divmtqtplsspvtlgqpasisfrssqslvhsdg-ntylswlqqrpgqpprlliyk
gi|12745541       eivmtqtplsspvtlgqpasisfrssqslvhsdg-ntylswlqqrpgqpprlliyk

O11-O1      (k2a) ls-----yrasgvpdrfsgsg--sgtdftlkisrveaedvgvyycmqriefp----
O14               ls-----yrasgvpdrfsgsg--sgtdftlkisrveaedvgvyycmqriefp----
A19-A3      (k2b) gs-----nrasgvpdrfsgsg--sgtdftlkisrveaedvgvyycmqalqtp----
A2          (k2c) vs-----nrfsgvpdrfsgsg--sgtdftlkisrveaedvgvyycmqsiqlp----
A23               is-----nrfsgvpdrfsgsg--agtdftlkisrveaedvgvyycmqatqfp----
A5                vs-----nrfsgvpdrfsgsg--sgtdftlkisrveaedfgvyycmqdaqdpp---
A7                vs-----nrfsgvpdrfsgsg--agtdftlkisrveaedvgvyyctqatqfp----
gi|12745541       vs-----nrfsgvpdrfsgsg--agtdftlkinrveaedvgvyyctqatqfp----
```

a

```
humlv801    (λ8)  qlvltqsps-asaslgasfkltctlssgshs----syaiawhqqqpekgprylmkl
DPL24             lpvltqpps-asallgasikltctlssehs-----tytiewyqqrpgrspqyimkv
V5-4              qpvltqsss-asaslgssvkltctlssghs-----syiiawhqqqpgkaprylmkl
V4-1              qpvltqpps-ssaspgesarltctlpsdinvg---syniywyqqkpgspprryllyy
V4-4              qpvltqpss-hsassgasvrltcmlssgfsvg---dfwirwyqqkpgnppryllyy
DPL22             qpvltqpps-asaslgasvtltctlssgys-----nykvdwyqqrpgkgprfvmrv
IGLV8             qtvvtqeps-fsvspggtvtltcglssgsvst---syypswyqqtpgqaprtliys
V1-20             qagltqpps-vskglrqtatltctgnsnivg----nqgaawlqqhqghppkllsyr


humlv801    (λ8)  nsdgsh-skgdgipdrfsgss--sgterylltisslqsedeadyycqtwgsgi----
DPL24             ksdgsh-skgdgipdrfmgss--sgadryltfsnlqsddeaeyhcgeshtidgqvg
V5-4              egsgsy-nkgsgvpdrfsgss--sgadryltisnlqfedeadyycetwdsnt----
V4-1              ysdsdk-gqgsgvpsrfsgskdasantgillisglqsedeadyycmiwpsnas---
V4-4              hsdsnk-gqgsgvpsrfsgsndasanagilrisglqpedeadyycgtwhsnskt--
DPL22             gtggivgskgdgipdrfsvlg--sglnryltikniqeedesdyhcgadhgsgsnfv
IGLV8             tn-----trssgvpdrfsgsi--lgnkaaltitgaqaddesdyycvlymggvw---
V1-20             nn-----nrpsgiserfsasr--sgntasltisglkseveanyhcslysssytf--
```

b

Fig. 4. ***a*** Aligned sequences of seven λ germline genes for which usage has not been documented in patients with multiple myeloma or primary amyloidosis, but which have been identified in the other data sets. Five of the seven germline genes are related to humlv801, which has been observed in multiple myeloma patients. ***b*** Aligned human κ2 germline sequences illustrating an unusual germline gene that encodes a variable domain in which the highly conserved Cys23 is replaced by a phenylalanine. Although this germline gene product has not been found in any of the datasets described in table 1 or figures 1–3, this light chain has been identified in a functional scFv extracted from a phage display library (gi|12745541).

a matrix representation of the amino acid variations between germline genes within the two groups. The minimum number of differences is found to be zero; the amino acid sequence encoded by the κ2 gene, O14, is indistinguishable from that encoded by the identical O11–O1 genes although O14 is distinctive at the DNA level. The maximum κ variation is 63 amino acid differences between L22 and both A7 and A23; no example of an L22 product has yet been found (as judged by the content of the NCBI database). The rarely expressed A10 gene

	L12a	O18	O12	A30	L1	L18	L5	L11	O11	O14	A19	A2	A23	A7	A5	L2	A27	A11	L6	L25	L10	B3	B2	L22
O18	17																							
O12	11	13																						
A30	13	18	11																					
L1	11	14	8	8																				
L18	11	14	11	13	10																			
L5	10	16	7	10	7	9																		
L11	16	16	10	6	10	10	11																	
O11	51	50	46	50	49	50	48	49																
O14	51	50	46	50	49	50	48	49	0															
A19	49	46	43	47	46	48	46	46	14	14														
A2	50	46	44	49	48	49	48	48	16	16	15													
A23	52	51	48	52	50	52	48	51	21	21	20	17												
A7	53	52	49	53	51	53	49	52	23	23	22	18	3											
A5	54	53	49	53	50	52	52	50	26	26	23	18	28	28										
L2	26	31	29	32	30	28	29	32	43	43	41	44	48	48	47									
A27	29	33	28	35	30	29	30	34	40	40	38	41	45	45	43	14								
A11	30	32	29	36	31	29	31	35	42	42	40	42	47	47	44	16	4							
L6	31	30	29	36	32	27	31	33	41	41	39	42	46	46	44	11	9	9						
L25	30	29	26	34	31	29	30	29	42	42	40	42	46	46	44	9	11	13	8					
L10	31	30	28	35	32	30	31	31	49	45	43	48	48	49	50	13	14	35	33	29				
B3	35	34	31	37	33	36	35	36	34	34	31	32	38	38	43	31	32	33	34	31	36			
B2	49	43	45	44	47	44	45	43	60	60	59	57	62	62	61	45	47	46	45	46	48	58		
L22	29	31	28	30	28	27	27	29	57	57	55	56	63	63	58	43	44	43	43	43	29	49	52	
A10	35	37	31	36	36	33	33	35	46	46	44	44	49	50	49	37	35	37	34	36	37	40	44	45

Fig. 5. Matrix representation of amino acid sequence variations in human κ variable domain germline genes. Germline identifications have been abbreviated to conserve space. Gaps between clusters separate subgroups.

differs from all other κ germline genes from a minimum of 31 sites to a maximum of 50 positions. Although this protein appears to be rarely used it may make a significant (unknown) contribution; the chimpanzee encodes a homolog (gi|55597677) that differs at only 7 positions, possibly suggesting selective pressure for conservation. The closest pairing of λ germline genes is found for the λ3 genes, V2–14 and humlv318, which differ at two sites. The greatest separation is found for the rarely expressed DPL24 and V2–8 genes, which differ at 71 positions. In both groups, the ‘youngest’ subgroups appear to be κ1 and λ1. It is possible that there is a loose inverse correlation between the overall usage, which facilitated discovery and definition of group, and apparent evolutionary age of the included genes. For instance, the rare κ4 and λ6 subgroups have no close counterparts among other proteins in their group. Both proteins are rarely expressed but are over represented in the myeloma database due to their clinical interest.

κ_{x1} vs. κ_{x2}; λ_{y1} vs. λ_{y2}

Three more well-known mechanisms add to the foundation of diversity provided by the menu of the inherited κ and λ germline genes. During the

	humlv114	122	1S2	111	2.1	L12	318	150	III	214	2-8	219	215	418	6S1	801	5-4	3-2	LV8	4-1	4-4	L24	L22	120
humlv122	20																							
IGLV1S2	20	18																						
V1-11	24	14	23																					
VL2.1	37	35	30	36																				
DPL12	34	33	29	36	10																			
humlv318	43	47	44	49	47	46																		
hsiggl150	42	45	42	47	44	42	27																	
VIII.1	41	45	45	47	44	42	23	19																
V2-14	43	47	44	47	47	46	2	27	23															
V2-8	48	51	48	50	50	50	14	32	26	12														
V2-19	47	48	49	50	46	44	34	14	25	58	37													
V2-15	47	51	52	53	49	47	34	22	27	62	37	24												
humlv418	39	42	37	46	43	41	34	26	29	34	36	32	35											
IGLV6S1	40	39	39	43	43	40	47	44	45	49	51	49	47	45										
humlv801	56	55	56	59	62	61	61	57	61	62	65	61	63	60	59									
V5-4	55	53	54	57	59	58	60	57	60	61	64	58	62	55	56	25								
V3-2	58	55	53	59	56	55	58	53	56	59	63	53	55	56	59	60	58							
IGLV-8	48	48	47	55	50	47	53	52	52	54	58	54	55	45	47	55	54	33						
V4-1	57	55	54	58	56	55	57	55	57	58	63	58	60	60	56	48	47	61	57					
V4-4	57	58	58	60	59	59	60	61	59	61	64	60	65	63	58	50	45	62	59	27				
DPL24	65	64	66	68	68	67	67	66	66	67	71	68	69	65	69	40	41	70	68	59	59			
DPL22	60	62	62	63	64	64	67	65	66	67	70	69	68	63	65	46	46	68	61	62	59	60		
V1-20	40	44	41	44	44	45	49	46	45	49	51	51	53	43	53	60	59	61	56	58	63	70	64	
V1-9	43	42	40	45	36	34	57	57	56	57	60	61	62	51	50	67	68	65	58	65	69	72	70	50

Fig. 6. Matrix representation of amino acid sequence variations in human λ variable domain germline genes. Germline identifications have been abbreviated to conserve space. Gaps between clusters separate subgroups.

maturation of the B-cell, three separate processes take place. A variable domain gene (exon) is combined with a joining segment (J) exon, providing combinatorial diversity. In the case of κ light chains for instance, approximately 25 variable domain exons can be combined with a repertoire of five J segments leading to 125 combinations. Additional forms are generated by nucleotide additions/deletions that can occur at the time of fusion of V and J exons. Finally, the rearranged gene undergoes somatic mutation, which can result in a dysfunctional light chain, which can lead to anergy or apoptosis of the cell, or can prompt receptor editing to attempt an alternative V–J pairing. Cells that produce light chain and heavy chain combinations that have significant autoimmune properties may be eliminated, or alternative V–J pairs may be formed by receptor editing [106–113]. Immune system diversification is the subject of many reviews [114–122].

There is no quality control system to protect against the synthesis of amyloidogenic light chains; it is a virtual certainty that every individual has many B cells that produce potentially amyloidogenic light chains. It is not unreasonable to expect that a sampling of cDNAs from human B cells would find that

```
     B3 DIVMTQSPDS LAVSLGERAT INCKSSQSVL YSSNNKNYLA WYQQKPGQPP KLLIYWASTR
 npth01 .......... ........V. .......TIS .GF......T .....S.... ..........
 npth02 .......... .......... .......... ....S..... .......... ..........
 npth03 N.......E. .......... .....AH.I. .AP...T... ....R..... R.........

