Macromolecular Properties That Promote Mesangial Binding and Mesangiopathic Nephritis

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ABSTRACT

The hydrodynamic size, electrostatic charge, and specificity are established determinants of the site of glomerular localization of macromolecules. Larger macromolecules or aggregates and anionic charge are associated with mesangial deposits, despite the fact that the mesangial matrix bears a negative charge similar to that of the capillary wall. Antigens such as Sendai virus, a model infectious pathogen, gliadin, a model dietary/environmental agent and fibronectin, a model endogenous macromolecule, bind to mesangial cells *in vitro* on the basis of cell surface glycoconjugates. Nonantibody immunoglobulin A, which does not bind to cells directly, binds to these elements via different carbohydrate specificities (simple sugar inhibition). Such binding promotes or augments macromolecular deposition in the mesangium. More significantly, mesangial deposits *per se* are not pathogenic, because normal renal function can be observed with florid deposits. Pathogenic deposits must have properties that alter mesangial cell metabolism or interaction with the matrix. Although complement activation is well recognized, complement-independent mechanisms related to cell surface modulation are being recognized. *In vitro*, antigen/immunoglobulin A aggregates alter mesangial cell eicosanoid synthesis. *In vivo*, large-lattice cross-linking by particulate antigen promotes hematuria. We conclude that the binding of macromolecules to cells and the cross-linking of cell surface molecules cause alterations in the mesangial cells and therefore in glomerular function. The mesangial cell, rather than a passive respondent, is an active participant in the genesis of glomerulonephritis.

Key Words: *Glomerulonephritis, lectins, virus, cross-linking, glyconjugates, mesangial cells*

Deposition of immune aggregates within the glomerular mesangium, uniquely or in association with capillary wall deposits, is a very frequent element of glomerulonephritis (1–5). Indeed, glomerulonephritides with only mesangial deposits include some of the most frequent patterns of glomerular disease worldwide, such as most cases of immunoglobulin A (IgA) nephropathy, IgM nephropathy, and mesangial proliferative lupus nephritis (1–3,6–8). Glomerulonephritides characterized by purely mesangial lesions were traditionally considered to be essentially benign and nonprogressive. Clinically, "essential" hematuria has been associated with mesangial deposits (9,10), seen in low-grade cases and the resolving or remission phases of acute postinfectious or lupus nephritides, or in other mild forms of glomerulonephritis. In some diseases, mesangial deposits do not elicit any symptoms at all in the majority of patients (1–10). More recently, mesangial proliferative glomerulonephritides were recognized to be potentially progressive; indeed, its prevalence and frequency of progression makes mesangial proliferative glomerulonephritis one of the most common underlying causes of end-stage renal disease in the world (4–8), even if lesions are confined to the mesangium (1–3,11,12).

The frequency at which mesangial proliferative glomerulonephritis occurs presupposes a predilection for mesangial, as opposed to extramesangial, immune deposition and/or heightened cellular responsiveness to immune stimuli by mesangial cells compared with other glomerular cells that lie beyond the mesangium. Mesangial immune deposits do seem to form more readily than deposits elsewhere within the glomerulus. The passive administration of preformed immune complexes to experimental animals results in mesangial deposits at relatively low doses; only at much higher extremes can capillary deposits be superimposed, unless there is some affinity of a component of the immune complex for the capillary wall (13–17). Likewise, no special properties of the antigen (*e.g.*, size, charge, planting) are required for the induction of active mesangial glomerulonephritis, in

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contrast to other active systems (18–21). In addition to immune complexes, macromolecules aggregated on a nonimmune basis also readily enter, and often accumulate in, the mesangium (1,22–25); in fact, specific pathways for influx from capillaries into the mesangium are recognized (see article by H. Latta in this issue for a review). Indeed, the mesangium, populated as it is by both resident bone marrow-derived phagocytes (26) and contractile cells with phagocytic potential (27–29), seems especially adapted to macromolecular influx.

Mesangial deposition became considered to be a "negative selection:" the fate of circulating macromolecules, including immune complexes, that escaped reticulophagocytic clearance but that also lacked features targeting these molecules to glomerular capillaries (5,6,11–19,30,31). In light of the ease of the induction of mesangial immune deposits, the well-established capacity for macromolecular penetration into the mesangium, and the phagocytic properties of mesangial cells (24,29), a perception of the mesangium as the "dumping ground" of the glomerulus arose. By this view, mesangial deposits represent either nonspecific transient accumulations of immune or nonimmune aggregates or more durable trapping of such aggregates when the rate of influx exceeds the combined rates of efflux and phagocytosis/catabolism.

