MINIREVIEW

Autoimmune Diseases of the Kidney: An Update[1] (44010)

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In 1984, *Proceedings of the Society for Experimental Biology and Medicine* published a Minireview entitled "Autoimmune Diseases of the Kidney" (1). The focus of the present update is on new antigen(s) (Ag[s]) and epitopes which incite the nephritogenic autoimmune response with or without tissue damage, their genetic requirements, and the evidence that T cells are involved not only in helping B cells synthesize autoantibodies but also in the development of tissue injury. As in the previous Minireview, we will consider the pathogenesis of spontaneous and experimentally induced autoimmune diseases in laboratory animals and the evidence, frequently partial or tentative, that similar pathogenetic mechanisms operate in humans.

Diseases Induced Mainly by Antibodies Reactive with Structural Antigens

Glomerular Basement Membrane. *Goodpasture disease. **Antigen.*** The nephritogenic epitope is in type IV collagen of the glomerular basement membrane (GBM), alveolar basement membrane (ABM), and testicular basement membrane and lens, as shown in humans, cattle, and small laboratory animals. Type IV collagen is the scaffold of basement membranes, in combination with other macromolecular components, such as laminin, heparan sulfate proteoglycans, and enctactin. Type IV collagen is formed by building block units (protomers) that are linked to one another by end-to-end bonds. The protomers are composed of six genetically distinct α-chains characterized by three structural domains, the amino terminus (7S), the central helical region, and the NC1 (non collagenous) domain at the carboxyl terminus. At the NC1 junction, the NC1 domains (monomers) of the six α-chains form a hexamer. The antibodies (Abs) of Goodpasture patients or animals with experimentally induced Goodpasture disease bind to the NC1 domain of the α3-chain (α3[IV]) (2–4). The epitope is tentatively localized in the carboxyl terminus of the NC1 domain of α3(IV), encompassing residues 198–233 as the primary interaction site (5, 6). Goodpasture Abs bind poorly to the hexamer, but the binding increases when the hexamer is dissociated into dimeric or monomeric forms, suggesting that the epitope is cryptic (7). Nevertheless, Goodpasture Abs bind to basement membranes in tissue sections not treated with dissociating buffers and *in vivo*, showing that under these conditions the epitope is accessible. The COL4A 3 gene encoding the Ag has been cloned and localized to Chromosome 2 bands g35–37 (8).

Goodpasture disease in laboratory animals. The first model of anti-GBM GN was developed in the early 1960s by Steblay, who immunized sheep with heterologous (human) or homologous GBM (9). In 1983–1986, Sado *et al.* demonstrated that immunization of Wistar-Kyoto rats with a trypsin-solubilized fraction of bovine GBM induced a dose-dependent rapidly progressive glomerulonephritis (GN) and hemorrhagic pneumonitis (10–12). Hudson and his colleagues showed that immunization of rabbits with 300 mg bovine α3(IV) NC1 dimer in CFA provoked a rapidly progressive and crescentic GN and hemorrhagic pneu-

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monitis characterized by linear deposition of rabbit IgG in renal and pulmonary basement membranes, proteinuria, decreased renal function, and signs of pulmonary distress. Anti-α3(IV) Abs were present in the circulation and eluted from the kidneys (13). Immunizations with the α1(IV)/α2(IV) dimer and NC1 hexamer induced autoantibody responses but no tissue damage.

Since the publication of the work of Sado *et al.*, Wistar-Kyoto rats have become animals of choice for the study of experimental Goodpasture disease (14, 15). Ten to fourteen days after immunization with a crude preparation of bovine GBM, the initial lesions were characterized by accumulation of LFA-1$^+$ T cell and monocyte/macrophages in glomerular capillaries, associated with upregulation of ICAM-1 in the glomerular endothelium. These changes were followed by infiltration of LFA-1$^+$ cells in Bowman's space, Bowman's capsule, and in the peri-glomerular area, with proteinuria and decreased renal function. After 4–5 weeks, circumferential cellular and fibro-cellular crescents were found in almost all glomeruli; the rats also developed a severe tubulo-interstitial nephritis (TIN) and died in uremia. Monoclonal Abs to rat ICAM-1/LFA-1 prevented or markedly reduced crescent formation with striking prophylactic and therapeutic effects. These results show that the interaction of mononuclear cells with the activated endothelium is an early and critical step in extravasation of inflammatory cells into Bowman's capsule and in formation of crescents (14).

Studies designed to test the effect of more-purified and better-characterized Ags were performed in Wistar-Kyoto rats immunized with 25–100 mg bovine GBM α3(IV), α2(IV), or α4(IV) chains dimer or monomer. Only α3(IV) induced Goodpasture disease. The glomerular lesions, however, were less severe than those seen in rats immunized with crude GBM preparations (14). Pulmonary lesions occurred in 20% and 30% of rats immunized with 25 and 100 mg α3(IV) dimer, respectively (Zoia C, Kalluri R, *et al.*, personal observations). α3(IV) Abs were present in the circulation and concentrated in the kidneys. Linear deposition of rat IgG was evident in the GBM but not in the ABM. In contrast, Wistar-Kyoto rats immunized with bovine testicular basement membrane hexamer consistently developed severe pulmonary lesions associated with linear deposition of rat IgG in the ABM (Brunmark *et al.*, personal communication). Brunmark's results, which differ from those obtained in rabbits (13), may be due to differences in preparation of the antigen and/or to higher concentration of α3(IV) in testicular basement membrane than in GBM.

In the early stage of anti-GBM GN of Wistar-Kyoto rats, T cells and macrophages were visualized in glomeruli, and the accumulation of T cells preceded that of macrophages. Moreover, when the disease progressed, T cells and macrophages infiltrated the interstitium. CD4$^+$ T lymphocytes from donor BN rats with anti-GBM GN transferred the disease to naive recipients, and administration of monoclonal Abs to CD4 prevented the development of nephritis (16).

