

Invited Review

Crescentic glomerulonephritis – a manifestation of a nephritogenic Th1 response?

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Summary. Crescentic glomerulonephritis (GN) is the histopathological correlate of the clinical syndrome of rapidly progressive glomerulonephritis. Glomerular crescent formation complicates proliferative forms of GN and indicates severe disease with a poor renal prognosis. In the past 10 years evidence from experimental models of GN and from human disease has accumulated suggesting that crescentic glomerulonephritis is a manifestation of a delayed type hypersensitivity (DTH)-like response to nephritogenic antigens. The elucidation of T helper 1 (Th1) and Th2 subsets in mice and in humans has led to the hypothesis that crescentic GN is a manifestation of a Th1 predominant DTH mediated immune response. Recent experiments performed mainly in a murine model of crescentic glomerulonephritis have tested this hypothesis. Crescent formation in this model is substantially interleukin (IL)-12 and interferon- γ (IFN- γ) dependent. Administration of IL-12, deletion of endogenous IL-4 or IL-10 results in enhanced disease, while administration of exogenous IL-4 and/or IL-10 reduces crescentic injury. These findings, together with the available evidence from human studies (examining the pattern of immune effectors in glomeruli, data on cytokine production by peripheral blood mononuclear cells and case reports of the induction of proliferative and/or crescentic GN by administration of IFN- γ or IL-2) suggest that human crescentic GN is manifestation of a Th1 mediated DTH-like nephritogenic immune response.

Key words: Th1 cells, Glomerulonephritis, Delayed type hypersensitivity, Macrophages, Cytokines

Crescentic glomerulonephritis

Glomerulonephritis (GN) is a group of diseases that result from a number of different immune mediated

processes directed against a variety of self or non-self antigens that are present in the glomerulus either by being intrinsic to the glomerular structure, being planted in the glomerulus, or by lodging in the glomerulus as immune complexes. The result of these varying pathogeneses is a variable histological pattern of injury. Therefore, unlike a number of other organ specific diseases GN may result from a number of different systemic or local diseases. The understanding of the pathogenesis of the various forms of GN is therefore likely to be important in the development of more effective treatments. The most acute and clinically worrying clinical syndrome in GN is rapidly progressive GN. Glomerular injury is rapid and progressive such that end stage renal failure occurs in a matter of weeks or months. Most patients with this clinical syndrome have crescentic GN on renal biopsy (Glasscock et al., 1996). Crescentic GN occurs as a complication of a number of different types of GN, usually proliferative forms. It takes its name from the accumulation of cells, fibrin and other matrix components within Bowman's space that under the microscope has the appearance of a crescent. Treatments for crescentic GN include immunosuppressive and/or cytotoxic agents such as corticosteroids and cyclophosphamide that are non-selective in their induction of immunosuppression and have the potential for significant side effects. The development of new, more targeted treatments based on a more complete understanding of the immunopathogenesis of crescentic GN has the potential to offer improved outcomes and lesser toxicity to people with crescentic GN.

Crescentic glomerulonephritis can be classified into three broad categories

Type I Anti-glomerular basement membrane (GBM) GN (or Goodpasture's disease) - associated with the presence of antibodies to the $\alpha 3$ chain of the NC1 domain of type IV collagen ($\alpha 3$ (IV) NC1), which is present in the glomerular basement membrane.

Type II Immune complex associated disease complicated by significant glomerular crescent formation - for example, IgA nephropathy, WHO Class IV lupus nephritis, membranoproliferative GN and post-

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infectious GN.

Type III "pauci-immune" often associated with the presence of anti-neutrophil cytoplasmic antibodies (ANCA) in the serum, and often with Wegener's granulomatosis or microscopic polyangiitis. Many patients with ANCA positive crescentic GN do not have evidence of vasculitis elsewhere. It is reasonable to assume that these patients have renal limited microscopic polyangiitis.

Depending on the series reported, Type I makes up 10-20% of cases, Type II 10-20% and Type III 50-70% of cases of crescentic GN (Glasscock et al., 1996). While components of DTH, (CD4+ T cells, macrophages and fibrin) are present in glomeruli of patients from all 3 classes of crescentic GN (Neale et al., 1988; Cunningham et al., 1999), antibody is not present in glomeruli of patients with Type III crescentic GN (Stilmant et al., 1979).