On the other hand, there are clearly patients who have mesangial deposits, sometimes copious and sustained, without renal dysfunction (2,3,6–8,12). Likewise, heavy and long-lived mesangial deposits can form in experimental animals without evidence of disease (17–20). Therefore, differences exist beyond the degree and longevity of immune deposits among individuals, whether patients or experimental animals, that determine whether and to what extent glomerular dysfunction occurs. The recent, growing cognizance of the potential role of the antigen component of an immune complex in eliciting biologic responses could explain the genesis of immune complex-mediated mesangial glomerulonephritis (15–18,32,33). Moreover, if functional differences among individuals with comparable amounts of immune deposits are related to properties of the antigen component of an immune complex, it is possible that nonimmune macromolecular aggregates with otherwise appropriate properties also incite functional derangements, potentially accounting for at least some patients with nonimmune "mesangiopathic" disease (8).

In this article, we present several distinct lines of investigation. These independent observations collectively support and suggest a conjecture: namely, that the mesangium can be an active participant in the genesis of glomerulonephritis. Given the multiphasic and pluripotent capacity of the mesangium demonstrated in this symposium, this comes as no particular surprise. However, although it is speculative at present, we believe that we can outline a conceptual framework as to *how* the mesangium actively participates in nephritogenesis from the data presented herein in conjunction with that of published reports.

LECTIN BRIDGES

Lectins are proteins, generally derived from plants, that bind to specific carbohydrate domains (34). The carbohydrate ligands to which lectins bind range from simple monosaccharides to complex branched-chain polysaccharides with defined sequences, branch points, and stereospecificity. Moreover, simple sugars can, in some instances, inhibit lectin binding to more elaborate carbohydrate structures. Hence, lectins represent a wide and complicated category of proteins related only by their affinity for carbohydrates.

Among a variety of dietary lectins, gliadin (GLI) is most notorious, because it has been recognized as the causal element of celiac disease. Recently, GLI has also been implicated in the genesis or exacerbation of IgA nephropathy (IgAN) (35,36). Although patients with clinical or subclinical gluten-sensitive enteropathy do develop IgAN at an increased frequency (37–39), other factors definitely enter into play. Dietary restriction of GLI ameliorates renal dysfunction in an appreciable number of IgAN patients, including those with normal gastrointestinal xylose absorption and no clinical evidence of gastrointestinal disease (35). Rechallenge of such patients by allowing normal diet is accompanied by more frequent and/or more severe exacerbation of renal disease. Gastrointestinal permeability is not affected. The levels of circulating immune complexes, containing IgA and GLI as components, and of IgA antibodies specific for GLI are also positively correlated with dietary GLI intake.

In cognizance of the lectin properties of GLI, the ubiquitous presence of glycoproteins on the extracellular aspect of cytoplasmic membranes, and the heavy glycosylation of IgA molecules, we reasoned that GLI might serve as a lectin bridge, promoting the binding of IgA and IgA immune complexes to the renal mesangium. In the first series of experiments, we coated polystyrene microwells with GLI (100 μg/mL in 6 mM acetic acid buffer; pH 3.4) as described previously (40). After the wells were washed, biotin-substituted IgA, prepared as described previously for other proteins (41), was added to wells at varying concentrations, in combination with unlabeled GLI and/or selected simple sugars, over a range of concentrations. After a 30-min incubation at room temperature and extensive washing of the wells, an excess of alkaline phosphatase-conjugated streptavidin

was added. Finally, after another incubation at room temperature for 30 min and further washing of the plates, paranitrophenylphosphate (4 mg/mL in 50 mM glycine, 1 mM $MgCl_2$; pH 10.5) was added. The chromogenic hydrolysis of the phosphate was followed by measuring the optical density of the wells at 410 nm in a microplate reader (40,41). At the end of the linear phase of the reaction, optical densities were recorded. Monotonic binding of biotinyl IgA was observed as a function of IgA concentration (Figure 1A). In these experiments, the half-maximal binding occurred at 20 μg/mL IgA, independent of the fraction of IgA which was biotinylated, and of the dilution of streptavidin employed. Moreover, galactosamine (Figure 1A), but not mannose, fucose, or glucosamine (data not shown), inhibited the binding, depending on the concentration of sugar added (half-maximal inhibition, 100 mM).

