Review Article

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Cellular Immunity in Glomerulonephritis

HOWARD M. FILLIT, MD,
and JOHN B. ZABRISKIE, MD

From The Rockefeller University, New York, New York

IMMUNOLOGIC MECHANISMS have been thought to be responsible for glomerular disease since the turn of the century.[84,89] Since then, thorough investigations have clearly defined a role for humoral immune mechanisms, including immune complexes and anti-glomerular basement membrane antibody, in the pathogenesis of glomerular disease.[16,27,104,151] These investigations were made possible by the application of a number of technologic advances in humoral immunology and their application to the study of animal models and the human disease. Quantitative methods for the measurement of antibodies have existed since the turn of the century.[66] The advent of immunofluorescence[18,153] and assays for the detection of immune complexes[12,53,140] have added a new dimension to our understanding of humoral nephroimmunology. As a result of advances in the study of humoral immune mechanisms, and because of the lack of clear evidence for an observable mononuclear cell infiltration in most forms of glomerulonephritis, investigators thought a role for cellular immune effector mechanisms in glomerular disease was unlikely.[26] Perhaps, when seen in a historical light, the prejudice of renal immunologists in assuming that all human glomerular disease is mediated by humoral mechanisms is therefore understandable. Indeed, assays for the measurement and detection of cellular immune sensitization to antigen were really introduced for the first time in the mid 1960s.[43,99] Furthermore, the exact role and importance of immune complexes and anti-glomerular basement membrane antibody in renal disease has recently been reexamined.[21,51] In all likelihood, both humoral and cellular immune mechanisms are important in the development of glomerulonephritis, as is true in many other chronic immune-mediated diseases.[143] Thus, the application of advances in cellular immunology to glomerular disease has just begun, and cellular nephroimmunology is in its relative infancy. In this review, we will present the current evidence and concepts regarding the hypothesis that cellular immune mechanisms are important in the pathogenesis of glomerular disease.

It would be beyond the scope of this review to provide the reader with a basic course in cellular immunology. Such reviews are available.[47,110,114] Readers probably have some understanding of the nature and scope of cellular immunity and its importance in both homeostatic and pathologic processes. In antitumor immunity, antiviral immunity and in immunity to other chronic infections such as tuberculosis, cellular immunity plays a crucial role. As in humoral immunity, the inflammatory cascades and resultant tissue destruction incited by cellular immune mechanisms may cause local pathologic changes in tissue, such as granuloma formation, fibrosis, and scarring. In addition, again as in humoral immunity, cellular autoimmunity directed against native or altered host antigens may occur.

However, in contrast to humoral immunity, the main effector function in cellular immunity is provided by the T lymphocyte, which is easily distinguished *in vitro* by its surface markers from the antibody producing B lymphocyte. T lymphocytes mediate cellular immune function in a number of ways. T lymphocytes may cause tissue injury directly. The T lymphocyte is capable of killing host cells by itself (lymphocyte-mediated cytotoxicity), in conjunction with antibody but not complement (antibody-dependent cell-mediated cytotoxicity), via the activation of macrophages, and via the release of a number of lymphokines with varied functions, such as lymphotox-

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Address reprint requests to Howard Fillit, MD, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

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ins (which are directly cytotoxic) and migration-inhibition factor and T-cell growth factor (which have recruitment and amplication roles in the development of a cellular immune reaction).

In addition, the T lymphocyte has important immunoregulatory functions. Helper and suppressor T lymphocytes directly regulate the function of B cells and thus antibody production. Abnormalities in T lymphocyte regulatory function may permit the development of autoimmunity (via humoral or cellular mechanisms) or lead to an inappropriate antibody response with pathologic consequences. Finally, the T lymphocyte has important regulatory effects on the function of macrophages, via the release of lymphokines, which direct the macrophage to an antigenic site and activate the macrophage. The macrophage may then perform a number of effector functions of its own, including phagocytosis and cytotoxicity.

The monocyte is the circulating precursor of the tissue macrophage. The macrophage is important in the initiation and development of the immune response by binding antigen and processing it for recognition by the T lymphocyte. The macrophage also releases a number of immunoregulatory molecules. Finally, like the polymorphonuclear leukocyte, the macrophage may cause local tissue destruction by the release of a number of enzymes during the process of phagocytosis.

The result of antigen-directed reactivity of T cells and macrophages in a cell-mediated immune reaction is a lesion known as the delayed hypersensitivity reaction. A full discussion of the characteristics of delayed hypersensitivity, or cell-mediated immunity, is beyond the scope of this review but may be found elsewhere.[118] Important differences exist between humoral reactions and cell-mediated reactions with regard to their mode of induction, the type of antigens to which these systems react, and the pace of the reaction. Histologically, the cell-mediated reaction is characterized by a predominance of mononuclear cells with varying proportions of macrophages and T cells, but not plasma cells. Depending on the pace of the cell-mediated reaction, the histologic reaction may vary from only a few mononuclear cells to a massive infiltration with true granuloma formation or necrosis. It is difficult to identify a cell-mediated reaction *in vivo*, although new methods involving the use of monoclonal antibodies may facilitate the identification of T cells and macrophages in tissue. The demonstration of *in vitro* reactivity of lymphocytes to an antigen does not prove that cell-mediated immunity is involved in vivo. Although the demonstration of antigen-specific cytotoxic lymphocytes *in vitro* is highly suggestive of cell-mediated immunity, a true pathogenic role for cell-mediated immunity can only really be proven by the transfer of T lymphocytes from a sensitized donor to a normal recipient, with subsequent tissue damage—a procedure that is obviously difficult to perform in man. Finally, it should be noted that immune-mediated diseases are often the result of a combination of both humoral and cell-mediated immunity, and the presence of one type of immunity does not exclude the other.

In summary, cellular immune effector mechanisms elucidated mainly over the past 20 years have clearly been shown to have important protective immune functions and to contribute to the development of pathologic immune processes. In this review, we will attempt to use cellular immunity as a general term referring to the role of T cells in immune reactions, including both their regulatory functions on humoral immunity and their effector functions. We will refer to cell-mediated immunity as those immune reactions which involve T cells and macrophages as direct agents of injury.

