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REVIEW

Immunological Role of IgG Subclasses

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ABSTRACT

The loss of tolerance to self-antigens is the unequivocal "red line" of autoimmunity: both development of autoreactive T and B cells and production of polyclonal autoantibodies represent seminal keys to the pathogenesis of protean autoimmune diseases. Most of these autoantibodies are immunoglobulins G (IgG), functionally distinguished in four subclasses named IgG1, IgG2, IgG3, and IgG4, due to structural differences in the hinge and heavy chain constant regions. Different studies analyzed serum levels of IgG subclasses in the course of different disorders, showing that they might have a pathogenic role by regulating interactions among immunoglobulins, Fc-gamma receptors, and complement. To date, the mechanisms promoting different IgG subclasses distribution during the natural history of most autoimmune diseases remain somewhat unclear. Evidence from the medical literature shows that the serum IgG profile is peculiar for many autoimmune diseases, suggesting that different subclasses could be specific for the underlying driving autoantigens. A better knowledge of IgG subsets may probably help to elucidate their pathological task, but also to define their relevance for diagnostic purposes, patients' personalized management, and prognosis assessment.

KEYWORDS

Immunoglobulin G; immunoglobulin G subclasses; autoimmune diseases; immunodeficiency; biomarkers; personalized medicine

Introduction

Autoimmune diseases result from an impaired balance between pathogen recognition and self-tolerance, which can be initiated by environmental stimuli working in genetically susceptible individuals, and the development of autoreactive T and B cells with production of polyclonal autoantibodies (autoAbs) represents the main phenomenon observed in most of these disorders (Lux and Nimmerjahn 2013). Antibody-mediated autoimmunity can be related to immunoglobulins (Ig) binding to the autoantigen and interference with one or more biological functions in the absence of inflammation or tissue damage (as in Graves' disease). Otherwise, when the autoAbs opsonize cells, activate phagocytosis or

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complement cascade with subsequent direct tissue damage leading to overt inflammation (as in immune-mediated glomerulonephritis).

IgG act by binding Fcγ receptors (FcγR) on target cells and/or activating the complement system (Kapur et al. 2014). In humans, IgG are the predominant antibody class (7–15 g/L), and the four IgG subclasses, named IgG1, IgG2, IgG3, and IgG4, functionally distinct because of different heavy chain genes, differ in their ability to fix complement (IgG3> IgG1> IgG2> IgG4) and bind Fc receptors (Engelhart et al. 2017) Figure 1(a,b). The biological activities of each subclass of IgG are not completely understood. IgG receptors are surprisingly abundant in humans, and they comprise high- and low-affinity ones (Sigal 2012; Vidarsson et al. 2014). The genes of IgG subclass constant regions are positioned in the order of IgG3, IgG1, IgG2, and IgG4 in the human IgH region (Lowe et al. 2013; Tan et al. 2015).

Human antibodies provide a wide protection against pathogens and infectious diseases, and each class displays a prominent role, more specific for eradicating viruses or bacteria, and/

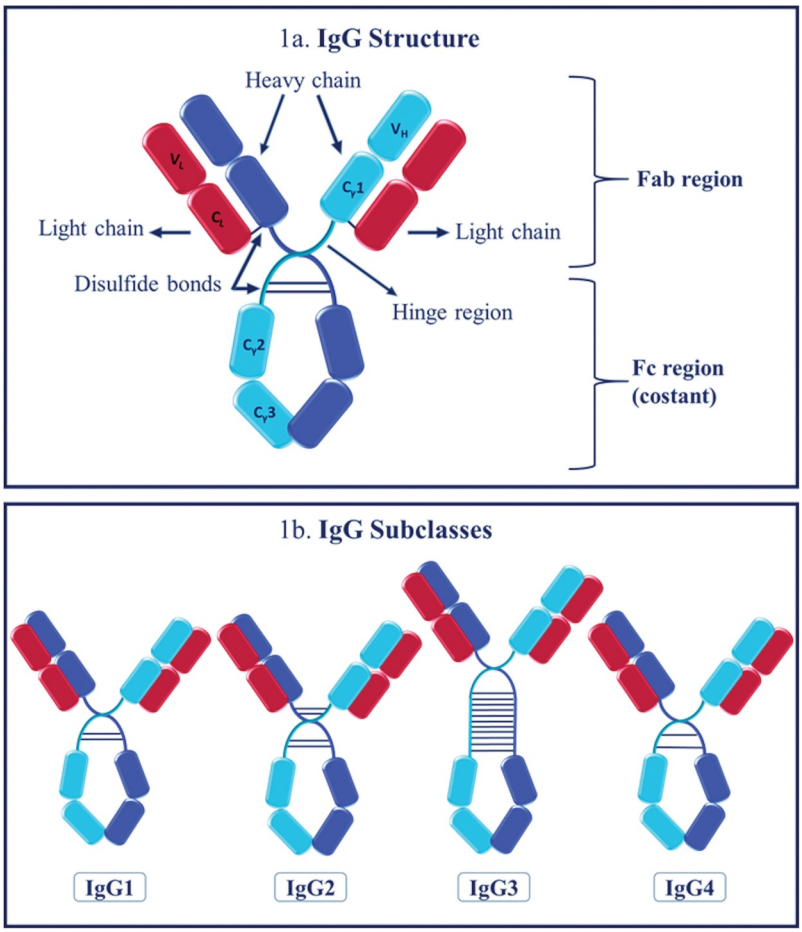

Figure 1. Structure of IgG and of different IgG subclasses. a. **IgG structure** The different domains of Fab and Fc regions of IgG are indicated. The constant (C) and variable (V) domains are also shown. b. **IgG subclasses** Structural variation of the different four subtypes of IgG.