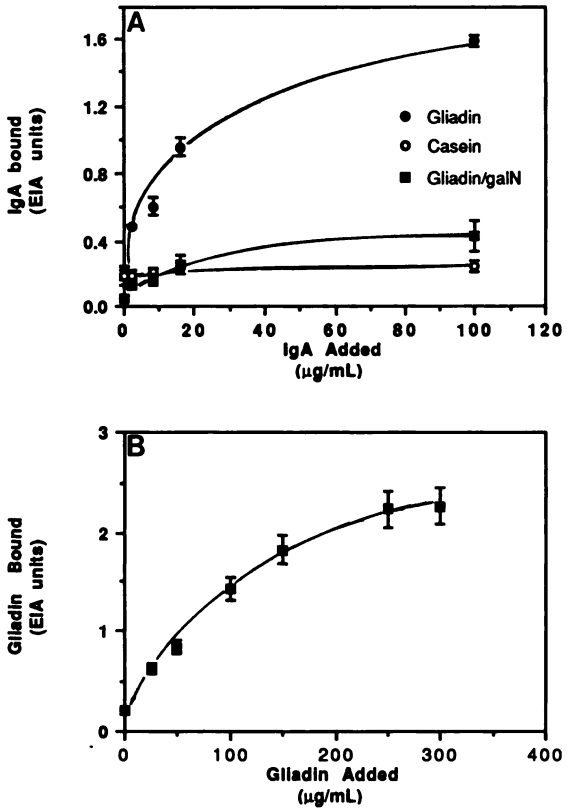

Figure 1. (A) The binding of dimeric murine IgA, purified from ascites of MOPC-315 plasmacytoma-bearing mice, to GLI adsorped to a polystyrene microwell plate is recognized as a saturable, monotonic function of the IgA concentration incubated. This binding, occurring at a half-maximal concentration of 20 μg of IgA/mL, is specific, because little or no IgA binds to casein, a protein very similar in molecular weight and amino acid composition to GLI. In addition, the binding appears to depend on a glycoconjugate present on the IgA, because the IgA binding to GLI is inhibitable by the addition of 100 mM galactosamine (Gliadin/galN) but not by glucosamine (not shown). Murine IgG, lacking galactosamine carbohydrate side-chains, does not bind to casein or GLI (not shown). (B) Saturable binding of GLI to murine mesangial cells in culture is demonstrated; again, the amount of protein added determines the amount bound, with a half-maximal binding of approximately 100 μg of GLI/mL in a culture of 200,000 cells. The addition of glucosamine but not galactosamine at 100 mM inhibits this binding (not shown). The ordinates are expressed in arbitrary enzyme immunoassay (EIA) units.

Using biotinyl GLI and unlabeled GLI in varying amounts and proportions, we similarly demonstrated monotonic binding of GLI to cultured mesangial cells (Figure 1B); the half-maximal binding occurred at a total GLI concentration of 100 μg/mL and was inhibited selectively by *N*-acetyl glucosamine, but not by galactosamine, fucose, or mannose (data not shown).

GLI evidently binds to IgA and mesangial cells via distinct lectin domains. Accordingly, we reasoned that GLI might serve as a lectin bridge to promote IgA binding to mesangial cells. To test this hypothesis, varying concentrations of IgA, trace labeled with biotin, were added to culture wells containing subconfluent mesangial cells, with or without GLI and/or simple sugars. A saturable binding of IgA to mesangial cells, inhibitable by either galactosamine or glucosamine, was observed with GLI coincubation (Figure 2). In the absence of GLI, very little IgA bound to cells nonspecifically. From these experiments, we conclude that, indeed, GLI may act as a bridge, promoting the binding of IgA to mesangial cells and perhaps to the extracellular matrix as well.

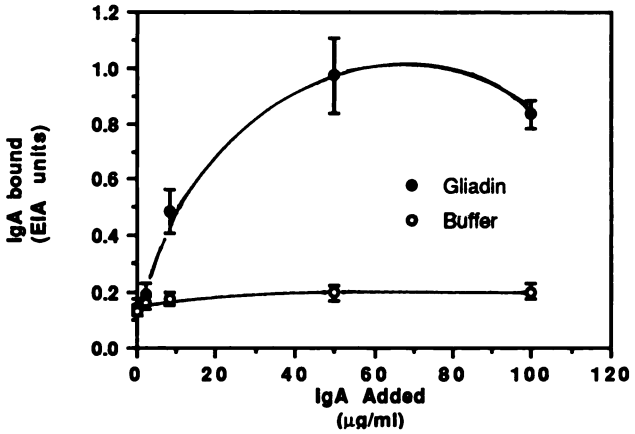

Figure 2. As predicted from Figure 1, dimeric murine IgA binds to murine mesangial cells in culture in the presence of GLI (100 μg/mL) as a plateau function of IgA concentration. The substitution of buffer for the GLI solution in the same buffer (Hanks' balanced salt solution) produced no significant binding. The binding of IgA to mesangial cells was inhibited by more than 90% if either glucosamine, which inhibits GLI binding to the cells, or galactosamine, which inhibits IgA binding to GLI, is added at 100 mM (data not shown).