Th1 and Th2 responses

The understanding of the pathogenesis of both protective and damaging immune responses was significantly advanced by the discovery that CD4+ cells can be defined functionally on the basis of their cytokine secretion patterns, known as Th1, Th2 and Th3. An immune response that is Th1 predominant has a different pattern of effector response (and therefore pattern of injury) to one that is Th2 predominant. Th1 cells tend to secrete IL-2, IFN- γ and tumor necrosis factor (TNF)- β (Mosmann et al., 1986). T cell production of TNF- α is also accepted as a marker of a Th1 response (Ludviksson et al., 1998). Th1 responses are characterised by DTH-like effector responses and immunoglobulin isotype switching to IgG2a and IgG3 in mice (in humans IgG1 and IgG3) (Abbas et al., 1996). Th2 cells secrete IL-4, IL-5, IL-10 (in mice) and IL-13 and induce allergic responses, IgE production and eosinophil mediated responses. They also regulate Th1 responses. Several factors determine the Th1/Th2 predominance of an immune response to a particular antigen. The nature of an antigen, its dose and route of administration, the genetic background of the host and the nature and presence of different costimulatory molecules and chemokines are all relevant to the Th1 or Th2 predominance of an effector immune response in an organism. In addition to these factors, the cytokine milieu in the context of antigen presentation has been shown to be particularly relevant (Seder and Paul, 1994; Abbas et al., 1996, Mosmann and Sad, 1996). IL-12 (and to a lesser degree IFN- γ) has been shown to be important in the development of a Th1 response (Trinchieri, 1995), and IL-4 (and recent evidence suggests IL-13) important in the generation of a Th2 response (Kopf et al., 1993; McKenzie et al., 1999). The existence of a Th3 subset, characterised by the production of transforming growth factor- β (TGF- β), also appears to have an important role in regulating Th1 responses (Mosmann and Sad, 1996). Despite the realisation that Th1/Th2 concepts are more

complex that originally recognized (Kelso, 1995), evidence continues to accumulate that the concept of Th1 and Th2 responses is relevant to the immunopathogenesis of many immune responses. When the available evidence is considered, concepts of Th1 or Th2 predominance are relevant to human conditions (reviewed by Romagnani (1995), Mosmann and Sad (1996) and Holdsworth et al. (1999)).

Many organ specific autoimmune diseases are Th1 predominant (Liblau et al., 1995), and the potential exists for selective modulation of this response to inhibit or limit injury (Racke et al., 1994). However, it is clear from studies in autoimmunity and allogeneic responses that Th2 responses, while having the potential to regulate Th1 responses, can be detrimental in some circumstances (O'Garra et al., 1997). The concept that either Th1 or Th2 responses drive the nephritogenic immune response in different forms of GN has not until recently been applied systematically to the study of GN. The function of the glomerulus as a filter renders it vulnerable to antigen trapping, autoimmune phenomena or immune complex deposition and in fact there is now evidence (discussed below) that either Th1 or Th2 responses drive different forms of GN (Holdsworth et al., 1999).

DTH, also known as Coombs and Gell type IV hypersensitivity, is a delayed reaction mediated by the ingress of cognate CD4+ T cells at the site of antigen challenge in a sensitized host (Janeway and Travers, 1996). Following challenge, antigen is processed and presented by local antigen presenting cells. Memory T cells recognise antigen and release cytokines and chemokines that act on the vascular endothelium, with resultant recruitment and activation of monocytes/macrophages, together with increased vascular permeability and fibrin deposition. DTH is considered a prototypic Th1 response (Janeway and Travers, 1996). It is mediated by Th1, but not Th2 clones (see below) (Cher and Mosmann, 1987). The DTH response is a standard marker for Th1 responses in a number of systems, including the study of immune responses in murine leishmaniasis (Reiner and Locksley, 1995) and in the evaluation of immune responses in phenotyping genetically deficient animals (Magram et al., 1996).

Experimental GN - evidence for DTH responses in crescentic injury

While classical early studies in an "anti-GBM" model of GN indicated an important role for antibody mediated injury in this disease (Lerner et al., 1967), there has been a substantial body of more recent work implicating cell mediated injury in this crescentic glomerular injury. Studies in experimental crescentic GN have demonstrated a functional role for CD4+ cells (Huang et al., 1994, 1997a). CD4+ cells have the potential to direct cell-mediated macrophage or CD8+ responses, and to provide help for B cells in antibody production. In accelerated autologous anti-GBM GN,

CD4+ cells appear in the lesion early and direct macrophage recruitment, prior to the influx of macrophages (Tipping et al., 1985). In this model, proliferative GN develops resulting from cognate immune responses to heterologous globulin planted in glomeruli. There is no evidence of an autoimmune response to the GBM (Unanue et al., 1965) and the use of term "anti-GBM GN" to describe autologous injury in this model is inaccurate (though still widely used and accepted). In its most severe form, this model results in glomerular crescent formation and renal failure. T cell activation requires antigen presentation, in the case of CD4+ cells via MHC Class II. Studies in a murine model of anti-GBM have demonstrated the absolute requirement for MHC II in crescentic GN (Li et al., 1998). Mice lacking MHC II did not develop significant renal injury, or skin DTH responses to the nephritogenic antigen, nor antigen specific serum IgG responses. In contrast, TAP-1 *-/-* mice (lacking functional Class I) developed a similar degree of glomerular injury to genetically intact mice (unpublished observations). CD4+ deficient mice do not develop significant glomerular injury in anti-GBM GN (Tipping et al., 1998). Like MHC II deficient mice, they did not develop significant cellular infiltrates, and only minimal antigen specific antibody formation.

