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PERSPECTIVES IN BASIC SCIENCE

Th1 and Th2 T helper cell subsets affect patterns of injury and outcomes in glomerulonephritis

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Th1 and Th2 T helper cell subsets affect patterns of injury and outcomes in glomerulonephritis. The recognition that human immune responses can be directed by two different subsets of T helper cells (Th1 and Th2) has been an important development in modern immunology. Immune responses polarized by either the Th1 or Th2 subset predominance result in different inflammatory effector pathways and disease outcomes. Many autoimmune diseases are associated with either Th1- or Th2-polarized immune responses. Although these different immune response patterns are relevant to glomerulonephritis (GN), little attention has been paid to the consequences of Th1 or Th2 predominance of nephritogenic immune responses for the pattern and outcome of GN. Unlike other autoimmune conditions, GN results from a variety of different immune responses and has a range of histologic features and immune effectors in glomeruli. This review assesses the data available from studies of experimental and human GN that address the Th1 or Th2 predominance of nephritogenic immune responses and their relevance to the different histopathological patterns and outcomes of GN. In particular, the evidence that Th1-predominant nephritogenic immune responses are associated with severe proliferative and crescentic GN is presented.

Renal injury in glomerulonephritis (GN) is characterized by injurious immune responses to self or foreign antigens [1]. Different immune responses lead to different patterns of injury with a variety of clinical presentations and disease outcomes. The development and maintenance of cognate immune responses generally involve the activation of T lymphocytes, which direct antigen-specific, cell-mediated effector mechanisms and promote antibody production and antibody-mediated effector mechanisms. It has long been observed that some immune responses are associated with prominent cell-mediated immunity and delayed type hypersensitivity (DTH), whereas others are characterized by stronger antibody responses and/or an allergic response [2]. For example, individuals infected with *Mycobacterium leprae* develop either tuberculoid leprosy, in which limited disease is accompanied by strong cellular immunity and granuloma formation, or lepromatous leprosy, with strong antibody production and more extensive disease [3]. Variable involvement of immune effector responses may also be relevant to human GN, in which there is variable evidence of DTH involvement and variable amounts and isotypes of immunoglobulin deposited [4, 5].

The concept that T helper cells, in rodents and in humans, differentiate into functionally distinct subsets (now termed Th1 and Th2) has altered the understanding of cognate immune responses, and has helped to explain the different immunologic patterns and outcomes in diseases such as leprosy. There is evidence to support the existence of at least one other population of Th cells, the Th3 subset, having immunoregulatory properties and characterized by the production of transforming growth factor-β (TGF-β) [6]. This review examines the roles of Th1 and Th2 subsets, as, currently, little is known about the role of the Th3 subset in GN.

The observations that Th1 and Th2 subsets produce distinct immune responses associated by distinct patterns of cytokine secretion have helped to explain the different immune effects of these cytokines. In 1986, Mosmann et al demonstrated that functionally distinct subsets of $CD4^+$ T cells could be defined by their pattern of cytokine production. This led to the concept of Th1 and Th2 T-cell subsets in mice [7] and in humans [8]. DTH is mediated by the Th1 subset of $CD4^+$ cells [9]. This Th1 subset is characterized by the production of interferon-γ (IFN-γ), interleukin (IL)-2, and lymphotoxin-α [tumor necrosis factor β (TNF-β)]. Th1 responses induce macrophage and cytotoxic T-lymphocyte activation and immunoglobulin IgG subclass switching to favor complement fixation and opsonization. Th1 responses favor effective

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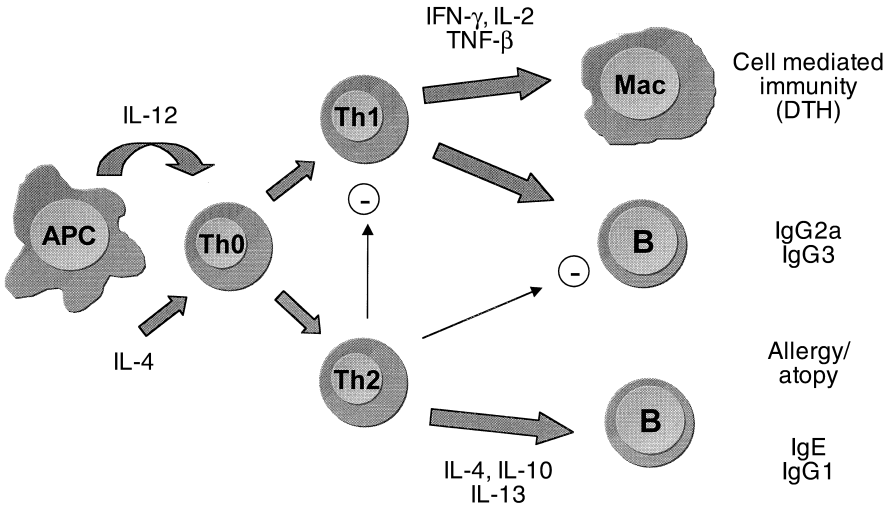

Fig. 1. T helper cell subsets, Th1/Th2. Interleukin (IL)-12 drives differentiation of T cells to the Th1 subset, which induces cell-mediated immune responses of which the classic example is the delayed type hypersensitivity (DTH) response. IL-4 induces Th2 cell development, humoral immunity, and components of immediate type hypersensitivity. The IgG subclasses listed are for the mouse.

clearance of intracellular pathogens and are likely to be important in organ-specific autoimmune diseases.

In contrast, Th2 cells, defined by their propensity to secrete interleukin (IL)-4, IL-5, and IL-10, are important in allergy, mast cell/IgE-mediated immediate type hypersensitivity responses, and helminth infections, in which protective responses are mediated by eosinophils. In addition, cytokines produced by Th2 cells act as regulators of the immune response. IL-4, IL-13, and particularly IL-10 regulate Th1 responses, suppress DTH, and have inhibitory effects on macrophages, especially in the context of the activation by Th1 cytokines such as IFN-γ [10–13]. Th2 responses are associated with high levels of antibody production promoted by cytokines such as IL-4 that stimulate B-cell growth. A simplified diagram of Th1 and Th2 response patterns is shown in Figure 1. There have been several detailed reviews on the characterization of Th1 and Th2 subsets and their relevance to a number of infective and inflammatory diseases in rodents and in humans [6, 8, 14, 15].

The profile of immunoglobulin isotypes is heavily influenced by the Th1/Th2 balance of the immune response. This has been studied extensively, particularly in the mouse [16–18], in which the levels of IgG1 (which has a weak affinity for Fcγ receptors) and IgG2a (which is strongly complement fixing and has a high affinity for Fcγ receptors) have been related to the Th1/Th2 predominance of immune responses. A Th2, IL-4–dominant response promotes a higher ratio of IgE and IgG1 to IgG2a, whereas Th1 response patterns (IL-12 and IFN-γ driven) have higher IgG2a to IgG1 and IgE ratios. In the mouse, IgG3 switching is also induced by Th1 responses, and this isotype is complement fixing with high Fcγ receptor affinity [18]. IgG2b is less extensively studied, but is induced by IL-12 [19] and by the absence of IL-4 [20]. The relevance of Th1/Th2 responses in isotype switching to IgA is less clear. TGF-β induces switching to IgA [21], and this may indicate that IgA is associated with a separate T helper cell subset response (Th3). Based on a comparison between the biological actions of the IgG subclasses and their up- or down-regulation in response to Th1 and Th2 cytokines, the human homologues of the murine IgG subclasses are given in Table 1.

Table 1. Human homologues of murine IgG subclasses, based on their complement fixing and opsonising abilities and on changes in isotype switching observed in response to Th1 and Th2 cytokines

	Mouse	Human
Th1	IgG2a	IgG1
	IgG3	IgG3
Probably Th1	IgG2b	IgG2
Th2	IgG1	IgG4

In vivo studies have demonstrated that immune responses and disease outcomes following antigenic stimuli are markedly influenced by the cytokine environment. This phenomenon has been most thoroughly explored in murine leishmaniasis [22]. Mice prone to Th1 responses (for example, the C57BL/6 strain) are protected from ongoing infection with *Leishmania major* by a response characterized by high levels of Th1 cytokines and DTH. However, BALB/c mice develop persistent disease with no DTH to *Leishmania*, hyporesponsiveness to IL-12, and high levels of IgE and IL-4 production [22, 23].