LECTIN-BASED MESANGIAL DEPOSITION

If lectins can serve as bridges to promote the binding of molecules such as IgA to mesangial cell cultures, then it follows that lectins may deposit in the mesangium *in vivo*, possibly as part of macromolecular aggregates. To explore the potential for lectins to augment the deposition of molecular aggregates within glomeruli, we prepared immunologically specific or nonspecific mixtures. We added one of several antibodies to solutions of BSA or GLI substituted with dinitrophenyl (DNP) haptens, containing 27 μg of DNP per milligram of protein as judged by the ratio of optical densities at 360 and 280 nm (40). This corresponds to approximately seven DNP residues per BSA molecule, as we reported previously. Although GLI is heterodisperse with regard to molecular weight, precluding expressing ratios on a mole basis, the degree of haptenation and amount of antibody were similar to that of BSA on a mass basis. Solutions containing 1 mg of antibody and 9 mg of DNP-BSA or DNP-GLI were injected into groups of five mice, in some cases in phosphate-buffered saline and in other cases in 0.01 M sodium phosphate (pH 7.5) containing 0.3 M glucosamine or 0.3 M galactosamine (as *N*-acetylated forms). After 1 h, mice were killed and the kidneys were harvested, snap-frozen, and cryostat sectioned. Direct immunofluorescence staining revealed varying intensities of antibody deposits in the mesangium; capillary deposits or extraglomerular deposits were not evident. Using a computer-assisted array processor to quantify fluorescence intensity, we estimated the amount of antibody deposited in each of 60 mice (5 each in 12 groups). Irrelevant IgA antibody (TEPC 15) mixed with DNP-BSA resulted in glomerular IgA intensities not significantly different from those of background, whereas IgA (MOPC 315) or IgG (NK1) specific for DNP mixed with DNP-BSA deposited at significantly higher concentrations (Figure 3). When GLI rather than BSA was used as a carrier for DNP, all antibodies deposited at higher concentrations, including TEPC 15. However, galactosamine, which, as previously seen, displaces IgA binding to GLI on a lectin basis reduced TEPC 15 binding to background levels. In contrast, galactosamine had no effect on the deposition of specific IgG and reduced specific IgA deposition only to a small degree (Figure 3). Glucosamine, which inhibits GLI binding to cultured mesangial cells (see above), reduced antibody deposition to levels similar to those observed with DNP-BSA, background in the case of the nonspecific TEPC 15. These data must be regarded as preliminary, because the quantification method, although repetitively linear with counts of ^{125}I-labeled antibody in isolated rat glomeruli in several model glomerulonephritides, is still new and because only one experiment with five mice in each group has been performed to date. Notwithstanding these caveats, we believe that there are three distinct components to the binding we observed. First, the