or for the recruitment and activation of additional cells of the immune system (Horns et al. 2016). Most antibodies are produced through a single dominant pathway that firstly involves the switching of antibody class, while IgG subclass switching occurs through differential temporal pathways that are driven by cytokines and epigenetic factors (Horns et al. 2016).

The four IgG subclasses are named in a descending order of amount: IgG1 is the most prevalent subclass, up to 60–70% of the total IgG, followed by IgG2 (20–30%), IgG3 (5–8%), and IgG4 that is the least frequent subclass (only 5%) (Schur 1987). Although IgG subclasses share more than 90% homology in the amino acid sequence, each of them has a specific profile with respect to antigen binding, immune complex formation, complement activation, effector cell triggering, half-life, and placental transport. These differences cause functional consequences of great significance (Sigal 2012). IgG1 and IgG3 are strong mediators of both FcγR and complement-mediated functions, and are the predominant subclasses involved in the response to protein antigens. IgG2 is a weak mediator of FcγR and complement-mediated functions and is involved in the response to polysaccharide antigens. IgG4 has a minimal ability to activate effector cells or fix complement. The circulating half-life of IgG1, IgG2, and IgG4 is about 22 days, because of recycling from pinocytic vesicles via binding to the neonatal Ig receptor. IgG3, which does not bind to receptor, has a half-life of 7 days (Roopenian and Akilesh 2007). All subclasses spread into the extravascular space through escape into the vascular system. Different methods and assays (by employment of polyclonal or monoclonal antibodies) have been developed for detection of Ig subclasses, such as radial immunodiffusion, radioimmunoassay, particle concentration fluorescence immunoassay, nephelometry, but the major problem is that their determination is "method"-dependent. Currently, turbidimetry and nephelometry are the most widely used methods for measuring IgG subclasses (Ludwing-Kraus et al. 2017). Serum protein concentration, including Ig and immune complexes, is determined as a measure of cloudiness of the sample solution through the transmission (turbidimetry) and scattering (nephelometry) of the light (Eleftherios and Christopoulos 1996). Moreover, it is unclear if the normal serum cut-off values for each IgG subclass vary in different populations. Despite technological advances, there are still only two assays available on the worldwide market, and limited knowledge about their performance with the literature reporting little and conflicting data in regards to reproducibility and harmonization of results between methods (Liu et al. 2018; Schauer et al. 2003). Differences occurring in IgG subclass measurement between two providers (The Binding Site, Birmingham UK; Siemens Healthineers, Munich, Germany) may be due to different calibration and reference materials employed in these assays (Wilson et al. 2013). The comparison of IgG subclass measurements by different methods has not been reached due to the lack of standardization of IgG subclass tests (Cho et al. 2018; Ludwig et al. 2017).

Total IgG and IgG subclasses can be also quantified by mass spectrometry-based assays, with a performance comparable to nephelometry (Ladwig et al. 2014). In this way, the inaccuracy due to the cross-reactivity of antibody reagents, as recently reported for nephelometry, can be overcome (Van der Gugten et al. 2018). Despite the potential advantages, this method still needs to be calibrated, and thus suffers from the lack of a standardized reference for IgG subclasses (Bernasconi et al. 2019). Different reports analyzed serum levels of IgG subclasses in the course of autoimmune diseases, underlying how these subclasses could contribute to pathogenesis of such disorders by regulating Ig, FcγR, and complement interactions. However, to date, the different alterations (increased or decreased levels) of

IgG subclasses in various autoimmune diseases suggest that they might be specific for the underlying driving autoantigens and that might have a pathogenic role (Zhang et al. 2015).

Subclasses of igG in the autoimmune diseases

A strikingly different serum IgG subclass distribution was detected in patients with autoimmune diseases compared with healthy controls (Zhang et al. 2015). The knowledge of IgG subclass distribution might help to understand their pathological contribution, establish diagnosis or disease prognosis and define management of the individual patient. In an immune response, IgG profile depends on the type of antigen and duration of antigen exposure, a concept known as "subclass restriction" (Engelhart et al. 2017). Moreover, it could reflect and be specific of the underlying driving autoantigen. Serum levels of IgG subclasses do not correlate with the amount of antibody deposition in tissues, suggesting that we cannot hypothesize a direct relationship between the elevated IgG subclass that we observe and the disease process (Engelhart et al. 2017).