In conclusion, these studies show that a Goodpasture disease can be induced in rabbits and in Wistar-Kyoto rats, and that the epitope eliciting the pathogenic autoimmune responses is localized in the NC1 domain of α3(IV). A preliminary report indicates that immunization with α3(IV) induces abnormalities with features of Goodpasture disease in SJL mice, which are genetically predisposed to develop autoimmunity (Section II, 2) (Kalluri R *et al.*, personal communication).

Goodpasture disease in humans. A strong association was found between MHC class II alleles DRB1*1502(DR2) and DRB1*04(DR4) and Goodpasture disease (88%). The genetic control of the immune response was also suggested by the occasional occurrence of the disease in siblings and in twins (16). In predisposed individuals, environmental agents, such as smoke, gasoline vapors, etc., may act to expose the cryptic nephritogenic epitope (17), although further evidence of this mechanism is needed.

Sera from large series of patients with anti-GBM GN, hemorrhagic pneumonitis alone, or Goodpasture disease, were studied using immunological assays with bovine α1–α6-chains (IV), or recombinant human type α1–α5(IV) collagen NC1 domains. All sera reacted with the α3(IV) NC1, and 85% of them exclusively with α3(IV). Reactivity to laminin, fibronectin, heparan sulfate proteoglycans, entactin, the 7S, or the triple helical fragments of type IV collagen was not observed (13, 18). A study of inhibition of Goodpasture Abs with a biotin-labeled monoclonal Ab to α3(IV) established that in 89% of the Abs were directed against the same epitope of the α3(IV) chain (18). These studies show that the immune response is highly restricted. Because the target epitope has been identified, it seems plausible to name "Goodpasture disease" the rapidly progressive GN, with or without hemorrhagic pneumonitis, associated with α3(IV) Abs. The reasons why GN and hemorrhagic pneumonitis without α3(IV) Abs should be termed "Goodpasture syndrome" are discussed below.

The response to α3(IV)/α4(IV) of autoreactive T cells obtained from blood of patients with Goodpasture disease has been investigated using a proliferation assay. T cells were stimulated by both α3(IV) and α4(IV) but T cells of some patients did not respond to the stimulus. Moreover, the interpretation of the data was impaired by the proliferative response of autoantigen-specific T cells of normal individuals and patients with other types of renal diseases (19).

Anti-GBM GN occurs in renal allografts of 5%–

10% patients with the Alport syndrome. The usual form of Alport syndrome is associated with mutations and deletions in COL4A5 gene (20), one of the six genes which constitute the α-chains of type IV collagen. The rare autosomal recessive form of Alport syndrome is characterized by mutations and deletions in the COL4A3 and COL4A4 genes, which encode for the synthesis of α3(IV) and α4(IV) chains, respectively (21). In patients with both forms of Alport disease, the target of alloantibodies is α3(IV) (22, 23), which is missing in Alport patients due to its defective assembly and incorporation into the basement membranes.

Membranous glomerulonephritis in rats injected with mercuric chloride. In rabbits, rats, and mice, mercuric chloride ($HgCl_2$) induces a genetically controlled autoimmune renal disease. BN rats carrying the RT-1n haplotype, and rats of the BN-derived DZB strain, are susceptible to the disease. In BN rats, one of the immunoregulatory abnormalities is the decreased number of $RT6^+$ T cells (presumably suppressor T cells), a phenomenon that is not observed in disease-resistant LEW rats (24). BN rats injected with $HgCl_2$ initially develop linear deposition of IgG in renal basement membranes, followed by granular immune deposits, corresponding to subepithelial deposits detectable by electron microscopy (membranous GN). The Abs present in the circulation or eluted from the kidneys react mainly with laminin (25). In DZB rats membranous GN occurs more rapidly and is more severe than in BN rats; the Abs react with the P1 fragment of the laminin 1 isoform (26). These finding suggest that laminin contains the immunizing epitope.

Tubular Basement Membrane. *Tubulo-interstitial nephritis.* Guinea pigs, rats, and mice immunized with heterologous tubular basement membrane (TBM) develop anti-TBM Abs and TIN. A similar disease is rarely observed in humans (1).

Antigen. Using monoclonal Ab to TBM and autoantibodies from patients with anti-TBM TIN a 48-kDa glucose-rich glycoprotein was isolated from collagenase-solubilized human and rabbit TBM. Sera from rats and mice with experimentally induced anti-TBM TIN also reacted with this Ag, and inhibited the binding of human Abs, thus showing that the target epitope is highly conserved among mammals (27). In other studies human Abs reacted with a 58-kDa human TBM glycoprotein (28). There was no immunologic cross-reactivity between the nephritogenic TBM Ag and other known basement membrane components. Moreover, peptide sequences derived from three different regions of the molecule—including a sequence of 173 amino acid—differed from those of known macromolecules (29). Recently cDNA encoding the TBM Ag was cloned and sequenced. The NH2-terminal region of the molecule contains a highly conserved epidermal growth factor–like repeat similar to several extracellular matrix glycoproteins, such as laminin A and S chains, α1-chain of type I collagen, von Willebrand's factor, and mucin. The COOH-terminal region shares extensive homology with the cathepsin family of cysteine proteinases. These findings show that the 48- to 58-kDa TBM Ag is a novel member of the family of basement membrane glycoproteins, restricted to renal proximal tubules, to the ileum, and, in small amounts, to corneal and epidermal basement membranes (30). The Ag does not interfere with the polymerization of type IV collagen but exerts a dose-dependent effect on the polymerization of laminin and preformed laminin polymers; it can also promote adhesion of cultured renal tubular cells and aortic endothelial cells, suggesting that *in vivo* it may contribute to the assembly of basement membranes and to cellular adhesion (30).