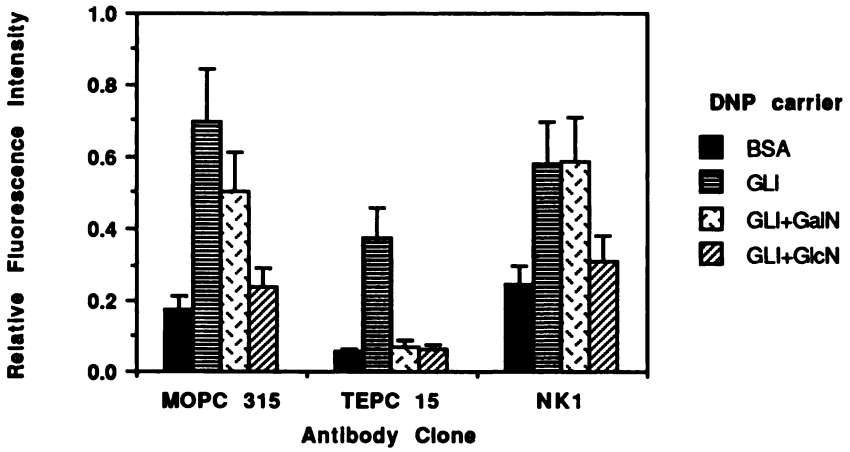

Figure 3. The potential biologic significance of Figure 2 is shown in this passive serum sickness experiment. Injection of immune complexes containing hapten-specific antibody of either IgA (MOPC 315) or IgG (NK1) class into normal BALB/c mice results in significantly more mesangial deposition of specific antibody than nonspecific IgA (TEPC 15), if a protein with no affinity for mesangial cells in culture (BSA) is used as the carrier for the hapten. If GLI is used as the carrier, the deposition of specific antibody of either class is likewise higher than the nonspecific IgA, but the nonspecific antibody (TEPC 15) shows appreciable deposition as well. Coinjection of galactosamine (which inhibits binding of irrelevant IgA to GLI *in vitro*; see Figure 1) with immune complexes containing a GLI carrier (GLI + GalN) reduces the deposition of both specific (MOPC 315) and nonspecific (TEPC 15) IgA but has no effect on IgG (NK1). Blocking of the binding of the GLI carrier to mesangial cells and/or matrix by simultaneous injection of glucosamine (which inhibits binding of GLI to mesangial cells *in vitro*; see Figure 1) with immune complexes containing a GLI carrier (GLI + GlcN) reduces the deposition of all Ig, regardless of class or specificity, to the levels seen if BSA is used as the carrier.

traditional immune lattice formation between antigen and specific antibody results in macromolecules that lodge within the mesangium on the basis of now well-recognized charge and size properties; in these, influx from capillary lumens into the mesangium exceeds clearance and efflux (H. Latta, elsewhere in this issue). Second, immunologically nonspecific binding of antibody, in this case, of the IgA class, to an antigen can promote mesangial deposition, at least if the antigen has affinity for the mesangium (*e.g.*, lectin bridging). The mesangial binding of TEPC-15 with DNP-GLI, inhibitable by either glucosamine (which inhibits binding of GLI to mesangial cells *in vitro*) or galactosamine (which inhibits binding of IgA to GLI), is evidence of this mechanism. The difference between MOPC 315 binding with DNP-GLI without galactosamine versus that with galactosamine is quantitatively similar and is likely accounted for on this basis as well. No effects are seen with IgG antibody, which does not bind to GLI *in vitro*. Third, the combination of the affinity of the antigen component of an immune complex for mesangial structures (lectin bridging) with traditional mechanisms promotes increased mesangial deposition of the complex relative to a similar complex lacking such affinity. Thus, the increased binding of antibody specific for DNP when DNP-GLI was used as the antigen relative to DNP-BSA seems to be due to the capacity of GLI to bind to mesangial cells and/or matrix; this increase is inhibited by the same simple sugar (glucosamine) that inhibits GLI binding to mesangial cells *in vitro* but not by a similar simple sugar (galactosamine). In conclusion, the lectin-based affinity of macromolecules for the mesangium may potentiate the deposition of nonimmune aggregates (TEPC-15/DNP-GLI), as well as enhance the deposition of immune aggregates (MOPC-315/DNP-GLI vs. MOPC-315/DNP-BSA) by binding to the mesangium via a glycoconjugate specificity.

TARGETING OF OTHER MACROMOLECULES TO THE MESANGIUM

Mesangial cells bind and internalize fibronectin via specific peptide sequences specified by an $\alpha_3\beta_1$ integrin receptor on the cells (42,43). We have also observed monotonic binding of bovine plasma fibronectin to cultured mesangial cells, totally displaceable by a specific peptide (GRGDSP) containing the relevant RGD sequence (data not shown). Beyond the very significant import this binding has for cell structure and function (see articles by Teti, Sterzel, and Border in this issue), such binding may have particular relevance to the localization and accumulation of immune complexes within the mesangium. Cederholm *et al.* reported some years ago that patients with a common mesangiopathic glomerulonephritis (IgA nephropathy) have appreciable levels of IgA-fibronectin aggregates in their serum (44). Several groups have recognized that IgA binds to fibronectin *in vitro* (45; A. Woodroffe and D. Schlondorff, independent personal communications), and some investigators posit that IgA-fibronectin aggregates are an important marker for IgA nephropathy (46,47). We have noted that immune complexes of monoclonal murine IgA (MOPC 315) specific for DNP with DNP-BSA bind monotonically to fibronectin, whereas the same monoclonal antibody itself binds much less well (Figure 4). This binding is partially inhibitable by galactosamine and, to lesser degrees, by glucosamine and mannose. It is conceivable, though we are not aware of any data at present, that the binding of IgA immune complexes to fibronectin promotes the mesangial deposition of IgA immune complexes. Perhaps alterations in IgA synthesis by patients with IgAN promotes the binding of IgA to fibronectin. Alternatively, fibronectin structure may be altered in IgAN patients. Such differences may explain why human immunodeficiency virus-positive patients with very high levels of circulating IgA-immune complexes do not have glomerular deposits of IgA, whereas IgAN patients, often with lower levels of IgA immune complexes, do have such deposits (48). If this is true, the administration of exogenous RGD peptides may help in the therapy of IgAN. Indeed, the administration of exogenous fibronectin has already been reported to have salutary effects in experimental proliferative glomerulonephritis (49).