 amyl01 ...L.....F .......... .......... .**.S..F.. ........*. ....*..NV.
 amyl02 .......... .TL....... .......... S....R...L .......... .....R..V.
 amyl03 .......... .S........ .......... L.FK.D.... ........S. .---......
 amyl04 ..A....... .......... .......... .......... .....S.... .....G....
 amyl05 .......... .......... .......... ...S...F.. .....AR... .....G....
 amyl06 .......... .......... .......... .......... .......... ..........
 amyl07 .......... .......... .K......L. .T...ES... ..L....... ..........
 amyl08 ---------- ---------- .......... .....Y.... ........S. N.......S.
 amyl09 ---------- ---------- ........L. .T.K..DY.N .......... ....H.....
 amyl10 ---------- ---------- M..------- ---------- .......... .....R....
 amyl11 ---------- ---------- L......... ....-..... ........S. .....S....
 amyl12 ---------- ---------- .S....H.I. .......... .......... N.........
 amyl13 ---....... .......... ........L. ..F....N.. ...L...... .VI..G.A..
 amyl14 EL........ .......... ......R... SD..SR.L.. .F........ ..........
 amyl15 .......... ....P..... .......NL. DA.FDT.T.. .......... ........S.
 amyl16 .......... .......... .......... .....R.... .....L.... ..........
 amyl17 .......... .V........ ...R...... .......... ........A. ...FS.....

 lcdd01 .......... .......... ......L..F F.P....... .......... ..........
 lcdd02 .......... .......... .......... .N.....F.. .......... ..........
 lcdd03 .......... .......... ...R.N..L. .....RE... ......R... ......S...
 lcdd04 .......... .......... ........L. ..TR..IC.. ........T. Q...S...S.
 lcdd05 --........ .......... ........l. ..F....N.. ...L...... .VI..G.A..
 lcdd06 .......... .......... .K......L. .T.D.ES... R.L....... ..........
 lcdd07 .......... .......... .......... SR....SC.T .......... R.........
 lcdd08 ---------- --L....... .........F H.PK..S... ....R..HR. ..........

 unkn01 --------.H ..A..A.... .......... H..TKR..F. .......... .V........
 unkn02 ---------- ---------- .......... .....Y.... ........S. N.......S.
 unkn03 ---------- ---------- ........L. .T.K..D..N .......... ....H.....
 unkn04 ---------- ---------. ...R...... ..A....N.. ...H...... ...F......
 unkn05 ---------- ---------. ....A..... .R.......G ...H...... R.......PW
 unkn06 .F........ .T........ .........F H.PK..S... ....R..HR. ..........
 unkn07 ---------- ---------- M......... A....N.N.. .......... .....R....
 unkn08 ---------- ---------- L......... ....-..... ........S. .....S....
 unkn09 V......... .......T.. ........L. .......... .F.....H.. ..........
 unkn10 N......... .......... .......T.. ...T...... .F........ ........S.
 unkn11 .......... .......... .S......L. .R.I..D... .......... ..........
 unkn12 ---------- ---------- .S....H.I. .......... .......... N.........
 unkn13 ...V...... .......... ....T..... F......... ....R..H.. ..........
 unkn14 .......... .......... .K......L. .T...ES... ..L....... ..........
```

Fig. 7. Illustration of sequence variability introduced by somatic mutation of a single germline gene (B3). Dots indicate identity with the germline encoded sequence; dashes indicate that no sequence information was provided. amyl = Amyloidogenic; npth = nonpathological; unkn = no clinical information.

approximately 10% are capable of forming fibrils. This representation may be somewhat higher, depending on the relative facility of in vivo and in vitro fibril formation processes.

As an example of the level of primary structure diversity that can arise from somatic mutations that occur during B cell differentiation and afterward, Figure 7 provides 42 sequences that originated from the human κ4 germline gene (B3). Six of the 42 sequences exhibit the addition of at least one amino

```
    B3 ESGVPDRFSG SG**SGTDFT LTISSLQAED VAVYYCQQYY STP****
npth01 .......... .......... ...T...... .........F TI.....HTF GQGTNLEI.K
npth02 .......... .......... .......... .......... .......YSF GQGTKLEI.K
npth03 Q....E.... .......... ....T..... ....F..... T......CTF GQGTKLEI.Q

amyl01 ........R. ....V..... ....N....L .......... .......YSF GQGGRLEI.K
amyl02 .......... ......S... .......... ..I....... NA.....YTF GQGTKLEI.K
amyl03 .......... .......N.. .......... .......... T......GTF GQGTKVEI.K
amyl04 .......... .......... .......... .......... G..P...YTF GQGTKLEI.K
amyl05 .....E.... .....A.... ...D...... ......H... .P.....RSF GQGTKLEI.K
amyl06 Q...S..... .......... ....G..... .......... G......LTF GQGTKVEI.K
amyl07 K......... .......... ....G..... .......... .SW....YTF GQGTKVEI.K
amyl08 .......... .......N.. ......HV.. .......... NS.....YTF GQGTKLEL.K
amyl09 QF..S...R. .......... .........- .......... DSS....PTF GQGTRVEL.K
amyl10 .......... .......... .......... .......... .......LTF GGGTNVEI.K
amyl11 ........R. .......... ...T...... .....-..H. .......FTF GPGTTVDI.K
amyl12 .......... .......... ....N..... A......... .G.P...YTF GQGTKLEI.K
amyl13 .......... .......... .......... .........S NS.....MTF GQGTRLEI.K
amyl14 V......... .......... .......... .......... ...PG..FTF GPGTKVEI.K
amyl15 .......... .......... .......... .......... .......PTF GGGTKVEI.K
amyl16 .......... .......... .......... ......H... .H.....QTF G----LEL.K
amyl17 .......... .......... ...PG..... .......... RI.....YTF GQGAK---.-

lcdd01 .......... .......N.. ....R..... .......... T.LS...WTF GQGTKVEI.K
lcdd02 G......... .......... ...TR..... .......... .......ITF GQGTRLEI.K
lcdd03 .......... ......AH.. .......... .......... AIP....LTF GGGTKVEI.K
lcdd04 .......... .........S .......... ..T......S TA.....WTF GQGTRVEI.R
lcdd05 .......... .......... .......... .........S NS.....MTF GQGTRLEI.K
lcdd06 K......... .......... ....G..... .......... SS*....WTF GQGTKVEI.K
lcdd07 .......... .......N.. ...T...... .......... N.L....LTR GGGTRVEI.K
lcdd08 H......... .........N ....G..... ..L.F..... T......LTF GGGTKLEI.K

unkn01 .......... .......N.. .....V.P.. ..L.S.H.C. .......FTF GPGTKVEI.K
unkn02 .......... .......N.. ......HV.. .......... NS.....YTF GQGTKLEL.K
unkn03 QF..S...R. .......... .........- .......... DSS....PTF GQGTRVEL.K
unkn04 .F........ .......... .......... .......... .......--- --------.-
unkn05 .F........ .......V.. ........D. .......... NI.....--- --------.-
unkn06 H......... .........N ....G..... ..L.F..... T......LTF GGGTKLEI.K
unkn07 G......... .......... .......... .......... .......LTF GGGTNVEI.K
unkn08 ........R. .......... ...T...... .....-..H. .......FTF GPGTTVDI.K
unkn09 ........I. .......... ...T...... .......... NN.....RTF GQGTKVER.K
unkn10 .Y........ .......... ...T...... .......... GS.....YTF GQGTKLEI.K
unkn11 .......... .......N.. .......... ..L....... L..P...YTF GQGTKLEI.K
unkn12 .......... .......... ....N..... A......... .G.P...YTF GQGTKLEI.K
unkn13 .......... .......... .......... .......... .S.....YTF GQGTKLGI.E
unkn14 K......... .......... ....G..... .......... .SW....YTF GQGTKVEI.K
```

Fig. 7. (continued).

acid at the V–J junction; in one case, two amino acids are added. In five of the cases, the amino acid added at position 95a is proline; we have shown that proline at this position is destabilizing, thus increasing potential risk for fibril formation (unpublished results). Two of the added prolines are found in proteins for which no clinical information is available. The reader is invited to deduce the causes of amyloidosis and LCDD from this data. While this is clearly impossible, useful hypotheses can be derived. A smaller set of B3 data was earlier found to be very useful in helping to provide support for the linkage between variable domain stability and risk of fibril formation [123].

A final source of diversification may be mentioned: allelic variation. Not all individuals inherit the same germline genes, which is true for many genes of course. A relevant example of allelic variation in human light chain variable domains is found in the κ1 subgroup in which the germline gene L12a encodes a serine at position 95; all other major human κ light chain germline genes, including an alternative κ1a allele, encode proline. Loss of proline at position 95 is destabilizing; therefore, the Ser95 allele may provide an inherited increased risk for amyloidogenesis. However, since the phenotypic consequence of any variable domain allele will be exhibited in the context of somatic mutation and V–J junctional diversification, it is unlikely that familial AL amyloidosis will be attributed to alleles in the explicit manner seen in other amyloid diseases such as those arising from transthyretin [124] and human lysozyme [125].

Light Chain Diversification

The phrase, light chain diversity, is used to describe the consequences of the mechanisms in place to provide rearranged light chain genes of heterogeneous sequence. Light chain diversification refers to the consequences of spontaneous mutations that occur during clonal expansion, be that as a result of normal immune response, an autoimmune response, or as a consequence of a neoplasm.

Light chain diversification (or clonal diversification) does not refer to biclonal myelomas that presumably arise from multiple neoplastic initiation events and are often revealed by the detection of two heavy chain classes or the presence of both a κ and λ paraprotein [126–134]. Two instances of apparent biclonality have been revealed by sequence analysis; both of these cases involved κ light chains. Therefore, immunological testing of the free light chain in the serum or urine would not reveal biclonality.

The most definitive case involves two proteins produced by a myeloma patient with amyloidosis. Hilschmann and co-workers [135] determined the amino acid sequence of the protein in fibrils deposited in the liver of the patient as well as the primary structure of the paraprotein purified from urine. The two proteins differed in amino acid content at no fewer than 21 positions (a 25-amino segment of the urinary protein was not sequenced). The two proteins were encoded by two different genes (fig. 8a). The sequenced portion of the urinary, non-amyloidogenic, protein contained six variations from the O12–O2 germline gene. The light chain that formed fibrils originated from the O8–O18 germline gene from which it differed at 13 sites.

Another probable biclonal myeloma was described by Frangione and co-workers [136] in a case in which the patient exhibited both IgG1 and IgG2

```
          0          1          2          2          3          4        5
          1          1          1          7d         5          5        4
  O12-O2  DIQMTQSPSS LSASVGDRVT ITCRASQSI. .....SSYLN WYQQKPGKAP KLLIYAASSL
  malu                        I        D        R---- ---------- ----------

          5          6          7          8          9      9
          5          5          3          3          3      9
  O12-O2  QSGVPSRFSG SG..SGTDFT LTISSLQPED FATYYCQQSY STP....--- --------.-
  malu  -         T        R                   H             YTF GEGTKLEI N   (6)

          0          1          2          2          3          4        5
          1          1          1          7d         5          5        4
  O8-O18  DIQMTQSPSS LSASVGDRVT ITCQASQDI. .....SNYLN WYQQKPGKAP KLLIYDASNL
  malf                        I                  D V

          5          6          7          8          9          9
          5          5          3          3          3          9
  O8-O18  ETGVPSRFSG SG..SGTDFT FTISSLQPED IATYYCQQYD NLP....--- --------.-
a malf    Q              Y  H   V   DR A           N  DF     PTF GGGTKVEI K   (13)

          0          1          2          2          3          4
          1          1          1          7d         5          5
  L12a    DIQMTQSPST LSASVGDRVT ITCRASQSI. .....SSWLA WYQQKPGKAP KLLIYKASSL
  wil1                          F   V   V        Y               N    Q  I
  wil2                              P  AF       G                E    RV

          5          6          7          8          9          9
          5          5          3          3          3          9
  L12a    ESGVPSRFSG SG..SGTEFT LTISSLQPDD FATYYCQQYN SYS....--- ---------
  wil1    DA         NI     D            N S R        R .    ..F GQGTKLEIK    (17)
b wil2    Q                                V       A  R .    ..F GQGTKLEIK    (12)