A number of models of organ-specific autoimmune diseases, including insulin-dependent diabetes mellitus in non-obese diabetic mice [24], experimental autoimmune encephalomyelitis (EAE), autoimmune thyroiditis [24], and experimental colitis [25], have been shown to be initiated by Th1-mediated responses. In some of these models, treatment with IL-4 [26] or IL-10 [27–29] has resulted in marked attenuation of disease with selective

reductions in Th1 responses. The transfer of memory T cells transfected to produce IL-4 [30] or IL-10 [31] ameliorated EAE. These results, plus analyses of the effects of attempts at oral tolerance, have led to the concept of "immune deviation," which suggests that modifying the immune response with IL-4 and/or IL-10 may lead to a diminution of inflammatory autoimmune injury [32].

The cytokine profile of antigen-stimulated $CD4^+$ T cells and the pattern of T-cell immune responses are determined by a number of factors, including the type, dose, and route of antigen presentation, the epitope T-cell receptor binding affinity, the nature and degree of costimulatory signals, and the genetic background of the animals. One of the most important and widely studied factors is the cytokine milieu at the time of antigen presentation [6, 14, 15]. IL-12 is crucial for the development of Th1 responses [33], whereas IL-4 is required for the generation of Th2 cells [15]. The presence of IL-12, which is not produced by T cells but by antigen-presenting cells such as macrophages and dendritic cells, polarizes uncommitted T cells toward a Th1 profile [33]. In the absence of IL-12 during the initiation of the immune response, T cells may lose future responsiveness to IL-12 because of a loss of the β2 subunit of the IL-12 receptor [34]. This early loss of responsiveness to IL-12 is linked to a region of chromosome 11 [35], homologous with the 5q31 region in humans, which is in the same area as the genes for a number of significant immunologic proteins, including IL-4, IL-5, and IL-12p40. Genetic loci for susceptibility to EAE [36] and for susceptibility to insulin-dependent diabetes mellitus in non-obese diabetic mice [37] have been identified in the same region of chromosome 11. Loci within the p31 region of human chromosome 5 have been associated with chronically dysregulated Th2 responses, such as total IgE levels [38] and bronchial hyperreactivity [39]. This suggests that genetic predisposition to some immunologic disorders is mediated by a tendency to produce exaggerated or persistent Th1 or Th2 responses [40].

Recent studies suggest that chemokines may influence or be influenced by Th1 or Th2 patterns of the immune response. Evidence exists that macrophage inflammatory protein-1α (MIP-1α), MIP-1β, and RANTES (regulated on activation, normal T cell expressed and secreted) are associated with Th1 responses [41, 42]. The evidence that monocyte chemoattractant protein-1 (MCP-1) plays a differential role in Th1/Th2 responses is less clear. Antibodies to MCP-1 diminished skin DTH, including T-cell and monocyte infiltration [43], and mice genetically deficient in CCR-2, the receptor for MCP-1, are deficient in Th1 responses [44]. On the other hand, there are data to support a role for MCP-1 in Th2 responses. MCP-1 induced Th0 cells to differentiate into a Th2 phenotype, and T cells cultured with MCP-1 could not transfer EAE [45]. MIP-1α had the opposite effect in the same study. MCP-1, but not MIP-1α, increased Th0 or Th2 IL-4 production [46], whereas MCP-1 decreased macrophage IL-12 production in granulomatous inflammation [47]. It is not clear whether these apparently contrasting effects of MCP-1 are due to differential effects on the direction in which Th0 cells are polarized or are due to the effect of MCP-1 on the accumulation of effector Th1 cells.

Although the concept of Th1/Th2 immune responses provides a useful framework, it is perhaps overly simplistic to consider that each immune response to an antigen will be strictly either Th1 or Th2, with one type of response being protective and the other harmful. The complexity of infectious and inflammatory responses implies that some cytokines (or a single cytokine) within a Th1 or Th2 grouping may have overlapping or, at times, opposing functions. The findings relating to the role of IFN-γ in humans with multiple sclerosis and animals with EAE attest to this statement [48–50]. At a cellular level, there is some heterogeneity in the pattern of cytokine secretion of Th1 and Th2 cells [51]. It has been suggested that the Th1/Th2 paradigm could be modified to include the concept that although an overall immune response to a specific antigen may be predominantly Th1 or Th2, antigen-specific T cells produce a spectrum of cytokines, with pure Th1 and Th2 cytokine profiles being at the extremes of a spectrum [52]. However, another review of the functional diversity of T helper lymphocytes refers to features that confirm the presence and relevance of the Th1/Th2 model in immune responses [14]. Two factors are cited: first, the clear pattern of antigen-specific Th1 or Th2 cell (and associated cytokine) predominance in a number of diseases in both mice and humans, and second, the tendency for T helper cell populations to become increasingly polarized and irreversibly committed with chronic immune stimulation. In addition to the extensive data pertaining to $CD4^+$ cells, there is now evidence for polarized Th1 and Th2 $CD8^+$ cells [53].

There is increasing evidence to support the relevance of Th1 and Th2 responses in human disease [6, 8]. Much of this evidence has been gathered using two methods. Antigen-specific $CD4^+$ T-cell clones secreting predominantly Th1 or Th2 cytokines *in vitro* have been isolated from affected tissues, fluid (for example, cerebrospinal fluid in multiple sclerosis or synovial fluid in arthritis), or associated lymphoid tissue. Panels of T-cell clones secreting either a Th1 or a Th2 profile of cytokines have been derived from humans with different infective and noninfective diseases. This approach has not been applied to the study of human GN. The second approach has been the *in vivo* assessment for Th1 or Th2 patterns of cytokine mRNA using reverse transcription-polymerase chain reaction (RT-PCR), Northern blotting, or *in situ* hybridization.

Table 2. Non-infective conditions associated with predominant Th1 or Th2 effector responses in humans

Th1	Th2
Multiple sclerosis [55–57]	Asthma [58–60]
Autoimmune thyroiditis [61]	Atopic dermatitis [62, 63]
Graves ophthalmopathy [64]	Vernal conjunctivitis [65]
Rheumatoid arthritis [66]	Normal pregnancy [67]
Lyme arthritis [68]	Idiopathic hypereosinophilia [69]
Reactive arthritis [70]	Omenn's syndrome [71]
Contact (nickel) dermatitis [72]	SLE [73]
Type 1 diabetes mellitus [74]	Scleroderma [75]
Erythema nodosum [76]	Chronic GVHD [75]
Recurrent abortion [77, 78]	
Psoriasis vulgaris [79]	
Renal allograft rejection [80]	
Primary biliary cirrhosis [81]	
Pulmonary sarcoidosis [82, 83]	
Crohn's disease [84]	

A number of host immune responses to infectious pathogens in humans demonstrate either Th1 or Th2 predominance. For example, the immune response in tuberculosis is Th1 predominant, whereas T-cell responses to *Toxocara canis* have been characterized as Th2 [54]. As well as the role of Th1 and Th2 subsets in infective immune responses, there have been numerous studies of Th1 and Th2 predominance in noninfectious diseases using clones and/or cytokine mRNA detection. These are summarized in Table 2. Organ-specific autoimmune diseases and granulomatous diseases have been linked to predominant Th1 responses, whereas allergy, pregnancy, and some systemic autoimmune diseases have been linked to Th2 patterns of immune responses.

Two recent studies provide data for a genetic basis for Th1 or Th2 predominance in human populations. Oro et al [85] found a lower incidence of IgE-mediated disease in people with multiple sclerosis than in normal individuals and Shirakawa et al [86] found an inverse relationship between tuberculin skin DTH and atopy in Japanese school children.

There are different patterns of immune effector response in different forms of GN. Many studies have addressed the roles of individual cytokines *in vivo* or *in vitro*. It is important to interpret the findings of these studies in the context of the underlying immune response and to assess whether the immune effectors and cytokines indicate either a predominant Th1 or Th2 pattern in individual forms of GN. The relevance of Th1 and Th2 pathological responses in a number of different nonrenal diseases is increasingly recognized. The initiation and regulation of these responses are currently being defined. If some or all of the patterns of GN suggest Th1 or Th2 predominance of the nephritogenic immune responses, this knowledge may allow rational and targeted biological therapies to be developed to manage the outcomes of GN more effectively.