Tubulo-interstitial nephritis in laboratory animals. The first model of immunologically-mediated TIN was developed in 1971 by Stebaly and Rudofsky, who immunized guinea pigs with rabbit TBM (31). At present, the antigen responsible for guinea pig TIN is still unknown. In contrast, the 48- to 58-kDa nephritogenic Ag has been identified in the TBM of BN, but not in the TBM of LEW rats. Upon active immunization, both BN and LEW synthesize anti-TBM Abs, but only BN develop TIN (17, 30). The gene determining the expression of the Ag is linked to the gene for albinism (32). The immune response is largely determined by a RT-1–linked gene (33).

The role of T lymphocytes in murine TIN was investigated. A monoclonal Ab to 48- to 58-kDa TBM Ag was used to select murine SJL tubular cells and to establish a clone secreting the TBM Ag and expressing it at their cell surface; the cells also expressed class I and II major histocompatibility complex (MHC) molecules and stimulated the proliferation of cultured TBM Ag–specific and class II MHC–restricted T helper cells (34), thereby suggesting that the cells of proximal tubules may be able to initiate and amplify a local T cell–mediated anti-TBM response.

In guinea pigs and rats, TIN was transferred by serum but not by immune T lymphocytes (1). In contrast, in mice the disease was transferable by immune T cells. The interstitial infiltrates were fully developed 4–6 weeks after cell transfer, and contained NK cells, T cells, and macrophages (35). Transfer of anti-TBM Abs induced TIN in SJL ($H\text{-}2^s$;$Igh\text{-}1^b$) mice, but not in B10.S(8R)($H\text{-}2^a$; $Igh\text{-}1^b$) mice, showing that the ability of TBM Abs to initiate the disease depends on genes susceptible to engage the cell-mediated immune response (36). Upon active immunization, both SJL and B10.S strains developed a nephritogenic T-cell response, which included $CD4^+$ and $CD8^+$ TBM-reactive T cells. Several days after immunization SJL mice preferentially expressed class I restricted $CD8^+$ cells, which were able to transfer the disease to naive

mice. In contrast, B10.S mice developed class II restricted $CD4^+$ effector cells which responded to TBM Ag but which were not effective in transferring the disease unless the recipients were pretreated with IFN-γ, an effect that apparently depends on augmented expression of class II MHC molecules in tubular cells (37). This enhancing effect was blocked by anti-TBM Abs which downregulated class II MHC transcription and gene product expression in tubular cells (37). These findings demonstrate the complexity of the nephritogenic TBM response in mice.

Tubulo-interstitial nephritis in humans. TIN with linear deposition of IgG in TBM was observed in about 70% of patients with anti-GBM GN. Immunoglobulins eluted from the kidneys of these patients bound to normal human kidneys with an immunofluorescence pattern similar to that observed in the nephritic kidneys. Some eluates, however, reacted only with some TBM, suggesting that Ags not readily accessible *in vivo* are exposed *in vitro* when the basement membranes are sectioned (17).

The specificity of TBM Abs obtained from patients with Goodpasture disease is unknown. However, since patients with GBM and TBM Abs seem to have more severe TIN than patients with GBM Abs alone, it is probable that TBM Abs contribute to the development of tubulo-interstitial lesions (38).

Anti-TBM TIN is a rare disease in humans, and occurs spontaneously or after renal transplantation (39–41). Deposition of IgG is strictly confined to the basement membranes of proximal tubules, and auto- and allo-Abs react with the 48- to 58-kDa TBM Ag, but not with α3(IV) or other basement membrane components (27, 28). TBM Abs were studied in two series of patients with a variety of renal diseases. The first study, performed by immunofluorescence and hemagglutination, included 99 patients. Eleven patients had GBM and TBM Abs, GN, and TIN; five had TBM Abs only. Though some of these patients had linear deposition of IgG in TBM, a correlation with the renal lesions was not established (42). The second study included 217 patients and was performed by ELISA, using the nephritogenic 58-kDa TBM Ag. TBM Abs were found in sera of 22% of patients with TIN. The pathogenic role of the Abs, however, was not established because immunohistochemical studies of the kidneys were not performed (43).

Plasma Membranes of Tubular and Glomerular Epithelial Cells. *Heymann nephritis.* Rats actively immunized with a plasma membrane enriched fraction (Fx1A) of rat proximal tubules develop Abs reactive with tubular brush border as well as with glomerular visceral epithelial cells, which results in immune deposits in the subepithelial part of the GBM (membranous GN); when proteinuria ensues, the Abs present in the glomerular filtrate react with the brush border and induce tubular damage (1). Similar lesions are found in rats injected with heterologous Fx1A Abs (passive Heymann nephritis). In both active and passive diseases the morphology of glomerular changes, their evolution, and the severity of the nephrotic syndrome are reminiscent of human idiopathic membranous GN (44, 45).