          0          1          2          2          3          4        5
          1          1          1          7d         5          5        4
  L1      DIQMTQSPSS LSASVGDRVT ITCRASQGI. .....SNYLA WFQQKPGKAP KSLIYAASSL
  ban1       L                         SV       Y  V                  D  T
  ban2       L                         SV       Y  V                  D  T

          5          6          7          8          9          9
          5          5          3          3          3          9
  L1      QSGVPSRFSG SG..SGTDFT LTISSLQPED FATYYCQQYN SYP....--- ---------
  ban1          N T           I                              YTF GQGTKVQIK    (10)
c ban2          N T           I                                       LE      (10)

                     1          2          2          3          4        5
          1          1          1          7d         5          5        4
  L2-L16  EIVMTQSPAT LSVSPGERAT LSCRASQSV. .....SSNLA WYQQKPGQAP RLLIYGASTR
  vas1       L             D                     N     F              A  V
  vas2       L             D                     N  T  F              A  V

          5          6          7          8          9           9
          5          5          3          3          3           9
  L2-L16  ATGIPARFSG SG..SGTEFT LTISSLQSED FAVYYCQQYN NWP....--- --------.-
  vas1    D VQG                  S      Q             Q      FTF GPGTKVDF K   (13)
d vas2    D VQG                  S      Q             Q      FTF GPGTKVDF K   (14)
```

Fig. 8. Multiplicity of primary structures in individual patients. ***a*** Co-expression of two germline genes, one yielding an apparently non-pathological protein while the other led to amyloidosis. ***b*** Probable dual expression of a single germline gene or, alternatively, possible example of extensive clonal diversification. ***c*** Example of clonal diversification of unknown consequence. ***d*** Example of clonal diversification that may have led to the development of amyloidosis.

paraproteins. The light chains in both IgGs were products of the L12a germline gene. However, they differed from the germline gene at 17 and 12 sites. Only at position 93 did the two light chains show usage of the same alternative amino acid, Arg (fig. 8b). Thus, both the presence of two IgG isotypes, and lack of significant commonality of light chain somatic mutations, support the notion that the two paraproteins arose from independent neoplastic events.

κ_{x11} vs. κ_{x12}; λ_{y21} vs. λ_{y22}

Light chain diversification arises from somatic mutation that occurs as inevitable replication errors during clonal expansion. The first example of this phenomenon was found by Benson's laboratory in analysis of the amyloidogenic κ light chain, BAN [137]. Sequencing of this protein revealed the presence of significant quantities of two different amino acids at adjacent positions in the J segment. Somatic mutation was inferred as probable. Since the quantitative presence of additional amino acids was considered significant during sequencing, rather than being attributed to noise, it is probable that these mutations occurred shortly after the neoplastic transition of the clone.

The most recent example of myeloma clonal diversification involves the possible spawning of an amyloidogenic clone from what may have been a non-amyloidogenic precursor. Westermark and co-workers [74] sequenced fibrils from a patient and identified two primary structures within the fibril. The two sequences differed at only one position. One of the sequences retained the L2–L16 germline-encoded Ala at position 34; the other sequence exhibited Thr at this location (fig. 8d). Both primary structures shared 13 mutations relative to the germline; thus, there is no doubt that the introduction of Thr represented a divergence from what had been a common lineage. In combination with the germline-encoded Asn at position 32, a glycosylation-acceptor site resulted from the introduction of Thr34. The presence of a glycosylation site has been previously suggested to represent a risk factor for fibril formation [138] and the possible contribution of glycosylation to fibrillogenesis has received considerable attention [75, 139–141].

Clonal diversification as deduced from light chain sequence diversification has been documented in various disorders including lymphoma [142], SLE, and ART. Figure 9 compares eleven similar primary structures derived from the V5–4 germline gene obtained from B cells of a ART patient [143, 144]. Figure 10 depicts a possible evolutionary path based on the amino acid variations observed, suggesting two evolutionary tracks. A more clear picture emerges when the DNA sequences are used as the basis for an evolutionary analysis, thus allowing the information content of silent base changes to be

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V5-4      qpvltqsss-asaslgssvkltctlssghs-----syiiawhqqqpgkaprylmklegsgsy-nkgsgvpd
AAC27305  qpvltqsss-asaslgssvkltctlssghs-----nyiiawhqqqpgkaprylmklegsgty-nkgsgvpd
AAC27306  qpvltqsss-asaslgslveltctlssghs-----nyiiawhqqqpgkaprylmklegsgty-nkgsgvpd
AAC27307  qpvltqsss-asaslgssvkltctlssghs-----nyiiawhqqqpgkaprylmklegsgty-nkgsgvpd
AAC27308  qpvltqsss-asaslgssvkltctlssghs-----nyiiawhqrqpgkaprylmklegsgty-nkgsgvpd
AAC27309  qpvltqsss-asaslgssvkltctlnyghs-----nyiiawhqqqpgkgprylmklegcgty-nkgsgvpd
AAC27310  qpvltqsss-asaslgssvkltctlssghs-----syiiawhqqqpgkaprylmklegsgsy-nkgsgvpd
AAC27311  qpvltqsss-asaslgssvkltctlssghs-----syiiawhqqqpgkaprylmklegsgsy-nkgsgvpd
AAC27312  qpvltqsss-asaslgssvkltctlssghs-----nyiiawhqqqpgkaprylmklegsgty-nkgsgvpd
AAC27313  qpvltqsss-asaslgssvkltctlssghs-----nyiiawhqqqpgkaprylmklegsgty-nkgsgvpd
AAC27314  qpvltqsss-asaslgssvkltctlssghs-----nyiiawhqqqpgkaprylmklegsgty-nkgsgvpd
AAC27315  qpvltqsss-asaslgssvkltctlssghs-----nyiiawhqqqpgkaprylmklegsgty-nkgsgvpd

V5-4      rfsgss--sgadryltisnlqfedeadyycetwdsnt
AAC27305  rfsgss--sgadryltisnlqsedeadyycetwdtnt-----vfgggtkltv
AAC27306  rfsgss--sgadryltisnlqsedeadhycqtwdtdi----avfgggtkltv
AAC27307  rfsgss--sgadryltisnlqsedeadyycetwdtnt-----vfgggtkl--
AAC27308  rfsgss--sgadryltisnlqsedeadyycetwdtnt-----vfgggtkl--
AAC27309  rfsgss--sgadryltisnlqsedeadyycetwdtnt-----vfgggtkltv
AAC27310  rfsgss--sgadryltisnlqfedeadyycetwdsni----rvfgggtkltv
AAC27311  rfsgss--sgadryltisnlqfedeadyycetwdsni----rvfgggtkl--
AAC27312  rfsgss--sgadryltisnlqsedeadyycetwdtnt-----vfgggtklt-
AAC27313  rfsgss--sgadryltisnlqsedeadhycqtwdtdi----avfgggtkltv
AAC27314  rfsgss--sgadryltisnlqsedeadhycqtwdtdi----avfgggtkl--
AAC27315  rfsgss--sgadryltisnlqsedeadyycetwdtnt-----vfgggtklt-
```

Fig. 9. Aligned sequences of light chain variable domains from cDNA obtained from a single ART patient. The rearranged genes originated from the λ V5–4 gene, which has not yet been observed in patients with multiple myeloma or primary amyloidosis.

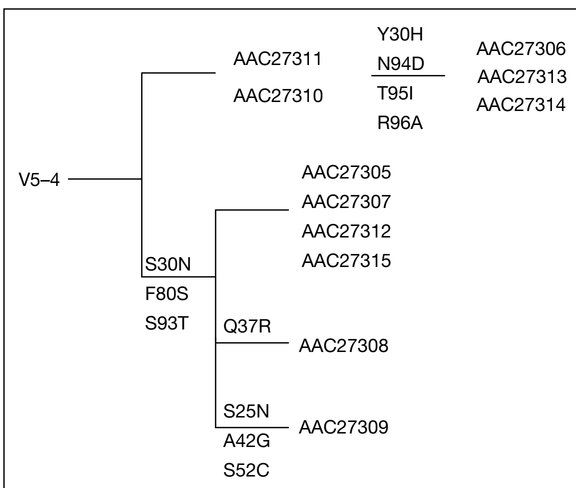

Fig. 10. Possible evolutionary tree for the sequences presented in figure 9 as inferred from the amino acid sequence.

accessed. As shown in figure 11, eight of the eleven samples had a clonal relationship, with two sets of duplicated sequences. The six distinct sequences allowed for the inference of two clonal intermediates that were not found in the data indicating that the initial B-cell progenitor clone diversified into a family

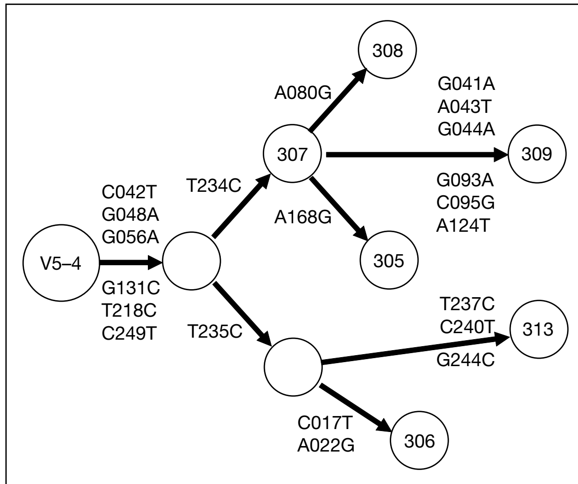

Fig. 11. Possible evolutionary tree for the sequences presented in figure 9 as inferred from the nucleotide sequence, which indicate the presence of at least 8 clones having evolved from a single rearranged V5–4 clone.

consisting of a minimum eight secondary clones. Secondary clones in which codon changes resulted in alteration of amino acids could potentially produce light chains having altered physicochemical properties with associated changes in pathological properties.

Amyloidogenesis by Proxy

Mutagenesis of light chains as a result of DNA replication during clonal expansion of antibody-producing cells during myeloma is inevitable and results in clonal diversification with the possibility of introducing amino acid variations. Although myeloma is usually a monoclonal disease, with exceptions such as illustrated in figure 8a and b, the clone is not likely to be homogeneous. Replication error frequency has been estimated to be on the order of as high as 1 error per 10^5 bases [145] in an in vitro study. If we assume a more conservative of one error per 10^6 bases, errors in light chain replication become inevitable by the time the clone has expanded to approximately 1,000–10,000 cells, considering that each replication of the light chain variable domain gene involves approximately 700 bases on the two strands of DNA. Since changes of the first or second base in a codon almost always are non-synonymous, a majority of the base errors are likely to result in amino acid substitutions. Some of

these replacements are likely to be innocuous or improve the stability of the domain. However, other mutations are certain to impair the stability of the variable domain.

Destabilizing mutations have the potential to create an amyloidogenic subpopulation of proteins in the midst of light chains that may not be prone to fibrillogenesis. If this subpopulation reaches a critical concentration, fibril nucleation could occur. If fibril formation occurs within the plasma cell, as has been observed [146], fibrils formed by this subpopulation could be expected to act as seeds [147–150] that have the capability to 'recruit' the non-amyloidogenic light chains (Stevens and Argon, unpublished results), if they are of intermediate stability such that domains that are partially unfolded are present. In such a scenario, the onset of amyloidosis could appear to be sudden as well as unexpected. In addition, the predominant protein in the fibrils, or the sequence of the cDNA in the prevalent B-cells, would be identified as the amyloidogenic species, although it was in fact the victim of amyloidogenesis by proxy, thus adding another level of complexity to the challenge of identifying the structural basis of AL.