EVIDENCE FOR TH1/TH2 POLARIZATION OF NEPHRITOGENIC IMMUNE RESPONSES IN GLOMERULONEPHRITIS

A number of criteria can be established to distinguish polarized Th1 or Th2 nephritogenic immune responses. The criteria rely on the ability of these Th cell subsets to be distinguished by their cytokine profile and the immune effectors they direct. These criteria are outlined in Table 3.

A Th1-predominant nephritogenic immune response should be associated with antigen presentation in the setting of IL-12 expression and the production of IFN-γ and IL-2 by Th1 cells. The effector responses would include the accumulation of T cells and macrophages, as well as the deposition of "Th1 type" IgG subclasses in glomeruli. Th2-predominant nephritogenic immune responses would be expected to demonstrate IL-4 and IL-10 production by antigen-specific T cells. DTH responses would be weak or absent in glomeruli, and "Th2 type" IgG subclasses would be deposited. This schema is complicated by the fact that counterregulatory cytokines, such as IL-10, are present within glomeruli of more severe glomerular lesions [99]. However, in this study, the IL-10 observed was not shown to be a product of Th cells within the lesion. Its presence may reflect counterregulatory mechanisms in the context of severe injury rather than indicating the nature of the cognate, T-cell–driven response to a nephritogenic antigen(s).

More direct proof of Th1 predominance would include the diminution of nephritogenic immune responses and associated GN by IL-4 and/or IL-10 administration or by the inhibition of Th1 cytokines IL-12, IFN-γ, or IL-2. The administration of Th1 cytokines or inhibition of IL-4 or IL-10 should enhance Th1 responses and exacerbate the severity of associated GN. Th2-predominant immune responses would be enhanced by IL-4 and/or IL-10. Blockade of these cytokines should diminish Th2-dependent immune responses and the associated antigen-specific Th2-type IgG response. Th2 immune responses might be diminished or deviated toward Th1, with potentially more severe glomerular injury if Th1 cytokines are administered. For these criteria to be completely assessed, the antigen inducing the nephritogenic immune response must be known. Because of the potential hazards of inappropriate *in vivo* cytokine administration, complete assessment of these criteria is likely to occur in only experimental GN.

A number of limitations constrain the evaluation of nephritogenic responses in human GN. In a vast majority of cases, the precise nature of the disease-initiating antigen is unknown, so the characteristics of the antigen-specific immune response cannot be clearly defined. The conditions influencing polarization of the immune response are usually unknown. Even in situations in which GN is associated with strong responses to identified antigens (for example, antinuclear antibodies or antibodies

Table 3. Idealized criteria used in this review for the assessment of Th1/Th2 predominance in GN. In part b), references from experimental models of GN that fulfill the criteria are quoted

(a) Observational data	Th1	Th2
DTH effectors in glomeruli	T cells, macrophages, fibrin present	T cells, macrophages, fibrin absent
IgG subclasses in glomeruli	IgG1, IgG3 (human) IgG2a, IgG3 (rodent)	IgG4 (human) IgG1 (rodent)
Cytokines in glomeruli[a]	IL-12, IL-2, IFN-γ	Absence of Th1 ± IL-4, IL-10
Antigen-specific systemic immune response	Skin DTH present IgG1, IgG3 (human) IgG2a, IgG3 (rodent) IL-2, IFN-γ	Skin DTH absent IgG4 (human) IgG1 (rodent) IL-4, IL-10
(b) Functional inventions	**Th1**	**Th2**
Administering IL-12 and/or IFN-γ (Th1 cytokines)	Augments GN [87]	Attenuates GN [88] or shifts to Th1 type GN [87]
Blocking Th1 cytokines	Attenuates GN [89–91]	
Administering IL-4 and/or IL-10 (Th2 cytokines)	Attenuates GN [92–94][b]	Augments GN [96]
Blocking Th2 cytokines	Augments GN [95]	Attenuates GN [97, 98]

[a] Assessment of this parameter is complicated by the possibility that counterregulatory cytokines produced by effector cells or intrinsic glomerular cells may be present within the lesion

[b] Also see (abstract; Fouqueray et al., *J Am Soc Nephrol* 7:1698, 1996)

against extractable nuclear antigens), proof of the causality of particular antigens in the induction of glomerular injury is indirect or circumstantial. Finally, Th1 or Th2 cytokines have not been administered or selectively inhibited in human GN. However, observations of the pattern of GN observed in people given IL-2 or IFN-γ for other diseases provide evidence for Th1 predominance in some forms of GN.

There is no comprehensive study of the Th1/Th2 predominance of an antigenic-specific nephritogenic human response in GN. There have been many studies of the cytokines produced by peripheral blood mononuclear cells in various types of human GN. Most of these use whole cell populations without antigen-specific stimulation, and results attributing the cytokine profile to the antigen-specific nephritogenic immune response must be interpreted with caution. Some attempts have been made, using biopsies of patients with GN, to determine the pattern of cytokines produced within kidneys. Although these are more relevant, many of these studies have examined the presence of only one or two cytokines and, therefore, do not provide sufficient data to allow a Th1/Th2 predominance to be determined. However, the immunohistological evaluation of renal biopsies has provided a great deal of information on the nature of the immune effectors in glomeruli. The immunoglobulin isotypes deposited in glomeruli and the presence or absence of DTH effectors permit an assessment of the predominance of Th1 or Th2 immune effectors in several subtypes of GN.

TYPES OF HUMAN GLOMERULONEPHRITIS LIKELY TO HAVE TH1-PREDOMINANT NEPHRITOGENIC IMMUNE RESPONSES

Crescentic glomerulonephritis

In crescentic GN, the pattern of glomerular immune effectors strongly suggests that injury results from Th1-predominant nephritogenic immune responses. A number of immunohistochemical studies have demonstrated the prominent participation of DTH effectors (macrophages, T cells, and fibrin) in this form of GN [100–107]. These observations apply to all forms of crescentic GN, regardless of the immunohistological category or the pattern of immunoglobulin participation. Crescent formation complicates many forms of GN but is most prominent in anti-glomerular basement membrane (GBM) GN and "pauci immune" antineutrophil cytoplasmic antibody (ANCA)-associated GN. Although both demonstrate significant T-cell and macrophage influx, the former demonstrates strong linear IgG deposition, whereas the latter is characterized by a paucity or absence of immunoglobulin in glomeruli. The variable participation of humoral immunity and the invariant appearance of effectors of DTH suggest that cell-mediated immunity is likely to play a predominant role in the development of crescentic GN. These observations are supported by specific experimental studies that confirm the potential for T-cell–directed immunity to account alone for the full expression of crescentic GN [89, 108–111]. The likely importance of DTH effectors in crescentic GN is consistent with Th1 direction of these forms of GN.

Anti-glomerular basement membrane glomerulonephritis

As well as the prominent participation of Th1 effectors in glomeruli, recent studies have confirmed the presence of circulating T cells specifically reactive with the nephritogenic epitopes derived from GBM [112, 113]. The specific antigen-stimulated cytokine profiles of these T cells have not been reported. Anti-GBM disease is strongly associated with (and clinically diagnosed by) the presence of anti-GBM antibody. Studies of the IgG subclasses of

these antibodies in the circulation and/or deposited in the kidney show the predominance of Th1 subclasses (IgG1 and/or IgG3), although IgG4 was also noted [114–117]. Further analysis provided in some of these reports supports a role for Th1 responses. In the study of Weber et al, only IgG1 (directed against GBM) was elevated out of proportion to the total IgG subclass levels in sera [114]. The presence of IgG4 anti-GBM antibodies has been noted in a minority of patients, predominantly older females [117]. The presence of Th2-type IgG4 anti-GBM antibodies was reported in a patient in which active disease did not recur after treatment of anti-GBM GN, whereas in another patient, recurrent GN was associated with the reappearance of IgG1 in the serum [115]. Taken together, these data suggest Th1 predominance of the injurious anti-GBM autoimmune response.

"Pauci immune" crescentic glomerulonephritis

Most patients with crescentic GN have glomerular injury characterized by the absence or paucity of glomerular antibody deposition. The demonstration of DTH effectors (macrophages, T cells, and fibrin) in glomeruli in the absence or paucity of immunoglobulin [100, 104, 107] provides strong evidence for a predominant Th1 nephritogenic immune response.