Antigens. The main antigen was purified from the tubular brush border using gel electrophoresis (46) or gel filtration (47), and its molecular weight was estimated 330 or 600 kDa, respectively. The 330-kDa component is considered to be a split product of the 600-kDa glycoprotein. The evidence that gp330/600 is the Ag of Heymann nephritis is based on the observations that actively immunized LEW rats develop subepithelial immune deposits; injection of anti-gp330/600 Abs induce similar lesions; and Abs eluted from nephritic kidney react with gp330/600. Partial cloning of rat cDNA demonstrated homology with the low-density lipoprotein receptor (LDL-R) (48), and LDL-R–related protein (LRP)/α_2-macroglobulin receptor (49, 50). The complete cDNA sequence of gp/330/600 was recently cloned and was found to encode a protein of 4660 amino acids with a molecular weight of 516.715 (without glycosylation) (51). Based on these data the term gp600, or "megalin" (51), seems the most appropriate. It has been also shown that megalin is a receptor with multiple ligands including low-density lipoproteins (52), plasminogen (53), plasminogen activator-inhibitor complex (54, 55), lipoprotein lipase and apoprotein E-enriched βVLDL (54–56), aprotin (57), lactoferrin (54) and Ca^{2+} (58). Most of the ligands also bound to LRP, but megalin and LRP were not co-localized in tissues, suggesting different specialized functions (59). Recently, it was shown that megalin serve as receptor for polybasic drugs, including gentamycin and polymyxin B. These aminoglycoside antibiotics, used for the treatment of gram-negative infections, are taken up in clathrin-coated pits by megalin-mediated endocytosis, and are subsequently delivered to lysosomes. Since these antibiotics are nephrotoxic and ototoxic, it is possible that megalin may be involved in the severe side effects of aminoglycoside therapy (60).

A 45-kDa rat protein, with cDNA distinct from the cDNA of megalin, is the second antigenic target of Heymann nephritis (61, 62). 39-kDa human (56) and mouse (57) homologs were also identified. Because of their ability to bind to LPR/α_2-macroglobulin receptor, the 39- to 45-kDa forms were named "receptor-associated protein" (RAP). Both megalin and RAP coprecipitated when Heymann Ag was purified by lectin chromatography or using antibodies eluted from nephritic kidney to screen an expression library. Moreover, both proteins precipitated either with anti-RAP or anti-megalin Abs. The interaction between megalin

and RAP is Ca^{2+} dependent since binding was abolished in presence of EDTA (63). Active immunization with RAP fusion protein made in bacteria, or injection of anti-RAP Abs, induced formation of subepithelial immune deposits (64, 65). Thus, it is evident that the antigen of Heymann nephritis is a complex of megalin and RAP, and the two macromolecules probably express distinct epitopes (64–66).

Megalin is mainly localized in the coated pits of glomerular and tubular epithelial cells, and co-localize with clathrin, in agreement with its role as receptor. Megalin is also found in the Golgi apparatus, in the endoplasmic reticulum, and in multivesicular bodies of glomerular visceral epithelial cells (67). RAP is found predominantly in rough endoplasmic reticulum (68). Studies of biosynthesis, assembly, and trafficking of megalin and RAP in cell cultures show that megalin is an N-linked glycoprotein that undergoes folding and maturation in the endoplasmic reticulum. Within 15 min after biosynthesis megalin associates with RAP, and this association continues in the Golgi during formation of the mature glycoprotein. Then, megalin and RAP move together from the Golgi to the cell surface, though RAP is not readily detectable on the plasma membrane by immunoelectron microscopy. However, the rapid development of subepithelial deposits after injection of anti-RAP Abs is in agreement with the hypothesis that *in vivo* RAP is expressed at the basal surface of glomerular epithelial cells. In conclusion, RAP seems to function as a molecular chaperone, interacting with megalin and assisting in its folding and oligomerization (66, 69).

Pathogenesis. The glomerular lesions responsible for proteinuria and nephrotic syndrome are initiated by interaction of circulating Abs with megalin mainly expressed in the coated pits at the base of the foot processes (70). Ag-Ab complexes are shed in the lamina rara externa. Concomitantly, there is an increased synthesis of megalin that allows its persistent expression at the cell surface, with continuous shedding of immune complexes (47). Whether anti-RAP Abs induce formation of subepithelial immune deposits by a similar mechanism is not known.

The mediators involved in the pathogenesis of active Heymann nephritis are largely unknown. In passive Heymann nephritis induced by Fx1A Abs, proteinuria is mediated by activation of complement and by assembly and insertion of the terminal lytic complex C5b-9 into the plasma membrane of glomerular visceral epithelial cells (71). Complement fixation and proteinuria depend on anti-glycolipid Abs contained in the anti-Fx1A serum, and glycolipids are present in glomerular immune deposits (72). Immunohistological studies have shown that C5b-9 is first inserted into the plasma membrane at the level of the coated pits, where immune complex formation initiates, and then is endocytosed and transported by multivesicular bodies through the epithelial cells, and exocytosed into the urinary space (73). During the heterologous phase of passive Heymann nephritis urinary escretion of C5b-9 correlates with the amount of Abs and C3 fixed to glomeruli. During the autologous phase C5b-9 present in the urine correlates with the amount of autologous Abs. Therefore, urinary excretion of C5b-9 may be an index of on-going immunologic glomerular injury (74). The sublytic lesions induced by C5b-9 promote activation of proteases and formation of oxygen species and other mediators which contribute to increase the permeability of the glomerular capillary wall (75, 76).

Human idiopathic membranous glomerulonephritis. Abs to megalin identify a cross-reactive Ag in human proximal tubules, but not in glomerular epithelial cells (77). With a few exceptions, anti-brush border Abs are not demonstrable in patients' sera. In human tubules, granular deposits of IgG, similar to those seen in Heymann nephritis, are absent. Therefore, there is no evidence implicating brush border Ags, and more specifically a megalin homolog, in the pathogenesis of human membranous GN. This conclusion is also based on failure of immunoglobulins eluted from kidneys of a few patients with membranous GN to react *in vitro* with normal human brush border or glomerular cells. Despite numerous studies of the Heymann model the etiology and the pathogenesis of human idiopathic membranous GN remain unknown.

Diseases Induced Mainly by Circulating Antigen-Antibody Complexes

The most important diseases to be discussed are systemic lupus erythematosus, drug-induced autoimmunity, graft-versus-host (GVH) and host-versus-graft (HVG) reactions. In laboratory animals and in humans, immune complexes and cryoglobulins are present in the circulation and in glomerular immune deposits, in agreement with the hypothesis that circulating immune complexes are the principal effectors. In recent years, however, it has been shown that autoantibodies of patients and animals with lupus or lupus-like syndromes can react with structural Ags of the kidney. Therefore, it is conceivable that autoantibodies and immune complexes cooperate in the development of the lesions.