CD4+ depletion in the effector phase of the immune response significantly attenuates injury. Depletion of CD4+ cells at the time of antigen challenge in previously sensitized mice 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 (Huang et al., 1997a; Li et al., 1997). Skin DTH to the nephritogenic antigen was reduced, but there was no difference in the serum levels of autologous antibody or the glomerular deposition of C3. Similar studies have been performed in rat anti-GBM GN (Huang et al., 1994). While autologous antibody is deposited in glomeruli in autologous anti-GBM GN, it is not essential for crescent formation. Mice with a genetic inability to produce antibody (immunoglobulin μ chain deficient mice) developed crescentic disease of similar severity to normal mice (Li et al., 1997). Together, these studies emphasise the importance of *effector* CD4+ directed, cell mediated responses in this model of crescentic GN.

Data from other models of crescentic GN support an important role for cell mediated immune responses in crescentic GN. Data from two models that plant a hapten in glomeruli of sensitized rats and demonstrating prominent DTH-like glomerular lesions (and hapten induced dermal DTH) argue for a role for T cell mediated effector mechanisms in the development of the glomerular lesion (Oite et al., 1989; Rennke et al., 1994). In model, one cell mediated immunity is prominent, while antibody deposition is absent (Oite et al., 1989). In the other, GN could be transferred by T cells (Rennke et al., 1994). A number of studies in models of experimental autoimmune glomerulonephritis (EAG)

support a role for CD4+ T cells in the pathogenesis of injury. In EAG in chickens, bursectomized birds (which therefore did not have B cells) developed crescent formation in 24% of glomeruli examined. Furthermore, GN could be transferred by spleen and kidney cells from nephritic birds. These cells had the light, electron and immunohistochemical appearance of T cells implying a role for effector T cells in this model (Bolton et al., 1988). Recent studies in murine EAG induced by immunisation with dimers of $\alpha 3(\text{IV})$ NCI collagen show that autoantibodies to the nephritogenic antigen occurred in all strains, but significant crescentic GN, proteinuria and renal failure developed only in strains that developed a mononuclear cell infiltrate in glomeruli (Kalluri et al., 1997). While crescentic GN could be transferred passively both with cells and with antibody, transfer with antibody required the recipient to be both of a susceptible strain and to possess an intact T cell receptor.

Macrophages are important effector cells in the DTH response. Macrophages have been implicated in proliferative and crescentic forms of GN for over 20 years (Atkins et al., 1976). In DTH responses, macrophages are recruited by CD4+ cells. They represent the predominant means by which CD4+ cells with glomeruli effect injury, although in vitro, T cell derived cytokines can activate and injure intrinsic glomerular cells (Schwarz et al., 1997). As major effector cells in crescentic GN, activated macrophages facilitate glomerular injury via a number of pathways. One result of T cell directed macrophage infiltration in DTH responses and in crescentic GN is glomerular fibrin deposition that is initiated by activation of the extrinsic coagulation pathway. Other pathways include the production and release of oxidants and proteases, the release of eicosanoids, and the production of cytokines and growth factors. Macrophages accumulating in Bowman's space damage matrix components, and effect injury to and stimulate proliferation of intrinsic glomerular cells. A definite functional role for macrophages in anti-GBM GN was first demonstrated in rat anti-GBM, where macrophage depletion by irradiation markedly reduced proteinuria (Schreiner et al., 1978). More recently, depletion of macrophages via the administration of clodronate-encapsulated liposomes limited the initial phase of disease in accelerated anti-GBM in rats (Huang et al., 1997b).