Considering the relevance that IgG subclass profile may play in the human immune system, this has been largely evaluated in patients with monoclonal gammopathies of undetermined significance, finding that IgG1 levels are often increased, while IgG3 as well as IgG4 are decreased. Conversely, in patients with multiple myeloma IgG1 is significantly increased compared to IgG2, IgG3, and IgG4 (Dolscheid-Pommerich et al. 2015). It remains unclear whether a potentially specific distribution pattern of Ig subclasses plays a pathophysiological role in the course of monoclonal gammopathies (Table 1.)

Table 1. Significant features of IgG subclasses in relationship with their most prominent involvement in autoimmune diseases.

Subclass	Significant features	Diseases involvement	Reference
IgG1	✓ The most abundant IgG subclass	MGUS and MM	Dolscheid-Pommerich, 2015
	✓ Principal IgG to cross the placenta	pSS	Zhang, 2015
	✓ Deficiency detectable as hypogammaglobulinemia	SARD	Gulli, 2020
		HIV infection	Banerjee, 2010; Kadelka, 2018
IgG2	✓ Defense against enveloped bacteria	PBC	Zhang, 2015
	✓ Humoral response against polysaccharide antigens	HT	Xie, 2008
	✓ Deficiency: weak antibody responses to polysaccharide antigens	IBS	Engelhart, 2017; Hussain, 1997
IgG3	✓ Potent pro-inflammatory effect	PBC	Zhang, 2015
	✓ Appear first during the course of infections	HCV-related MC	Gulli, 2016; Gulli, 2018; Basile, 2017
IgG4	✓ Produced in response to chronic exposure to antigens in non-infectious setting	Pancreatic-cholangiopathy	McAlister, 2019
		IgG4-RD	Hamano, 2001; Hamano, 2002
		RA	Chen, 2014
		MuSK-MG	McConville, 2004; Niks, 2008

MGUS: monoclonal gammopathy of undetermined significance; MM: multiple myeloma; pSS: primary Sjögren syndrome; SARD: systemic autoimmune rheumatic diseases; HIV: Human immunodeficiency virus; PBC: primary biliary cholangitis; HT: Hashimoto's thyroiditis; IBS: irritable bowel syndrome; HCV: hepatitis C virus; MC: mixed cryoglobulinemia; IgG4-RD: IgG4-related disease; RA: rheumatoid arthritis; MuSK-MG: myasthenia gravis with anti -muscle-specific tyrosine kinase antibody

IgG1

IgG1 is the most abundant subclass and the main IgG to cross the placenta. In fact, neonatal serum levels are similar to the maternal ones, though they drop over few months following birth (Burtis et al. 2012). One of the most relevant cause of hypogammaglobulinemia can be IgG1 deficiency, due to its higher level respect to other subclasses. IgG1 deficiency is associated with increased susceptibility to recurrent infections, and respiratory tract infection is the most common manifestation of undiagnosed IgG subclass deficiency. The diagnosis of IgG deficiency is made, on average, after 7 years of frequent or severe respiratory tract infections. Adults may be diagnosed to have IgG deficiency after longer intervals of infections than observed in younger patients (Barton et al. 2019).

Recent data show that deficiency of IgG1 (and IgG3) is associated with shorter overall survival and treatment-free survival in patients with chronic lymphocytic leukemia: immune dysfunction in chronic lymphocytic leukemia is not steady, and Ig levels are related to both disease duration and stage (Molica 1994). These observations indicate that patients with an advanced disease stage at the time of Ig assessment may have a higher incidence of both single and multiple Ig and IgG subclass deficiencies. Immune dysfunction correlates with disease severity, and can be considered as a predictor marker of treatment requirement. Monitoring Ig levels may be fruitful in these patients and provide a clue of disease progression (Crassini et al. 2018).

A significant reduction in serum IgG1 (and IgG3) levels has been also found in women with pregnancy-induced hypertension (pre-eclampsia) (Sarween et al. 2018). Considering the implication of immune mechanisms in the pathogenesis of pregnancy-induced hypertension, Sarween et al. evaluated markers of humoral immunity and their relationship with circulating markers of inflammation, angiogenic factors, and renal function. They observed changes in free light chains and IgG1 concentrations at the time of pregnancy-induced hypertension and appeared to be independent from other markers (Sarween et al. 2018). Moreover, on a multivariable analysis, IgG1 was found to be independently associated with pregnancy-induced hypertension (Sarween et al. 2018). The reduction of IgG levels could be related to proteinuria, reduced half-life as a result of lower systemic neonatal Fc receptor recycling, increased transport through the placenta to the fetus, or a combination of these factors. Besides, pregnancy-induced hypertension causes renal dysfunction and nephrotic syndrome. Although its large molecular weight, IgG can leak into the urine in course of severe nephrotic syndrome, resulting in hypogammaglobulinemia (Sarween et al. 2018).