Systemic Lupus Erythematosus. *Mice with spontaneous lupus-like disease.* The most revealing insight into the pathogenesis of murine lupus nephritis derives from genetic and immunologic studies of MRL and MRL-lpr/lpr mice. Wild-type MRL mice develop a late and mild GN. In contrast, in MRL mice with mutations at the lpr or gld locus, progressive accumulation of $CD4^-CD8^-TCR\alpha\beta^+CD3^+$ (double negative) T cells in lymphatic organs, anti-nuclear Abs, rheumatoid factors, and severe immune complex GN and TIN

develop at 3–4 months of age, and lead to death within 6 months (78). The basic abnormality is a failure of apoptotic deletion of $CD4^+$ T cells and B cells, resulting in loss of self-tolerance and autoimmunity (79). In normal conditions some mature autoreactive T cells migrate in peripheral tissues where they encounter self-Ags and undergo apoptotic death triggered by interaction of complementary ligand-receptor pairs proteins, CD95 (Fas/APO-1) and Fas ligand (FasL). Fas is a 45-kDa protein expressed by mature T cells and other cells in various organs, including thymus, liver, ovary, lung, and heart. FasL is a 40-kDa trimer expressed by activated lymphocytes and, to a lesser degree, activated thymocytes. The Fas-FasL interaction induces apoptotic cell death by cytoplasmic signals involving several cysteine proteases activating lytic enzymes (80).

MRL-lpr/lpr mice carry a mutation in the second intron of the Fas gene, which inhibits Fas expression (81). The manifestations of the disease do not occur in MRL-lpr/lpr transgenic mice with a normal Fas gene (82). Mice heterozygous for the gld gene, which codes for a point mutation in the FasL gene, develop a disease similar to that of MRL-lpr/lpr mice (83). Failure of Fas-mediated deletion of mature $CD4^+$ T cells at the periphery allows survival of autoreactive T cells with inappropriate stimulation of B cells. The role of $CD4^+$ T cells in the pathogenesis of the disease is confirmed by the prophylactic effect of CD4 monoclonal Abs (84), whereas CD8 Abs are ineffective (85). Reagents which interfere with T cell activation, such as CTLA-4 fusion protein (86), or which block T cell help to B cells, as anti-CD40 Abs (87), also exert beneficial effects. Moreover, the disease does not occur in MHC-deficient MRL-lpr/lpr mice devoid of $CD4^+$ T cells (88).

Another mechanism of autoimmunity is the failure to delete autoreactive B cells. Normal B cells exposed to autoantigens during development carry a "desensitized" immunoglobulin receptor at their surface (anergic B cells); upon interaction with Ag-specific $CD4^+$ T lymphocytes these B cells are eliminated by Fas-mediated apoptosis. In contrast, in lpr/lpr Fas-deficient B cells exposed to autoantigens and Ag-specific $CD4^+$ lymphocytes, apoptosis does not occur, and proliferation of B cells and synthesis of high affinity autoantibodies ensue (89). Failure to delete Fas-deficient autoreactive B cells may be associated with an upregulation of Bcl-2, an oncogene that encodes a mitochondrial membrane associated-protein which inhibits some forms of apoptosis (90).

Nucleosomes (formed by eight histone proteins and two spherical loops of 146 bp of DNA) elicit the main autoimmune response (91, 92). Apoptosis is the most likely source of nucleosomes (93). Nucleosomes and anti-histone/DNA Abs are present in the circulation, free or as immune complexes (91, 94) which can be entrapped in glomerular capillaries. Since histones are cationic, they can also bind to the fixed negative charges of the GBM, and fix polydisperse anionic DNA fragments, thereby forming the nucleus for *in situ* formation of histone/DNA immune complexes (95–97). Moreover, monoclonal Abs derived from MRL-lpr/lpr spleen cell hybridomas bind to Ags localized on the plasma membranes of glomerular cells or in the mesangial matrix, and can induce local formation of immune deposits (98). A direct interaction of autoantibodies with structural antigens of the glomerular capillary walls may increase their permeability and cooperate in the deposition of circulating histone/DNA immune complexes.

Cryoglobulins and rheumatoid factors participate in the development of glomerular lesions. The subendothelial deposits of immunoglobulins—corresponding to the "wire-loop" lesions of the old literature—are mainly formed by cryoprecipitable IgG_3 (99, 100). "Wire-loops," deposition of mouse C3, and proliferation of glomerular cells develop in wild-type mice injected with IgG_3 monoclonal Abs synthesized by MRL-lpr/lpr spleen cell hybridomas. In these mice, the development of GN is the consequence of physicochemical rather than immunologic properties of IgG_3, and occurs without formation of immune complexes and rheumatoid factors (101). Nevertheless, in mice with spontaneous lupus-like nephritis circulating IgG anti-nuclear Abs possess rheumatoid factor activity; nephritic glomeruli contain anti-nuclear IgG and IgG rheumatoid factors, which probably cross-react, forming multilayer aggregates that increase the size of immune deposits (102).

Another mechanism of tissue damage is the *in vivo* binding of Abs to the nuclei of renal cells. When tissue from nephritic mice is examined by immunofluorescence, deposits of mouse IgG are frequently found in the nuclei, indicating that the Abs are internalized. This phenomenon is reproduced by transfer of anti-DNA hybridoma cells or monoclonal anti-DNA Abs in naive mice; the nuclear binding of mouse IgG is associated with proliferation of glomerular cells and proteinuria (103).