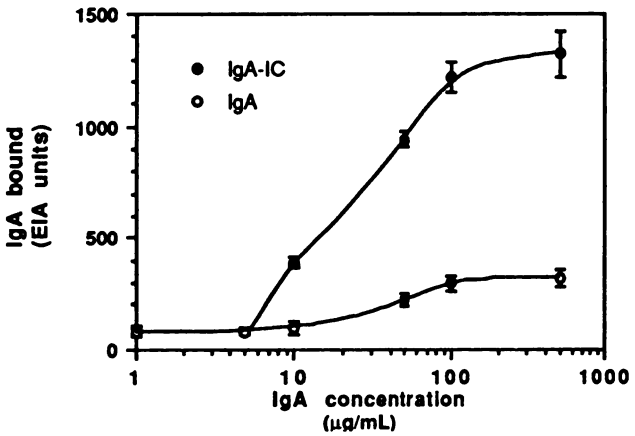

Figure 4. Immune complexes (IgA-IC) of dimeric murine antidinitrophenol (MOPC 315) and dinitrophenyl BSA bind to fibronectin adsorped to a polystyrene dish, as a saturable function of the amount of IgA added. Although the dimeric monoclonal antibody itself (IgA) also binds to fibronectin monotonically, the magnitude of such binding is significantly less than that observed if fivefold equivalent antigen is added.

INDUCTION OF EXPERIMENTAL IgAN BY VIRUS

Sendai virus is a common parainfluenza pathogen for rodents, closely related to similar human pathogens (50–53). We have observed, via qualitative immunofluorescence, that Sendai virions bind to cultured mesangial cells (unpublished observations). This binding is largely inhibited by sialic acid and therefore appears to be related to the hemagglutin/neuraminidase component of the viral envelope. Therefore, on the basis of the foregoing data with lectin-based deposition, we speculated that mesangial deposition of immune complexes of virus-specific antibody and viral particles would likely be augmented by virtue of this binding (see above). Interestingly, although Sendai virus binds to mesangial cells, there is no increase in viral titer in mesangial culture as late as 4 days after viral inoculation, thereby suggesting that mesangial cells do not support viral replication. In this regard, it seems that the fusion viral protein requires specific cellular conditions (54), lacking in mesangial cells. The potential for favored attachment without infection makes Sendai virus a valuable antigen for probing mesangial cell responses to infectious agents, because infection would confound interpretation of experiments with infectious virus as an antigen.

We immunized 129J mice intranasally with 10^4 and 10^7 UV-inactivated Sendai virus on days 0 and 7, respectively, followed by intranasal challenges with 10^4, 10^6, and 10^8 infectious virions on days 14, 28, and 35 (55). Subsequently, mice were challenged i.v. with infectious or intact virions or a soluble viral sonicate; all preparations contained 1 mg of protein (55). Forty-eight hours after challenge, mice were individually housed in metabolic cages for 16 h and then killed. Hematuria, proteinuria, glomerular immunofluorescence for murine Ig and C3 and viral antigens, and light and electron microscopy were assessed as described previously (40,55). Age-matched nonimmune controls, both challenged and unchallenged, were also maintained, and some immune mice were left unchallenged.

Most (80 to 100%) immunized mice developed granular mesangial deposits of IgA, IgG, IgM, C3, and viral antigen, comparable in intensity among the groups, regardless of the nature of the challenge (data not shown). Nonimmune mice had low background levels of immunofluorescence. However, immunized mice challenged with intact virions, whether infectious or not, developed microhematuria, including erythroycte casts (Figure 5). In contrast, immunized mice challenged with viral sonicates or not challenged at all did not develop hematuria or proteinuria. In addition to the dichotomy between immune deposits and glomerular function among the immunized mice, there was no correlation between glomerular C3 deposits and hematuria. This is distinct from