As recently reported, the specific IgG subclass responses (mainly IgG1 and IgG3) induced by a recombinant protein-based malaria vaccine (RTS, S/AS01B) were protective in infants and young children (RTS, S Clinical Trials Partnership 2015). Interestingly, vaccine efficacy against malaria is still below the target of 75%, as set by the World Health Organization (Ubillos et al. 2018). Kurtovic et al. have recently demonstrated that naturally acquired human antibodies or antibodies generated by repeated experimental inoculation with sporozoites can promote fixation of the complement factor C1q and activate the classical complement pathway against the *Plasmodium falciparum* sporozoite (Kurtovic et al. 2019; White et al. 2015).

IgG1 is generally the prevalent subclass in the infection caused by human immunodeficiency virus (HIV) (Banerjee et al. 2010), which can differ depending on the HIV antigen type, disease progression and immunization regimen. IgG1 responses show the highest

reactivity across all antigens, followed by IgG3 and IgG2. Elevated IgG1 levels may result from conditions that favor subclass switching to IgG1, increased frequencies of IgG1-specific cells, and/or high level of IgG1 secretion. The broad spectrum of IgG functions, evaluated through the switching of subclasses, is crucial for the best protective response to various pathogens (Horns et al. 2016). Analyzing host, viral, and disease parameters associated with broadly neutralizing Ab development in HIV-1 patients, Kadelka et al. reported that IgG subclass responses are differently regulated, leading to distinctive patterns of neutralizer and non-neutralizer antibodies. They showed that all parameters significantly linked with the rate of neutralization (viral load and diversity, infection span, and ethnicity) were predominant in anti-HIV-1 antibody responses in a selective antigen-driven and IgG subclass-dependent manner (Kadelka et al. 2018).

Zhang et al. evaluated serum IgG subclass levels in primary Sjögren syndrome (pSS), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and primary biliary cholangitis (PBC): the authors observed that serum IgG1 (IgG1/IgG) and/or IgG3 (IgG3/IgG) were significantly higher in these patients than in healthy individuals, and identified distinct characteristics for each autoimmune disease group (Zhang et al. 2015). Patients with pSS display higher levels of IgG and selective increase of IgG1, IgG2, and IgG3, while IgG4 are significantly decreased; moreover, IgG1 levels are higher in patients with positive anti-Ro or anti-La extractable nuclear antigen antibodies, while IgG4 is lower in comparison with the negative ones. In addition, a positive correlation between IgG3 levels and disease duration has been identified. These findings support a pathogenic role of IgG1 and IgG3 subclasses in pSS (Liu and Li 2011).

A subnormal (defined as a concentration below the corresponding lower reference limit) distribution of IgG1 and IgG3 subclasses was observed in 46.4% of 28 patients with autoimmune sensorineural hearing loss (Bertoli et al. 2014).

Recently, Basile et al. explored IgG subclass levels in patients with myasthenia gravis (MG) in comparison with patients affected by other systemic autoimmune diseases and healthy blood donors, finding that only patients with systemic autoimmune diseases displayed a mean serum IgG1 value above the normal range and significantly different from the MG and healthy groups (Basile et al. 2018). These observations further strengthen the hypothesis that serum IgG subclass distribution might have peculiar characteristics in different autoimmune diseases.

IgG2

IgG2 subclass represents 20–30% of total serum IgG, with an average serum concentration of 3 mg/ml in adults. These antibodies are active against enveloped bacteria and have a crucial role in the humoral response against polysaccharide antigens. For this reason, individuals with IgG2 deficiency are particularly susceptible to invasive infections by *Haemophilus influenzae, Neisseria meningitidis* and *Streptococcus pneumoniae* (Preud'homme and Hanson 1990; Umetsu et al. 1985). Patients with IgG2 deficiency, selective or combined with one or more subclass deficiency, often display weak antibody responses to polysaccharide antigens and/or IgA deficiency. The Canadian Respiratory Research Network recently reported that low levels of IgG subclasses such as IgG1 and IgG2 represent risk factors for exacerbations and/or hospitalizations in patients with chronic obstructive pulmonary disease (Leitao Filho et al. 2018).

Yamazaki et al. evaluated serum level of total IgG as a marker in patients receiving allogeneic hematopoietic stem cell transplantation, since they noted a higher susceptibility to infectious complications in the early post-transplant period, before neutrophil engraftment, and 2 years after transplantation (Yamazaki et al. 2019, 2018). In particular, the authors found that suboptimal serum IgG2 levels might be considered a possible risk factor for late-onset bacterial pneumonia after allogeneic hematopoietic stem cell transplantation. Moreover, they demonstrated that peri-transplant rituximab administration significantly reduces total IgG and even IgG2 levels after transplantation. Through the analysis of the IgG2/IgG ratio they also found an association between low ratios and increased incidence of bacterial pneumonia (Yamazaki et al. 2019, 2018).