Conclusions

The possibility of amyloidogenesis by proxy does not imply that a large proportion of light chain primary structures thought to be amyloidogenic are incorrectly assigned. Certainly the variable domains of the κ4 proteins, Rec and Sma, were found to be of low thermodynamic stability and readily formed fibrils in vitro [123]; however, more studies of the effects of all mutations found in other amyloid associated light chain variable domains would be of value. The case described by Karimi et al. [74] might be a candidate for 'amyloidogenesis by proxy'; stability studies of this variable domain could be informative. Systematic studies of cDNA extracted from multiple B cells in patients with myeloma and monoclonal gammopathy of undetermined significance may also be of interest to evaluate the possible extent of clonal diversification. Such studies may provide a basis of methods to eventually detect a risk of 'late-onset' amyloidosis in patients prior to presentation of symptoms.

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High-Dose Therapy in Patients with Plasma Cell Dyscrasias and Renal Dysfunction

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Abstract

Multiple mycloma causes a disproportionate amount of the malignancy-related renal insufficiency. Acute renal insufficiency in myeloma patients can occur due to dehydration, hypercalcemia, side effects of medications (NSAIDs) or tumor lysis syndrome in addition to cast nephropathy, amyloidosis and light chain deposition disease. Patients on hemodialysis have traditionally been excluded from antineoplastic therapy due to fear of side effects and lack of studies addressing benefit. Melphalan is the most effective chemotherapeutic agent in myeloma and its PK (pharmacokinetics) are not adversely affected by impaired renal function. Because of more pronounced toxicity of Melphalan 200 mg/m^2 conditioning regimen, Melphalan 140 mg/m^2 has become the standard of care. 24% of patients become dialysis-independent at a median of 4 months after autotransplantation. Favorable factors for becoming dialysis independent were duration of dialysis $\leq$6 months and pretransplant creatinine clearance $>$10 ml/min. While no good data are available on the use of thalidomide in the presence of renal failure, it is our experience that severe neuropathy, constipation, lethargy and bradycardia are more frequent in patients with creatinine $\geq$3 mg/dl. It has become apparent that bisphosphanates-zoledronic acid more than pamidronate-cause renal dysfunction. If patients remain dialysis-dependent after autotransplantation, we recommend to delay considering a renal transplant until at least 3 years after the first transplant.

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Multiple myeloma causes a disproportionate amount of the malignancy-related renal insufficiency. Despite representing only 1% of all malignancies, it contributes to over half of malignancy-related end-stage renal disease [1]. The histology of renal dysfunction in myeloma has been reviewed elsewhere [2, 3]

and in other sections of this book. Acute renal insufficiency in myeloma patients can occur due to dehydration, hypercalcemia, side effects of medications (NSAIDs) or tumor lysis syndrome (more common in patients with high tumor burden in the setting of modern, highly effective antitumor therapy), in addition to cast nephropathy, amyloidosis and light chain deposition disease. As a general rule, in cast nephropathy there is a large amount of Bence Jones proteinuria. In amyloidosis and light chain deposition disease, there is minimal Bence Jones proteinuria, but in amyloidosis there is usually significant albuminuria. The degree of reversibility of different renal insults depends on the degree and extent of sclerotic tissue changes. Hypercalcemia, if the only cause of renal failure, is rapidly reversible. Cast nephropathy is usually reversible, if treated promptly. Amyloidosis is less likely to reverse, with light chain deposition disease the least reversible. Renal biopsies are extremely helpful as diagnostic and prognostic tools. They should no longer be considered only of academic interest. A retrospective study of 118 patients showed that mortality in patients with amyloidosis was often related to extrarenal involvement (heart failure, cachexia and arrhythmias); patients with cast nephropathy more commonly died of infections, and that patients with light chain deposition disease had a longer survival than the other two groups, even on maintenance hemodialysis [4].

Patients on hemodialysis have traditionally been excluded from antineoplastic therapy due to fear of side effects and lack of studies addressing benefit. This situation is now changing with data from retrospective studies [5] and pharmacokinetic (PK) studies on that population [6]. Coordination between the Nephrology and Hematology teams is important for the care of this group of patients with special challenges [7].

Treatment of Multiple Myeloma

Standard Therapy

Symptomatic MM (presence of renal failure, hypercalcemia, anemia or lytic bone lesions) requires initiation of systemic therapy, historically performed with melphalan-prednisone (MP) [8] or combination chemotherapy [9]. Several studies have shown unequivocally that combination chemotherapy with addition of other alkylating agents such as cyclophosphamide or nitrosureas and anthracyclines has not improved the survival of patients with MM beyond results obtained with standard MP. Furthermore, even the relatively low doses of oral melphalan used in the MP regimen, result in doubling (36 vs. 18%) of the incidence of grade 3–4 hematologic toxicities depending on GFR ($>$50 vs. $<$30 ml/min, respectively) [10].

High-Dose Therapy

When high-dose therapy with melphalan was introduced in the mid 1980s, complete remission's (CR) were observed more frequently and attainment of CR became a primary trial objective as a potential prelude to long-term disease control [11–15]. Extensive phase II studies, initially with autologous bone marrow transplant and, subsequently, with mobilized PBSC support, indicated that drug resistance could be largely overcome by dose escalation. Thus, in newly diagnosed disease up to 50% of patients achieved a stringently defined CR (immunofixation negative) [16–21]. With PBSC, the duration of severe bone marrow aplasia was shortened to <1 week, so that treatment-related mortality was well-reduced to under 5% [22]. Two randomized trials by the Intergroupe Francais du Myélome (IFM) and the Medical Research Council (MRC) showed better outcome with high-dose therapy than with standard chemotherapy among patients with newly diagnosed disease [23, 24]. Similarly, a better outcome was observed in transplanted patients when compared to case controls [25] and in a population based study [26].

Tandem Transplants

The superiority of tandem transplants over a single transplant has been strongly suggested by different observations during the last years. In newly diagnosed multiple myeloma patients, researchers at the University of Arkansas observed an increase in CR rate from 32% after the first autograft to 48% in those who completed tandem transplants [27]. In the French IFM-94 study overall survival (OS) at 7 years was significantly better in the tandem transplant when compared to a single transplant (42 vs. 21%) (Attal NEJM Dec 2003). In a non-randomized study, Lemoli et al. observed a 14 vs. 41% CR rate respectively in patients receiving a single vs. tandem transplant ($p = 0.04$). The median event-free survival (EFS) for the two groups was 17 and 35 months, respectively ($p = 0.03$) [28].

High-Dose Therapy in Patients with Renal Dysfunction

The Arkansas Experience

Since melphalan is the most effective chemotherapeutic agent in myeloma, it was important to investigate how PK of this drug differed in the presence of renal failure. Most patients with severe renal failure had been excluded from high-dose therapy. Twenty patients were studied prospectively. Five patients were on chronic hemodialysis. Melphalan was given in two equal doses of 100 mg/M^2 on 2 consecutive days, followed by administration of peripheral blood stem cells. Melphalan PK were not adversely affected by impaired renal function (fig. 1). There was no negative impact of renal failure on quality of

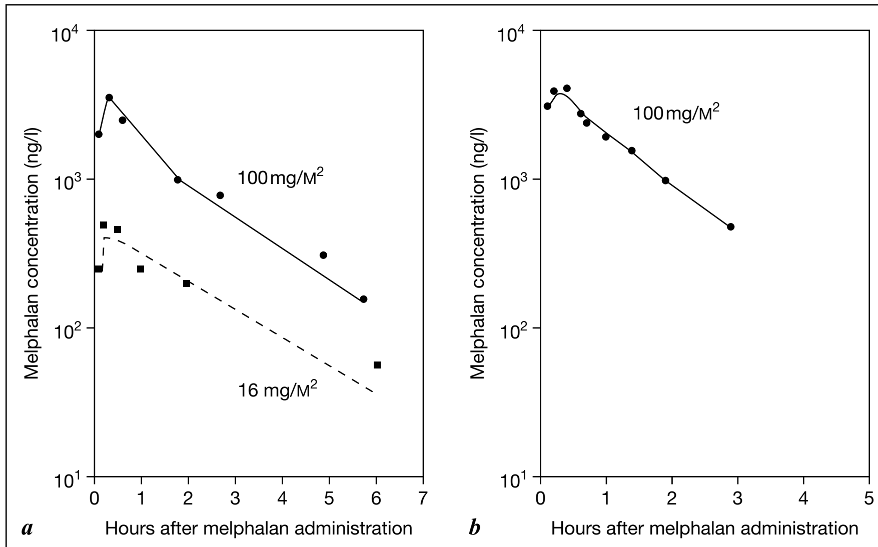

Fig. 1. MEL concentration (ng/l) over time in two individual patients. ***a*** Renal failure patient on hemodialysis (■), data points obtained after a test dose of 16 mg/M^2. (●) = Data points obtained after 100 mg/M^2 MEL. ***b*** Patient with normal renal function. MEL clearance of 100 mg/M^2 was not impaired in the patient hemodialysis (***a***) compared to the patient with normal renal function.

peripheral blood stem cell collection or post-transplant engraftment. However, renal failure patients had more days of fever ($p = 0.0005$) and hospitalization ($p = 0.004$) compared to patients with normal renal function [6].

Clinical Transplant Results

The Myeloma Institute for Research and Therapy at the University of Arkansas for Medical Sciences (UAMS) group reported in 2001 data on 81 patients with CR >176.8 μmol/l of which 38 patients were on dialysis. 26% had received more than 12 months of prior chemotherapy [29]. Fifty-one patients were mobilized with G-CSF for CD34+ cell collection with a median yield of 10×10^6 CD34+ cells/kg and 27 patients were mobilized with chemotherapy plus G-CSF, yielding a median of 16×10^6 CD34+ cells/kg. Melphalan 200 mg/M^2 conditioning regimen was administered to the first 60 patients (27 on dialysis). Because of more pronounced toxicity, the following 21 patients received Melphalan 140 mg/M^2. Median time to ANC recovery to 0.5×10^9/l was 11 days and to platelet recovery to $>50 \times 10^9$/l was 41 days. A 26% CR rate after first transplant and 38% after tandem transplant was achieved. Two patients were able to discontinue dialysis after transplant. The

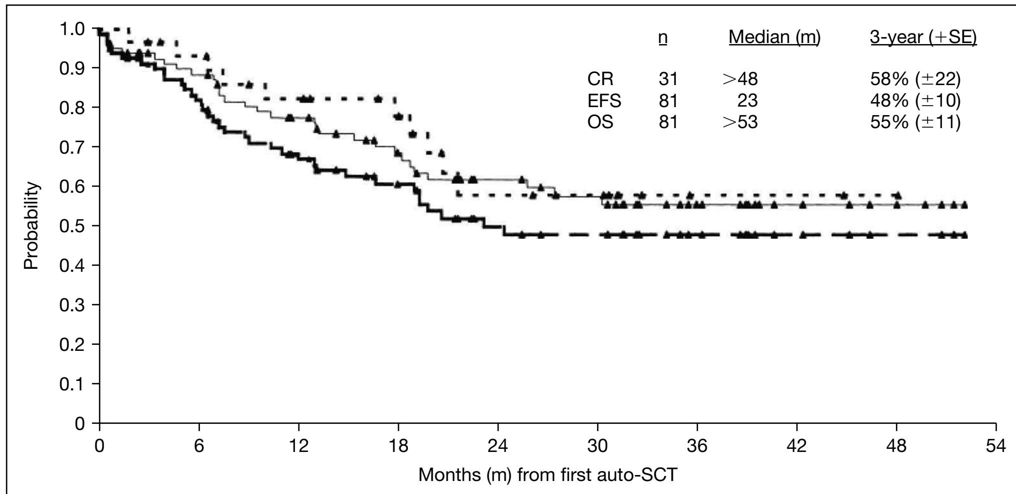

Fig. 2. Actuarial survival for myeloma patients with renal failure after auto-SCT.

outcome of these patients is depicted in figure 2. In 2004, 59 patients on hemodialysis at the time of autotransplant were analyzed. Thirty-seven patients had been on dialysis for ≤6 months. Five year EFS and OS after autotransplant was 24 and 36%, respectively. Fifty-four patients were evaluable for renal function improvement, of which 13 (24%) became dialysis-independent at a median of 4 months after autotransplantation. (Range = 1–16 months). Favorable factors for becoming dialysis independent were duration of dialysis ≤6 months and pre-transplant creatinine clearance >10 ml/min. Twelve of 36 (33%) patients on dialysis ≤6 months vs. one of 17 patients (6%) recovered renal function. Ten of 26 (38%) patient with creatinine clearance >10 ml/min vs. 3 of 28 (11%) < 10 ml/min recovered renal function (fig. 3).

