Immunopathological Aspects of Immunoglobulin A Nephropathy and Other Mesangial Proliferative Glomerulonephritides

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ABSTRACT
Immunoglobulin A nephropathy (IgAN) is an immune complex (IC) glomerulonephritis (GN) that represents one of the most common forms of primary glomerular disease. Proliferation of mesangial cells and the increase of mesangial matrix are histological hallmarks of mesangioproliferative GN. Increased serum levels of IgA, polymeric IgA, IgA rheumatoid factor, IgA-IC, and spontaneous or pokeweed mitogen-induced production of IgA by peripheral blood mononuclear cells are major humoral immune alterations reported in IgAN. Recently, we focused on the role of cytokines and growth factors in the mediation of glomerular injury. Platelet-derived growth factor, transforming growth factor beta, interleukin (IL)-1 and IL-6 are expressed by and act on mesangial cells. Increased expression of platelet-derived growth factor was found in both an active model of IgAN and in renal biopsies of patients with proliferative GN. A strict correlation between increased expression of B-chain mRNA and mesangial proliferation was found. Cytokines such as IL-1, interferon gamma, and IL-6, released by infiltrating mononuclear cells or produced locally by mesangial cells, affect the glomerular response to IgA-IC. In a passive murine experimental model of IgAN, IL-1 and interferon gamma increased mesangial hypercellularity, whereas IL-6 was highly pathogenic when associated to IL-1. In conclusion, classical immunological mechanisms in mesangial GN could interact with other pathways involving cytokines and growth factors in the progression of glomerular injury.

Key Words: Immunoglobulin A, nephropathy, immunopathology, cytokines, growth factors

Proliferation of mesangial cells and increased production of matrix material represent basic glomerular responses to immune injury. A common type of mesangiopathic glomerulonephritis (GN) is immunoglobulin A nephropathy (IgAN), which is one of the most frequent primary glomerular diseases worldwide (1). Secondary IgAN can also be associated with other diseases such as systemic lupus erythematosus, chronic alcoholic liver disease, and Schönlein-Henoch syndrome. The presence of predominant IgM deposits and mesangial proliferation is thought to represent a different GN (IgM nephropathy) (2). Moreover, the mesangiproliferative GN can also occur without immune deposits (3).

Intense and diffuse mesangial IgA deposits are common to all patients with IgAN. Such deposits are granular and electron dense; hence, glomerular deposition of circulating IgA immune complexes (IgA-IC) is thought to be a central pathogenetic factor (4). However, despite this uniformity of immunopathological findings, the glomerular histological aspects and clinical outcome vary widely among patients. The latter consideration leads us to hypothesize that variable additive factors cooperate with the basic pathogenetic mechanism to modulate the glomerular response to immune injury.

SYSTEMIC IMMUNOPATHOLOGICAL FINDINGS
Clinical studies dealing with the immunobiology of the IgA system have reported several alterations in IgAN. Also, alterations of IgA production were detected in serum or in supernatants of peripheral blood mononuclear cell cultures of healthy relatives of IgAN patients (5).

Although not common to all patients, serological features of IgAN are increased levels of IgA, the presence of circulating IgA-IC, and an increased fraction of polymeric IgA molecules, which in some patients, have rheumatoid factor activity (6).

Spontaneous or pokeweed mitogen-induced increased IgA synthesis by peripheral blood mononuclear cells was first reported by Egido et al. (7) and then confirmed by others (8–11) and our group (12). We have also observed that during episodes of macroscopic hematuria there is a polyclonal B cell activation that leads to increased production of IgG, IgM, and IgA and to the presence of IgG-IC (12).
The abnormal production of polymeric IgA has a peculiar relevance. In fact, as experimentally demonstrated (13) nephritogenesis of IgA-IC depends upon the molecular form of IgA. The polymeric fraction is responsible for the formation of IgA immune deposits (13, 14). Moreover, polymeric IgA may indirectly modulate renal deposition of IgA and IgG-IC (15). We have in fact demonstrated that polymeric IgA purified from IgAN patient sera inhibits the solubilization of preformed IC in vitro by normal human serum (16). This means that IC that would form in circulation are not adequately disrupted by a mechanism involving complement activation and then are not efficiently cleared by the reticuloendothelial system. As a consequence, IC tend to deposit in glomeruli and activate inflammatory pathways.