It has been known for many years that glomeruli of animals with experimental proliferative GN contain fibrin (Vassalli and McCluskey, 1964). Glomeruli from rabbits with crescentic GN were found to express augmented procoagulant activity (Holdsworth and Tipping, 1985), later demonstrated to be due to tissue factor (Tipping et al., 1995). Tissue factor is the central player in the extrinsic pathway of coagulation, now recognised to be the relevant coagulation pathway in vivo (Broze, 1995). Defibrinogenating rabbits with anecrod reduced renal impairment and prevented glomerular crescent formation (Naish et al., 1972; Thomson et al., 1976). Studies using an anti-tissue factor

antibody (Erlich et al., 1997), or alternately infusion of tissue factor pathway inhibitor (Erlich et al., 1996) have prevented fibrin deposition and markedly attenuated renal injury in experimental crescentic GN when given prior to the initiation of glomerular injury. Plasminogen is activated to plasmin, which limits fibrin deposition by cleaving fibrin. Endogenous plasmin is important in regulating glomerular fibrin deposition and glomerular crescent formation (Kitching et al., 1997a). Prevention of plasmin formation in GN via the use of mice genetically deficient in plasminogen or alternately both tissue-type plasminogen activator (tPA) and urokinase-type PA (uPA) increased glomerular fibrin deposition and increased glomerular crescent formation and renal impairment (Kitching et al., 1997a). Mice deficient in tPA alone also developed increased injury, but not to the extent of plasminogen deficient mice.

The demonstration of the functional relevance of the classical features of DTH - namely Class II and CD4+ dependency, mediation by macrophages, with important roles for tissue factor and fibrin in experimental crescentic GN strongly implicate cell mediated, DTH like injury in the pathogenesis of this condition.

Evidence from studies in murine "anti-GBM" GN that experimental crescentic GN is a manifestation of a Th1 nephritogenic response

The effector CD4+ cell dependent, DTH-like, antibody independent nature of experimental anti-GBM GN suggests the hypothesis that Th1 responses are important in the pathogenesis of crescentic renal injury. The use of autologous phase models of anti-GBM in mice results in crescentic injury in which the role of Th1/Th2 subsets can be dissected. 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 (Reiner and Locksley, 1995). When sensitized C57BL/6 mice were challenged with anti-mouse 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 (Huang et al., 1997a). The accumulation of DTH effectors implies a Th1 driven immune response in glomeruli.

BALB/c mice produce IL-4, have reduced DTH responses and do not heal when infected with *Leishmania major* due to an ineffective Th1 immune response to this pathogen (Reiner and Locksley, 1995). Accelerated anti-GBM GN in BALB/c mice induced glomerular injury with only occasional crescent formation and absent cutaneous DTH to the nephritogenic antigen. Their nephritis was humorally mediated and was not CD4+ dependent in the effector phase (Huang et al., 1997a,c). Only the minor crescentic component was blocked by CD4+ depletion (Huang et al., 1997a). An advantage of using autologous anti-GBM GN in C57BL/6 mice to study the pathogenesis of crescentic GN is that the cellular and humoral mechanisms of injury are now relatively well characterised and allow interpretation of intervention studies. The relevant mediators of this model in the crescent and Th1 prone C57BL/6 strain and the relatively crescent resistant BALB/c strain are summarised in Table 1.

A number of recent studies performed in this model have examined the role of Th1 subsets in inducing crescentic GN by examining the effects of deleting (or blocking) and administering cytokines that induce or modulate Th1 responses. Specific T cell subset defining/effector/modulating cytokines evaluated in this

Table 1. Characteristics of anti-GBM GN in the C57BL/6 strain and the BALB/c strain.

	C57BL/6 STRAIN	BALB/c STRAIN
Crescentic glomeruli	25-40%	5-10%
Glomerular CD4+ cells	0.5-2 c/gcs	0.2-0.5 c/gcs
Glomerular macrophages	2-5 c/gcs	1.5-2 c/gcs
Glomerular fibrin	+++	+
Glomerular autologous antibody	++	+++
Glomerular C3	+	++
Functional injury	moderate to severe	moderate to severe
MHC Class II dependent	yes	ND
Effector CD4+ dependent	yes	no (only crescents)
MHC Class I dependent	no	ND
Effector CD8+ dependent	no (not crescents)	ND
Autologous Ig dependent	no	ND
Complement dependent	no (early autologous phase)	yes (early autologous phase)
Skin DTH to sheep Ig	present	absent
IFN- γ production	high	low
IL-4 production	low	high

Values for glomerular crescent formation, CD4+ and macrophage infiltration are in severe accelerated GN at day 10. c/gcs: cells per glomerular cross section; ND: not done. References: Huang et al., 1997a,c; Li et al., 1997, 1998; unpublished data.

model include IL-12, IL-18, IFN- γ , IL-4 and IL-10.