Mononuclear Cells and Glomerulonephritis

With this brief background in mind, let us return to our theme concerning the role of cellular immunity in glomerular disease. An increase in the cellularity of glomeruli of patients with glomerulonephritis (GN) is a well-established observation.[54] However, whether this increased cellularity is due to proliferation of intrinsic glomerular cells or to invading mononuclear cells from the circulation has long been controversial. As early as 1929, cells that were thought to be invading mononuclear cells were noted, especially in cases of chronic proliferative glomerulonephritis.[103] In 1951, Jones[65] concluded that invading mononuclear cells were present in glomeruli of patients dying of acute poststreptococcal glomerulonephritis and postulated that these cells were related to the disease process. Perhaps the first experimental investigations on the origin of the proliferating mononuclear cells was by Okumura[111] in 1971. Applying electron microscopic (EM) examination to the study of the passive serum sickness model of glomerulonephritis, he demonstrated that the proliferative changes occurring in the first 1–2 hours consisted mainly of mesangial hypercellularity, endothelial swelling, and accumulation of polymorphonuclear cells. However, between 4 and 12 hours these polymorphonuclear cells disappeared, and the predominant intraluminal cell was mononuclear in appearance. Extending these observations to the nephrotoxic model of glomerulonephritis, Kondo[72,73] noted that the predominant cell type associated with the onset of proteinuria was a monocyte, identified on EM examination by its numerous lysozymes, vacuoles, and ves-

icles and a prominent Golgi apparatus. In addition, lymphocytes and plasma cells were identified in the crescents of Bowman's space. Other investigators have made similar observations[67,109] Thus, these studies attempted to identify clearly the presence of monocytes and lymphocytes in the glomeruli of man and in animals with experimentally induced glomerulonephritis.

Within the decade a series of articles appeared that more clearly defined the role these mononuclear cells play in glomerular lesions. Using an accelerated form of nephrotoxic nephritis (NTN), Schreiner et al[127] showed that in the *early* stage of the disease a prominent infiltration of polymorphonuclear cells was seen in association with the onset of early proteinuria. However, by 4 days only mononuclear cells were seen in the capillary lumen, coinciding with a second phase of proteinuria. The mononuclear cells were identified as monocytes by EM criteria. To determine whether these monocytes played an active role in glomerular pathology, these investigators exposed animals to whole-body irradiation (which kills mainly monocytes and not polymorphonuclear cells). Early proteinuria persisted in association with polymorphonuclear cells, but *late* proteinuria and monocytes were now absent. To demonstrate that the proliferating mononuclear cells were extrinsic to the glomeruli, the investigators preimmunized animals with tritiated thymidine, which radiolabeled proliferating cells (predominantly monocytes of bone marrow origin) prior to the induction of nephritis. The results demonstrated that in 1–2 days a significant number of labeled cells were in the glomeruli of the experimental animals and not in the controls. Thus, in this model, invading extrinsic mononuclear cells that were identified as monocytes contributed to glomerular hypercellularity, and these cells appeared to be intimately associated with late-phase proteinuria.

The role of mononuclear cells in causing glomerular damage was emphasized further in the studies of Bhan et al.[7,8] In the accelerated NTN model[7] they showed that when lymph node cells from animals immunized to rabbit IgG were injected into animals that had received subnephritogenic doses of rabbit anti-rat kidney serum, glomerular lesions developed, while the controls that received lymphocytes from animals immunized to albumin did not have glomerular lesions. The lesions consisted of segmental hypercellularity, with necrosis in some of the glomeruli. Tritiated thymidine experiments demonstrated the presence of proliferating cells in the glomeruli of animals receiving transferred lymph node cells from donor animals sensitized to the rabbit IgG. Both intrinsic glomerular cell proliferation and invading mononuclear cells appeared to account for glomerular hypercellularity. In another series of experiments these investigators established that transferred T cells from lymph nodes of immunospecifically sensitized animals were capable of inducing the hypercellular reaction.

Using another paradigm,[8] these investigators injected preformed immune complexes containing homologous antibodies and foreign antigen into rabbits intravenously and then demonstrated localization of the complexes to the glomeruli. Subsequently, lymphocytes, and in other experiments purified T cells, from donor animals sensitized to the injected foreign antigen were injected into recipient rabbits and were followed by an injection of tritiated thymidine. By histologic criteria, mesangial hypercellularity was seen only in animals given immune complexes and appropriately sensitized lymphocytes or purified T cells. Autoradiographs revealed increased numbers of labeled cells in the mesangial regions and the glomerular capillary loops of animals that received immune complexes and appropriately sensitized cells. Electron micrographs suggested that the increase in cellularity was mainly due to an influx of circulating mononuclear cells. Unfortunately, these experiments were not correlated with the actual development of proteinuria. In summary, Bhan's studies[7,8] demonstrated that in the accelerated NTN model, and in an immune complex model, immunologically specific sensitized lymphocytes are necessary for the induction of glomerular hypercellularity and probably are involved in the development of cell-mediated reaction in the glomeruli.

Other investigators[11,50] have employed the nude mouse, which lacks a thymus and therefore an effective T-cell system, to demonstrate the importance of an intact cellular immune system for the development of glomerulonephritis. In both an immune complex and an autoimmune model, these investigators have shown that nude mice have fewer proliferative changes in their glomeruli and less severe or no glomerulonephritis, probably because of a decline or absence of antibody production to relevant antigens in each model. Thus, an important role for the thymic-dependent T-cell system in the development of glomerulonephritis is suggested by these experiments. The thymic effect may be mediated via T-cell–dependent antibody responses or perhaps via thymus-dependent cellular immune effector mechanisms that remain to be elucidated.

Another approach to the problem of an important role for cellular mechanisms in renal disease have been experiments that have reexplored the acute serum sickness model of glomerulonephritis. By histologic observations, an increase in glomerular cellular-

ity was clearly noted to be associated with the immune clearance phase somewhat preceding the onset of proteinuria. At this point intraluminal monocytes were identified by EM study, and histochemical nonspecific esterase staining confirmed these findings.[64] Studies employing colloidal carbon suggested that the invading cells were phagocytic and had an immune clearance role.[123] Another recent study[80] demonstrated that anti-macrophage serum reduces the degree of glomerular hypercellularity and proteinuria in the acute serum sickness model.

However, in a heterologous immune complex model of GN, Bakker[5] suggested that cell-mediated immune responses were not critical to the development of proteinuria. In this model, cell-mediated responses to relevant antigens were detected by macrophage-inhibition factor production and delayed-type hypersensitivity skin reactions and were then suppressed by immunosuppression of the animals. Such immunosuppression given only during the autologous phase of the disease did not prevent proteinuria. However, immunosuppression given during the induction of the disease did prevent the development of cell-mediated sensitization and the autologous phase of proteinuria and glomerular deposition of autologous IgG. These results suggest that cellular immunity may be important during the induction phase in this particular model.

Striker[136] employed an interesting approach to the question of whether the mononuclear cells seen in hypercellular glomeruli were derived from resident mesangial cells or invading blood monocytes. Taking advantage of the morphologically obvious giant lysosomes of Chediak-Higashi (CH) mice, cross transplantation of bone marrow cells between normal mice and CH mice were performed. Monocytes with giant lysosomes became apparent in the peripheral blood of the normal mice. These animals were then given preformed immune complexes. When the kidneys were subsequently examined by electron microscopy, mesangial electron-dense deposits were noted. Infiltrating cells containing giant lysosomes were observed. These cells entered mesangial regions and contained the electron-dense deposits in their giant lysosomes. In addition, it was noted that normal CH mesangial cells did not contain giant lysosomes, suggesting they were not monocyte-derived cells. In summary, the results suggested that invading monocytes play an important role in the clearance of immune complex from the glomerulus.