Increased levels of IgG2 have been described in the course of different immune-mediated diseases. Hashimoto's thyroiditis represents the most frequent organ-specific autoimmune disease, and the anti-thyroid peroxidase antibody (TPOAb) is its serologic hallmark. Levels of TPOAb found in the sera of Hashimoto patients are principally IgG, and IgG2 is the most represented subclass. IgG subclass distribution of TPOAb is different based on the thyroid functional status (Xie et al. 2008). It has been shown that T helper (Th) lymphocytes infiltrating the thyroid gland are mainly Th1 cells in Hashimoto's thyroiditis, while interferon gamma, a major Th1 cytokine, is able to induce IgG2 expression and cause IgG2 increase in the serum. Increased IgG2 levels may represent a higher risk of developing a frank hypothyroidism (Kawano et al. 1994). Conversely, other studies have reported a prevalent level of IgG1 in this thyroid disorder (Antonelli et al. 2015; Kotani et al. 1986). Hashimoto's thyroiditis is characterized by chronic inflammation and follicular destruction that should reach about 90% of the thyroid gland to induce hypothyroidism: in the early stage of this thyroiditis (which is euthyroid stage) immune impairment has already started, although patients display no symptoms. The predominant TPOAb IgG subclasses in the sera from patients with Hashimoto's thyroiditis were IgG1 and IgG4. Patients with higher levels of TPOAb IgG2 and IgG4 subclasses may present an increased risk of developing an overt hypothyroidism (Xie et al. 2008).

Recently, a significant association between irritable bowel syndrome (IBS) and isolated elevated levels of serum IgG2 has been described, confirming the need to explore further disease associations in patients with isolated IgG subclass elevations (Engelhart et al. 2017). Interestingly, elevated serum IgG2 concentrations were detected in IBS patients in the presence of *Blastocystis hominis* infection, suggesting that carbohydrate antigens of this parasite could be implicated in the pathogenesis (Hussain et al. 1997).

IgG3

IgG3 subclass represents 4–8% of total IgG in the serum. These Ig are potent pro-inflammatory antibodies, and their shorter half-life may limit the potential of excessive inflammation. In general terms, the course of viral infections is characterized by an increase of IgG3, followed by IgG1 (Vidarsson et al. 2014).

Different studies indicate that hepatitis C virus (HCV) infection with type III (polyclonal IgM and IgG) or type II cryoglobulins (CGs) is correlated with specific IgG subclasses and IgG-RF activity (Gulli et al. 2018). Different patterns of autoAbs and IgG3 levels are evident between the two groups: IgG3 subclass and IgG-RF are significantly higher in patients with type III cryoprecipitates, probably due to a progressive evolution of mixed

cryoglobulinemia. Therefore, decreasing levels of IgG3 appear to be reliable predictors for disease worsening. Low temperature and IgG3 seem to trigger a reversible cryoprecipitation, possibly by inducing steric modifications of Ig molecules. The presence of IgG3 in cryoprecipitates of HCV and antinuclear antibody-positive patients is likely the main factor activating autoimmune mechanism in the long term (Gulli et al. 2016). Basile et al. assessed IgG3 in cryoprecipitates of HCV-positive individuals to look for a potential relationship with mixed cryoglobulinemia, which represents the main extrahepatic manifestation of HCV infection. They hypothesized that CGs could be part of a progressive clonal selection process, in which B cells are initially stimulated to produce more clones of oligoclonal IgG3 with rheumatoid factor (RF) activity *versus* IgG1 (Basile et al. 2018; Gulli et al. 2018). In this scenario, the persistence of the antigenic stimulus could elicit the production of polyclonal IgM-RF with the development of a cryoprecipitate characterized by oligoclonal IgG/polyclonal IgM that represents the marker of a worsening evolution (Basile et al. 2018).

Furthermore, to evaluate HCV-related disease deterioration, the METAVIR score system was used as an indicator of inflammation and liver fibrosis: a score of F0 or F1 means that no significant fibrosis is present; a score equal or greater than F2/F3 indicates mild or moderate fibrosis, while F4 correlates with cirrhosis and suggests that treatment should be started. Indeed, patients with CGs and IgG3 have a METAVIR score between F0 and F1, while worsening patients with IgG3-negative type II CGs, including monoclonal IgM and polyclonal IgG, have a higher score (F2-to-F4) (Basile et al. 2018).

Zhang et al. assessed serum IgG subclass distribution in patients with different autoimmune diseases, reporting a prominent increase of IgG3 in primary biliary cirrhosis compared with other autoimmune disorders, strengthening the hypothesis of a pathogenic role of IgG3 in liver-related autoimmunity (Zhang et al. 2015).

IgG4

According to a decreasing order of frequency, IgG4 is the least abundant among IgG subclasses, with only 5% of total IgG (Schur 1987). IgG4 is generally considered benign and non-inflammatory, as it cannot exert many of the aforementioned effects of the other IgG subclasses because of steric hindrance of its Fc portion and hinge region. There are studies that show that IgG4 is not really immunologically inert. In fact, IgG4 is able to bind FcγR under distinct conditions and a therapeutic IgG4 antibodies caused major side effects due its Fc domain binding to FcγRs (Koneczny 2018). Despite its apparent immunological inertness, a growing number of autoimmune diseases has been related to IgG4, such as pemphigus, MG, thrombotic thrombocytopenic purpura, and chronic inflammatory demyelinating polyneuropathy.