Human disease. Defects in expression or function of Fas have not been found, but apoptosis appears abnormal, as suggested by increased concentrations of soluble Fas, increased expression of bcl-2, and increased apoptosis when lymphocytes are cultured *in vitro* (104). In some children with lymphoproliferative and autoimmune manifestations, not including renal disease, the gene encoding Fas has a large deletion, the activated T lymphocytes do not express Fas at their surface (or express it in reduced amount), and Fas-mediated apoptosis is impaired (105). Therefore, further studies are required to establish whether a ge-

netic deficiency of Fas-FasL—similar to that of murine lpr mutation—provides a molecular basis for human lupus.

Oligonucleosomes (106) and anti-histone/DNA Abs (107) are present in the sera of patients with active nephritis. Moreover, histones and anti-histone/DNA antibodies—presumably immune complexes passively entrapped from the circulation—have been visualized in glomeruli by immunohistochemical methods (108). As in mice with spontaneous lupus-like nephritis (95, 96), histones may contribute to *in situ* formation of immune complexes.

Drug-induced Autoimmunity. Mercuric chloride ($HgCl_2$), gold and D-penicillamine can provoke autoimmune renal diseases in man. Mercury was formerly used for the treatment of syphilis and may have induced membranous GN in some patients. More recently membranous GN and nephrotic syndrome have been reported in a few persons who applied mercury to their skin or absorbed it *via* gastrointestinal tract (109, 110). The disease was presumably autoimmune in nature, but the antigens and the pathogenetic mechanisms remain unknown. Gold and D-penicillamine are used in the therapy of rheumatoid arthritis, and this treatment is occasionally associated with membranous or rapidly progressive GN (17); the etiology and the pathogenesis of these disease are also unknown. Other drugs, such as hydralazine, procainamide or isoniazid may generate antibodies reactive with DNA, histones, and other nuclear antigens but, in general, these autoimmune responses are not associated with renal diseases.

The effect of $HgCl_2$ has been studied in laboratory animals. As described above, rats injected with non-toxic doses of $HgCl_2$ develop antibodies to laminin and other components of the extra-cellular matrix. Mice carrying the H-2d haplotype are resistant to $HgCl_2$. In contrast, mice with the H-2a haplotype, such as A.SW, SJL, and B10.S, develop an autoimmune response to nuclear antigens, especially to a 34-kDa nucleolar protein of the U3 RNP particle, named fibrillarin (111, 112). Anti-nuclear and -nucleolar antibodies are associated with granular deposition of mouse IgG in the walls of the capillaries and larger vessels in many organs, in agreement with the hypothesis that the lesions are induced by circulating immune complexes. However, the possibility that the autoantibodies recognize structural antigens, such as those of the mesangial matrix, cannot be excluded. It is noteworthy that the autoantibodies of patients with scleroderma are specific for fibrillarin (113).

T lymphocytes exert a critical role in the pathogenesis of drug-induced autoimmune response, as shown by the ability of $HgCl_2$ autoreactive T cells to transfer the disease to irradiated syngeneic recipients, and by failure of T cell–deficient mice to develop lupus-like lesions (114). Several lines of evidence indicate that $HgCl_2$ preferentially stimulates Th2 lymphocytes. $HgCl_2$ increases the expression of IL-4 transcripts in T cells of susceptible mice, and injections of anti–IL-4 mAbs inhibits the development of the disease. *In vitro* $HgCl_2$ inhibits the production of IL-2, and the development of T cell–mediated hypersensitivity reactions. Moreover, in rats $HgCl_2$ prevents the development of uveoretinitis, which is Th1-mediated, and increases the number of IL-4 producing cells in the spleen, whereas the production of IL-2 and IFN-γ is suppressed. In addition, $HgCl_2$ increases the expression of MHC class II molecules on B cells (115).

There is only a partial understanding of how certain drugs initiate lupus-like autoimmune reactions, and preferentially stimulate Th2 cells. *In vitro* these drugs exert a cytotoxic effect only in presence of activated neutrophils. The drugs undergo chemical transformation to cytotoxic products through the enzymatic action of myeloperoxidase. Studies of inhibition kinetics suggest that the drugs serve as substratum for the enzymatic reaction. The resulting metabolites may act as ligands of lymphocyte receptors, inducing a dysregulation of the immune response (116). Murine $CD4^+$ T cells treated *in vitro* with DNA methylation inhibitors and adoptively transferred to syngeneic recipients generate anti-DNA Abs and severe immune complex GN and pneumonitis (117, 118). In mice, $HgCl_2$ apparently induces denaturation of fibrillarin and presentation by antigen-presenting cells of a novel Hg^{2+}/fibrillarin epitope, upregulation of the presentation of unaltered fibrillarin epitopes, with consequent activation of Hg^{2+}-specific, as well as autoreactive $CD4^+$ T cells (119). Other studies show how cytokines modulate the acquisition of T helper phenotype. Th2 cells are preferentially stimulated by IL-4 and IL-10, but not by IFN-γ, because the inhibitory signal transduction factor (STF-IFN-γ) is activated. In contrast, Th1 cells proliferate in the presence of IFN-γ because the second chain of IFN-γ receptor (IFN-γRb) is absent, and the inhibitory STF-INF-γ is not activated (120).

Allogeneic Interactions. The study of allogeneic interactions, mainly focused on transplantation of bone marrow and higher vascularized organs (121), provides a better understanding of the pathogenesis of autoimmune renal diseases. Murine GVH and HVG reactions are characterized by autoimmune responses similar to those of murine lupus, including anti-nuclear Abs, membranous and proliferative GN and TIN, progressive glomerulosclerosis, proteinuria and renal failure (1). The glomerular and vascular lesions are probably due to deposition of circulating DNA-anti-DNA complexes and to Abs binding to structural Ags of the vessels. Abs to laminin and type IV collagen are present in the circulation and eluted from glomeruli of nephritic mice (122). Other Abs react with megalin and

CD26, which are expressed on the plasma membranes of the renal brush border; CD26 is also expressed on the plasma membrane of glomerular endothelial and epithelial cells, and is the main antigenic target of murine membranous GN (123).