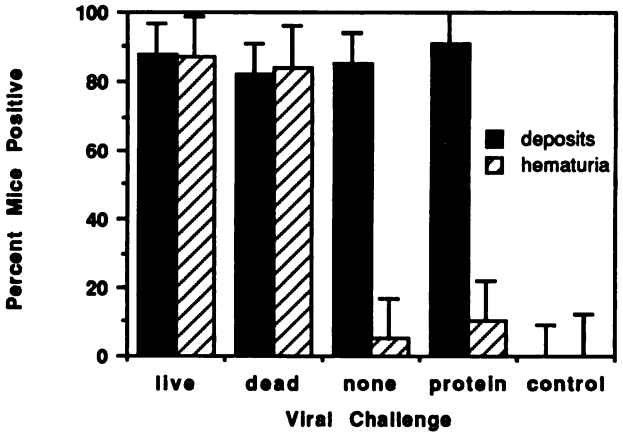

Figure 5. Mice actively and repetitively immunized with Sendai virus via an intranasal route develop significant and comparable mesangial immune deposits of primarily IgA, with IgG and antigen, whether parenterally (i.v.) challenged with infectious (live) or inactivated (dead) virions, not challenged at all (none), or challenged with an unfractionated extract of viral protein (protein), compared with controls. On the other hand, hematuria is observed only after i.v. challenge with intact virions, whether infectious or not. This emphasizes a dichotomy between mesangial immune deposits and glomerular functional alterations such as hematuria.

several other IgAN model systems, in which hematuria and glomerular C3 deposition are closely related (40,56).

Traditionally, glomerular immune deposition has been considered causal of glomerular dysfunction. Recently, in the case of experimental IgAN induced by inert macromolecular antigens, IgG or IgM codeposits were invoked as causal of hematuria, through their strong activation of the classical pathway of complement (40). In the system induced by Sendai virus, glomerular immune deposits are present in mice with normal glomerular permselectivity, and mice with hematuria and proteinuria have, on average, no more Ig or C3 deposits than do those without these signs of glomerulonephritis. The difference in glomerular permselectivity is apparently associated with the nature of the antigen: immunized mice challenged with particulate antigen, infectious or not, develop hematuria and proteinuria. We agree with Rifai *et al.* that in immune complex glomerulonephritis, the quality of the antigen influences the propensity for glomerular damage and, perhaps, the mechanism (complement-dependent or not) of that damage (32,33). Specifically, in the model just described, we propose that the binding of particulate antigen within an immune complex to the cells within the mesangium subtends intracellular signals that alter cellular function, thereby promoting hematuria. Because mesangial cell contraction appears to be responsible for hematuria in a rat model of IgAN (57), we consider

calcium influx and cellular contraction to be likely responses of mesangial cells to viral particles but not to solubilized viral macromolecules. If this speculation is valid, then a mechanism parallel to complement- and Ig-mediated cellular stimulation exists that can augment or supplant the more widely appreciated mechanisms of glomerular injury. Indeed, Camussi, Andres, and coworkers have already documented analogous pathways in a variety of cell-ligand systems (58–60).

COMMENT

We have presented here several nascent concepts. First, a variety of materials, including environmental and endogenous macromolecules and infectious agents, can bind to the mesangium. Second, the affinity of particular components of a macromolecular aggregate for mesangial structures can promote or augment glomerular deposition of the aggregates. Third, the quality of the antigen, and particularly its capacity to instigate cellular responses, is a critical determinant of glomerular injury, independent of the amount of immune deposition that ensues.

Collectively, these concepts lead to a new appreciation of the mesangium in the genesis of glomerular immune deposits and of the physiologic responses to such deposits. The binding of macromolecules to the mesangium and any resultant affinity of aggregates for the mesangium are as much a function of the mesangium as they are properties of the ligand. By the synthesis, modulation, and catabolism of acceptor sites, the mesangial cell can actively influence the propensity for immune or nonimmune aggregates to deposit within the mesangium. If true, this speculation can explain why some individuals develop a renal disease, whereas others in an apparently identical immunologic and hemodynamic situation do not. Obviously, genetic specifications may provide a ligand for binding some antigenic component in one host but not in another. Perhaps, for example, the glycosylation patterns underlying the differences between blood group antigens in black and white patients apply to differences in mesangial cell glycosylation or IgA glycosylation as well. This difference, manifest as a difference in lectin binding, could account for the different frequency of IgAN in Americans of these races, despite apparently identical environments (61). More subtly, however, reactive metabolic differences can alter glycation acutely. Potent cytokines such as interleukin (IL)-4, IL-5, IL-6, and transforming growth factor beta impact heavily on IgA synthetic rates, and IL-6 and transforming growth factor beta affect the mesangial cell as well (61). Although not yet assessed, these factors may influence glycation to promote mesangial IgA deposition. Those with higher rates of synthesis of such factors would be at higher risk for IgAN; patients with IgAN, for example, have higher levels of IL-4 in lymphocyte supernatants than do patients with other forms of glomerulonephritis (62,63).