In the context of IgG4-related autoimmunity, the pathogenicity of IgG4 is associated with alternative mechanisms: experimental evidence demonstrated that IgG4 can block enzymatic activity or protein–protein interactions of the target antigen, and that can activate the complement cascade by the alternative (lectin) pathway. Particularly interesting for IgG4 are the Fc portion and hinge region: while there is a high degree of homology between IgG subclasses, there are single amino acid differences that as immediate consequence have the inability to activate the classic complement pathway or activate immune cells (Koneczny 2018).

The production of IgG4 is stimulated by interleukin (IL)-4, IL-13, and IL-10 in response to chronic exposure to non-infectious antigens (Adjobimey and Hoerauf 2010; Hussain et al. 1992; Jeannin et al. 1998; Punnonen et al. 1993). There is increasing evidence that IgG4 can exhibit anti-inflammatory properties and therefore be involved in tolerance mechanisms (Ludwig et al. 2017; Nimmerjahn and Ravetch 2008). IgG4 is often elicited after long-term exposure to antigens in a non-infectious setting and becomes the dominant subclass in allergic individuals who underwent immune therapies (Reichert 2014). In immunotherapy, relief of symptoms appears to correlate with IgG4 induction (Mishra et al. 2019).

Higher levels of circulating IgG4 have been associated with asymptomatic infections caused by helminths and specifically with filariasis, which induces the formation of IgG4 (Mishra et al. 2019). A positive correlation between anti-filarial IgG4 and IgG4 against autoantigens has also been observed, suggesting the synergistic role of poly-specific IgG4 with anti-filarial IgG4 in blocking the disease pathogenesis in asymptomatic patients with microfilariasis (Mishra et al. 2019).

Significant attention has been focused on the detection of serum IgG subclasses since IgG4-related disease (IgG4-RD) was described in 2010 as a novel disease entity (Takahashi et al. 2010). The discovery of IgG4-RD coincided with the observation of isolated elevations of IgG4 in the serum of patients with autoimmune pancreatitis and extra-pancreatic lesions (Hamano et al. 2002; Kamisawa et al. 2003). Kamisawa et al. reported IgG4-positive cells infiltrating the pancreas of patients with autoimmune pancreatitis, and proposed to name this entity as IgG4-RD (Kamisawa et al. 2003; Kubo and Yamamoto 2016; Mahajan et al. 2014; Pagliari et al., 2019b; Stone et al. 2012; Takahashi et al. 2010; Umehara et al. 2012a, 2012b). In particular, the terms lymphoplasmacytic sclerosing pancreatitis and idiopathic duct-centric pancreatitis refer to the different histologic patterns of autoimmune pancreatitis, named type 1 and type 2, respectively. Type 1 autoimmune pancreatitis can be distinguished by raised levels of serum IgG4 and should be considered as part of systemic IgG4-RD, while type 2 autoimmune pancreatitis is frequently reported in younger patients and has less clear immune-mediated pathogenetic mechanisms (Pagliari et al. 2019a, 2019a). Serum IgG4/IgG ratio may be helpful in confirming the diagnosis of IgG4-RD (Umehara et al. 2012b). In addition to elevated serum IgG4 concentrations and pancreas involvement, IgG4-RD may affect a wide variety of organs and is characterized by tissue infiltration with IgG4-positive plasma cells, storiform fibrosis, obliterative phlebitis, and mild-to-moderate eosinophilia (Chen et al. 2014; Umehara et al. 2017). Although autoreactive IgG4 are elevated in IgG4-RD, there is no evidence that they are directly pathogenic (Huijbers et al. 2013; Shiokawa et al. 2016, 2018).

A potential correlation between higher levels of IgG4 and clinical manifestations of rheumatoid arthritis (RA) or therapeutic response in RA patients has also been studied: Chen et al. analyzed patients with RA having elevated levels of IgG4 in the serum, showing that this association may depict a specific clinical phenotype characterized by higher disease activity, higher level of autoAbs, and poorer response to therapy (Chen et al. 2014). Moreover, elevated IgG4 correlated with increased synovial IgG4-positive plasma cells, which also correlated with RA-typical histological synovitis (Chen et al. 2014). Overall, the role of IgG4 in RA remains elusive and further studies are needed to clarify its pathogenetic role. MG is an autoimmune disorder that affects the neuromuscular junction, caused by anti-acetylcholine receptor (AChR) antibodies impairing the efficacy of neuromuscular transmission. Anti-muscle specific tyrosine kinase (MuSK) antibodies are present

in a high proportion of myasthenic patients without anti-AChR antibodies and are associated with a clinically well-defined subtype of MG (MuSK-MG). Additionally, MuSK antibodies correlate with disease severity and treatment response (Bartoccioni et al. 2006; McConville et al. 2004). Niks et al. investigated the longitudinal association between anti-MuSK specific IgG subclasses and disease severity, finding that only IgG4 was related to disease severity (Niks et al. 2008). Notably, also the pathogenic effect of MuSK antibodies was almost exclusive for the IgG4 subclass, as demonstrated *in vitro* with purified IgG4 and in experimental animals via passive transfer of purified IgG4 (Huijbers et al. 2013; Klooster et al. 2012; Koneczny et al. 2017; Raibagkar et al. 2017). At the best of our knowledge, only one case of MuSK-MG associated with lymphadenopathy and histopathology consistent with IgG4-RD has been described: this patient showed an excellent response to B cell depletion, though still maintaining high levels of serum IgG4. Recent evidences, reporting a selective effect of anti-CD20 depleting therapy on specific MuSK_IgG4 without affecting total IgG4, suggest that specific anti-MuSK antibodies probably represent only a fraction of the total IgG4 (Marino et al. 2020). Further investigations are necessary to elucidate the link between MuSK-MG and IgG4-RD.