Authors from other institutions have also reported results of high-dose therapy in patients with renal dysfunction, showing the feasibility of such approach [30–32].

Pharmacologic Considerations of Commonly Used Agents in the Treatment of Myeloma

While anthracyclines, etoposide and cyclophosphamide do not require reduction in the presence of renal failure when given at commonly used doses, the dose of cisplatin needs to be reduced in the presence of renal failure.

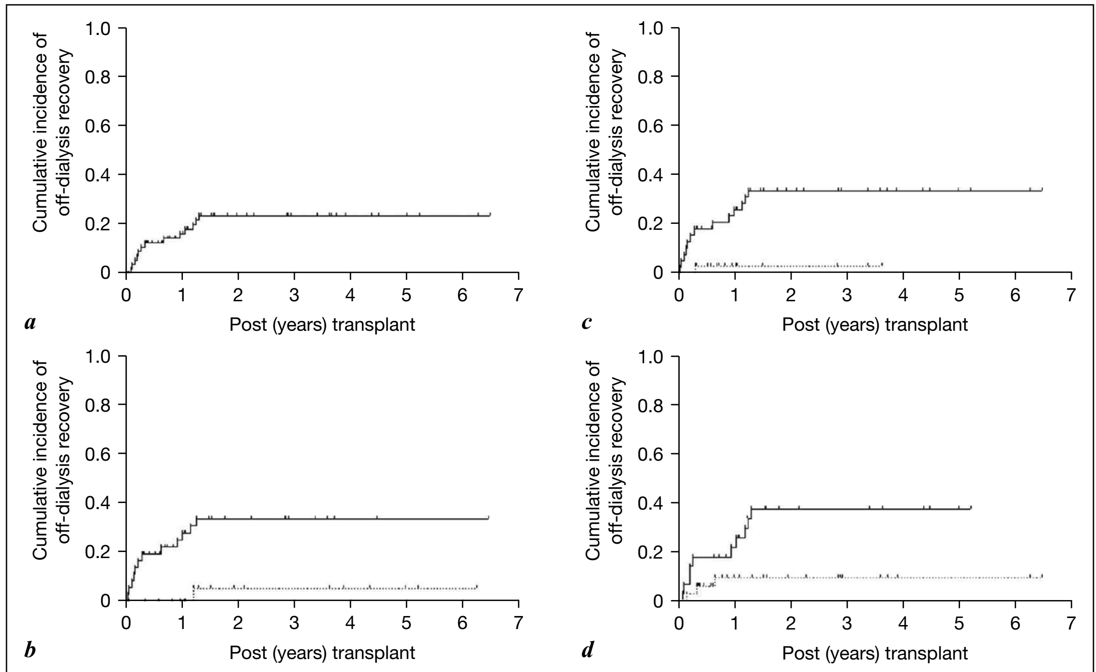

Fig. 3. Cumulative incidence of off-dialysis recovery: ***a*** whole group; ***b*** prior dialysis duration <6 months (solid line) vs. six months (dashed line); ***c*** CR+nCR (solid) vs. <nCR (dashed) post-transplant; ***d*** creatinine clearance >10 ml/min (solid) vs. 10 ml/min (dashed). Bone Marrow Transplant 2004;8:823–828.

As a general rule, we give full-dose cisplatin if creatinine clearance is ≥50 ml/min; we reduce the dose by 50% if the clearance is ≥30–50 ml/min and omit cisplatin if patients have a clearance of <30 ml/min. Although vincristine use has decreased considerably, its dose needs to be adjusted for renal function, because of its increased neurotoxicity under such circumstances. We reduce the dose by 50% with a creatinine clearance between 30 and 50 ml/min and omit it if clearance is <30 ml/min.

While no good data are available on the use of thalidomide in the presence of renal failure, it is our experience that severe neuropathy, constipation, lethargy and bradycardia are more frequent in patients with creatinine ≥3 mg/dl. These patients in general will not tolerate more than 50–100 mg of thalidomide a day. Jagannath et al. [33] have reported that no dose reduction is required for bortezomib (velcade) in patients with renal failure, although the study included only a relatively small number of myeloma patients.

It is generally recommended to reduce the dose of lovenox, given either to prevent or treat thrombo-embolic complications which are frequently observed with combination chemotherapies including thalidomide. Although investigators initially recommended not to reduce dosing of bisphosphonates in the presence of renal failure, it has become apparent that bisphosphanates-zoledronic acid more than pamidronate-cause renal dysfunction. The first manifestation of renal toxicity of pamidronate is non-specific proteinuria (albuminuria). Once urine total proteinuria exceeds 1,000 mg/24 h in the absence of $>$100 mg of Bence Jones proteinuria, bisphosphonate therapy needs to be discontinued to see if the proteinuria is reversible. If reversible, pamidronate should be given at a lower dose with a larger interval between administrations. Monthly administration of bisphosphonate, in the presence of renal failure will result in rapid increase in parathormone level due to severe hypocalcemia. In such patients, it is important to follow parathormone levels and, in general, when the creatinine clearance is between 50 and 70 ml/min, we give pamidronate 90 mg every 2 months; when between 30 and 50 ml/min, pamidronate is administered at a reduced dose of 60 mg over 4 h every 3 months and with a creatinine clearance $<$30 ml/min, pamidronate is given every 6 months at a dose of 60 mg over 6 h. Especially with a clearance $<$70 ml/min, pamidronate should be preferred over zoledronic acid, which is clearly more nephrotoxic, partly related to the short time of administration.

A Potentially New Effective Drug for Transplantation: Busulfex®

Busulfan (Bu) is a bifunctional alkylating agent currently used almost exclusively as a component of conditioning regimens for both autologous and allogeneic stem cell transplants. PK studies of the oral form have shown great interpatient variations due to age, circadian rhythm, underlying disease and drug–drug interactions. Bu toxicity (pulmonary, neurologic, hepatic) correlates with a high systemic exposure, manifested as area under the plasma concentration–time curve (AUC). Therefore, attempts have been made to reduce toxicity by individualizing the dose based on therapeutic drug monitoring. However, even this approach is problematic since PK data cannot be obtained in up to 25% of patients because of delayed absorption and/or elimination, or because of loss of part or the entire intended dose due to vomiting. The advent of intravenous Bu (IV Bu) was anticipated to solve the problem of bioavailability variation and to ensure a safer dosing regimen.

Data from phase I studies of IV Bu determined the dose of 0.8 mg/kg of body weight to yield PK parameters similar to those obtained after a standard oral dose of 1 mg/kg of body weight. These PK data were confirmed in a

recently completed phase II trial which showed the safety and efficacy of IV Bu given at 0.8 mg/kg × 16 doses every 6 h along with two daily doses of cyclophosphamide. Since the original prescription design of oral Bu (every 6 h) was intended to improve patient compliance, alternative dosing schedules of the drug were investigated. Preclinical data on once/day IV dosing in animal models and orally in children have not shown increased toxicity. In a study of 70 patients with hematologic malignancy receiving allogeneic stem cell transplant, IV Bu (3.2 mg/kg/day as a single infusion over 3 h × 4 days) was combined with fludarabine. Hepatic toxicity was transient; there was no clinically diagnosed veno-occlusive disease. Grade II stomatitis occurred in 70% of the patients; only one patient, with subtherapeutic phenytoin levels, developed convulsions. With a median follow-up of 16 months, the treatment-related mortality at 100 days and 2 years was 2 and 5%, respectively. No difference in plasma half-life, clearance and AUC were detected in PK studies performed after the first and the fourth dose of the IV drug. In a study of 36 CML patients conditioned with IV Bu and cyclophosphamide prior to allogeneic transplant, the risk of death and the incidence of regimen-related toxicity and acute GvHD were analyzed as a function of the per dose IV Bu AUC. No patient developed veno-occlusive disease. The probabilities of developing GI toxicity, hepatotoxicity, mucositis and acute GvHD all increased with increasing AUC. The risk of death was significantly lower for patients having a per-dose AUC ranging between 950 and 1,520 μmol-min; this risk of treatment failure increased sharply with either lower (due to relapse) or higher AUC levels (due to toxicity), suggesting the presence of an optimal therapeutic 'window', based on per-dose AUC.

We recently opened a phase I/II open label, dose escalating study of IV Bu in MM patients who are either >65 years of age or have serum creatinine >3.0 mg/dl (or creatinine clearance <30 ml/min), to be followed by an autotransplant. PK studies are performed to evaluate interpatient variability and relationship to toxicities.

Patients with high risk of treatment-related complications such as renal failure age ≥ 65 have more severe problems with mucositis than other patients. Since Bu causes less mucositis, we feel these patients may benefit more from this drug. We will compare toxicities from the first transplant when Bu will be given to the second transplant when melphalan will be given. Moreover, Bu introduces profound cytopenia much more slowly than melphalan while the time to recover after transplantation is the same and depends upon the dose of stem cells administered. The duration of cytopenia with Bu should therefore be shorter. Bu given in myeloma patients with renal failure has proven to be effective [34]. The initial proposed dose level in our study is 3.2 mg/kg of body weight over 4 h × 2 days. The next levels will be 3.2 mg/kg of body weight over

6 h × 3 days, 3.2 mg/kg of body weight over 6 h × 4 days, 4.3 mg/kg of body weight over 6 h × 3 days, 5.6 mg/kg of body weight over 6 h × 2 days and 6.4 mg/kg of body weight over 6 h × 2 days. Three patients per dose level will be entered.

Supportive Care

Plasma Exchange

Although there is ancetodal evidence of success with plasmapheresis in myeloma patients with cast nephropathy, in general this intervention is unsuccessful in reversing renal failure [31].

Dialysis Considerations

Peritoneal Dialysis: Peritoneal dialysis gives patients more flexibility with their daily activities, but it has its own problems during high dose chemotherapy treatments. Gastro-intestinal side effects from melphalan (diarrhea, upper-lower GI mucositis) during the periods of neutropenia would make the performance of peritoneal dialysis a challenge during that time. Moreover, after stem cell transplantation, myeloma patients are very catabolic and usually require daily hemodialysis to deal with these toxicities. Therefore, patients on peritoneal dialysis may benefit from a transient switch to hemodialysis until the gastro-intestinal toxicities and risk of infection is resolved after high-dose therapy. Furthermore, if a patient on peritoneal dialysis experiences severe gastro-intestinal problems, the peritoneal reaction to infection/inflammation may transiently or permanently impair the exchange properties of the peritoneal membrane.