Patients with active Wegener's granulomatosis have HLA-DR$^+$ CD4$^+$ cells in the peripheral blood that produce increased IFN-γ (but not IL-4, IL-5, or IL-10) compared with normal donors or to those with inactive disease [118]. This increased IFN-γ production was inhibited by exogenous IL-10. Monocytes from these patients produced increased IL-12, although this increase also occurred in those with inactive disease. Increased levels of markers of cell-mediated immunity, including neopterin, TNF receptor, and IL-2 receptor, are present in active disease and correlate with disease activity [119].

This form of GN is strongly associated with the presence of circulating ANCAs. The role of this antibody in the development of glomerular injury remains unclear. The subclasses of these antibodies have been variably reported to be Th1 type or Th2 type. The ANCAs most potent in inducing neutrophil activation are the Th1 IgG3 subclass [120].

To complicate this analysis further, the predominant ANCA subclass has been reported to vary according to the particular neutrophil antigen to which it is directed [121]. The subclass of ANCA also may vary according to the time of assessment in the disease process and activity of disease. IgG3 is most commonly reported as the predominant subclass involved. IgG3 subclass ANCA is associated with the onset of disease and correlates with presence and severity of renal disease [122].

In summary, pauci immune GN is distinguished from other forms of GN by the predominance of glomerular DTH effectors and paucity of antibody. These data suggest that the immune response relevant to renal injury in this disease is Th1 predominant. Moreover, in other granulomatous diseases, such as tuberculosis, antibody plays a relatively minor role.

Membranoproliferative glomerulonephritis

Few studies are available to allow assessment of the polarization of Th responses in membranoproliferative GN. The published reports suggest that Th1 responses may direct injury in this condition. IgG3 (Th1-type subclass) is the predominant subclass in glomeruli [123, 124], and autoantibodies to solid phase C1q are restricted to IgG3 [125]. The presence of glomerular DTH effectors [104] is also consistent with a predominant Th1 response directed toward as yet unidentified antigens in glomeruli.

Th1 cytokine-induced crescentic/proliferative glomerulonephritis

The increasing application of cytokine therapy for malignant and autoimmune diseases has produced a number of outcomes, suggesting that Th1 cytokines direct crescentic GN in humans. The Th1-associated cytokines IFN-γ and IL-2 used for the treatment of rheumatoid arthritis and malignancy, respectively, have been associated with the development of crescentic nephritis. Two patients treated with IFN-γ for rheumatoid arthritis developed systemic lupus erythematosus (SLE) with proliferative and/or crescentic GN [126, 127]. Another patient who retrospectively probably had IgA disease developed crescentic GN following IL-2 therapy [128]. Two further patients developed either crescentic or severe proliferative GN *de novo* in association with IL-2 treatment [129]. These data strongly support the argument that Th1 cytokines direct crescentic GN in humans.

SUBTYPES OF HUMAN GLOMERULONEPHRITIS WITH EVIDENCE FOR PREDOMINANT Th2 NEPHRITOGENIC IMMUNE RESPONSES

Membranous glomerulonephritis

This form of GN is characterized by IgG and complement deposition in glomeruli. The analysis of renal biopsies for DTH effectors is consistently negative. A number of reports have shown that IgG4 (Th2-type subclass) predominates in renal biopsies [123, 130–133]. This pattern is observed in both idiopathic- and lupus-associated membranous GN [131, 132]. Although it is possible that the affinity and/or size of immune complexes is relevant to their subepithelial localization in membranous GN, there is evidence that systemic immune responses in this disease are characterized by the presence of IgG4. IgG4 is overrepresented in the circulating immune complexes [134] and predominates in serum cryoglobulins [132] in membranous GN. Although limited, these data would suggest that the initiating glomerular antigen in membra-

nous GN initiates Th2-type predominantly humoral immune responses in membranous nephropathy.

Minimal change glomerulonephritis

The absence of humoral and cellular immune effectors in glomeruli makes it difficult to argue strongly that this form of GN results from immune responses directed toward endogenous or planted/deposited exogenous antigens. However, many reports suggest systemic immune activation in this condition with evidence of the production of a humoral substance with significant effects on vascular permeability [135–137]. The reports of production of Th2-type cytokines by peripheral blood mononuclear cells and association with IgE, IgG4 production, and atopy [138, 139] suggest that the systemic immune activation associated with minimal change GN is the Th2 type.

TYPES OF HUMAN GLOMERULONEPHRITIS WITH EVIDENCE FOR HETEROGENEITY OF Th1/Th2 PREDOMINANCE

IgA nephropathy

There is no clear evidence for either Th1 or Th2 predominance in IgA-associated GN. Although this disease is characterized by glomerular IgA deposition, IgG deposits are also observed. The subclasses of these deposits are predominantly IgG1 and IgG3 (Th1 isotypes) [140]. The participation of DTH effectors is seen in only a minority of patients, typically those with crescentic IgA disease [106]. The pattern of renal cytokine expression suggests both Th1 and Th2 involvement, with IL-4 expression emphasized in some reports [141–143] and IFN-γ expression emphasized in other reports [144, 145]. The cytokines found in serum or produced by peripheral blood mononuclear cells also suggest participation of either Th2 [146, 147], Th1 [148–150], or both Th1 and Th2 involvement [144, 151, 152].

There is evidence that abnormal glycosylation of IgA is relevant to the glomerular deposition of IgA in IgA nephropathy [153, 154]. A recent report has implicated Th2 responses in this process [155]. The addition of IL-4 and IL-5 to B cells significantly altered the terminal glycosylation of IgA, which may promote deposition of IgA in glomeruli.

Given the wide spectrum of severity and outcome in IgA nephropathy, it may be simplistic to assume that all nephritogenic immune responses in this disease are similar. It may be possible that the pattern of immune responses in this condition differs among individuals. Polarization toward a Th1 response may be most prominent in the subgroup with strong evidence of glomerular DTH, that is, crescentic IgA GN. The occurrence of synpharyngitic crescentic GN may be induced by intercurrent antigen stimuli (for example, sepsis) polarizing the nephritogenic immune responses in susceptible humans toward Th1.

Lupus nephritis

It is difficult to ascribe the autoimmune systemic responses of SLE to either purely Th1 or Th2, although the majority of evidence suggests that dysregulated IL-10 might be important. Particular immunoglobulin isotypes in serum or the predominant cytokines of blood mononuclear cells vary with the severity of disease, whether unstimulated or mitogen-stimulated cells are studied, or with the particular autoantigen studied. Blood mononuclear cell cytokine profiles (unstimulated or mitogen stimulated) have been more frequently reported to be Th2 rather than Th1. Dysregulated and increased production of IL-10 from B cells and monocytes [73, 156–159] have been demonstrated and may have a genetic basis [160]. IL-4 has been reported as being increased [158, 161, 162], whereas reduced IL-12 [163] and IFN-γ [158, 164] have been commonly reported. However, less frequent reports of increased production of IFN-γ and IL-2 have been noted together with reduced IL-4 [165]. Elevation of serum neopterin levels [166] and soluble IL-2 receptor levels correlating with disease is indirect evidence of involvement of cell-mediated autoimmunity.

Despite these observations, the profile of autoantibody subclasses in lupus is more often reported to be of the Th1 pattern with predominant IgG1 and IgG3 [167–171] and reduced levels of IgG2 and IgG4 [172]. Some reports have noted a Th2 (IgG2, IgG4 predominant) response to some autoantigens, whereas the same sera show Th1 (IgG1, IgG3) responses to others. Serum cryoglobulins in proliferative lupus nephritis are IgG3 and IgG4 in membranous lupus nephritis [132]. Reports suggest that in patients with lupus nephritis, IgG1 and IgG3 autoantibodies correlate with activity [173].

In the kidney, the pattern of IgG subclasses varies with the histologic pattern of disease. Proliferative and crescentic forms of GN are associated with Th1 immunoglobulin subclasses [132] and prominent influx of DTH effectors, macrophages, T cells, and fibrin. Nonproliferative lupus nephritis (membranous) is characterized by the deposition of IgG4 [132] and the absence of DTH effectors [174]. IL-4 mRNA has been detected in the glomeruli of patients with lupus nephritis, and its expression was inversely correlated with the degree of glomerular injury [143].