Interleukin-12 is a heterodimeric cytokine produced by antigen presenting cells (Trinchieri, 1995). It is crucial to the development of Th1 responses by polarizing uncommitted T cells towards a Th1 profile (Hsieh et al., 1993) and by acting as a costimulatory molecule for maximal IFN- γ secretion by committed Th1 cells (Gately et al., 1998). IL-12 plays a central role in protective and harmful Th1 responses (Trembleau et al., 1995; Trinchieri, 1995). Neutralisation of IL-12 in accelerated GN in C57BL/6 mice using a monoclonal anti-IL-12 antibody reduced the severity of injury (Kitching et al., 1999a). Glomerular crescent formation and DTH effectors (CD4+ cells, macrophages and fibrin) were reduced in glomeruli. While there was no net alteration in the humoral immune response, antigen specific IgG2a (Th1 associated) subclasses titers were reduced. Further experiments using IL-12 -/- have confirmed these observations (unpublished observations), with additional effects in reducing proteinuria and protecting mice from the development of renal failure. Administration of recombinant IL-12 to genetically normal C57BL/6 mice in which relatively mild proliferative, but non-crescentic GN had been induced enhanced GN by inducing glomerular DTH with marked crescent formation (Kitching et al., 1999a). This was associated with amplification of antigen specific Th1 responses. Recent experiments in our laboratory have shown that IL-18, considered an important cofactor in the generation of Th1 responses (Ahn et al., 1997), amplifies glomerular injury in this model (unpublished observations).

Interferon- γ is produced by Th1 cells (Mosmann et al., 1986). It promotes macrophage activation, induces MHC Class I and Class II expression and regulates immunoglobulin isotype switching (Dalton et al., 1993; Huang et al., 1993). Mice deficient in IFN- γ develop decreased skin DTH, suggesting an important role for IFN- γ in effector Th1 responses (Dalton et al., 1993). While IFN- γ is necessary for the expression of experimental hypersensitivity pneumonitis, a granulomatous lung disease (Gudmundsson and Hunninghake, 1997), studies in organ specific autoimmune diseases such as experimental autoimmune encephalomyelitis, have not confirmed a pathogenetic role for IFN- γ (Duong et al., 1994; Ferber et al., 1996). A regulatory role for IFN- γ in allogeneic responses is suggested by the development of increased cytotoxic activity in the mixed lymphocyte reaction using cells from IFN- γ -/- mice (Dalton et al., 1993) and by studies in experimental allogeneic heart transplantation showing that deficiency of endogenous IFN- γ did not permit engraftment (Saleem et al., 1996; Raisanen-Sokolowski et al., 1997). However, IFN- γ is necessary for rejection of MHC II mismatched skin grafts (Ring et al., 1999b). Studies in murine crescentic GN have demonstrated that IFN- γ plays an effector role in experimental crescentic injury in this lesion (Huang et al., 1997a; Kitching et al., 1999b). The use of a neutralising anti-IFN- γ monoclonal

antibody (Huang et al., 1997a) or mice genetically deficient in IFN- γ (Kitching et al., 1999b) resulted in diminished crescentic injury. In the latter study, examination of the disease at a later timepoint (day 22 compared to day 10) showed some catch up in injury, implying that there is some redundancy within the system (consistent with the concept that IFN- γ is not the only activator of macrophages) and/or that IFN- γ is capable of playing a regulatory role in limiting T cell expansion, as has been suggested (Ring et al., 1999a).

IL-4 and IL-10 are two Th2 cytokines that have the capacity to regulate Th1/DTH responses at several levels, including antigen presentation, Th1 subset differentiation and function and macrophage activation (Paul, 1991; Bogdan and Nathan, 1993; Mosmann, 1994). Mice genetically deficient in either of these cytokines develop more severe glomerular injury and renal impairment. DTH effectors in glomeruli were increased, as was dermal DTH to the nephritogenic antigen. These findings demonstrate a regulatory role for endogenous Th2 cytokines in this lesion. They support the hypothesis that crescentic GN is a manifestation of a Th1 driven DTH like injury (Kitching et al., 1998, 2000). While the absence of either of these cytokines resulted in enhanced Th1 responses, IL-4 deficiency resulted in suppression of titers of the Th2 associated IgG1 subclass, suggesting a relative Th2 deficiency. However, IL-10 deficient mice had normal IL-4 levels and no suppression of IgG1, implying that endogenous IL-10 is more important in regulating Th1 responses than inducing Th2 responses.

Immune modulation with exogenous IL-4 and/or IL-10 can inhibit crescent formation with selective inhibition of Th1 responses (Tipping et al., 1997). When cytokine administration began prior to the initiation of injury, crescent formation was prevented and proteinuria was reduced. IL-10 alone and the combination of both IL-4 and IL-10 prevented the development of renal failure. Antigen stimulated/specific Th1 responses were reduced. Dermal DTH was inhibited and splenocyte IFN- γ production was reduced in treated mice. Although total serum antigen specific titers were unaffected, antigen IgG2a and IgG3 titers were diminished by treatment. These cytokines also have inhibitory effects when treatment is initiated after the initiation of glomerular injury (Kitching et al., 1997b). While IL-4 or IL-10 alone had some effect on disease, synergism between IL-4 and IL-10 was required to have significant effects on crescent formation. IL-4 and IL-10 co-administration preserved renal function and limited the extent of crescent formation. The effects of IL-4 and IL-10 in these studies seemed to be at the effector cell level, with direct suppressive effects on local T cell/macrophage recruitment and function likely. These findings have been supported by studies involving IL-4 administration after the initiation of GN in a crescentic anti-GBM model in the rat (Cook et al., 1999). Table 2 summarises the results of studies in murine autologous phase crescentic anti-GBM models in C57BL/6 mice.