A similar approach was taken by Schiffer.[125] Taking advantage of the presence of Y bodies in male cells, but not in female cells, these investigators searched for the presence of cells positive for Y bodies in a man who had received a transplanted human kidney from a woman. Y-body-positive cells were found in glomerular crescents, but not in transplanted kidneys with only mesangial hyperplasia, again suggesting that invading monocytes contribute to crescent formation but are not normally part of the mesangium.

Further confirmation regarding the presence of monocytes in glomerular pathology has recently come from tissue culture experiments. In this approach, glomeruli isolated by graded sieving are grown *in vitro*. Employing a classic nephrotoxic nephritis model, investigators[58,141] have induced the formation of crescents in rabbits by giving sheep anti-rabbit glomerular basement membrane (GBM) antiserum. In addition, sheep were immunized with rabbit GBM in order to induce an autoimmune (Steblay type) GN. Subsequently, by Day 14, crescents were observed. The glomeruli from these animals were cultured *in vitro*. Normally, only two cell types grow from cultured glomeruli: mesangial and epithelial cells.[42] However, a third cell type was reproducibly grown from crescentic glomeruli, and this cell was the predominant cell type in culture. Using a number of cell identification techniques, the cell was identified as a macrophage. The importance of deposited fibrin to the participation of macrophages in glomerular crescents was also demonstrated.[59] Defibrinated animals failed to develop crescents in this model. Although macrophages were cultured from both noncrescentic and crescentic glomeruli with proliferation, macrophages were only identified by EM examination in Bowman's space in animals that had not been defibrinated, suggesting that fibrin deposition in Bowman's space is an important stimulus for macrophage migration in crescent formation, but not with regard to macrophage accumulation within capillary loops.

These authors have also applied their technique to the study of human glomerular outgrowths obtained from biopsy material.[2] A number of different histologic lesions were studied. Generally, only glomeruli from patients with crescentic forms of GN grew cells that resembled macrophages. These cells were rarely seen in outgrowths of normal glomeruli. The cells were identified as macrophages by time lapse cinematography, ability to ingest yeast, and electron microscopy. By time lapse cinematography, the cells were seen to grow from the crescents and were the predominant cell type of the crescent.

More recently investigators have applied the techniques of glomerular cell culture to the study of acute and chronic serum sickness[60] as well. These studies demonstrated an important association of glomeru-

lar hypercellularity with the onset of proteinuria, immune elimination of antigen, and the deposition of IgG and C3 in glomeruli. The hypercellularity of the glomeruli was shown to be due to the presence of macrophages by glomerular cell culture, as well as by histochemical staining for nonspecific esterase and by electron microscopy, in both acute and chronic serum sickness models.

The question of whether mesangial cells are tissue macrophages with immune function has long been debated. Early studies by Farquhar[33] suggested that the mesangial cells have phagocytic abilities. However, more recent studies suggest that resident mesangial cells do not have phagocytic function. The advent of glomerular cell culture has permitted new approaches to this problem (reviewed in Foidart et al[42]). Mesangial cells in tissue culture do not appear to have phagocytic abilities, nor to express monocyte/macrophage cell surface markers. Indeed, the mesangial cells appear more closely related to smooth muscle cells and are seen to contract in response to vasoactive agents such as angiotensin and vasopressin.[3] However, a recent study suggests that a small subpopulation of mesangial cells express Ia markers and Fc receptors and have phagocytic abilities.[128] This observation, if confirmed, will have important implications for our understanding of local glomerular immunity, both cellular and humoral.

In an interesting and perhaps highly practical application of the search for elements of the cellular immune system in glomerulonephritis, Monga et al[107] studied 28 patients with chronic glomerulonephritis and histologic proliferation. These authors used the nonspecific esterase technique to stain the human biopsy specimens for the presence of monocytes. Patients with cryoglobulinemia and membranoproliferation, idiopathic membranoproliferative glomerulonephritis, and diffuse proliferative glomerulonephritis associated with systemic lupus erythematosus were studied. Monocytes were identified in biopsy material in a number of cases, particularly in patients with cryoglobulinemia. Another study by Magil[92] had similar results. An interesting conclusion in this study was the correlation of nonspecific esterase-positive cells in glomeruli with subendothelial deposits, but not in glomeruli with subepithelial or predominantly mesangial deposits. Thus, these studies showed that monocytes can be identified in the glomerular lesions of biopsy material from patients with GN.

In summary, studies during the past 10 years have clearly shown that circulating mononuclear cells infiltrate the glomerulus in a number of forms of experimental and human glomerulonephritis. The presence of monocytes was first suggested by histologic and electron-microscopic studies and more recently confirmed by glomerular cell culture techniques and other *in vivo* experimental manipulations, including tritium labeling of circulating mononuclear cells. By these techniques, monocytes have been identified in the glomeruli of animals with nephrotoxic serum nephritis, acute and chronic serum sickness, and in persons with crescentic and proliferative GN associated with cryoglobulinemia and systemic lupus erythematosus. Thus, macrophages probably contribute to glomerular hypercellularity. In addition, experimental manipulations, including the removal of monocytes by irradiation or anti-macrophage serum, clearly suggest a role for macrophages in causing proteinuria in both nephrotoxic serum nephritis and in acute and chronic serum sickness. Macrophages also appear to play a role in clearing immune complexes from the glomerulus, and this is probably their physiologic role. However, the primary stimulus for monocyte infiltration of the glomerulus is unknown. Possibilities include nonspecific infiltration mediated by complement-induced chemotaxis, monocyte Fc receptor activity associated with immune complex deposition, collagen peptide-induced chemotaxis,[116] or an antigen-specific event related to classic cellular immune processes mediated via the T lymphocyte. The observation of an Ia-positive mesangial cell has important implications for the development of a local antigen-specific cellular immune response and will probably be important to future investigations in this area. As will be noted in the next section, both organ-specific and antigen-specific cellular immune reactivity is found in human glomerulonephritis. Investigations of the role of such immunospecific cells in causing glomerular cell cytotoxicity are now possible because of the advent of glomerular cell culture *in vitro*. Future studies will also probably investigate the role that invading macrophages and lymphocytes play in the development of glomerular sclerosis and the proliferation of intrinsic glomerular cells via the release of soluble mediators.[19,20,45,81,146] Indeed, preliminary studies investigating the interaction of inflammatory mediators and mesangial cell matrix production in tissue culture have been reported.[136]

Cellular Autoimmunity to Glomerular Antigens in Glomerulonephritis

The possibility that certain histologic forms of human glomerulonephritis might be associated with autoimmunity to specific glomerular antigens was first suggested by the demonstration of anti-fetal kidney antibodies in patients with acute and chronic