These observations suggest that the Th1/Th2 profile of the immune response is heterogeneous in SLE and that immune cells other than T cells are producing cytokines, particularly IL-10, which may be important in disease pathogenesis. However, the reactants in nephritic glomeruli support the argument that Th1 responses induce DTH effectors and proliferation and crescentic GN, whereas Th2 responses lead to less severe nonproliferative, predominantly humorally-mediated renal injury.

EXPERIMENTAL MODELS OF GLOMERULONEPHRITIS

Much of our knowledge of the immunopathogenesis of GN comes from the study of experimental models. Evidence suggests that similar events are likely to occur in various forms of human GN. Many studies provide data relevant to the evaluation of the role Th1 and Th2 subsets in determining the pattern of injury and outcome of disease. Many of the criteria for Th1/Th2 predominance in GN (Table 3) have been tested in experimental GN.

Glomerulonephritis induced by anti-glomerular basement membrane globulin or glomerular basement membrane extracts

Proliferative GN may be induced passively by the injection of heterologous anti-GBM antibodies into naive animals. This results in transient glomerular injury mediated by activation of complement and recruitment of neutrophils [175]. As there is no involvement of an active immune response, there is no role for cognate T cells, or T helper cell subsets. The autologous phase of anti-GBM is characterized by cognate autologous immune responses to an antigen (heterologous immunoglobulin) planted in the glomerulus. Sensitization to the heterologous immunoglobulin prior to the administration of anti-GBM globulin accelerates the induction of GN. Autoimmune forms of anti-GBM GN can be induced by immunization with GBM antigens and involve loss of tolerance with immune responses against the GBM itself. The latter two models of GN result in severe crescentic injury in which the role of Th1/Th2 subsets can be dissected.

One approach has been to use strains of mice with different Th1/Th2 predominance in their immune responsiveness. Only mice with Th1 predominance develop severe crescentic GN. C57BL/6 mice develop healing responses following *Leishmania major* infection because they develop Th1-dominated immune responses [22]. When sensitized C57BL/6 mice were challenged with antimouse GBM globulin, they exhibited strong DTH responses and predominant IFN-γ production by antigen-stimulated T cells consistent with a Th1 response. Their pattern of glomerular injury showed crescent formation, glomerular accumulation of $CD4^+$ cells, macrophages, and prominent fibrin deposition (Fig. 2) [89]. The accumulation of effector of DTH implies a Th1-driven immune response in glomeruli. The presence of fibrin, a product of this Th1 response, may amplify the inflammatory response via its chemotactic effects on macrophages [176]. Although autologous antibody was deposited in glomeruli in this model, it is not essential for crescent formation because mice with a genetic inability to produce antibody (immunoglobulin μ chain gene knock-out mice) still developed crescentic disease of similar severity to normal mice [111]. Furthermore, the lesion was $CD4^+$ dependent in the effector phase. Depletion of $CD4^+$ cells at antigen challenge (that is, after the establishment of the immune response) resulted in the abrogation of crescent formation and a marked reduction in the severity of GN without affecting the titers or glomerular deposition of antibody [89].

Murine models provide an opportunity to test the relevance of Th1/Th2 predominance to the pattern of GN by *in vivo* manipulation of key Th1 and Th2 cytokines. The contribution of a number of the cytokines involved in the initiation and maintenance of Th cell responses (IL-12, IFN-γ, IL-4, and IL-10) has been explored. Several studies using the anti-GBM model support the hypothesis that crescent formation in C57BL/6 mice is a manifestation of predominant Th1 responses to nephritogenic antigens [87, 89, 91–93, 95]. Interleukin-12, produced by antigen-presenting cells, acts on uncommitted T cells to induce differentiation into Th1 cells [33]. Blockade of IL-12 by a neutralizing monoclonal antibody attenuated crescent formation and glomerular injury (but had little overall effect on humoral responses), whereas the administration of recombinant murine IL-12 to mice with mild GN accelerated this disease and induced severe crescentic GN [87].

Interferon-γ, produced by Th1 cells, activates macrophages and may play a role in the induction and maintenance of the Th1 response. Neutralization of IFN-γ with a monoclonal antibody attenuated crescent formation and diminished renal injury in an accelerated murine model of anti-GBM GN, emphasizing the importance of Th1 effectors in this crescentic disease. This was associated with fewer glomerular macrophages but no effect on the circulating titers of antigen-specific antibody [89]. These findings have been supported by recent experiments demonstrating that mice genetically deficient in IFN-γ developed fewer crescents, diminished glomerular $CD4^+$ T cells, macrophages, and fibrin together with a lesser degree of functional injury [91]. Haas et al also reported lesser renal injury in the same model of crescentic GN in IFN-γ receptor-deficient mice [90], although the extent of some of the reductions in disease parameters, including glomerular crescent formation, did not reach statistical significance.

Interleukin-4 is produced predominantly by T cells and is one of the determinants of the Th1/Th2 balance. When GN was induced in Th1-prone mice genetically deficient in IL-4, they developed antigen-specific immune responses that were more polarized toward Th1 (increased skin DTH, decreased IgG1, and increased IgG3) compared with genetically normal, strain-matched mice. These changes translated into markedly increased renal impairment, increased glomerular crescent formation, and increased accumulation of glomerular effectors of DTH [95]. These data suggest that in Th1-prone mice,

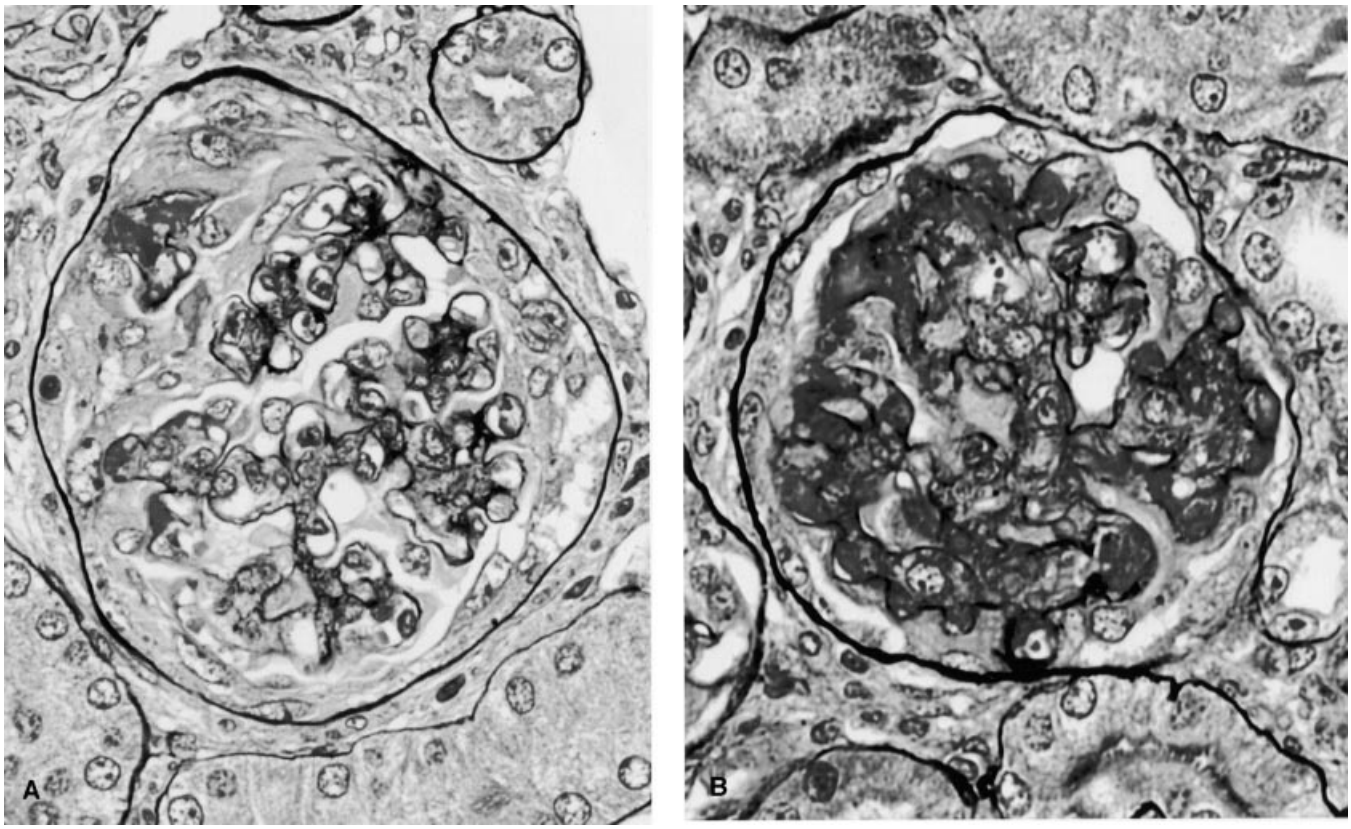

Fig. 2. Different patterns of glomerular injury in C57BL/6 and BALB/c mice with anti-glomerular basement membrane glomerulonephritis (anti-GBM GN). Th1-prone C57BL/6 mice develop proliferative GN with frequent glomerular crescent formation (*A*). BALB/c mice (Th2 prone relative to C57BL/6 mice) develop GN with paucity of DTH effectors and sparse crescent formation (*B*; silver methenamine/acid fuchsin stain; magnification ×400).

endogenous IL-4 attenuates crescentic GN by modulating the Th1/Th2 balance of the immune response.