The initial event of GVH reaction is the recognition of MHC class II alloantigens of host B cells by donor $CD4^+$ T cells with consequent proliferation of B cells and synthesis of high-affinity autoantibodies (115, 124). Interference with T and B cells costimulatory molecules CTLA-4 (125) or CD40 (126) prevent or ameliorates the lethality of GVH reaction. An increased amount of IL-4 and IL-10 mRNA transcripts is found in the spleen of GVH mice, and IL-4 enhances the expression of class II molecules on B cells. *In vitro* the amount of IL-4 produced by T cells is increased, whereas the production of IL-2 and IFN-γ is diminished. *In vivo* IFN-γ or mAbs to IL-4 decrease the levels of serum IgE and IgG1, but not of IgG2a; moreover, the beginning of proteinuria is delayed and the time of survival is prolonged. In contrast, mAbs to IFN-γ increase serum IgE and IgG1 and decrease IgG2a. These results show that the donor $CD4^+$ cells interacting with host B cells are mainly Th2 (115). Other changes occur in the epithelial and lymphoid compartments of the thymus with abnormal maturation and function of thymocytes, decreased expression of class II molecules, defective TCR upregulation on $CD4^+CD8^-$ cells, and alterations in TCR usage, reflecting an aberrant thymic selection (115).

The serological and histological manifestations of the HVG reaction occurring in BALB/c mice neonatally injected with spleen cells of (C57BL/6 × BALB/c) F1 hybrids are similar to those of the GVH reaction. The recipients develop partial tolerance to donor alloantigens, with clonal deletion of donor-specific cytolytic T cells and some Th1 clones. Other T cells, however, escape deletion and stimulate B cells to synthesize anti-nuclear and anti-basement membrane Abs. Surviving T cells synthesize IL-4, but not IL-2, suggesting that they are Th2 (127). Additional evidence for the prevalent pathogenic role of Th2 cells is provided by the beneficial effect of mAbs to IL-4 (128). B cells express increased amount of class II molecules, and preferentially synthesize IgG1 and IgE isotypes. As in drug-induced renal diseases, the apparent resistance of Th2 cells to develop a complete HVG neonatal unresponsiveness may be due to a cytokine-mediated selective activation of inhibitory signal transduction factors (120).

Glomerulo-vascular Diseases Associated with Anti-neutrophil Cytoplasmic Antibodies

The association of necrotizing GN and anti-neutrophil cytoplasmic Abs (ANCA) was first described by Davies *et al.* in 1982 (129). In 1985–1988, it became apparent that ANCA were sensitive markers for Wegener's granulomatosis (130) and microscopic polyarteritis (131). In 1988 Falk and Jennette showed that anti-myeloperoxidase (MPO) Abs are frequently found in the circulation of patients with necrotizing and crescentic GN characterized by minimal or no immune deposits (pauci-immune GN) (132). In 1989 Goldschmeding *et al.* (133) and Niles *et al.* (134) reported that some ANCA recognize proteinase 3 (PR3) as antigenic target. Anti-MPO and anti-PR3 Abs identify a category of vasculitides that can be distinguished from other diseases, and include Wegener's granulomatosis, microscopic polyarteritis, pauci-immune necrotizing crescentic GN and vasculitis with or without hemorrhagic pneumonitis, allergic granulomatosis (Churg and Strauss syndrome), and overlapping forms of these vasculitides. Anti-PR3 Abs are more frequent in Wegener's granulomatosis, and anti-MPO Abs in pauci-immune idiopathic crescentic GN and Churg-Strauss syndrome (135). Some patients have ANCA and anti-GBM Abs (136–139). In a recent study including 36 patients with Goodpasture syndrome, 90% had circulating autoantibodies: 45% were PR3-ANCA, 20% MPO-ANCA, and 5% PR3 + MPO-ANCA (total 70%); and 7.5% were anti-GBM + MPO-ANCA, and 15% anti-GBM. Anti-GBM antibodies were associated with a poor outcome (140). In another study including 88 patients, 48 had ANCA, six had anti-α3(IV) Abs, and seven had both. In patients with ANCA, the prevalent lesions were pauci-immune necrotizing and crescentic GN and pulmonary capillaritis. Only eight patients had evidence of Wegener's granulomatosis (141). These findings show that Goodpasture syndrome is more frequently associated with ANCA than with anti-α3(IV) Abs, but anti-GBM antibodies are associated with a worse prognosis.

Antigens. MPO, a neutrophil cationic protein with a molecular weight of 146 kDa, plays an important role in the generation of oxygen radicals (142). Anti-MPO Abs recognize native, but not denatured, MPO (143, 144) and are directed against multiple epitopes, as shown by studies of inhibition of ANCA with different monoclonal Abs (145). The relevant epitopes are not yet identified, and most MPO Abs do not inactivate the enzyme (143, 144).

PR3 is one of the three (the other two being elastase and cathepsin G) serine proteases present in the azurophilic granules of granulocytes and monocytes. PR3 is a 29-kDa glycoprotein (146) physiologically inhibited by α_1-antitrypsin (147). PR3 Abs inhibit the inactivation of PR3 by α_1-antitrypsin, suggesting that they recognize epitopes near the site of enzymatic activity (148).

Sera from some patients with ANCA do not react with MPO or PR3 but with other constituents of neutrophil granules, such as lactoferrin (149), lysozyme

(150), elastase (133), cathepsin G (151), or β-glucoronidase (152). In general, these Ags were identified by ELISA and not by additional Western blotting and immunoprecipitation. For the most part, these Abs have not been shown to be specific for patients with vasculitic syndromes associated with Abs to MPO or PR3.