GN during the 1950s.[74,79,88] The subsequent demonstration of circulating anti-adult (collagenase solubilized) GBM antibodies in a small percentage of patients (<5%) with glomerulonephritis has been well documented.[95,152] Thus, the presence of a humoral immune response to a particular glomerular antigen (the GBM) has been well documented in human and experimental GN.[135]

The first study to suggest the occurrence of cellular autoimmunity to glomerular antigens in GN was reported by Bendixen in 1968.[6] This report followed relatively closely in time the development of the migration inhibition assay for detecting cellular hypersensitivity to specific antigens, first described in 1962 by George and Vaughan.[43] This assay is based on the observation that in response to an antigen, sensitized T lymphocytes release a lymphokine (migration-inhibition factor), which immobilizes macrophages to the site of the antigen. The inhibition of macrophage movement is then measured experimentally. Bendixen showed that white blood cells from 13 of 15 patients with biopsy-proven glomerulonephritis exhibited migration inhibition to a crude fetal kidney homogenate, while patients with pyelonephritis and normal controls showed no migration inhibition to the kidney homogenate. Subsequently, Rocklin,[120] using a modification of the migration inhibition assay and collagenase-solubilized adult GBM obtained by sonication of glomeruli as antigen, showed that while only 1 patient of a total of 30 normal and "non-nephritic" controls exhibited cellular reactivity to GBM, 7 of 14 patients with glomerular disease were found to have cellular sensitization to GBM.

Bendixen's and Rocklin's early studies[6,120] were soon elaborated on by Mahieu,[93] using a more defined patient population with biopsy-proven GN. In Mahieu's study,[93] the leukocyte migration inhibition (LMI) assay was used to detect cellular sensitivity to GBM and tubular basement membrane (TBM) solubilized by collagenase. A passive hemagglutination assay for the coexistence of circulating antibodies to basement membrane antigen was also employed. The results demonstrated that 4 of 14 patients with focal proliferative GN and 5 of 15 patients with diffuse proliferative GN (including three with poststreptococcal disease) had cellular immune sensitivity to GBM. None of 16 controls and none of a total of 40 patients with minimal changes, membranous lesions, membranoproliferative lesions, pyelonephritis, and congenital nephropathies had cellular sensitization to GBM. However, 5 of 15 patients with major vascular lesions (including polyarteritis and cortical necrosis) also had positive results. Only 2 of the patients with glomerulonephritis who had cellular sensitivity to GBM had linear IgG staining. The remainder had granular IgG on immunofluorescence. Further, circulating antibodies to the GBM were detected in only 50% of the patients with cellular sensitivity to the GBM. Clinically, all the patients with cellular and humoral sensitivity to GBM had low creatinine clearance rates and rapidly progressive courses.

Three other studies have confirmed observations regarding cellular reactivity to GBM[24,91,101] in proliferative GN, using similar GBM antigenic preparations and the LMI assay. In summary, the salient points of these studies include the following: 1) nearly all patients with circulating antibodies to GBM, or linear deposits on the GBM by immunofluorescence, can be shown to have cellular immune reactivity to GBM; 2) some patients with granular deposits by immunofluorescence and no circulating antibodies to GBM have cellular reactivity to GBM; 3) patients with clinically active and progressive proliferative GN have more cellular reactivity than patients with inactive disease. Thus, these studies suggest that cellular autoimmunity to the GBM may play an active role in the pathogenesis of proliferative GN, both in the presence and in the absence of detectable humoral GBM autoimmunity.

The importance of the antigenic preparation of GBM used for detection of cellular immunity to GBM in idiopathic GN was first noted by Mallick.[98] While previous studies (except for Bendixen,[6]) used *adult* GBM, Mallick used a crude human *fetal* kidney antigen in the migration inhibition assay to detect cellular sensitivity to renal tissue among patients with biopsy-proven GN and the nephrotic syndrome. Again, 6 of 7 patients with diffuse idiopathic proliferative lesions (5 of whom had granular immunofluorescent IgG deposits) and 2 of 4 patients with focal proliferative lesions had positive cellular reactivity to the fetal antigen. However, in contrast to previous studies, 8 of 8 patients with minimal-change lesions (all adults) were also reactive to the fetal kidney antigen, as were 3 of 6 patients with membranous lesions. Only 2 of 23 controls were positive reactors. Although again confirming previous reports of cellular reactivity to GBM among patients with idiopathic proliferative lesions, the use of fetal kidney antigen demonstrated a wider spectrum of *in vitro* cellular immunologic reactivity to renal antigens among patients with other histologic forms of GN, including minimal-change and membranous lesions. A further discussion regarding cellular immunity in minimal-change and membranous nephropathy will be presented later. Mallick's and

Bendixen's work emphasizes the importance of the type of antigenic preparation used in the detection of cellular immunity in various forms of GN.

Our own studies[35-38] have confirmed the presence of cellular reactivity to GBM with the use of the lymphocyte blastogenesis assay in patients with proliferative GN. These studies have further emphasized the importance of the antigenic preparation used for the detection of cellular sensitivity to GBM, and have provided a possible mechanism for the development of GBM autoimmunity in the previously nonimmune host.

To begin with, it should be noted that all the previously described studies used a form of the migration inhibition assay to detect cellular immunity to GBM. A study by Gotoff[48] in a small number of patients used the lymphocyte blast transformation assay to detect cell-mediated immunity to adult, trypsin solubilized GBM. Significant lymphocyte blastogenesis in response to GBM was found in a single patient with Goodpasture's syndrome.

Our first study[35] employed three preparations of human GBM, prepared not by sonication of whole glomeruli (as in the previous studies), but by detergent extraction.[105] A batch of GBM prepared in this manner was briefly sonicated for a workable suspension and was considered native GBM. A portion of the native GBM was then "altered" by either collagenase (resulting in a soluble preparation) or mixed glycosidases, which altered the carbohydrate of the GBM without altering the protein backbone. The rationale for these treatments was based on the observation that polymorphonuclear leukocytes[148] and macrophages[126] release these enzymes extracellularly during attempted phagocytosis of immune complexes. Thus, we postulated that the deposition of immune complexes along the GBM, followed by the intrusion of phagocytic cells which release enzymatically active compounds to the local milieu, might chemically alter the GBM and expose new immunogenic sites. This process could initiate an ongoing autoimmune process.