The administration of Th2 cytokines provides another way of modulating Th1 responses and examining the effects on the development of GN. Treatment with either IL-4 or IL-10 or both selectively inhibited Th1 systemic and nephritogenic immune responses [92] when given prior to the initiation of anti-GBM GN. Skin DTH was reduced, as was IFN-γ production, by splenic T cells. Total circulating antigen-specific immunoglobulin was unaltered, but levels of IgG2a and IgG3 were selectively reduced. Glomerular crescent formation was abrogated, and glomerular effectors of DTH ($CD4^+$ T cells, macrophages, and fibrin) were diminished. Proteinuria was decreased in all treated groups, and the combination of IL-4 and IL-10 prevented the renal impairment, whereas IL-10 alone provided partial protection of renal function.

The beneficial effects of IL-4 and/or IL-10 treatment prior to disease initiation raise the important prospect of using these cytokines to treat established disease. This prospect has now been addressed by five studies using rodent models. Combined treatment with IL-4 and IL-10 commenced 72 hours after the administration of anti-GBM globulin to sensitized C57BL/6 mice reduced glomerular crescent formation and preserved renal function [93]. In the same study, IL-10 treatment showed a trend to reduced crescent formation, whereas IL-4 alone had no effect on crescent formation or renal function. Although all treatments had some effect on the accumulation of glomerular $CD4^+$ cells and macrophages, there were no changes in the systemic immune response to sheep globulin (IgG subclasses, splenic T-cell IFN-γ), suggesting that the Th1/Th2 balance between the responding subsets has not been altered. The synergistic effect of IL-4 and IL-10 has been observed in other models of Th1-mediated immune responses [177, 178].

The diminution of GN is likely to be due at least partially to immunomodulatory properties of IL-4 and IL-10 on the cellular effectors of injury. Support for this conclusion comes from a study by Tipping et al that assessed the T-cell independent effects of IL-10 in a macrophage-dependent model of anti-GBM GN induced in Wistar-Kyoto rats by passive administration of autologous antibody (abstract; Tipping et al, *Nephrology* 3:S231, 1997). In this model, rats develop glomerular macrophage accumulation and proteinuria, which were attenuated by

IL-10 treatment. Phenotypic markers of glomerular macrophage activation were also reduced in IL-10–treated rats.

Three further studies have examined the effects of IL-4 or IL-10 on accelerated anti-GBM GN in inbred Sprague-Dawley rats, a strain in which the Th1/Th2 balance of the responding T-cell subsets has not been defined. In these studies, cytokine treatment was commenced after sensitization but immediately prior to antigen challenge. Tam et al reported that rat IL-4 reduced proteinuria and histologic indices of renal injury in a noncrescentic model, but had no effect on the systemic immune response to the nephritogenic antigen [94]. They observed a modest reduction in glomerular macrophage numbers and an up-regulation of mRNA for IL-1 receptor type II, which acts as a decoy receptor for IL-1. Interleukin-10 gene transfer into rats either by transfer of transfected mesangial cells or by transferring the IL-10 gene into skeletal muscle reduced proteinuria in the autologous phase of the disease (abstract; Fouqueray et al, *J Am Soc Nephrol* 7:1698, 1996). Chadban et al administered murine IL-10 to inbred Sprague-Dawley rats and found no change in crescent formation [179]. Treated rats developed an immune response against murine IL-10, which the authors state may have contributed to the renal injury observed in the treated animals. Renal expression of MCP-1 and IL-1β mRNA was reduced by high-dose IL-10, but glomerular macrophage numbers were increased. High-dose treatment also increased antigen-specific antibody levels in the serum but not the immunoglobulin deposition in glomeruli. Although skin DTH was reduced, the accumulation of T cells in glomeruli was not assessed. Although it is not clear how this study contributes to the understanding of the nephritogenic immune response, it indicates the need for careful dose selection in the pharmacological use of IL-10 in GN.

Other studies support the hypothesis that Th1 responses initiate proliferative and/or crescentic GN in anti-GBM models. Coelho et al studied the role of cellular immunity in a noncrescentic model of anti-GBM GN in two inbred rat strains [180]. Lewis rats are susceptible to Th1-mediated organ-specific immune diseases such as EAE, whereas the Brown-Norway strain develop Th2 based autoimmunity when injected with $HgCl_2$. Four days after the induction of GN in sensitized rats, both strains had developed similar humoral responses to sheep globulin; however, the degree of renal injury was more pronounced in Lewis rats. They developed more skin DTH and increased numbers of glomerular T cells and macrophages than Brown-Norway rats. The authors conclude that the increased susceptibility of Lewis rats to this disease was due to their capacity to mount a Th1 immune response.

Kalluri et al demonstrated that susceptible strains of mice immunized with α3(IV) NC1 collagen developed an autoimmune response directed against the Goodpasture antigen and crescentic GN with mononuclear infiltrates and antibody deposition [181]. This disease could be transferred to T-cell receptor-deficient mice by lymphocytes from nephritic mice. Susceptibility to nephritis was associated with a Th1-like Th cell response, with IL-12 and IFN-γ detectable in glomeruli (but not IL-4 or IL-10) and antigen-specific IgG2a. Nonsusceptible strains did not develop glomerular cellular infiltrates, and neither IL-12 nor IFN-γ was detectable in glomeruli. In resistant mice, although antibody was deposited in glomeruli and total serum levels of antigen specific antibody were unaltered, IgG2a was barely detectable, indicating a poor Th1 response. A susceptible strain, SJL/J, could be tolerized orally to α3(IV) NC1 collagen, with resulting abrogation of crescent formation. The development of tolerance was associated with reduced serum IgG2a levels, whereas serum IgG1 levels were unaffected. IL-12 was undetectable in glomeruli. These findings in autoimmune anti-GBM GN support the data from other models that crescent formation results from Th1 responses to nephritogenic antigens.

BALB/c mice produce IL-4, have reduced DTH responses, and do not heal when infected with *Leishmania major* because of an ineffective Th1 immune response to this pathogen [22]. Accelerated anti-GBM GN in BALB/c induced glomerular injury with only occasional crescent formation (5% to 10% of glomeruli; Fig. 2) and absent cutaneous DTH to the nephritogenic antigen. Their nephritis was humorally mediated and was not $CD4^+$ dependent in the effector phase [89, 182]. Only the minor crescentic component was blocked by $CD4^+$ depletion [89]. Marked crescent formation in this strain was induced by administration of IL-12 [87], but IL-4–deficient mice did not develop severe crescentic GN (in contrast to the findings in mice that do make normal Th1 responses) [87]. Therefore, in mice with intact IL-12 responses, the observed Th1-predominant nephritogenic immune response is negatively regulated by endogenous IL-4 [95]. In this context, the findings in BALB/c IL-4 −/− mice suggest that in mice that do not generate predominant Th1 responses, endogenous IL-4 has little regulatory effect on cell-mediated nephritogenic immune responses. These experiments show that the BALB/c strain that is resistant to Th1 responses, cell-mediated glomerular injury, and crescent formation is genetically deficient at the level of the IL-12/IL-12 receptor system and imply that the presence of normal IL-12 production and responsiveness is critical to nephritogenic Th1 responses.