Th1 responses in crescentic GN

The roles of IL-12 and IL-4 have been assessed in the relative crescent resistance that occurs in the BALB/c strain in this model (Kitching et al., 1999a). It is still controversial whether the Th2 proneness (particularly with regard to leishmaniasis) of this strain is related to overproduction of IL-4, or to a genetically determined abnormality of the IL-12/IL-12R system (Guler et al., 1996; Launois et al., 1997). Administering IL-12 induced Th1 responses (glomerular DTH effectors, splenocyte IFN- γ production and antigen specific IgG2a) and crescentic GN of similar severity to that seen in C57BL/6 mice. Suppression of the Th2 associated IgG1 subclass provided some evidence of a diminution in the Th2 response. However, induction of accelerated GN in IL-4 -/- BALB/c mice had little effect on the severity of injury. Specifically, although there were alterations in the pattern of IgG subclasses consistent with IL-4 deficiency, enhancement of cell mediated Th1 responses at either a glomerular or splenocyte level did not occur and significant glomerular crescent formation did not ensue. These studies suggest that the immune deficit in these mice exists at the level of the IL-12/IL-12R system. They implicate IL-12 in the genesis of severe crescentic GN. The findings in BALB/c IL-4 -/- mice with GN are in contrast to those in Th1 prone C57BL/6 x 129Sv mice (Kitching et al., 1998). They suggest that in mice that do not generate predominant Th1 responses, endogenous IL-4 has little regulatory effect in this model of GN.

While there is considerable evidence that experimental crescentic GN is a manifestation of a Th1 nephritogenic immune response, other models of GN, particularly models such as HgCl₂ induced membranous nephritis, are clearly associated with IL-4 driven Th2 responses (Prigent et al., 1995; Druet and Pelletier, 1996; Holdsworth et al., 1999).

Other evidence addressing the concept that Th1 responses are important in experimental crescentic GN

"Anti-GBM" models

In a detailed study of murine experimental autoimmune glomerulonephritis induced by immunising mice with the Goodpasture antigen (α 3(IV) NC1), Kalluri et al. (1997) demonstrated that T cell effector responses were central to the pathogenesis of this autoimmune experimental crescentic lesion that has many similarities to human anti-GBM GN (Kalluri et al., 1997). Although all strains of mice studied developed anti- α 3(IV) NC1 antibodies, significant disease ensued only in those strains that developed an IL-12 and IFN- γ positive (but not IL-4 or IL-10 positive) cellular infiltrate in glomeruli. All mice had measurable anti α 3(IV) NC1 antibodies of the Th2 associated IgG1 subclass, but only those with crescentic disease developed significant IgG2a titers. A susceptible strain could be tolerated by oral administration of antigen, a process associated with

the suppression of crescentic GN, glomerular IL-12 protein and serum IgG2a titers.

A recent study by Ring et al. (1999a) in a model of mild autologous anti-GBM induced injury showed that, in contrast to studies in models that were severe enough to induce significant glomerular crescent formation (Haas et al., 1995; Huang et al., 1997a; Kitching et al., 1999b), the absence of endogenous IFN- γ resulted in increased disease. The mechanism behind this finding is not clear, although it seems to indicate that while IFN- γ mediates severe crescentic injury, it may paradoxically facilitate milder disease. Two studies in rat anti-GBM GN have suggested that IL-4 has the capacity to modify effector immune responses via suppressing macrophage effector function (Tam et al., 1997; Cook et al., 1999). Another study found that administering recombinant murine IL-10 to Sprague-Dawley rats did not significantly alter the expression of crescentic GN when injections were commenced immediately prior to challenge four days after sensitising rats (Chadban et al., 1997).

Murine models of systemic lupus erythematosus (SLE) with crescent formation

Humans with SLE develop a variety of histological patterns of renal injury, including crescentic GN. Lupus-like syndromes develop spontaneously in several strains of mice (including MRL/lpr, NZB/W and BXSB 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 (Andrews et al., 1978). While the deposition of antibody and complement is emphasised in these models, several lines of evidence suggest a role for cell mediated immune responses in these mice. Firstly, macrophages and T cells are present both in glomeruli and in the interstitium (Schwartz et al., 1998). Deletion of the IFN- γ R attenuates disease and reduces cellular infiltrates as well as autoantibody production

Table 2. Results of experiments in murine anti-GBM GN that have used manipulation of Th1 or Th2 cytokines in the Th1 and crescent prone C57BL/6 mouse strain.