A novel association has been recently described between celiac disease and isolated elevation of IgG4 in the serum. While until now a confirmation of this occurrence is still lacking, different reports suggest (Mahajan et al. 2014; Xie et al. 2008) that IgG4 plays a role in the immune response to many food protein antigens, and that there is a relatively high number of IgG4-producing cells in the intestinal Peyer's patches, which may in part explain this finding (Engelhart et al. 2017).

IgG subclasses have been rarely studied in patients undergoing liver transplantation, but attractive results are emerging: an increase of serum IgG4 after transplantation has been related to pancreatic-cholangiopathy responsive to corticosteroids. The detection of donor-specific IgG3 before transplantation or its expansion afterwards seem to be associated with rejection and liver graft loss. Conventional immunosuppressive regimes, especially with a combination of tacrolimus and sirolimus, reduce the production of all IgG subclasses after transplantation, but actually it is not known if they deviate the humoral response. Future studies on IgG subclass responses in liver diseases and liver transplantation will probably suggest novel tailored treatment pathways (McAlister 2019).

Hybrid igG4

The classic antibody is produced by a single mature plasma cell and is constituted by Ig heavy chain (α, γ, δ, ε, or μ in humans) that is associated with one type of light chain (κ or λ). The type of heavy chain defines the class of antibody, IgA, IgD, IgE, IgG, and IgM. Each heavy chain has two regions, the constant region, and the variable region. Moreover, each antibody contains two light chains that are always identical; only one type of light chain, κ or λ, is present per antibody in mammals (Burtis et al. 2012; Nezlin 2019). Light chains have variable sequences that allow the complete antibody to stick to specific bacteria or allergens.

Human IgG4 molecules are dynamic and can exchange half molecules *in vitro* to become bi-specific (with two different fragment antigen-binding arms) and functionally monovalent antibodies (Liu and May 2012; Nirula et al. 2011). Approximately 50% of IgG4 molecules consists of heavy chains linked weakly by noncovalent forces. This process leads to forming new disulphide bonds in the hinge region but without disruption of the heavy-light chain

disulphide bond (Young et al. 2014). In an IgG4 molecule without disulfide bonds between the heavy chains, dissociations of the noncovalent bonds permit the chains to separate and recombine randomly (Fab-arm exchange) (Aalberse and Schuurman 2002; Van der Neut Kolfschoten et al. 2007). Hence, an asymmetrical or "hybrid" Ig with two different antigen-binding domains may exist with both κ and λ chains present on the same IgG4 molecule Figure 2. Experimental data indicate that hybrid IgG4 κ/λrepresents a relevant portion of IgG4 in the normal human serum, suggesting a physiological role of this molecule. Naturally