Recently it was reported that sera from some ANCA-positive patients with necrotizing and crescentic GN reacted with 170- and 80- to 110-kDa membrane protein fractions prepared from human neutrophils, and with a 130-kDa glycoprotein isolated from human glomeruli. gp170/80–110 is the lysosomal-associated membrane protein 2 (h-lamp-2), expressed in the granule membrane of resting neutrophils. gp130 is localized at the surface of endothelial cells in human glomeruli and interstitial capillaries. h-lamp-2 and the endothelial Ag appeared immunologically cross-reactive (153).

Pathogenesis. The close association between anti-MPO and anti-PR3 Abs and necrotizing vasculitis, and the rise in titer that precedes most clinical flares of the disease (though not all rises in titer are accompanied by a flare) argue for a pathogenic role of ANCA. This role, however, is not easily reconcilable with histologic changes more reminiscent of cell-mediated than humoral hypersensitivity reactions. Furthermore, some patients with limited Wegener's granulomatosis have negative tests for ANCA. Conversely, some patients who are in prolonged remission following immunosuppressive therapy continue to have high titers of ANCA. Several, not mutually exclusive, hypotheses are considered. Binding of PR3 Abs to their targets impairs the inactivation of the enzyme by its natural inhibitor, α_1-antitrypsin. In patients with Wegener's granulomatosis the activity of the disease correlates with the amount of inhibitory activity in the serum rather than with the level of Abs (135). The deficiency, or inactivation of α_1-antitrypsin could inhibit natural inactivators of chemotactic factors, with inordinate delivery of neutrophils and their enzymes to the sites of inflammation (154). The hypothesis that interference with enzyme inactivation contributes to the pathogenesis of vasculitis is supported by the notion that necrotizing and crescentic GN and vasculitis occur in patients with α_1-antitrypsin deficiency (155, 156).

Other studies have been focused on the interaction of ANCA with neutrophils, and leukocytes with vascular endothelium. Experiments performed *in vitro* showed that pro-inflammatory cytokines, such as TNF-α, IL-1, and IFN-γ, induce expression of PR3 (157) and gp170/80–110 (153) at the surface of neutrophils. Circulating neutrophils with surface PR3 were detected in patients with septic shock or active Wegener's granulomatosis (158). The interaction of ANCA with neutrophil surface targets enhanced the formation of oxygen radicals, activated protein kinase C, and increased the exocytosis of cytoplasmic granules (135, 157). The Ag-Ab interaction required both Fab and Fc portions of the Ab molecule because blocking of neutrophil FcII receptors inhibited the activation of neutrophils (159). Release of MPO and PR3 apparently did not occur in the circulation but rather in the extravascular space. In rats injected with a small amount of nephrotoxic serum anti-rat MPO Abs enhanced the severity of GN by releasing MPO from neutrophils; the enzyme was found in glomerular structures (160). In patients with Wegener's granulomatosis PR3, MPO, and elastase were visualized in tissues, in proximity of degranulated cells at the site of the lesions (161). Released cytoplasmic granules may induce tissue damage by direct enzymatic activity, by activation of the MPO-hydrogen peroxide-halide system which generates hypoclorous acid (162, 163), and by formation of immune complexes (160).

Extravasation of leukocytes requires their interaction with activated vascular endothelium (164). Endothelial cell activation and damage may be induced by anti-endothelial Abs that are frequently associated with ANCA (165, 166). One of such Ab is h-lamp-2, cross-reactive with a 130-kDa endothelial glycoprotein (153). Endothelial cell activation and injury might also be elicited by fixation of anti-PR3 Abs to PR3 on endothelial cells. Studies performed by polymerase chain reaction, Western blotting, and cyto-ELISA, showed that pro-inflammatory cytokines increase the expression of PR3 in the cytoplasm of human endothelial cells in culture, and promote PR3 translocation at their surface (167). When PR3 is accessible, specific Abs may induce endothelial damage by a mechanism of Ab-dependent cellular cytotoxicity (168).

The observation that—besides neutrophils—cellular infiltrates contain monocytes and $CD4^+$ T cells, the formation of granulomas in patients with Wegener's granulomatosis, and the scant deposition of immunoglobulins, suggest that cell-mediated immunity is involved in the initiation and amplification of the lesions. Elevated levels of soluble IL-2 receptors were found in patients with Wegener's granulomatosis (169). The proliferative response of circulating T lymphocytes to PR3 and MPO was stronger in patients with Wegener's granulomatosis than in controls, suggesting presence of autoreactive, PR3-specific, T cells (170, 171). Other investigators, however, failed to confirm these findings (172), and further studies are necessary.

Progress in understanding the pathogenesis of ANCA-associated vasculitis has been hampered by lack of experimental models. Laboratory animals actively immunized with MPO or PR3 have failed to develop necrotizing vasculitides. Injections of ANCA in suitable experimental animals have also failed to induce lesions (135). In rats, active immunization with

MPO and subsequent perfusion of one kidney with MPO, has provided controversial results (173, 174). Rapidly progressive GN and small vessel vasculitis was observed in mice (SCG/Kj) derived from (BXSB/Mp × MRL/Mp-lpr/lpr) F1 hybrid mice by brother × sister mating, according to a selection based on the highest frequency of glomerular crescents. Crescent formation involved 97% of glomeruli at 9 weeks of age, and the life span was reduced. Vascular lesions were found in many organs but spared the kidneys. Immune deposits were minimal or absent, as in patients with pauci-immune rapidly progressive GN and vasculitis (175). If ANCA are detected in their sera, SCG/Kj mice may become a valuable model for the study of human autoimmune GN and vasculitis.

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