These studies in murine GN support the available human data on Th1 predominance of crescentic GN. Mouse strains prone to predominant Th1 nephritogenic immune responses develop crescentic GN, which is $CD4^+$ effector dependent, antibody independent, feature prominent DTH effectors, and are associated with the presence of Th1 cytokines in glomeruli. In these strains, cytokine

manipulations, which inhibit Th1 responses attenuate GN, whereas those that amplify Th1 or inhibit Th2 responses exacerbate disease. In a strain without Th1 predominance (BALB/c), enhancing Th1 responses via IL-12 administration can induce crescentic GN.

A number of studies have examined Th1 and Th2 cytokines in the heterologous phase of anti-GBM GN in which active cognate immune responses are not involved. The presence of IL-4, IL-10, and IL-13 mRNA in renal tissue in a rat model has been suggested to be linked to the production of anti-inflammatory eicosanoids [183]. IL-10 [184], but not IL-4 administration [94], was protective in rats, whereas IL-4–deficient mice developed increased proteinuria [185]. These observations suggest potential effects of Th1/Th2 cytokines on the response of intrinsic glomerular cells to injury or may be explained by effects of these cytokines on neutrophils. IL-12 did not increase proteinuria in the heterologous phase of anti-GBM–induced injury in naive mice (abstract; Kitching et al, *J Am Soc Nephrol* 8:458–459, 1997).

Some cytokines integral to the Th1 or Th2 systemic immune response may be produced by or may affect intrinsic glomerular cells. However, there is no evidence that Th1 or Th2 cytokines produced by intrinsic glomerular cells are involved in the initiation of nephritogenic immune responses. Although intrinsic glomerular cell-derived Th1- or Th2-type cytokines may interact with immune effector cells, there is no evidence that the pattern of effector responses is regulated by cytokines produced in the target organ. Although there is the potential for significant interaction between intrinsic glomerular cells and immune cells, there is no evidence that the overall pattern of immune response is dictated by these interactions, as opposed to the systemic immune response.

Experimental immune-complex glomerulonephritis

Murine lupus nephritis. In experimental anti-GBM GN, Th1-driven nephritogenic immune responses induce glomerular DTH and crescent formation, whereas antibody responses play only a minor role. In contrast, in mice developing lupus-like syndromes, antibody production is essential for development of GN [186]. In these models, injury is mediated substantially by autoantibodies and immune complex deposition, and a major role for DTH responses has not been demonstrated. The available data on the role of Th1 and Th2 cytokines in lupus nephritis suggest that both Th1 and Th2 immune responses are required for maximal autoantibody production and full expression of GN. Lupus-like syndromes develop spontaneously in the MRL/*lpr* strain [including MRL/*lpr* mice carrying an autoimmune acceleration gene (*Yaa*) on the Y chromosome] and NZB/W F_1 hybrid mice. These syndromes are associated with development of autoantibodies and lymphoproliferation. Mice develop proliferative GN that may result in development of crescents, as well as lymphadenopathy, vasculitis, and arthritis.

Although somewhat conflicting, studies of the cytokine and antibody isotype profiles in lupus mice provide evidence of involvement of both Th1 and Th2 subsets. Increased expression of IL-4 mRNA in the thymus and spleen has been reported during the early stages of disease in MRL/*lpr* mice [187]. Mitogen-stimulated T cells from NZB/W mice produce higher levels of Th2 cytokines (IL-4 and IL-10) and lower levels of Th1 cytokines (IFN-γ and IL-2) than C57BL/6 mice [188]. These data suggest Th2 involvement. On the other hand, involvement of Th1 immune responses is suggested by a number of studies. The ratio of IFN-γ to IL-4 secreting cells in MRL/*lpr* mice is increased compared with MRL +/+ mice [189]. IL-12 levels are increased in sera of MRL/*lpr* mice [190]. Nucleosomal peptide epitopes stimulated production of Th1 cytokines and induced severe lupus nephritis in SWR × NZB F_1 mice [191]. In the kidney, Th1 cytokine mRNA has been detected. IL-12 protein and mRNA is up-regulated in tubular cells and macrophages [192], whereas IFN-γ is overexpressed and is associated with enhanced MHC II [193].

Although the pattern of cytokine expression in lupus mice does not clearly identify a predominant role for either Th1 or Th2 subsets, there has been considerable work in MRL/*lpr* mice suggesting a pathogenic role for IgG3, a Th1-type IgG subclass [194–197]. In four strains of MRL mice, including those with the *Yaa* gene, disease progression correlated with increased production of IgG2a and IgG3 anti-DNA autoantibodies and increased IFN-γ production but not with IgG1, IL-4, or IL-10 production [195]. Although IgG2a is the dominant subclass of autoantibodies in the serum of MRL/*lpr* mice, IgG3 autoantibodies show a greater tendency to form immune complexes, and their kidney deposition is higher than IgG2a [194]. IgG3 monoclonal antibodies with cryoglobulin activity derived from lupus-prone mice induce "wire loop" lesions in glomeruli [196] and are nephritogenic independent of their capacity to form immune complexes [196, 197].

Studies of the effects of administration of recombinant cytokines and monoclonal antibodies and the use of gene knock-out and transgenic mice have produced data that support a role for both Th1 and Th2 subsets in lupus nephritis. The transfer of either IL-4– or IL-12–stimulated splenocytes to NZB/W mice promoted immunoglobulin synthesis and anti-dsDNA antibody production [198]. A majority of studies suggest that blocking or deleting either Th1 (IFN-γ, IFN-γR) [199–203] or Th2 cytokines (IL-4 or IL-10) [199, 204] diminished disease, with variable effects on total autoantibody levels but with the appropriate shifts in IgG subclasses. Particular emphasis has been placed on the role of IFN-γ, mainly in the generation of autoantibodies. However, one study

in particular has addressed its role in promoting cell-mediated effector responses in renal injury, finding that MRL/*lpr* IFN-γR–deficient mice had reduced T cells and macrophages in glomeruli [203]. In a study of IL-10 in NZB/W mice, anti-TNF-α antibodies abolished the protection afforded by blocking IL-10, suggesting that up-regulation of endogenous TNF-α may also play an important role [204]. The administration of IL-12 [190], IFN-γ [202], or IL-10 [204] enhanced disease. IL-10 administration accelerated the development of autoimmunity, but IFN-γ or IL-12 did not alter autoantibody production. These studies demonstrate that augmentation of either Th1 or Th2 responses can increase the severity of murine lupus nephritis.

Mercuric chloride-induced glomerulonephritis. Mercuric chloride induces polyclonal B-cell activation that is associated with a self-limited syndrome of GN, vasculitis, and arthritis in mice, rats, and rabbits [205]. These animals develop hypergammaglobulinemia with high circulating levels of IgG1 and IgE [206], driven by Th2 $CD4^+$ T cells [207]. They also exhibit a range of autoantibodies that include rheumatoid factors, anti-DNA antibodies, anti-myeloperoxidase [208], and anti-GBM antibodies [209]. Brown-Norway rats develop a biphasic self-limited GN with marked proteinuria and nephrotic syndrome. The initial phase of glomerular injury is associated with IgG1 and IgG2a anti-GBM antibodies, which are deposited in glomeruli in a typical linear fashion [210]. However, crescent formation is not a feature [211]. This is followed by the development of circulating immune complexes, which deposit in glomeruli in a granular pattern, typical of human membranous nephritis [212].

The analysis of profiles of cytokine mRNA expression in susceptible Brown-Norway rats shows pronounced up-regulation of IL-4 mRNA, but only modest up-regulation of mRNA for IFN-γ, IL-2, and IL-10 [213]. *In vitro* studies of the effects of $HgCl_2$ show that splenocytes and purified T cells from Brown-Norway rats also express high levels of IL-4 mRNA. Lewis rats, which are resistant to this disease, have higher baseline levels of IFN-γ [213] and IL-12 mRNA [214] and do not increase IL-4 mRNA production after $HgCl_2$ treatment [213, 215]. T-cell lines produced from Brown-Norway rats showing a Th2 phenotype (IL-4 producing) or a Th0 phenotype (expressing IL-4 and some IFN-γ) can transfer the disease to CD8-depleted Brown-Norway rats [96]. These studies implicate IL-4 in the pathogenesis of this model. Further to this, T-cell lines from Lewis rats producing IL-2, IFN-γ, and TGF-β protected Lewis/Brown Norway F_1 hybrids from $HgCl_2$-induced autoimmunity [216].

