the key enzyme glucosaminyl N-deacetylase. A decreased activity of this enzyme has been found in liver and glomeruli of experimental diabetic rats [5].

Since GBM heparan sulphate is also thought to be important for the maintenance of the integrity of the GBM through its chemical and electrostatic interactions with other GBM constituents, qualitative alterations in heparan sulphate may subsequently alter the molecular assembly and ultrastructure of the GBM. This is further enhanced by non-enzymatic glycation of laminin and collagen IV, which reduces the binding to heparan sulphate. Finally, the importance of heparan sulphate for proteinuria in diabetic nephropathy is also demonstrated by the fact that therapies (angiotensin-converting enzyme inhibition, calcium entry blockade, and heparin analogues), which prevent or reduce albuminuria in diabetic nephropathy, also lead to a restoration c.q. normalization of heparan sulphate in the GBM [5].

Concluding remarks

Based on these data, we propose that the development of microalbuminuria, and a decreased charge selectivity of the GBM is the consequence of an undersulphation and later on a decrease of heparan sulphate in the GBM. The loss of size-selectivity of the GBM is probably more related to the non-enzymatic glycation of the GBM and the increased production of collagen IV and laminin which both lead to a more loosely organized molecular architecture of the diabetic GBM.

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References


Is type II mixed cryoglobulinemia an essential part of hepatitis C virus (HCV)-associated glomerulonephritis?

G. D’Amico
Division of Nephrology, San Carlo Borromeo Hospital, Milan, Italy

Cryoglobulinemic GN and HCV infection

Up to the end of the 1980s, around 30% of mixed cryoglobulinemias (MC) of both type II (with a monoclonal immunoglobulin as the anti-IgG rheumatoid factor component) and type III (in which the rheumatoid factor was polyclonal) were called ‘essential’, since they were not associated with well-characterized diseases, such as lymphoproliferative disorders, connective-tissue autoimmune diseases or infectious diseases [1–3]. A peculiar membranoproliferative exudative glomerulonephritis (MPGN), caused by glomerular deposition of the circulating cryoglobu-
bulin, and referred to as ‘cryoglobulinaemic GN’, has been found frequently in type II essential mixed cryoglobulinaemia, especially when an IgMk was the monoclonal rheumatoid factor bound to IgG in the cryoglobulin [4–8]. In these last 5 years, availability of serological markers for hepatitis C virus (HCV) infection has revealed that nearly all patients with essential type II and type III mixed cryoglobulinaemia did in fact have this viral infection, showing positivity not only for HCV antibodies, but also for HCV RNA, and therefore evidence of active viral replication. It therefore became evident that cryoglobulinaemic glomerulonephritis is an HCV-associated glomerulonephritis [9–13].

Non-cryoglobulinaemic GN and HCV infection

In 1993 in the USA Johnson et al. [14] described positivity for this viral infection even in a few patients with membranoproliferative GN (3 of 8) in whom no concomitant mixed cryoglobulinaemia could be detected, and more recently the same investigators [15] reported 34 patients with HCV infection and membranoproliferative or acute proliferative GN, five of whom did not have cryoglobulinaemia at the time of the biopsy or over the ensuing months. In addition, Japanese investigators [16] in an abstract presented to the American Society of Nephrology in 1993 have described circulating HCV antibodies in 6 of 10 random patients with MPGN, only one of whom had cryoglobulinaemia. Furthermore some anecdotal cases of membranous nephropathy associated with HCV infection in patients without mixed cryoglobulinaemia in both the USA [13,17] and in Italy [18] have been reported. Johnson et al. in their recent editorial [13] expressed their impression that the association between HCV infection and glomerulonephritis, mainly membranoproliferative type I GN without concomitant cryoglobulinaemia, may be more common than is documented in literature.

However, the experience in Southern Europe, where there is a relatively high prevalence of HCV infection, does not validate this impression. An ongoing survey among the Renal Units in Italy has revealed that only three of 128 adult patients with biopsy-proven ‘idiopathic’ type I MPGN and absence of signs of a concomitant mixed cryoglobulinaemia had HCV infections (unpublished observation). In France the prevalence of HCV antibodies was null in 110 adult patients with primary glomerular disease [19] and in 35 patients with idiopathic type I MPGN [20]. In Spain only one patient with IgA nephropathy out of 70 adult patients with biopsy-proven chronic primary glomerular diseases had HCV antibodies [21].

Pathogenesis of HCV-associated GN: an hypothesis

We think the existing evidence points to the prominent role of type II mixed cryoglobulinaemia and the presence of an IgMk rheumatoid factor in inducing HCV-associated glomerulonephritis (Figure 1). If HCV antigens are directly involved in mediating immune complex glomerular lesions without a concomitant cryoglobulinaemia, as are HBV antigens in chronic hepatitis B infection, this mechanism is in any case rather infrequent.