In our initial study we found significant lymphocyte blastogenesis in response to glycosidase-treated and not to native or collagenase-treated GBM in patients with proliferative GN. In addition, less significant cellular reactivity was seen in patients with focal segmental sclerosis and membranous nephropathy. Normal controls and patients with nonglomerular renal disease were not reactive. In a subsequent study, we have confirmed these findings[38] in a separate group of patients, with more stringent criteria for positive cellular reactivity. The serum from patients with chronic GN and lymphocyte reactivity to altered GBM were also tested for the presence of circulating immune complexes[37] by the method of Hay[53] and for the presence of circulating antibodies to collagenase-solubilized native GBM by immunodiffusion[37] and by radioimmunoassay.[36] Neither circulating immune complexes nor circulating autoantibody to GBM were found in patients with cellular reactivity, suggesting that in chronic GN, cellular reactivity to glomerular antigens is present in the absence of humoral reactivity. Recently, Matsumoto[102] confirmed that patients with glomerulonephritis—and, again, particularly the proliferative group—have cellular reactivity to glycosidase-altered GBM using the leukocyte adherence inhibition assay. Thus, these studies demonstrated lymphocyte reactivity to altered GBM antigens in some patients with idiopathic proliferative GN, adding to the previously discussed evidence gathered when the migration inhibition assay was used and, further, suggesting that cellular immune sensitization and effector mechanisms may play a role in the pathogenesis of idiopathic proliferative GN, perhaps in the absence of humoral immunologic reactivity.

There are a number of hypotheses regarding how GBM autoimmunity might develop in a previously apparently normal host. Concepts regarding the development of autoimmunity and the concept of immunologic reactivity to altered self antigens are not unique to GN and may be viewed as general immunologic phenomena.[139] One theory postulates that a primary event involves alterations of host antigens that may occur in a number of ways. For example, microbes secrete a number of enzymes capable of altering host antigens.[25] In addition, microbial toxins may release or uncover normally hidden antigens. Virus-infected cells may express viral antigens on the cell surface, causing these cells to be seen as foreign by the host because of the expression of altered cell surface antigens.[122] Environmental agents, including toxins and drugs, may also cause alterations of host antigens.[138] Finally, cross-reactions between microbial antigens and either native or altered host antigens may be important in genetically predisposed individuals.[90,156]

In addition, autoimmunity may be a physiologic phenomenon whose function is to recognize altered host antigens and serve as a mechanism for their removal. For example, senescent red blood cells accumulate altered cell surface antigens during the aging process. These altered antigens are recognized by host immunoglobulin, and immunoglobulin-coated aged blood cells are rapidly removed by the reticuloendothelial system.[68] It is thought that sialic acid, a

normally terminal carbohydrate of glycoproteins, may play a role in this and a number of other regulatory processes, both biologic and immunologic.[121] Indeed, the role of glycoprotein carbohydrates as regulatory moieties is a new and fascinating area of ongoing biologic research[1] with potentially important implications for research in glomerulonephritis. In fact, recent studies have dealt with this subject in the context of immune complex glomerulonephritis.[40]

A final point of view regarding the development of autoimmunity focuses on altered immunoregulation, which perhaps may play a role in certain forms of more generalized autoimmune diseases involving the kidney, such as sytemic lupus erythematosus,[10] and perhaps in minimal-change nephrotic syndrome and membranous nephropathy.

In the case of proliferative GN, there have been few studies directly addressing the question of how glomerular autoimmunity develops. With regard to altered host GBM antigens, Mahieu[94] studied the specific antigenic determinant responsible for cellular reactivity as measured by the LMI assay in patients with proliferative GN. These studies showed that the disaccharide glycoprotein of the GBM appeared to contain the major antigenic sites. Further, the major immunodeterminant appeared to be galactose. However, galactose is not normally an exposed terminal carbohydrate of the GBM. Similar findings have been noted during experimental immunization with GBM.[29] Thus, these studies suggest that perhaps normally hidden carbohydrate moieties of the GBM may be the major antigenic foci for autoimmunity and experimental immunization.

Our own studies have focused on the use of altered GBM antigens as test antigens for the detection of cellular immune reactivity in patients with GN. Thus, we have found that cellular immune reactivity in patients with proliferative GN is directed primarily against glycosidase-altered GBM. Studies using periodate reduction have been used for investigation of the immunochemical determinants of the GBM to which cellular reactivity in proliferative GN is directed. Periodate treatment breaks the six-carbon-ring structure of carbohydrates and destroys their antigenicity. Periodate reduction of glycosidase-altered GBM eliminates lymphocyte reactivity to glycosidase-altered GBM.[39] These studies suggest that in patients with proliferative GN, cellular reactivity is directed against a carbohydrate antigen of the GBM exposed after glycosidase treatment and destroyed by periodate reduction. We have also used specific glycosidases in a sequential fashion to cleave carbohydrates from the GBM, on the basis of a knowledge of the probable structure of the heteropolysaccharide glycoprotein of the GBM.[32,69,133] Removal of terminal sialic acid alone with neuraminidase does not result in lymphocyte reactivity.[36] However, subsequent removal of galactose with β-galactosidase (after neuraminidase treatment) does result in significant lymphocyte reactivity in patients with proliferative GN. The release of sialic acid and galactose from human GBM by these enzymes has been confirmed by the thiobarbituric acid assay,[147] and by gas-liquid chromatography.[39] Amino acid analysis of altered GBM showed no differences when compared with native GBM prepared by us and others.[149] A continued sophisticated biochemical approach to this problem will, we hope, help to demonstrate definitely that cellular immune reactivity in proliferative GN is directed primarily against altered GBM antigens.

In summary, a number of studies have demonstrated that cellular autoimmunity to glomerular antigens in various forms of GN can be found with the use of different assays for the detection of cellular immunity. The particular antigen employed in these studies may be crucial. The possibility that glomerular autoimmunity may be directed against altered, and not native, glomerular antigens has been suggested by a few studies. Future investigations will, it is hoped, further characterize the etiology and mechanisms of cell-mediated glomerular autoimmunity in various forms of GN. In this context, the relationship of glomerular altered antigens to glomerular fetal antigens may be important. In addition, the role of lymphocytes specifically sensitized to glomerular antigens in mediating macrophage function, as discussed in the previous section, and directly on glomerular cell function (for example, basement membrane collagen production[146]) and toxicity will probably be areas of fruitful research. Some of these phenomena may be mediated by polypeptide lymphokines, which may have therapeutic implications, particularly in proliferative glomerulonephritis and in glomerular sclerosis.[134] The advent of glomerular cell culture techniques makes these experiments currently feasible.[42]

Cellular Sensitivity to Streptococcal Antigens in Glomerulonephritis

A number of investigators have observed heightened sensitivity to streptococcal antigens in chronic glomerulonephritis.[24,35,38,91,101,154] These studies suggest that those cases with heightened sensitivity to streptococcal antigens are a result of an initial acute poststreptococcal GN. However, other possible explanations are that heightened sensitivity to streptococcal antigens may 1) result in immunologic reac-

tivity to cross-reactive native or altered GBM antigens and thus exacerbate the autoimmune response[155] or 2) represent some other underlying abnormality of the immune system that contributes to the development of GN. Thus, these observations suggest that the streptococcus plays a permissive role in certain forms of glomerulonephritis, regardless of initial etiology; so that heightened sensitivity to the streptococcus may be a marker of autoimmune sensitization to cross-reactive GBM antigens[62] or may possibly represent some other role for streptococcal sensitivity in the disease process.