Mice expressing $H\text{-}2A^s$ are also susceptible to polyclonal B-cell activation following exposure to $HgCl_2$. They develop increased serum levels of IgG1 and IgE and immune complex GN [217]. This susceptibility in B10.S mice and the resistance to $HgCl_2$ in B10.D2 mice are associated with preferential activation of Th2 and Th1 T cells, respectively [218]. Treatment with anti-IL-4 monoclonal antibody abrogated the IgE increase, attenuated the IgG1 response to $HgCl_2$, and shifted the subclasses of antinuclear antibodies to increased levels of IgG2a, IgG2b, and IgG3 [98]. Resistance to auto-antibody formation in $HgCl_2$-treated B10.D2 mice was reversed by anti-IFN-γ monoclonal antibody, but GN was not induced [219]. In B10.S mice, treatment with rIFN-γ limited the increase in serum IgE but did not prevent $HgCl_2$-induced autoantibody formation and GN [219].

Chronic graft versus host disease. Graft versus host (GVH) disease results when parental spleen cells are injected in semi-allogeneic F_1 hybrids and induces different manifestations of disease according to the haplotypes involved. Chronic forms of GVH disease are associated with the development of GN. The immune responses are Th2 in type with hypergammaglobulinemia, high serum levels of IgE, and lesser increases in IgG1 and IgG2a. Mice develop antibodies to nuclear antigens, erythrocytes, thymocytes and skin basement membrane and a membranous GN with granular immunoglobulin deposition and nephrotic syndrome [220]. Treatment with anti-IL-4 antibodies or IFN-γ decreased IgE and IgG1 without reducing IgG2a. Anti-IL-4 antibody treatment diminished proteinuria and prolonged survival [97], indicating a pivotal role for this Th2 cytokine in this experimental model of membranous GN.

Similarly, host versus graft disease (HVG) is associated with the development of a membranous pattern of GN [221]. This disease is also associated with increased serum levels of IgE and IgG1, indicating a predominant Th2 response, which is attenuated by blocking IL-4 [222]. Attenuation of Th2 responses by treatment of neonatal mice developing HVG disease with IFN-γ also reduces the serum IgG1 levels, autoantibody formation, and glomerular immune-complex deposits [88].

Taken together, these data demonstrate that in $HgCl_2$-induced GN, GVH and HVG glomerular injury result from Th2-predominant nephritogenic immune responses. Overall, the immune response shows Th2-predominant Ig isotypes. The presence of local DTH effectors has not been specifically sought, but the lack of "proliferation" and the absence of crescent formation argue against a significant glomerular T-cell involvement. This form of GN has immunohistological features similar to human membranous GN. Similar forms of Th2-mediated autoimmunity can be induced in Brown-Norway rats by the administration of gold salts or D-penicillamine [207]. These observations in experimental GN, together with the fact that human $HgCl_2$, gold or penicillamine associated nephropathy results in a membranous pattern of injury [5, 223] support the evidence that human membranous GN results from Th2-predominant nephritogenic immune responses.

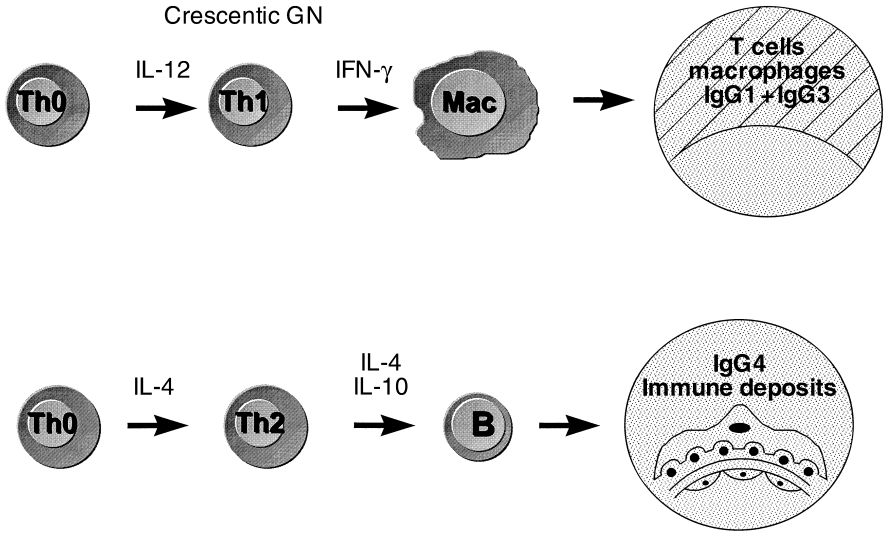

Fig. 3. Hypothesized immune response patterns in crescentic GN (Th1 predominance) and membranous GN (Th2 predominance).

Table 4. Hypothesized Th1/Th2 responses in glomerulonephritis (GN), based on currently available human and experimental data

A. Histological patterns of GN with dominant Th1 immune response
Anti-GBM GN
"Pauci immune" (ANCA associated) GN
Membranoproliferative GN
B. Histological patterns of GN with dominant Th2 immune response
Membranous GN
Minimal change GN
C. Histological patterns of GN with heterogeneous Th1/Th2 predominance
IgA nephropathy (crescentic, Th1; non-crescentic, Th2/indeterminant)
Lupus nephritis (crescentic, Th1; non-crescentic/membranous, Th2/indeterminant)

SUMMARY

It is generally accepted that GN results from cognate immune responses. Over the last decade, the recognition that two major subsets of T helper cells direct different patterns of immune effectors has revised our understanding of host immune responses and autoimmune diseases. However, the consequences of variable Th1/Th2 predominance in immune responses leading to GN have not been widely considered. GN exhibits a variety of histopathological subtypes with different outcomes. These subtypes have variable deposition and accumulation of Th1 and Th2 immune effectors. The available evidence suggests that some forms of human GN, including crescentic GN and membranoproliferative GN, are directed by Th1-predominant nephritogenic immune responses (Fig. 3 and Table 4). The strongest evidence for this comes from the renal biopsy demonstration of prominent Th1-directed DTH effectors in these forms of GN. Experimental models of GN support this view, as Th1-prone strains are more sensitive to the induction crescentic GN with prominent glomerular deposition of DTH effectors. Glomerular T cells are the predominant initiators of injury, and manipulation of Th1 and Th2 cytokines confirms Th1 predominance. The administration of Th1 cytokines exacerbates injury, whereas Th2 cytokines (IL-4 and IL-10) attenuate the nephritogenic immune responses and the severity of GN.

Human membranous GN is associated with IgG4 deposition without DTH effectors, suggesting Th2 predominance. Animal models of GN associated with polyclonal B cell activation and autoimmunity ($HgCl_2$-induced GN and GN associated with chronic GVH and HVG disease) with Th2-predominant immune responses. They develop GN with similar immunopathological features to human membranous GN. The inhibition of Th2 cytokines and administration of Th1 cytokines in these experimental models attenuated both the nephritogenic immune response and the associated GN.

In IgA nephropathy and lupus nephritis, the evidence suggests heterogeneity of Th1/Th2 predominance. Crescentic subgroups, however, show evidence of Th1 polarization, whereas membranous lupus has immunopathological features similar to idiopathic membranous nephritis, consistent with Th2 polarization of the associated immune responses. Murine models of lupus nephritis demonstrate that both Th1 and Th2 subsets contribute to autoantibody production and the consequent immune complex nephritis. Th1 immune effectors, however, appear to induce more severe glomerular injury in these models.

The variable predominance of Th1 or Th2 nephritogenic immune responses helps explain the different participation of immune effectors and the pattern of histopathology seen in several forms of GN. Understanding the predominance of either subset suggests the mechanisms of cytokine regulation of injury. Such knowledge may provide a rational basis for biological manipulation of

the outcomes of GN and planning potential therapeutic strategies.

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APPENDIX

Abbreviations used in this article are: ANCA, antineutrophil cytoplasmic antibody; DTH, delayed type hypersensitivity; EAE, experimental autoimmune encephalomyelitis; GBM, glomerular basement membrane; GN, glomerulonephritis; GVH, graft versus host; HVG, host versus graft; IL, interleukin; IFN-γ, interferon-γ; MCP-1, monocyte chemoattractant protein-1; MIP-1α, macrophage inflammatory protein-1α; SLE, systemic lupus erythematosus; TNF, tumor necrosis factor; TGF-β, transforming growth factor-β.

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