The mesangial cell may also be an active participant in the injury process. As exemplified by the Sendai virus model we describe here, comparable levels of immune deposits, whether augmented by affinity for the mesangium or not, can elicit different degrees of mesangial injury, depending on the nature of the antigen. We believe that the cross-linking of cell surface glycoproteins, promoted by intact virions but not solubilized viral proteins, underlies glomerular pathophysiology in the Sendai model. In this case, the mesangial *response* to signals elicited by the binding or cross-linking of an antigen with the cell surface is a requisite for the pathophysiologic response. In an analogous situation, consider the effect of the cross-linking of GLI on the mesangial cell. We have already observed that monomeric GLI binds to mesangial cells in culture (Figure 1B). Although GLI, with or without antibody, inhibits prostaglandin production by mesangial cells, the addition of GLI or polyclonal rabbit anti-GLI to mesangial cells in culture has little effect on thromboxane synthesis (Figure 6) or tumor necrosis factor release (not shown). However, coincubation of both elicits a significant increase in thromboxane synthesis, the thromboxane:prostaglandin ratio, and tumor necrosis factor production. Presumably, antibody-mediated cross-linking of the GLI bound to the cell surface underlies this effect. This difference in antigen

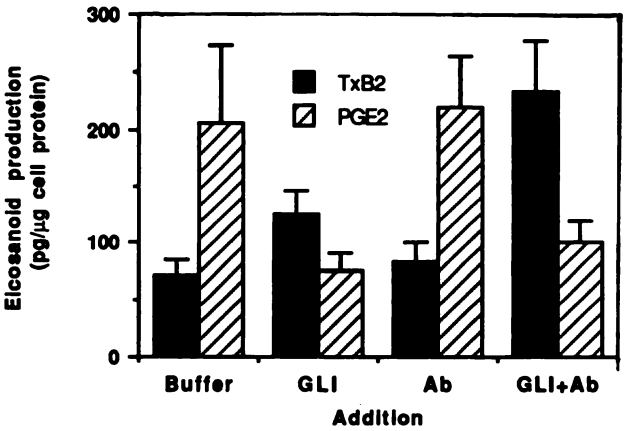

Figure 6. GLI, which binds to mesangial cells in culture (Figure 1B), has no effect on thromboxane synthesis (TxB_2) but significantly inhibits prostaglandin synthesis (PGE_2) by these cells compared with parallel cultures exposed only to buffer. Polyclonal rabbit antibody specific for GLI (Ab) by itself has no effect on either eicosanoid, relative to the control cells exposed to buffer. Soluble immune complexes prepared with GLI and rabbit anti-GLI (GLI + Ab) increase thromboxane production; the inhibition of prostanglandin seen with the addition of GLI alone is recapitulated.

binding to mesangial cells versus cross-linking may explain observations in other experimental systems as well, parallel to mechanisms documented for glomerular epithelial cells (58,59) and postulated for the Sendai virus system just described. Although not yet established experimentally, it is possible that the metabolic state of the mesangial cell influences such responses. For example, membrane fluidity and the polymerization state of cytoskeletal actin are under cellular metabolic control and in turn would affect the capacity for the cross-linking of cell surface glycoproteins. Finally, phagocytic functions recognized in contractile mesangial cells and intracellular signal transduction associated with such phagocyte function would influence further metabolic responses of the cell.

In summary, the mesangial cell emerges as a determinant of mesangial immune deposition and of mesangial response to such deposition. Rather than simply a passive responder to Ig, complement, and/or antigen placed at its doorstep by extramesangial processes, the mesangial cell appears to actively modulate glomerular macromolecular accumulation. This comes as no surprise, given the pluripotent functional capacity of mesangial cells (64,65). As such, we can no longer consider the mesangium a victim of mesangial immune deposition. Instead, we must review the mesangium as a partner in the genesis of glomerular injury and pathophysiology. Even though such a view does not posit defects exclusively in mesangial cells, it nonetheless fully justifies the concept of "mesangiopathic" glomerulonephritis, in that the mesangial cell is an active participant in nephritogenesis. Consequently, therapy directed to altering mesangial cell function and metabolism may be appropriate as a logical extension of the appreciation of the mesangium as a partner in nephritogenesis. Hence, refinement of our appreciation of the role of the mesangium is not simply semantic but has implications for our comprehension of the dynamics of glomerulonephritis as well as novel approaches to treatment of glomerular disease.

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