Hemodialysis: Careful control of fluid status is important particularly during the high-dose chemotherapy period (peri-transplant). Hemodialysis should not be performed during the first 6 h after stem cell transplantation not to endanger engraftment. One challenge is the DMSO, a cryopreservative for the stem cells product. The DMSO can cause side effects that are increased in patients with renal insufficiency. When a patient will receive more than 2 stem cell bags, it is better to administer the stem cell infusion over two days.

Renal Transplantation

If patients remain dialysis-dependent after autotransplantation, we recommend to delay considering a renal transplant until at least 3 years after the first transplant. The majority of myeloma relapses occur during that period and a myeloma relapse will almost certainly destroy the function of the transplanted kidney.

Coagulation Considerations

Patients with nephrotic proteinuria have hypercoagulability based on antithrombin III urinary loss. Patients with amyloidosis acquire factor X deficiency from adsorption of factor X by the amyloid deposits.

Conclusion

Myeloma is an important cause of renal insufficiency which frequently can be reversed with early appropriate therapy. For that reason an expedited diagnostic phase is necessary. Hypercalcemia, dehydration and medication side effects need to be promptly treated to define the contribution of the myeloma to renal dysfunction. A renal biopsy is a very useful diagnostic and prognostic procedure. The best treatment for the renal manifestations of myeloma is to treat the underlying malignant clone and eliminate the nephrotoxic monoclonal protein production. Our initial treatment approach is to start with dexamethasone therapy 40 mg for days 1–4, 9–12 and 17–20 and closely follow free light chain concentration in the blood and creatinine levels. If by day +12, there is no reduction in free light chain concentration in the blood, it is unlikely that patient will respond to corticosteroids. In that case, corticosteroids should be discontinued and patient should be started on combination chemotherapy with DT-PACE without cisplatin (dexamethasone, thalidomide, adriamycin, cyclophosphamide and etoposide) followed by stem cell collection. Patients with renal failure should not be excluded from autotransplant protocols, but such transplants should only be performed in centers with experience in treating such patients. A sizable fraction of patients will show a clear improvement in renal function and approximately 25% will become dialysis independent. While patients with persistent renal failure need to receive reduced doses of melphalan, those with clearly improved renal function may be able to receive full dose melphalan, which is more effective. We usually recommend one cycle of consolidation therapy with DT-PACE without cisplatin, followed by 2 year of maintenance therapy with thalidomide and dexamethasone. During the period of intensive therapy, hemodialysis is preferred. Once maintenance therapy starts, patients should consider peritoneal dialysis. We would not recommend proceeding with a renal transplant until patient has been in remission for at least 3 years after the first transplant. Bisphosphonate therapy should be given but adjusted for the degree of renal failure and always in conjunction with vitamin D and calcium supplementation. Such an approach as outlined above has clearly improved outcome of renal failure patients, who had a median survival of 14 months with conventional chemotherapy [35].

With the arrival of the newer drugs such as revlimid and bortezomib, as well as the many drugs now being tested in Phases I and II trials, further improvement in outcome of such patients appears likely.

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Current and Emerging Views and Treatments of Systemic Immunoglobulin Light-Chain (AL) Amyloidosis

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Abstract

Amyloidosis is a disease in which abnormal proteins form toxic intermediates and fibrillar tissue-deposits that compromise key viscera and lead to early death. In order to treat amyloidosis, the type of abnormal protein must be identified. The most common type is monoclonal immunoglobulin light chain or AL amyloidosis. One-third to one-half of patients with systemic AL amyloidosis has renal involvement in the form of glomerular, vascular and interstitial deposits of amyloid causing progressive proteinuria. Less than 5% of AL patients present with renal failure requiring dialysis; patients with renal involvement usually present with fatigue, peripheral edema, proteinuria and hypoalbuminemia. The aim of therapy in systemic AL amyloidosis is to reduce the amyloid-forming monoclonal light chains, measured with the serum free light chain assay, by suppressing the underlying plasma cell dyscrasia, while using supportive measures to sustain organ function. Amyloid deposits can be resorbed and organ function restored if the amyloid-forming precursor light chain is eliminated. The most effective treatment for systemic AL is risk-adapted melphalan with peripheral blood stem cell transplant; oral melphalan and dexamethasone is the most effective therapy for patients who are not stem cell transplant candidates although it carries a risk of myelodysplasia and leukemia. Novel therapies currently under study include thalidomide, bortezomib and lenalidomide. With therapy, a majority of patients can achieve long-term durable remissions with stabilization or recovery of organ function. The use of novel antibody-based approaches for imaging amyloid and possibly for accelerating removal of deposits is under active investigation.

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Amyloid is an abnormal insoluble extracellular fibrillar protein that resists protease digestion and has the pathognomonic characteristic of red-to-green

dichroism when stained with Congo red and viewed microscopically under polarized light [1–3]. Over 20 different proteins have the propensity to form amyloid fibrils and cause systemic amyloid disease; these 'fibril–precursor' proteins determine the type of amyloid that a patient has. All amyloid, no matter the type, has as its distinctive component linear non-branching haphazardly arranged protein fibrils about 7–10 nm in diameter [4]. The fibrils are the final products of a pathway that remains rudimentarily understood.

With few exceptions, the fibril–precursor proteins are made by normal cells. In some cases, such as β_2 microglobulin amyloid associated with hemodialysis or wild-type transthyretin amyloid associated with senile systemic amyloidosis, the fibril–precursor protein paradoxically is a normal protein. In the most common type of systemic amyloidosis, immunoglobulin (Ig) light-chain or AL amyloidosis, the fibril–precursor protein is the product of a clonal B-cell disorder, usually of the terminally differentiated clonal plasma cells of a monoclonal gammopathy. Therefore, in systemic AL amyloidosis, both the fibril–precursor protein (an aberrant Ig light chain) and the cells producing it (clonal plasma cells) are abnormal.

Systemic AL amyloidosis is a rare disorder with an incidence estimated to be 8 per million person-years. The median age at diagnosis is about 60 and the median survival of patients seen within 1 month of diagnosis is about a year without treatment. Prior to the use of autologous stem cell transplant (SCT), $<$5% of patients lived 10 years or more from diagnosis [5, 6]. Renal involvement is common in AL amyloidosis and contributes significantly to the morbidity of the disease. For example, of 445 patients with systemic AL amyloidosis reported by the Italian Amyloidosis Study Group, 53% had renal protein losses exceeding 3 g/day and 19% had serum creatinine levels $\geq$2 mg/dl [7]. Patients with renal involvement presented most frequently with fatigue and edema.

In this chapter, we discuss new views of AL amyloidosis with emphasis on various aspects of the Ig light chain fibril–precursor protein as toxic intermediate, renal-tropic protein and target of therapy, and focus on clinical aspects of renal involvement and on current and emerging ways to treat AL disease.

Amyloid Fibrils and Precursor Protein Toxic Intermediates

The steps of fibril formation have been inferred from the study of protein fragments derived from deposits and from in vitro studies [8]. The current view is that fibril–precursor monomers, usually comprised of N-terminal fragments of the whole precursor protein, bind each other hydrostatically into stable intermolecular stacks of β-sheets perpendicular to the fibril axis, forming a filament [9–11]. Four to six filaments then twist about each other like the strands of a rope to form the fibril. No matter the type, all amyloid fibrils share this structure,

a curious happenstance given the diverse native conformations of fibril–precursor proteins with respect to the presence or absence of β-strand domains or β-sheets. Minor components of the amyloid deposits include heparan sulphate proteoglycans, serum amyloid P (SAP) protein and apolipoprotein E. The roles of these minor components have not been fully elucidated but experimental evidence, including knock-out studies, indicate that SAP likely protects fibrils by impairing proteolysis [12]. Proteoglycans and apolipoprotein E polymorphic variants may provide important surface features or niches that enable fibril propagation [13].

How amyloid fibrils cause organ dysfunction is not well defined. Suggested mechanisms include mechanical disruption, oxidative stress and formation of aberrant ion channels [14–16]. How fibrils are metabolized and organs improve is no less mysterious. The liver, e.g., can tolerate a large burden of AL amyloid without failing and, once treatment eliminates the supply of fibril–precursor Ig light-chains, the liver can respond with restoration of normal synthetic function and dramatic regression to normal size. These changes can happen over a period of 6–12 month. This is attributed to the regenerative capacity of the liver but begs the question as to how the fibrils are removed.

In contrast, 1 year after elimination of the clonal Ig light-chains, only 20% of patients with cardiac amyloidosis show measurable reduction in left ventricular wall thickness [17]. Indeed, a patient's clinical status with AL is not simply determined by the amyloid burden; patients with peripheral nervous system or cardiac involvement can have minimal tissue burdens of amyloid but advanced symptomatology and organ-system failure. The use of I^{123}-labeled SAP to image amyloid tissue burden with total body scintiscans, although limited in availability, has revealed how labile amyloid deposits can be; however, a significant fraction of patients who experience elimination of the clonal Ig light-chains do not demonstrate reduction in amyloid burden despite clinical improvement and abatement of symptoms [18].

Response of the plasma cell disease after treatment in large measure determines amyloid organ response. Patients with renal amyloid and normal renal function prior to SCT who achieve a complete hematologic response (CR, immunofixation negative with normal serum free light chains [FLC]) post-SCT are after 1 or 2 years highly likely to experience a significant reduction in proteinuria and an increase in serum albumin (with no loss of renal function) (fig. 1) [19]. Similar patients who fail to achieve a CR post-SCT are likely to have stable proteinuria for a period of time but can progress to end-stage renal disease and dialysis-dependence in the 5 years post-SCT. How and why improvement in proteinuria occurs over 1–2 years in complete responders remains obscure; repeat biopsies of surrogate sites such as abdominal fat continue to show amyloid deposits. It is unlikely that glomerular amyloid deposits

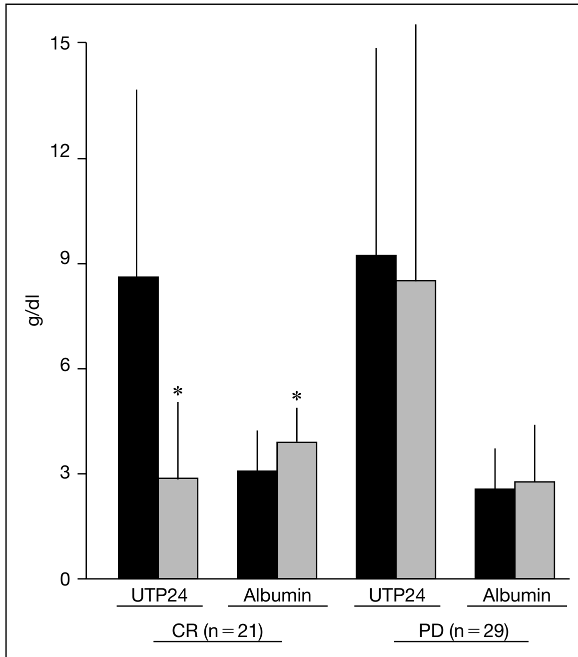

Fig. 1. This figure depicts the outcomes in 50 consecutive AL patients with dominant renal involvement (spilling > 1 g/day) who underwent autologous SCT as described in reference [19]. Twenty-one patients experienced a CR while 29 had persistent clonal disease (PD) at 1 year after SCT. The drops in UTP24 and increases in serum albumin were significant for patients with CR by two-tailed paired t-test (means ± standard deviations shown). There was no change in either measure for patients with PD (*$p < 0.05$). UTP24 = 24-hour total urinary protein.

are resorbed and therefore it is more likely that effective treatment controls the toxic effects of Ig light chains.