The initial observation of heightened cellular reactivity to streptococcal antigens in GN was made by Zabriskie.[154] In this study, 13 patients with progressive glomerulonephritis demonstrated significant direct migration inhibition and lymphocyte blastogenesis to particulate streptococcal antigens, including streptococcal walls and membranes from nephritogenic and nonnephritogenic strains, but not soluble streptococcal antigens, including M and T proteins, group-specific carbohydrate, mucopeptide, cytoplasmic materials, and bacterial nucleic acids. Normal controls and patients with nonglomerular renal disease exhibited minimal migration inhibition in reaction to the streptococcal antigens. Reaction to a control antigen was similar in controls and patients with glomerulonephritis, demonstrating the specificity of the heightened response to the streptococcal antigen.

These results were subsequently confirmed by Macanovic[91] and Dardenne,[24] using the migration inhibition assay. In general, the reactivity found to streptococcal membrane preparations correlated with reactivity to GBM among patients with proliferative GN. In Dardenne's study,[24] patients with Goodpasture's syndrome reacted only to GBM and not to streptococcal antigens. Our own studies[35,36] employing the blastogenesis assay also found heightened cellular reactivity to streptococcal antigens among patients with heightened reactivity to glycosidase-altered GBM. However, no differences in the response to PHA or BCG were noted between patients who responded to GBM and those who did not, again confirming the specificity of such streptococcal reactivity.[38]

Bhat[9] suggested that patients with poststreptococcal chronic GN had depressed blastogenesis in response to streptococcal antigens. It should be noted that this study is the only study specifically employing patients with documented poststreptococcal GN, while the previously reported studies employed idiopathic proliferative GN. Thus, the patients in Bhat's study[4] generally had progressive glomerular sclerosis, not active proliferative lesions. These patients did not differ from controls in their response to PHA, PPD, or SKSD, ruling out the possibility of generalized immune deficiency. Further, they found similar results in both autologous and homologous serum, suggesting that a serum blocking factor was not responsible for the observed depression of reactivity to streptococcal antigens. Similar findings have been reported by Kryzmanske.[76] The possibility exists that streptococcal specific suppressor cell hyperactivity is present in patients with chronic poststreptococcal GN, and that this may be either a physiologic or a pathologic response.

Williams[150] studied T-lymphocyte surface markers and streptococcal antigen-binding cells during acute poststreptococcal glomerulonephritis (APSGN) in man. They found depressed proportions of T cells during the acute disease, with an increased number of streptococcal binding cells. Depressed levels of T gamma and T mu (suppressor and helper) cells were also found when compared with those of patients with uncomplicated streptococcal infections. These results may have implications for understanding cellular immune events during APSGN in man.

In summary, although the role of streptococcal antigens in idiopathic proliferative GN is not yet defined, many of these patients have heightened reactivity to streptococcal antigens. The migration inhibition assay and the blastogenesis assay have both demonstrated similar findings and have shown a correlation between GBM reactivity and streptococcal reactivity. In general, such heightened reactivity does not appear to be nonspecific. Further, it has been suggested that the basis of this phenomenon is a cross-reaction between streptococcal antigens and either native or altered GBM antigens. In the specific case of chronic poststreptococcal GN, with progressive glomerular sclerosis, depressed cellular reactivity to streptococcal antigens has been demonstrated. Further studies are clearly required to delineate the role of altered cellular immunity to streptococcal antigens in different forms of GN.

Cellular Studies of Minimal-Change Nephrotic Syndrome

Minimal-change nephrotic syndrome (MCNS) is a form of glomerular disease of unknown etiology, characterized by normal glomerular histologic characteristics, epithelial cell swelling, and foot process fusion on electron-microscopic examination, and highly selective proteinuria. A number of clinical immune abnormalities have been observed in this disease. These include the often striking steroid responsiveness,[15]

the induction of remission by measles infection and the susceptibility to certain bacterial infections, depressed serum IgG and IgA, and elevated serum IgM[44,129] with normal serum complement,[83] and other abnormalities (reviewed in Glassock,[45] Mallick[97]). Shaloub[130] (1974) suggested that MCNS may be related to a T-cell disorder, particularly a disorder of suppressor T cells. The basic postulate here is that the glomerulus is an innocent bystander in MCNS, and that glomerular injury in MCNS is related to the presence of a soluble circulating factor, released because of a primary cellular immune abnormality, that has the observed pathologic effects in the glomerular capillary.

Recent observations have also suggested generalized cellular immune abnormalities in MCNS that may be related to the presence of a factor in the serum of patients with nephrotic syndrome that inhibits lymphocyte proliferation.[108,142] Similar observations of serum factors that inhibit lymphocyte proliferation have been found in uremia from any cause, and the question of whether the inhibiting serum factor is specific to MCNS, or related to uremia, has been raised.[100,124] Indeed, a number of important changes in both humoral and cellular immunity occur in uremia.[28,119]

A vascular permeability factor derived from lymphocytes of patients with MCNS has been described.[77,78] However, recently this factor has also been found in nephrotic syndrome of any cause,[132] again raising the question of specificity for MCNS versus the nephrotic syndrome in general. In any case, it has been postulated[63] that this factor might be a lymphocyte-derived product with toxicity for the glomerular permeability barrier. Of interest in this context, Kreisberg[75] (1979) has suggested that macrophages are capable of inducing loss of glomerular polyanion and proteinuria in an experimental model of GN.

Circulating immune complexes have been found in MCNS.[14,82,117] These complexes could be a cause of nonspecific activation of lymphocytes, resulting in circulating lymphokine production, could have blocking abilities that induce lymphocyte paralysis, or could simply represent an epiphenomenon that could contain important clues to the antigen responsible for or related to abnormal cellular immune dysfunction. In any case, classic immune complex injury mechanisms related to their glomerular deposition do not appear operative in MCNS, since there is no evidence for glomerular deposition by immunofluorescence or electron microscopy.

A recent observation by Kerpen[70] suggested an abnormality in T-cell markers in patients with MCNS. They demonstrated the presence of circulating T cells (by sheep RBC rosetting techniques) that also had receptors for complement, normally a B-cell marker. In addition, a decreased number of null cells were observed, with a normal number of B cells as determined by the presence of cell-surface immunoglobin. Smith[131] found a decreased number of lymphocytes with complement receptors in adenoid tissue from patients with MCNS. These studies suggest that MCNS patients may have an abnormal lymphocyte subpopulation.