INHIBITS CRESCENT FORMATION/ GLOMERULAR INJURY	ENHANCES CRESCENT FORMATION/INJURY
Monoclonal anti-IL-12 antibody ¹	Recombinant murine IL-12 ¹
IL-12 gene deletion ²	Recombinant murine IL-18 ³
Monoclonal anti-IFN- γ antibody ⁴	
IFN- γ gene deletion ⁵	
Recombinant murine IL-4 ^{6,7}	IL-4 gene deletion ⁸
Recombinant murine IL-10 ^{6,7}	IL-10 gene deletion ⁹

¹: Kitching et al., 1999a; ²: unpublished observations; ³: unpublished observations; ⁴: Huang et al., 1997a; ⁵: Kitching et al., 1999b; ⁶: Tipping et al., 1997; ⁷: Kitching et al., 1997b; ⁸: Kitching et al., 1998; ⁹: Kitching et al., 2000.

(Schwartz et al., 1998). Secondly, in the SCG/Kj (spontaneous crescentic glomerulonephritis strain, bred from BXSB mice crossed with MRL/lpr mice and breeding pairs selected from parents that had prominent crescent formation) mouse, 58% of female mice (34% of males) developed more than 50% crescents by 3.5 months of age (Kinjoh et al., 1993). Interestingly, despite their high levels of glomerular crescent formation, SCG mice have little Ig or C3 in glomeruli. However, fibrin deposition and lymphocytes were reported in glomeruli suggesting that cell mediated immune responses are important for crescent formation in these lupus prone mice selected and bred only for their susceptibility to crescentic GN. The paucity of humoral response in glomeruli in these crescent prone mice is intriguing considering the prominent Ig and C3 deposition of the lupus prone BXSB and MRL/lpr background strains from which SCG/Kj mice are derived. Thirdly, While B cells are essential for the development of disease in the MRL/lpr strain, there is some evidence that it is not the effector function of the B cells (i.e. antibody secretion) that is important in severe renal injury, as lupus prone mice with B cells that are unable to secrete antibody but express membrane Ig and have intact antigen presentation and cytokine production still developed renal injury (Chan et al., 1999).

Interpretation of data on Th1 and Th2 cytokines in murine lupus nephritis would suggest that both Th1 and Th2 immune responses are required for maximal autoantibody production and full expression of GN. A number of studies have emphasised the role of endogenous IFN- γ , while the role of IL-4 is less clear, with variable results depending on the technology used (i.e. knockout, cell type overexpressing IL-4) (Erb et al., 1997; Peng et al., 1997; Santiago et al., 1997). Dysregulated overproduction of IL-10 by B cells and monocytes but not CD4+ cells (Llorente et al., 1994) is likely to be important in the generation of the systemic autoimmunity that is responsible for this condition. Interestingly, the protection afforded NZB/W mice by blocking IL-10 was dependent on TNF- α (Ishida et al., 1994). This finding is at odds with studies on the role of TNF- α in crescentic GN (Karkar et al., 1995; Le Hir et al., 1998), suggesting upstream effects of TNF- α in the development of autoimmunity rather than in the effector phase of glomerulonephritis.

Direct application of results of these studies in murine lupus to the pathogenesis of crescentic GN is difficult. In the more commonly employed models of lupus, mortality is a frequent endpoint. The renal lesion is considered to correlate with mortality, which may not always be the case (Lloyd et al., 1997). The patterns of glomerular injury and particularly the extent of glomerular crescent formation are only infrequently assessed in studies of immune manipulation in lupus prone mice. Humans with renal lupus are known to express a number of patterns of renal injury, at least two of which (membranous lupus and crescentic lupus nephritis) vary markedly in the deposition of immune

reactants in glomeruli. Therefore results of studies that do not address *patterns* of renal injury, while potentially relevant, should be assessed with caution when considering the pathogenesis of crescentic GN.

Is human crescentic GN is a manifestation of a Th1 mediated, nephritogenic DTH-like response?

Studies in experimental crescentic glomerulonephritis demonstrate a role for Th1/DTH responses in crescentic glomerulonephritis. While studies involving cytokine depletion and administration have not been performed in human disease, and antigen specific Th1 clones have not been isolated from humans with crescentic GN, there is considerable evidence available to support a role for Th1/DTH responses in several forms of human crescentic GN.