Such observations have led to the view that amyloid fibrils per se may not be the sole pathologic basis of amyloid disease [20]. Experiments in vitro with several fibril–precursor proteins have shown that intermediate forms or oligomeric aggregates may be more toxic to cells than fibrils, hence the term 'toxic intermediates.' Indeed fibrils may represent a less toxic form of the fibril–precursor protein and may provide relative protection [21]. The relationship between fibrils and intermediates in the various types of amyloidosis remains undefined and the role of intermediates in disease is unclear. However, if experimental systems can be developed to elucidate the mechanisms of toxicity, an opportunity to pursue novel protective therapies will likely be created.

Immunoglobulin Light Chains and the Kidneys

Immunoglobulin light chains are produced in slight excess by normal plasma cells and in significant excess at times by aberrant clonal plasma cells. In the absence of Ig light chain variable regions, Ig heavy chain variable regions tend to aggregate because they possess a large hydrophobic surface area that remains exposed without the cover of light chain partners. Hence, unassociated Ig heavy chains are usually retained intracellularly and degraded while unassociated Ig light chains usually fold more adeptly and attain secretory competence.

Ig light chains circulate in normal individuals and can be measured with the recently available serum FLC assay [22]. Over 95% of patients with systemic AL amyloidosis have abnormal levels of serum FLC, and the abnormally elevated FLC usually is the fibril–precursor protein. The pathologic Ig light chain level in AL is probably proportional to the size of the abnormal plasma cell clone; however, there is likely inter-patient variability in Ig light chain production per clonal plasma cell that has not been well-described or explained. In addition, the AL disease process has a significant though difficult to measure claim on the elimination kinetics of serum FLC. In normal individuals without renal insufficiency, serum free κ light chain levels are lower than λ. In normal bone marrow and the normal expressed repertoire of immunoglobulins, the κ-to-λ ratio is 3:2. The κ light chain is usually a monomer of ~25 kDa while the λ light chain is usually a dimer of ~50 kDa and is cleared more slowly by the kidneys [23]. The slower clearance of λ light chains likely explains the higher free λ levels observed in normals, but is not thought to play a major role in systemic AL amyloidosis.

The serum FLC assay has significantly changed the way that patients with AL amyloidosis are diagnosed and monitored during therapy. The FLC assay is quantitative, unlike immunofixation, and appears more sensitive to the presence of clonal disease than immunofixation in some cases [18, 24]. A series of FLC values in newly diagnosed patients with amyloidosis is depicted in figure 2a. The FLC can help to distinguish hereditary from AL amyloid and localized from systemic AL, although FLC values are minimally elevated in about one-quarter of patients who have localized AL disease. In figure 2b, the proteinuria and serum FLC levels of an AL patient during treatment are shown, illustrating how renal response follows FLC response.

It is important to note that rare patients with amyloid can have two possible sources of fibril–precursor proteins, a monoclonal gammopathy, including elevated serum FLC, and an hereditary amyloid protein [25, 26]. At minimum, African-Americans with amyloid and patients presenting with peripheral neuropathy should be checked for the presence of both possible sources and referred to specialized centers for tissue-typing studies if both precursor proteins are identified.

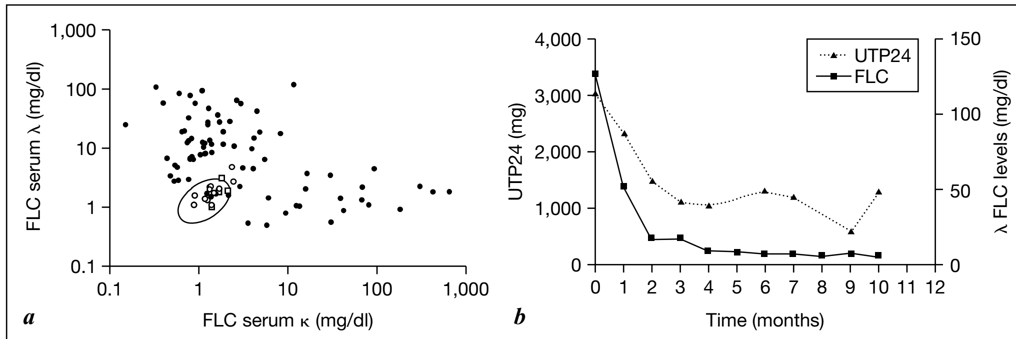

Fig. 2. ***a*** shows the serum FLC levels with axes in $\log_{10}$ format for 110 newly diagnosed patients with amyloidosis seen at Memorial Sloan-Kettering from 2002 to 2004. The solid circles (n = 92) are systemic Ig light chain (AL) patients, open circles are localized AL patients (n = 11) and open squares are hereditary (ATTR, n = 6) patients. The oval near the origin is the normal range for the ratio of κ-to-λ. ***b*** shows the UTP24 and λ FLC levels for a 73-year-old man with renal, cardiac and autonomic nervous system involvement who received monthly oral melphalan and dexamethasone with a response. Over the 10 months of therapy the brain natriuretic peptide level also decreased from 3,280 to 1,670 pg/ml. The graph illustrates how renal response follows FLC response. UTP24 = 24-Hour total urinary protein.

Because of their small size, Ig light chains are filtered by the glomeruli, and resorbed and metabolized in the proximal tubules by a receptor-mediated process. In states of light-chain overproduction, light chains appear in the urine because tubular reabsorption is overwhelmed. In light-chain myeloma, proteinuria is selective and predominantly Ig light chains. In AL amyloidosis, proteinuria is non-selective, and the dominant protein, albumin, is bound to fatty acids. Progression of proteinuria in AL portends end-stage renal disease and may both indicate and accelerate worsening renal function. Ig light chains can aggregate in tubules causing cast nephropathy or can damage glomeruli and tubules by depositing amorphously or as fibrils, causing glomerulosclerosis and tubulointerstitial scarring. In AL amyloidosis, the pattern of fibril deposition is usually glomerular, interstitial and vascular. Tubular deposition patterns, though rare, do occur and are usually associated with rapidly progressive renal insufficiency with minimal proteinuria. Why some light chains cause AL renal involvement and others do not is not known. Indeed, the organ tropism of AL amyloidosis remains poorly understood.

We hypothesized that immunoglobulin germline gene use played a role in organ tropism and several years ago tested the hypothesis that the light chain variable region (Ig V_L) germline genes used by AL clones influenced organ

tropism. The clonal Ig V_L genes of patients with B cell disorders can be amplified, sequenced, assigned germline gene donors and assessed for homology to the germline sequences. This type of analysis allows one to show whether or not the germline genes used in a particular B cell disorder vary preferentially from the normal repertoire.

To test our hypothesis regarding germline gene use and organ tropism, we amplified, cloned and sequenced the clonal Ig light chain genes from 60 AL patients [27]. To assess the renal effects of some light chains, we used an in vitro renal mesangial cell model of amyloid formation [28, 29]. In the Vλ cases, there was preferential germline gene utilization. The 1c gene was used in 8 of 15 VλI cases, 2a2 in 6 of 7 VλII cases, 3r in 7 of 8 VλIII cases, and the 6a gene in all 18 VλVI cases. In the normal expressed repertoire, 7–8% of λ light chains are derived from 1c, 20–35% from 2a2, 7–8% from 3r, and <5% from 6a [30]. Light chains of the rare VλVI subtype have been found frequently in AL [31]. In the Vκ cases, there was also preferential utilization. The relatively rare LFVK431 germline gene was used in 4 cases and the more common O18–O8 gene in 7. All of the Vκ genes used by the clones in this series were members of the VκI subtype. In normal usage, genes of the VκIII subtype dominate [32].

With respect to organ tropism, we learned that the 1c, 2a2 and 3r germline genes were associated with dominant cardiac and multisystem disease (10 of 21 patients), while the 6a gene was associated with dominant renal disease (16 of 18 patients). Indeed, the association between the 6a donor and dominant renal involvement was striking and a comparison of the frequency of dominant renal involvement in 6a patients versus all others was highly significant ($p < 0.01$, $\chi^2 = 12.61$, d.f. = 1, relative risk = 2.5, 95% CI = 1.56–4.02). Furthermore, in the in vitro model, unlike all other light chains, 6a light chains formed amyloid rapidly both with and without amyloid-enhancing factor. These observations regarding the renal tropism of 6a light chains, though striking, remain undeveloped. The mechanisms and pathways involved in the renal-tropic toxicity of 6a light chains are not yet known.

Renal Involvement in AL Disease

In general, one-third to one-half of AL patients will have renal involvement at diagnosis, often presenting with nephrotic syndrome without hypertension and findings of peripheral edema, frothy urine, low serum albumin and elevated cholesterol. Hypotension may also be a presenting sign and may be due to hypoalbuminemia and plasma volume depletion, reduced cardiac output due to severe diastolic dysfunction or autonomic neuropathy. Many patients with renal involvement are diagnosed after proteinuria is serendipitously revealed;

a minority will have daily proteinuria of 10 g or more. AL amyloidosis infrequently presents as renal failure.

Typically the finding of excess proteinuria leads to renal biopsy, securing the diagnosis; amyloidosis is found in about 3% of renal biopsies. Anasarca and massive proteinuria with hypoalbuminemia but normal serum creatinine and blood urea nitrogen may occur, although evidence of mild renal dysfunction is most commonly found. In patients with malignant nephrosis, the use of diuretics and albumin may be poorly tolerated and bilateral embolization of the renal arteries may be considered; such cases are rare. The presence of pleural and pericardial effusions can give the picture of heart failure. Patients are salt-avid but may be volume-depleted, causing nausea and vomiting and complicating attempts at diuresis. Volume-depletion can also cause orthostasis mimicking autonomic neuropathy.

In AL, the urine sediment is usually unremarkable, containing variable numbers of red blood cells and fatty droplets. Patients have kidneys that by ultrasound are normal in size but usually demonstrate echo findings consistent with medical renal disease. Rarely, patients may have both amyloid and light-chain nephropathy or light-chain deposition disease on renal biopsy. Continuous proteinuria leads to worsening renal function and eventually to end-stage renal disease requiring dialysis. Historical patient series indicate that the median time to dialysis-dependence for AL patients presenting with nephrotic-range proteinuria is about 15 months, and the major determinant of survival is the presence or absence of amyloid involvement of the heart [33, 34].

Current Treatments

For patients with systemic AL amyloidosis, the FLC assay provides a direct measure of the fibril–precursor protein, whether the aberrant B cell factory for FLC production is a plasma cell dyscrasia or a non-Hodgkin lymphoma [35]. A major problem in treating AL patients has been gauging response and titrating therapy, since the response of amyloid organ disease lags behind the reduction in the fibril–precursor protein. This is a critical clinical point. Therefore, integration of the FLC into clinical practice allows the titration of therapy to determine when the optimal response of the clonal disease has been attained. This is usually before an organ response and clinical improvement may be evident. Stable declines in the FLC of >50% from baseline values have been shown to be associated with prolonged survival in systemic AL patients after treatment [36].