A few experimental investigations have observed organ-specific cellular imune abnormalities in MCNS. Eyres[31] has suggested that patients with MCNS have lymphocyte-mediated cytotoxicity directed against an epithelial cell culture derived from human kidney. Ooi[113] has suggested the presence of lymphocytotoxins in the serum of patients with MCNS. Mallick[98] demonstrated the presence of cellular reactivity directed toward a fetal kidney antigen preparation, as measured by the MIF assay. Cabilli[13] suggested lymphocyte transformation to glycosidase-altered GBM could be demonstrated in patients with MCNS. This observation may be of interest, since glycosidase-altered GBM may be similar antigenically to fetal GBM. These observations suggest that organ specific cellular reactivity to fetal kidney antigens could be related to the development of MCNS. Alternatively, reactivity to fetal antigens could also represent a generalized abnormality of the cellular immune system.

In summary, a number of studies have suggested abnormalities in the cellular immune system in patients with MCNS. At this time, we do not have a clear understanding of the immune defect in these patients. However, most evidence appears to suggest that some abnormality in the T-cell system leads to the release of a soluble factor that alters glomerular permeability. It is also possible that a circulating soluble factor leads independently to both altered glomerular permeability and a dysfunction of the cellular immune system.

Cellular Immune Studies in Membranous Nephritis

Heymann's nephritis is induced by immunizing rats with a kidney suspension in Freund's adjuvant.[56] The prominent pathologic characteristic of the resultant glomerulonephritis is a membranous lesion with basement membrane thickening and no cellular proliferation or invasion. Clinically, the animals develop the nephrotic syndrome. The mechanism of injury in this model was investigated by Glassock[46] and thought to be at least partly immune-complex–mediated. The responsible antigen was isolated by Edgington[30] and found to be derived from renal proximal tubular

epithelial cells. It was postulated that the immunization of rats with heterologous renal tubular antigen (RTE) resulted in the termination of tolerance to autologous RTE and the development of autoimmunity to autologous RTE. The presence of circulating autologous RTE and antibody to RTE would result in chronic membranous nephropathy, via circulating immune complex mechanisms. However, circulating immune complexes have not been demonstrated in human membranous glomerulonephritis.[12] Further, although RTE was demonstrated by immunofluorescence in the glomeruli of the animals, circulating RTE was not found by Glassock.[46] There is conflicting evidence for the role of RTE in human membranous GN.[17,106] It has subsequently been suggested that *in situ* formation of immune complexes might account for the lack of circulating immune complexes in membranous nephropathy.[23] It has also been proposed that circulating antibodies to cross-reactive glomerular antigens may be involved in the pathogenesis of Heymann's nephritis.[145] These and other investigations[22,34,41,96,145] employing the passive Heymann nephritis model have shown that heterologous serum, and indeed heterologous IgG, directed against the brush border antigen responsible for classic Heymann's nephritis (Fx 1A) can induce a model of membranous nephropathy in animals, clearly demonstrating a role for humoral mechanisms in the development of this disease via *in situ* immune complex formation.

Earlier studies by Hess[55] and Heymann[57] demonstrated that the transfer of serum alone from animals with ongoing Heymann's nephritis did not result in renal disease, while the transfer of lymphoid cells alone from affected animals did result in renal disease, similar to the original disease. Both Hess[55] and Heymann[57] used a number of experimental manipulations designed to control for immune reactions against organism-specific antigens (or histocompatibility antigens) by using inbred strains of animals. Transfer of cells from normal animals and control animals that were immunized with irrelevant antigens did not result in renal disease. These results suggested that a cellular immune reaction might be involved in the pathogenesis of this model. However, these experiments have been criticized, and evidence against a role for lymphoid cells alone causing Heymann's nephritis was summarized by Unanue.[144] Thus, whether Heymann's nephritis is actually transferable by lymphoid cells alone is controversial.

Investigations of cellular immunity in membranous nephropathy have been provoked by the possibility that the disease is transferable by lymphoid cells alone. Grupe[49] studied the role of cellular immunity in Heymann's nephritis by applying the technique of migration inhibition, using the immunizing renal suspension as antigen. In these experiments, animals were immunized to Heymann's antigens in Freund's complete adjuvant, and the majority of animals developed the nephrotic syndrome. Significant migration inhibition was seen to the kidney antigen in these animals at 8–12 weeks. Some reactivity was also seen to control liver antigen, but no reactivity was seen to lung antigen. Control animals, immunized with either Freund's adjuvant alone or given aminonucleoside of puromycin to induce the nephrotic syndrome, had no cellular reactivity to any antigens. Of 8 animals developing Heymann's nephritis, all had significant migration inhibition to kidney antigen, while only 3 of 6 animals had precipitating circulating antibodies to kidney antigens when tested by immunodiffusion. These results suggest that lymphocytes from animals with Heymann's nephritis are sensitized to kidney antigen and secrete migration-inhibition factor in the presence of this antigen.

Studies by Mallick[98] and our own work[35,36] have also suggested that organ-specific glomerular autoimmunity can be found in membranous nephropathy, using GBM antigens as test stimuli. Using a fetal kidney antigenic preparation and the migration inhibition assay, Mallick[98] found that 3 of 6 patients with membranous nephropathy demonstrated cellular immunologic reactivity. Our own studies using glycosidase-altered GBM[35,36] also showed low-grade lymphocyte blastogenesis responses in a few patients with membranous nephropathy. Interestingly, one of these patients became a quite strong reactor to altered GBM in our assay during a period when his disease entered a more rapidly progressive stage toward uremia.[39] Whether his disease had actually transformed from membranous nephropathy to a glomerular autoimmune disease, as has been previously observed,[71] is a provoking possibility.

A study by Holm[61] suggested that lymphoid cells from animals with Heymann's nephritis were cytotoxic to an *in vitro* grown kidney cell line, which appeared to be predominantly an epithelial cell type. An elaborate method for determining cytotoxicity essentially measured ^{14}C-thymidine release from labeled kidney cells. Lymphocytes were obtained from the peripheral blood and lymph nodes of nephrotic and control rats that had been immunized to liver and lung homogenates and adjuvant alone. Microscopic examination revealed adherence of nephrotic lymphocytes to cultured cells within 3 hours and the appearance of degenerative cellular changes by 48 hours. Somewhat similar findings were found with liver-sensitized cells, but not in other control groups.

Radioisotope cytotoxicity experiments further suggested the nephrotic lymphoid cells were cytotoxic to cultured kidney cells, some specificity being demonstrated. Serums from nephrotic rats were also tested for cytotoxicity of cultured kidney cells in the presence of fresh complement, but no cytotoxicity was observed.