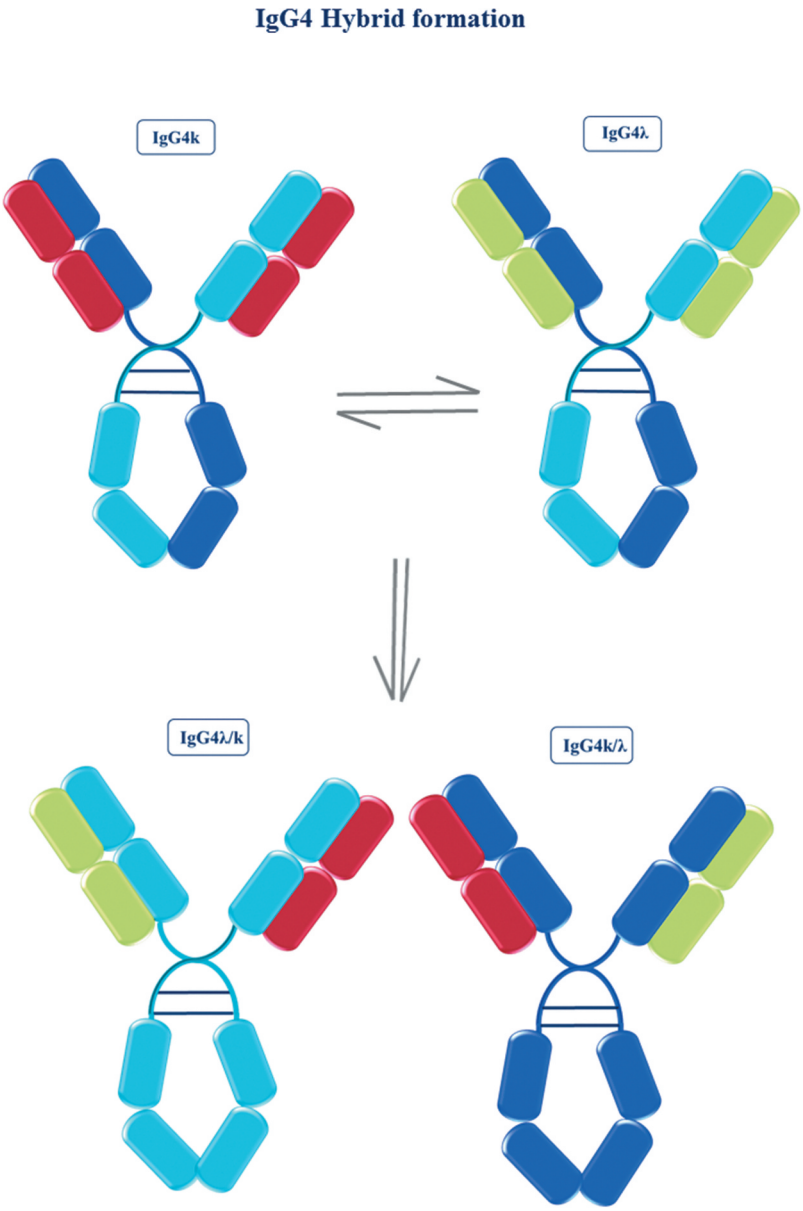

Figure 2. IgG4 hybrid formation.

produced hybrid molecules cannot cross-link antigen or elicit lymphoid responses, decreasing the inflammatory response (Young et al. 2014).

Conclusive remarks

Aimed to elucidate IgG subclass involvement in the immune-pathogenesis of diseases, this manuscript reviews the current literature data related to IgG subclasses distribution in a number of disorders. Different reports have shown a different distribution in patients with autoimmune diseases (Basile et al. 2018; Bijl et al. 2002; Engelhart et al. 2017; Gulli et al. 2020; Hamano et al. 2001; Niks et al. 2008; Zhang et al. 2015). Many reports suggest that the nature of antigens may drive and regulate the production of each IgG subclass (Valenzuela and Schaub 2018; Zhang et al. 2015). Each IgG isotype displays different biological and functional properties: the subclass distribution may therefore regulate the outcome of immune-mediated and autoimmune diseases (abolhassani H, 2015). However, it should be noted that serum levels of IgG subclasses do not necessarily correlate with the amount of antibody deposition in the tissues.

The upgraded role of FcγR in the outcome of an inflammatory/immune response has renewed interest for IgG subclasses. It has been reported that polymorphisms in the *FCGR* gene can affect the interaction with IgG subclasses and, in the end, immune complex deposition, determining the individual susceptibility to infectious diseases, response to antibody-based therapeutics and autoimmune diseases (Bournazos et al. 2009; Wu et al. 2014).

Although the constant regions of the four IgG subclasses are more than 90% homologous, their effector functions and affinity for antigens can vary significantly: subsequently, IgG subclasses differ in their potential to induce an inflammatory response as they interact differentially with complement and Fc*γ*R (Bijl et al. 2002). In particular, studies aimed to optimize effector functions of therapeutic antibodies have pinpointed key amino acid residues, which control the affinity of the Fc region for FcγRs and for complement components (Valenzuela and Schaub 2018). The comprehension of molecular and cellular mechanisms that regulate Ig subclass profile might play an important role in the rational design of immunogens and therapeutics, aimed at enhancing protective immunity and reducing the pathological effects of autoimmune responses.

The introduction of a certified reference standard for IgG subclass detection could largely improve the reliability of all the scientific reports that analyze IgG subclasses and their different expression in autoimmunity. Currently, an international reference preparation for IgG subclass quantification is still lacking. The consequence is that there are differences in calibration among assays and a variety of reference intervals with difficulties in comparing results.

Owing to their distinctive immune characteristics, IgG subclasses proved to be potential marker to control the multifaceted evolution of B cell immune response. Therefore, in the precision medicine era, assessment of IgG subclasses represents an attractive tool that could open a new scenario in patient's personalized drug treatment: it can concur to the definition of a disease-related "immunological fingerprint" that could be very useful in the prevention of diseases at early stages, and in predicting the course of many diseases.

Take-home messages

- The four IgG subclasses display different physical and biological properties, and their distribution depends on the type of antigen and duration of antigen exposure.
- The deep comprehension of molecular and cellular mechanisms that regulate IgG subclasses expression may impact on the rational design of immunogens for therapeutic purposes.
- The introduction of IgG subclass assessment in a diagnostic routine could ameliorate the general management of patients with immunological diseases, but a certified reference material is still lacking for a better standardization.
- Serum IgG subclasses show different patterns in various immuno-mediated diseases, concurring to define a distinct fingerprint, and might be correlated to the pathogenesis of such conditions.

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