Oral melphalan and prednisone were studied in phase III trials and increased overall survival from 12 to 18 months [37, 38]. Responses (>50% reductions) occurred in 25% of patients with a median time to response of

12 months; renal disease improved in 17%. For patients who survived more than 3.5 years after receiving oral melphalan, there was a high risk of myelodysplasia often leading to secondary leukemia [39]. The Southwestern Oncology Group (SWOG) conducted a multi-center phase II trial testing pulse dexamethasone, followed by maintenance therapy with dexamethasone and α interferon, in patients with AL [40]. Responses were seen in 53% of evaluable patients with complete responses (CR) in 24%. Organ responses occurred in 45% of patients. Median survival of the entire cohort was 31 months, with estimated 2-year overall and event-free survivals of 60 and 52%.

Recently, the Italian Amyloidosis Center completed a phase II trial combining oral melphalan with dexamethasone in patients with AL not eligible for SCT [41]. This combination led to a response in 67% of patients with a 33% CR rate and a median survival that had not been reached with nearly 4 years of follow-up (G. Merlini; personal communication). In 48% of the responsive patients organ responses were observed. FLC measurements were not available. There were 2 treatment-related deaths in the first 100 days of therapy and 2 patients also subsequently developed myelodysplasia.

Oral melphalan and dexamethasone are clearly an active regimen based on these data, are easily administered and can be considered equivalent in some respects to melphalan-based SCT except for the risk of myelodysplasia and secondary leukemia. Time to response is an important variable in treating systemic AL amyloidosis. The combination of melphalan and dexamethasone appears significantly more active than melphalan and prednisone with a time to response of 3 months.

The effectiveness of high-dose melphalan with autologous SCT in reversing the clinical manifestations of AL in most surviving patients has been documented at numerous centers [42–44]. As the production of amyloid stops, the quality of life of AL patients improves [45]. Objective criteria for assessing organ responses have recently been defined [46]. In the largest series of patients reported, the median overall survival post-SCT was 4.6 years with 47% survival at 5 years [47]. For patients without cardiac involvement at SCT, the median survival was 6.4 years with 60% 5-year survival, while for those with cardiac involvement at SCT the median survival was 1.6 years with 29% 5-year survival. Treatment-related mortality was 19%, including toxicities of stem cell mobilization. Forty-four percent of evaluable patients achieved organ responses at 1-year post-SCT. Complete hematologic responses occurred in 40% of patients surviving at 1 year post-SCT and were associated with an 82% 5-year survival compared to 55% for those not achieving CR. Controversial aspects of this single-center experience include the high treatment-related mortality and the fact that many patients at the time of SCT were more than 1 year from diagnosis and had prior treatments.

Renal Transplantation

The use of solid organ transplantation (liver, heart and kidney) in patients with AL amyloidosis is frequently deemed inappropriate because of the likely accumulation of amyloid in the grafted organ. Liver transplant, in contrast, is a therapy of choice for hereditary amyloidosis and has even led to the use of 'domino' grafts in which the otherwise normal mutant-TTR producing liver is used as an allograft [48]. This situation is slowly changing. In the past decade a number of AL patients have successfully undergone cardiac allograft and then SCT; the feasibility of this approach is established and a phase II trial needs to be performed to demonstrate safety and efficacy in a systematic fashion [49]. Renal transplantation has also been shown to be effective, and renal allografts survive for lengthy periods in many recipients especially after SCT [50]. Renal failure requiring dialysis develops in about a third of patients after 2 years despite standard oral therapy but only in about 10% of patients after dose-intensive therapy with stem cell support.

Emerging Therapies

New agents with novel mechanisms of action are being evaluated for the treatment of multiple myeloma and other plasma cell dyscrasias. These include the immunomodulators thalidomide and lenalidomide as well the proteasome-inhibitor bortezomib. These agents and novel monoclonal antibody approaches to amyloidosis are entering clinical trials. Phase I/II clinical trials testing the single-agent activity of lenalidomide and bortezomib are on-going at this time; preliminary data indicate that both have activity.

Two small phase I/II clinical trials have been conducted testing the toxicity and efficacy of thalidomide in AL amyloidosis. Both demonstrated that thalidomide had significant toxicity and minimal activity [51, 52]. Two other phase II trials, however, tested the combination of thalidomide and dexamethasone as therapy for AL. In one, the combination was used as salvage therapy, while in the other the combination was used as adjuvant therapy after melphalan-based SCT. The results of these studies indicate that thalidomide and dexamethasone should likely be regarded as first-line salvage therapy at this time pending the results of the lenalidomide and bortezomib trials.

In the Italian Amyloidosis Center's phase II trial of thalidomide and dexamethasone combined as salvage therapy [53], thalidomide was dosed at 100 mg/day with increments up to 400 mg and dexamethasone at 20 mg/day on days 1–4 every 3 weeks. Thirty-one patients with AL refractory to or in relapse after first-line therapy were enrolled. Eleven patients (35%) tolerated 400 mg/day of thalidomide for

a median of 6 months; 14 could take no more than 100 or 200 mg/day for a median of 3 months. Fifteen (48%) achieved a hematologic response with 19% complete remissions and 26% organ responses; the response rate was higher in those taking higher doses of thalidomide. Overall median time to response was 3.6 months. There were no treatment-related deaths but two-thirds experienced severe toxicity. Fluid retention and symptomatic bradycardia without QT prolongation were common adverse reactions while neuropathic and thromboembolic complications were rare.

At Memorial Sloan-Kettering Cancer Center, we recently conducted a phase II clinical trial for newly diagnosed AL patients eligible for autologous SCT, combining melphalan-based SCT with the use of thalidomide and dexamethasone. To be eligible for autologous SCT, patients had to have only 1 or 2 major organs significantly involved and could have cardiac involvement that was not advanced. After SCT, patients with persistent plasma cell disease received thalidomide and dexamethasone as adjuvant therapy [54]. Our goals were low treatment-related mortality and optimal hematologic and organ response rates. To be eligible, patients had to be untreated and diagnosed within 12 months of enrollment.

Patients received autologous SCT with melphalan dosed at 200, 140 or 100 mg/M^2 based on age, renal function, and cardiac involvement. Those not achieving a CR at 3 months post-SCT were treated with 9 months of thalidomide (50–200 mg nightly) and dexamethasone (20 mg/M^2, 1–3 pulses monthly), or with only dexamethasone if they had prior deep venous thrombosis or neuropathy. Aspirin was used for prophylaxis against thromboembolic complications.

Forty-five untreated patients (23 men) enrolled, a median of 57 years old (range = 34–73) and 2 months from diagnosis. Over two-thirds had renal involvement (31/45) with median proteinuria 5.95 g/day (range = 0.51–22.1 g/day) compared to 0.184 g/day (range = 0–0.475 g/day) for those without renal involvement. Two-thirds of those with renal involvement (21/31) had been diagnosed with renal biopsy, the remainder with surrogate site or other involved-organ biopsies. Two patients presented at diagnosis with creatinine clearance $<$20 ml/min, one requiring hemodialysis and the other soon to require it, making the incidence of systemic AL amyloidosis presenting as renal failure in this cohort 4.4% (2/45).

Dominant organ involvement in these 45 patients was renal in 58% (n = 26), cardiac in 24% (n = 11), and liver/GI or peripheral nervous system in 18% (n = 8). A third (n = 15) had 2 organ systems involved. At baseline 53% had elevated serum brain natriuretic peptide levels, suggestive of cardiac involvement. Dose-assignments were 200 (n = 15), 140 (n = 24) and 100 mg/m^2 (n = 6) of melphalan. Treatment-related mortality was 4.4% (2/45). At 3 months post-SCT, persistent clonal disease was found in 34 patients; 1 refused and 2 were too ill for

adjuvant therapy. Twenty-two patients received thalidomide and dexamethasone, and 9 received dexamethasone alone. With adjuvant therapy 48% had an improved response at 12 months including 6 who achieved CR. There were no treatment-related deaths in the adjuvant phase and toxicities were manageable. The hematologic response rate at 12 months was 77% (38% CR) with no significant difference based on the dose of melphalan. Fifty-two percent had organ responses at 12 months, while 29% had stable and 19% worsened organ function. With a median follow-up of 20 months, overall survival is 76% and median survival is not yet reached.

In the 31 patients with renal involvement the hematologic response rate at 1 year was 77% with 45% complete responders; 71% (22/31) received adjuvant therapy post-SCT. At 2 years post-SCT, 45% of the patients (14/31) had improvement in renal disease defined as a >50% reduction in proteinuria with no loss of renal function, while 29% (9/31) had stable renal disease. In the 14 patients with renal responses, mean (± standard deviation) 24-h proteinuria declined from baseline 7,270 ± 6,582 to 1,993 ± 1,759 mg ($p < 0.01$ by paired t-test). All but one of those with a renal response had a hematologic response as well. Two patients required hemodialysis shortly after diagnosis (serum creatinine >7.0 mg/dl in both cases) and beginning treatment, as noted earlier; one successfully received a renal allograft from a spouse donor 1 year post-SCT. An additional 2 patients experienced toxicities of SCT (veno-occlusive disease, sepsis) that led to dialysis-dependence. Overall survival for these 31 patients at 2 years post-SCT is 87% (27/31); three patients died of progressive amyloidosis and one died of an unrelated illness.

These results promise improved outcomes as novel agents are incorporated into the platform provided by melphalan-based therapy; indeed, lenalidomide or bortezomib combined with dexamethasone may challenge melphalan-based approaches as initial therapy for AL. Systematic multi-center clinical trials will be needed to define the optimal roles of emerging treatments for AL.

Monoclonal antibody therapy has had significant impact on the treatment of non-Hodgkin lymphoma, and breast and lung cancers. The development of amyloid-reactive antibodies was based on the premise that an immune reaction to amyloid deposits might help to stimulate fibril disassembly [55, 56]. Amyloid tumors formed in the skin of healthy mice resolved within several weeks after the generation of anti-amyloid antibodies that recognized antigenic determinants on AL amyloid fibrils. 'Amyloidolysis' occurred in treated but not untreated animals. The antibody localized within the amyloid tumors and caused rapid resorbtion of this material by neutrophils. The manufacture of a chimeric antibody is currently complete and clinical trials are beginning in the near future. One of the first applications will be imaging amyloid deposits by PET/CT with I^{124}-labeled antibody, a collaborative clinical trial undertaken by

investigators at the University of Tennessee and Memorial Sloan-Kettering Cancer Center.

Active research is underway at several centers to find unique plasma cell antigens that can provide targets on the surface of the aberrant clonal plasma cells that cause AL and other disorders such as multiple myeloma. The availability of effective cytolytic antibody therapy would likely result in a significant improvement in survival for AL patients, possibly even for those with multiorgan failure whose clinical status would preclude current therapies because of toxicity.

Conclusions

Our views of systemic AL amyloidosis and our approaches to its treatment are rapidly evolving. The serum FLC assay has made diagnosis and treatment more focused since it provides a measure of the fibril–precursor protein. The growth of autologous SCT and the use of novel agents to treat AL has created further incentives for physicians to diagnose the disease earlier in its course. A majority of patients diagnosed today can be helped significantly with approaches designed to eliminate the supply of fibril–precursor Ig light chains. Ideally, patients with AL should be treated on clinical trials whenever possible. Hopefully, in the near future novel imaging and monoclonal antibody approaches will be available for evaluation, and in the not-too-distant future rationally designed cytoprotective agents will also be in clinical trials as well.

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Erratum

In Contributions to Nephrology, 2006, vol. 150, pp 70–76, the name of one of the authors was incorrectly spelled. An DeVriese should be An De Vriese.

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