In addition to altered complement-mediated solubilization of IC, a defect in the plasma clearance of macromolecular IgA in IgAN patients has been hypothesized as responsible for glomerular IgA-IC deposition. We first performed clearance studies in IgAN patients with cross-linked macromolecular human IgA as a model of IgA-IC (17). The rate of removal from the circulation in IgAN patients was not statistically different from that in normal volunteers. Recently, however, Roccatoello et al. (18) have found a defect in the clearance of IgA1-IgG-IC. The different form of IC used may explain the controversial results.

Defects of mucosal immune response in IgAN are strongly supported by the strict association between upper respiratory tract infections and clinical relapsing of GN. Altered antigen processing in the lamina propria of mucosa can generate IgA-IC–containing viral or dietary antigens that, once gaining access to the circulation, would deposit in the glomeruli (19). The mesangium is the elective area of IgA-IC deposition. Mesangial cell activation can be triggered by both the antibody and antigenic part of IC. In this regard, although the IgA is presumably constant in all patients, the antigen may vary, thus being responsible for a wide range of histopathological glomerular responses. Experimental studies support this hypothesis. Montinario et al. (20) established an experimental model of IgAN in mice, whereby glomerular IgA-IC composed of IgA only did not induce significant histopathological changes. By comparison, foreign antigens captured by the same glomerular deposits of IgA-IC induced a spectrum of histological lesions. Furthermore, the extent and severity of glomerular damage and pathophysiological alterations strictly correlated with the nature and amount of antigen administered (20).

Indirect evidence of viral antigens deposits (adenovirus, herpes simplex, varicella-zoster, and parainfluenza 3) have been demonstrated by Tomino et al. (21) not only in the renal sections but also in the nuclear region and/or cytoplasm of HeLa cells after incubation with the extract of pharyngeal cells from IgAN patients. In addition, some other conflicting data have been reported about the presence of human cytomegalovirus antigen within the renal immune deposits (pros, references 22–24; cons, references 25–29).

An alteration of oral tolerance also seems to contribute to the pathogenesis of IgAN. The phenomenon of oral tolerance is an important feature of the mucosal immune system and appears to protect against immune-mediated diseases by inhibiting the production of systemic IgG and IgM antibodies directed toward immunogens chronically present at mucosal surfaces. Gesualdo et al. (30) showed that blunting of oral tolerance with cyclophosphamide and/or estradiol induces nephritis in chronically orally immunized mice and that glomerular IC containing IgG and/or IgM promote complement deposition and hematuria. These findings are clinically supported by the increased production of IgG, IgM, and IgG-IC observed during episodes of macroscopic hematuria in IgAN patients (12).

### RENAL IMMUNOPATHOLOGICAL FINDINGS

Genetic factors, exposure to various antigens, and systemic and/or mucosal immunological abnormalities likely represent the primus movens (early or initial events; Figure 1) of the pathogenesis of IgAN and other mesangiproliferative GN (31). However, complement activation, the production of inflammatory mediators, and the activation of intrinsic glomerular mechanisms could perpetuate the renal damage towards sclerosis.

In the 1970s and 1980s, a large amount of experimental observations indicated that complement activation plays a key role in renal damage alone or in association with platelets, coagulation, and cellular inflammatory products (32, 33). After these acute events, the renal damage can resolve totally or progress toward sclerosis. The latter events (Figure 2) leading to sclerosis are likely common to various renal diseases and seem to be related to the activation of intrinsic glomerular cells (epithelial, endothelial, and mesangial cells).

In the 1990s, research is being aimed at dissecting out the glomerular components of the nephritic process leading to sclerosis. Among diffusible factors produced by glomerular cells and probably involved in sclerosis, a primary role seems to be played by cytokines and growth factors (34). These substances exert "paracrine" and "autocrine" effects and can be both positive and negative modulators of cell growth.

From in vitro studies, we know that many cytokines are involved in tissue remodeling and reparative growth during and after inflammation. These factors have distinct but overlapping actions.
Figure 1. Summary of early pathogenetic events involved in IgAN and other mesangioproliferative GN. PAF, platelet-activating factor; TNF, tumor necrosis factor.

Figure 2. Involvement of cytokines, as later pathogenetic events, in the progression of renal damage to sclerosis.