We [22] have proposed that the role of hepatitis C virus in inducing the glomerular lesions is an indirect one, and consists of infecting B lymphocytes and triggering the hyperproduction of polyclonal IgM rheumatoid factors by them (type III MC). Additional but as yet uncharacterized events (duration of the HCV infection? superimposed infection with other viruses, such as HBV or EBV?) might induce in some of these patients with polyclonal B cell activation due to viral infection a shift to abnormal proliferation of a clone of B cells producing a monoclonal IgM rheumatoid factor (type II MC). As we said, this monoclonal IgMk rheumatoid factor is necessary to induce the renal lesions in cryoglobulinaemic GN, and even in MPGN without cryoglobulinaemia Johnson et al. [14] found an IgM RF which bound anti-HCV IgG.

We have recently induced an MPGN very similar to human cryoglobulinaemic GN in mice by intravenous injection of solubilized type II mixed cryoglobulins from patients with this renal disease and HCV infection [23]. The monoclonal IgMk rheumatoid factor isolated from such mixed cryoglobulins and certainly not containing any viral antigenic component was able when injected separately to deposit in the glomerulus and induce the same lesions caused by the mixed cryoglobulin, suggesting a specific affinity of IgMk RF component of the cryoglobulins for some glomerular structure (such an affinity has already been demonstrated by Solomon for light chains in light chain nephropathy [24]).

Very recently we demonstrated that the same purified IgMk binds to cellular fibronectin, a known constituent of mesangial matrix, but this property was not displayed by purified monoclonal IgM from patients with Waldenström disease, polyclonal IgM from patients with rheumatoid arthritis, or polyclonal IgM from normal subjects (unpublished).

In conclusion we suggest that the prevalent pathogenetic mechanism in HCV-associated GN is the deposition in the glomerulus of a monoclonal IgM rheumatoid factor with particular affinity for the glomerular matrix, which is produced by permanent clones of B lymphocytes infected by the virus. The IgM RF can deposit alone or as a mixed IgG-IgM cryoglobulin, not necessarily bound to HCV-derived antigens.

It is possible that in a minority of cases immune complexes composed of HCV antigens and anti-HCV IgG antibodies can deposit directly in the glomerular structures in the absence of a concomitant type II mixed cryoglobulin with a monoclonal IgM RF, to give an immune complex GN similar to those described in patients infected with hepatitis B virus. However, neither HCV antigens nor HCV RNA have been detected in the glomerulus up to now, in spite of the
fact that laboratory technology for revealing the virus in the tissues more easily gives false-positive results than false-negative [25,26]. Non-cryoglobulinemic HCV-associated GN seems to be quite infrequent, if it exists at all, in the countries of Southern Europe, where both HCV infection and mixed cryoglobulinemia are rather frequent, and the search for cryoglobulins is a 'routine' diagnostic procedure for all patients with glomerular diseases. In our opinion the evidence that non-cryoglobulinemic GN is more frequently associated with HCV infection in other geographical areas, such as the USA and Japan, needs to be confirmed.

References


Fig. 1. Proposed pathogenetic mechanisms in HCV-associated GN. Description in the text.


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The TH1/TH2 concept and its relevance to renal disorders and transplantation immunity

M. Goldman¹ and P. Druet²

¹Department of Immunology, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium; ²Unite INSERM U28, Hôpital de Purpan, Université Paul Sabatier, Toulouse, France

**Introduction**

T cells expressing CD4 molecules on their membrane (CD4⁺ T cells) play an essential role in the orchestration of immune defences, as dramatically illustrated by the profound immunodeficiency caused by the HIV virus, which preferentially infects CD4⁺ cells. Indeed, CD4⁺ T cells are required for the induction of cell-mediated immune responses involving activated macrophages or cytotoxic CD8⁺ T cells as well as for the activation of B cells and their differentiation into antibody-producing cells. Most of these CD4⁺ T cell functions are exerted through the secretion of cytokines.

The definition of two major populations of CD4⁺ cells designated as TH1 (type 1 helper T cells) and TH2 (type 2 helper T cells) derived from the pioneer studies of Mosmann *et al.*, demonstrating that mouse CD4⁺ T cell clones generated *in vitro* produce distinct sets of cytokines [1]. The TH1 clones preferentially synthesize interleukin-2 (IL-2), which is a major growth factor for all T cells, and interferon-gamma (IFN-γ), which is responsible for the activation of macrophages, whereas the TH2 clones preferentially secrete IL-4, IL-5, IL-6, IL-10, and IL-13, which are growth and differentiation factors for B cells, and IL-5, which is involved in the differentiation and activation of eosinophil polymorphonuclear cells (Figure 1).

TH1 and TH2 cells were subsequently identified in *vivo* in experimental models of infectious diseases. The best example is that of murine leishmaniasis in which CD4⁺ T cells differentiate into either TH1 or TH2 cells, depending on the genetic background [2]. Mouse strains developing TH1 cells display strong protective cell-mediated immunity against the parasite, resulting in a self-limiting cutaneous disease, whereas mouse strains developing TH2 cells display high levels of non-protective antibodies associated with low-grade cell-mediated immunity, and die from a systemic form

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Correspondence and offprint requests to: Michel Goldman MD, Hôpital Erasme, Department of Immunology, 808, route de Lennik, B-1070 Brussels, Belgium.