Studies of the development of cellular immunity to the probable antigen responsible for Heymann's nephritis have been done by Litwin.[85] In these studies, the semipurified antigens Fx 1A derived from renal cortex in complete Freund's adjuvant was injected intradermally into rats. A similarly derived, nonnephritogenic fraction, known as Fx 1B, was used as a control. Rats injected with Fx 1A developed proteinuria by 8 weeks, with glomerular lesions resembling membranous nephropathy, while control rats remained normal. Intradermal skin testing with Fx 1A in animals developing proteinuria were positive in 9 of 14 animals, and histologic sections showed typical delayed hypersensitivity reactions in the skin. Control intradermal skin testing with Fx 1B was negative. Lymphocyte cultures done at the same time revealed significant blastogenesis to the Fx 1A in 10 of 13 proteinuria rats but was negative to Fx 1B. In a subsequent study, Litwin[86] investigated the sequential kinetics of the onset of humoral and cellular immunity in the Heymann model. For these experiments, the responsible purified antigen (RTE) identified by Edgington[30] was also employed for the detection of cellular immunity. In this study, at the time of onset of proteinuria, all rats had positive delayed hypersensitivity to RTE by skin testing. At the same time, most rats had significant lymphocyte blastogenesis to RTE and Fx 1A. Precipitating antibodies to normal rat proximal tubular cells were detected in most rats by about 6 weeks after immunization. A $C1_q$ test for circulating immune complexes also became positive in most animals, although this generally occurred after the onset of proteinuria. Histologically typical membranous nephropathy with granular IgG deposition on immunofluorescence examination was noted. These results suggest that the onset of membranous nephropathy in Heymann's nephritis is associated not only with a humoral immune response to the responsible antigen, but also with a cell-mediated immune response, which has been demonstrated by intradermal skin testing, migration-inhibition assays, and lymphocyte blastogenesis. Two recent studies[87,52] have focused on the role of suppressor cells in Heymann's nephritis, suggesting that the induction of Fx 1A-specific suppressor T cells may modify antibody production and prevent or inhibit the development of glomerulonephritis.

Finally, a study by Ooi[112] in patients with idiopathic membranous nephropathy showed that lymphocytes from these patients have a diminished ability to produce immunoglobulin *in vitro*. Specifically, B cells were incubated with a nonspecific activator, pokeweed mitogen, and the production of IgG and IgM was measured. Lymphocytes from patients with membranous nephropathy produced significantly less IgG and IgM than controls. In co-culture experiments, lymphocytes from patients with membranous nephropathy suppressed the production of immunoglobulin by control lymphocytes. However, by removing the T cells or monocytes from the lymphocytes of membranous nephropathy patients, the suppressor was eliminated. These results suggest that patients with membranous nephropathy have a primary cellular immune defect, namely, an abnormal suppressor cell. This defect could lead to diminished ability to produce immunoglobulin, resulting in prolonged antigenic stimulation and circulation, and particularly in prolonged antigen excess. This could lead to a propensity for the development of *in situ* immune complex disease and could account for the development of membranous nephropathy in these patients.

In summary, a number of studies have investigated the role of cellular immunity in membranous nephropathy, in both animal models and in human disease. Early studies of Heymann's nephritis have suggested that membranous nephropathy is transferable by lymphoid cells alone, and not by serum. However, recent studies suggest Heymann's nephritis is transferable by serum, and particularly IgG directed against appropriate brush border antigens. Other studies have demonstrated cellular immune reactivity to the responsible RTE antigen in Heymann's nephritis. Still other studies of organ-specific cellular immunity in membranous nephropathy have shown cellular reactivity to GBM antigens and cellular cytotoxicity to renal cells. These studies are difficult to evaluate in view of the clear lack of cellular infiltration in the glomeruli in membranous nephropathy. Whether such reactivity reflects an underlying abnormality in cellular immunity or indicates transformation of membranous nephropathy into a progressive autoimmune stage cannot be determined at this time. Finally, the possibility that an abnormal suppressor cell may be found in patients with membranous nephropathy has been suggested. These studies provide highly provocative evidence that, despite the lack of cellular infiltration or proliferation in the glomeruli of patients with membranous nephropathy, a primary defect in the cellular immune system may lead to glomerular pathology via abnormal regulation of the humoral system. In the animal models, it appears

that the induction of suppressor cells prevents membranous disease, while in man it has been suggested that suppressor cells are overactive and permissive for the development of membranous disease. We may speculate that relatively complete suppression of antibody production prevents disease in animals, while partial suppression is permissive for disease in man. Normal immunoregulation would presumably result in full antibody production, complete immune clearance, and no disease.

Summary and Conclusions

In this review, we have attempted to present both data and hypotheses that have accumulated predominantly during the last 20 years that suggest a role for cellular immunity in the development of glomerulonephritis. These facts and ideas were made possible by advances that have occurred in cellular immunology during this time. Thus, it appears that cellular immune mechanisms play an important role in classic models of glomerulonephritis, such as the immune complex models, nephrotoxic serum nephritis, and Heymann's nephritis. Data has also accumulated in man suggesting that cellular immune mechanisms are important in the development of human glomerulonephritis, perhaps in the absence of humoral mechanisms. Although humoral effector mechanisms clearly play a role in the development of certain forms of glomerulonephritis, we may speculate on the basis of recent data that the expression of abnormal humoral immunity in certain forms of glomerulonephritis may be the result of a primary abnormality in the cellular immune system, which regulates antibody production. Thus, our perspective should include not only cell-mediated effector mechanisms in glomerulonephritis, but also cellular immune mechanisms of sensitization to antigen and immunoregulation of antibody production.

In summary, investigators have demonstrated that macrophages are important effector cells in animal models and are present in the glomeruli of patients with certain forms of GN. Immunospecific T cells may play a role in attracting macrophages to the glomerulus and the site of foreign or altered host antigen. In some cases, these immunospecific T cells may be autosensitized to native or altered glomerular antigens. The mechanism by which such glomerular autoimmunity occurs is not known, although hypotheses have been presented. Cellular sensitization to streptococcal antigens in human glomerulonephritis may be an important clue to the role of microbial influences in GN and serve as a model for possible mechanisms by which exogenous stimuli may cause, exacerbate, and contribute to the progression of glomerular disease. In minimal-change nephrotic syndrome and membranous glomerulonephritis, it appears that more generalized cellular immune abnormalities may contribute to the development of GN. In MCNS, an abnormality in suppressor cells appears to result in the release of a factor, in response to an unknown stimulus, which induces glomerular capillary permeability. In membranous nephropathy, abnormal immunoregulation of the humoral immune system by the cellular immune system may be permissive for the development of *in situ* immune complex formation.

Since the cellular immune system can be therapeutically manipulated, we can express hope that advances in cellular nephroimmunology will not only lead to an understanding of why and how glomerulonephritis occurs, but also to effective modes of treatment. However, a great deal of work by investigators in the field of cellular nephroimmunology needs to be done before the practical implications of this work come to fruition. Finally, although glomerulonephritis is really a rather uncommon disease, despite its implications for the individual patient, glomerular disease serves as an excellent model of capillary injury and probably vascular injury in general, as well as a model for organ-specific autoimmune disease and the effects of generalized immune disorders on the capillary. In this light, although cellular nephroimmunology is still a young field, recent and future investigations will, we hope, make an important contribution to the body of knowledge and understanding of glomerular disease and vascular disease and cellular immunology in general.

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