Rat and human mesangial cells express platelet-derived growth factor (PDGF) (35–36), transforming growth factor beta (TGF-β) (37), interleukin-1 (IL-1) (38,39), interleukin-6 (IL-6) (40), and several other cytokines' mRNA (41) and proliferate in response to these factors (35,40,42). Mesangial cells synthesize and release PDGF in culture medium; on the other hand, PDGF is a potent mitogen for mesangial cells (35), therefore indicating a possible autocrine regulation of cell proliferation. In addition to its potent mitogenic effect, PDGF possesses different biological activities including chemoattraction, activation of inflammatory cells, and vasoconstriction (43). These features suggest that PDGF might play an important role in the pathophysiology of mesangioproliferative GN. Recently, the importance of this inflammatory mediator in the pathogenesis of certain forms of GN has been demonstrated (44). Gesualdo et al. (45) observed increased glomerular expression of PDGF B-chain mRNA in mice with IgAN induced by parental immunization with DEAE-dextran or dextran-sulfate. The increased PDGF expression correlated with the degree of hypercellularity and pathophysiological alterations of the disease. Moreover, PDGF was demonstrated immunohistochemically in glomeruli of patients with GN characterized by mesangial proliferation, including IgAN. Finally, we accumulated preliminary data on 18 normal and 20 pathological human renal biopsies by polymerase chain reaction amplification, which further confirmed these findings.

Alterations in the expression of growth factor receptors have also been reported. Felling et al. (46) demonstrated increased expression of PDGF receptors in different forms of GN, especially in proliferative GN. Iida et al. (44), investigating PDGF and PDGF-receptor expression in another experimental model of mesangial proliferative GN induced with antibody to the Thy-1 antigen (which is normally expressed by rat mesangial cells), showed a marked increase in both PDGF A-chain and B-chain as well as in PDGF-receptor β-subunit mRNA. Furthermore, the specific protein, PDGF-B, was localized principally in mesangial cells by immunoperoxidase techniques.

TGF-β is also a growth factor elaborated during the inflammatory reaction. It can inhibit mesangial cell proliferation and stimulate the synthesis and secretion of extracellular matrix proteins, such as collagen and fibronectin (47). Recently, increased production and activity of TGF-β has been reported in an animal model of acute mesangioproliferative GN. Moreover, it has been shown that the administration of anti-TGF-β at the time of the induction of the extracellular matrix deposition attenuates the histological manifestations of the disease (48).

IL-1 is a potent polypeptide produced primarily by macrophages in settings of injury or antigen stimulation. IL-1 release is an important mediator of local inflammation and injury (49). Recently, it has been shown that glomerulonephritic rat kidneys express a twofold to threefold increase in IL-1 mRNA compared with that in normal kidneys (38).

Like IL-1, IL-6 has a broad spectrum of cell targets and thus can induce an equally wide array of immune
and inflammatory responses in vitro as well as in vivo (50). Cultured mesangial cells express IL-6 mRNA, and by immunohistochemical staining, it has been shown that patients affected by mesangioloproliferative GN express more IL-6 than do patients with membranous nephropathy, minimal change nephrotic syndrome, or normal kidneys. Furthermore, urine samples from patients with mesangioloproliferative GN were found to contain significant IL-6 activity, strictly correlated to the progressive stage of GN (51). In contrast, urine samples from patients with minimal change disease and normal volunteers did not contain any detectable IL-6 activity.

Montinaro et al. (52) induced IgAN in mice with IgA antiphosphorylcholine and either phosphorylcholine-BSA or pneumococcal C-polysaccharide (PnC). At the same time, mice were treated with IL-1, interferon gamma, or IL-6 alone or in double combination. Results from this study show that the glomerular injury induced by two antigens with different nephritogenic potential can be further modulated by cytokines. IL-1 and, to a lesser extent, interferon gamma aggravated glomerular injury induced by IgA-PnC complexes. IL-6 had a differential effect: alone it was slightly beneficial to the glomerular injury induced by IgA-PnC complexes, whereas associated to IL-1, it synergized to impair the IgA-PnC-induced glomerular damage. Thus, from the experimental observations reported above, it seems reasonable to think that IL-6 could represent a second signal working in concert with other mechanisms.

Taken together, these findings suggest that cytokines and growth factors may be major determinants of mesangial cell proliferation in these forms of GN. Although the specific stimuli triggering the autocrine production of these factors remain to be elucidated, major candidates are immune complexes, activated components of complement (C3b, C5b-9), platelets, and other polypeptide regulatory factors.

CONCLUSIONS

The pathogenesis of IgAN (as a paradigm of mesangioloproliferative GN) is likely dependent on systemic immunological alterations. However, increasing evidence of local glomerular mediation of tissue injury has surfaced in recent years. Several diseases with comparable histopathological patterns may encompass the same pathways of glomerular damage. A major role in inducing mechanisms of hypercellularity, synthesis of matrix, and evolution to sclerosis seems to be played by cytokines and growth factors.

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