A number of studies in the last 15 years have collectively demonstrated that DTH effectors - CD4+ T cells, macrophages and fibrin are present within glomeruli in all classes of human crescentic GN (Stachura et al., 1984; Bolton et al., 1987; Nolasco et al., 1987; Muller et al., 1988; Neale et al., 1988; Cunningham et al., 1999). However, immunoglobulin is not present (or only sparsely present) in over 50% of cases (type III or vasculitis/ANCA associated) of crescentic GN (Stilmant et al., 1979). These forms of GN were previously known as "pauci-immune" crescentic GN. The universal presence of DTH effectors in crescentic GN implies that DTH is relevant to the pathogenesis of this lesion and argues for a role for Th1 responses. There is evidence that IFN- γ is produced by glomerular T cells in human crescentic GN. Patients with ANCA-associated crescentic GN have mononuclear cells in their glomeruli that are positive by immunohistochemistry and by in situ hybridisation for IFN- γ (Waldherr et al., 1993). In human anti-GBM GN, the only form of GN in which the antigen is known, circulating T cells that react with nephritogenic epitopes have been identified (Derry et al., 1995; Merkel et al., 1996). A recent trial of lymphocytopenia in the treatment of crescentic GN showed CD3 depletion was effective in reducing glomerular injury (Furuta et al., 1998).

The pattern of IgG1 and/or IgG3 predominance in glomeruli and/or sera of patients with Type I (anti-GBM) (Bowman et al., 1987; Weber et al., 1988) or Type II ("immune complex" GN) (Roberts et al., 1983; Imai et al., 1997) favour a Th1 response. The Th2 associated IgG4 subclass is in fact associated with the non-crescentic and non-proliferative membranous GN (including membranous lupus nephritis) (Haas, 1994; Imai et al., 1997).

Systemic immune responses in patients with ANCA associated GN and WHO class IV lupus nephritis support a role for Th1 responses. Patients with active Wegener's granulomatosis have activated CD4+ T cells in the blood that are producing excessive amounts of IFN- γ , but not IL-4, IL-5 or IL-10. (Ludviksson et al.,

1998) and monocytes that produced more IL-12. The increased production of IFN- γ could be inhibited by IL-10. People with SLE have high IL-10 production by B cells and monocytes (Llorente et al., 1994) and low levels of IL-12 (Liu and Jones, 1998). However, it is possible that those with severe diffuse proliferative and crescentic lupus nephritis have shifted their immune response towards Th1. Supporting this concept is a recent study of the cytokine profile of peripheral blood Th cells in patients with SLE. It reported that a Th1 cytokine profile was predominant in those with Class IV lupus nephritis (diffuse proliferative, including crescentic GN) compared to normal controls or patients with SLE with without proteinuria (Akahoshi et al., 1999).

While cytokine therapy of crescentic GN has not been trialed, case reports document the development of proliferative and crescentic GN in people treated with Th1 cytokines. IFN- γ has been administered to humans as treatment for rheumatoid arthritis/SLE, and was reported to induce proliferative/crescentic GN (Machold and Smolen, 1990; Graninger et al., 1991). Similarly, IL-2 treatment of malignancy in a patient who in retrospect is likely to have had mild IgA nephropathy resulted in the development of crescentic GN (Chan et al., 1991).

Summary and conclusions

In recent years a considerable body of evidence has emerged that cell mediated nephritogenic immune responses are important in the development of crescentic glomerulonephritis. More recently, the Th1 subset has been implicated, particularly in murine crescentic GN. These studies collectively demonstrate that experimental crescentic GN induced by a planted antigen is a manifestation of a Th1 mediated DTH-like nephritogenic immune response. Available evidence from human studies that examine the pattern of immune effectors, cytokines in glomeruli, data on stimulated peripheral blood mononuclear cells and case reports of the induction of proliferative and/or crescentic GN by administration of IFN- γ or IL-2 suggest that these findings are relevant to human crescentic GN.

Defining crescentic GN as a manifestation of a Th1 nephritogenic immune response raises the question as to whether this bias in the immune response can be exploited in the treatment of at least some forms of human crescentic GN. One of the pitfalls of potential treatments of immune mediated injury that involve "immune deviation" is that the induction of a sufficiently strong Th2 response would be in itself damaging. At a theoretical level the question is really whether it is possible to deliver (or block) a cytokine in a manner and dose that will have suppressive effects on Th1 responses without deleterious enhancement of Th2 responses. Studies presented in this review offer proof of the concept that significant attenuation of Th1 nephritogenic immune responses without the emergence of deleterious Th2 responses in crescentic GN is in fact possible. To

translate this finding into new treatments for human crescentic GN requires careful consideration of the pharmacodynamics and pharmacokinetics of biological therapies that alter Th1 responses.

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Th1 responses in crescentic GN

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