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EDITORIAL REVIEW

Cryoglobulins in chronic hepatitis C virus infection

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Soon after the discovery of hepatitis C virus (HCV) by Choo and colleagues [1] and the establishment of serological tests [2] for the detection of antibodies against HCV (anti-HCV) it became apparent that 'mixed essential cryoglobulinaemia' was, in most cases, due to chronic HCV infection [3–7].

The hallmarks of cryoglobulins in HCV infection are that they appear only many years after the initial infection, and that they are mixed. That means that they contain IgM antibodies directed against the Fc portion of IgG, i.e. rheumatoid factors (RF) and polyclonal IgG as the antigen [5,7]. According to the nomenclature of Brouet, one refers to type III cryogloblins when the IgM is polyclonal and type II when the IgM is monoclonal [8]. However, Tissot *et al*. have described that in many patients the IgM RF is in fact oligoclonal (type II/III) [9]. In addition, there have been many reports suggesting that HCV leads to the formation of low-grade lymphoma. Although these observations suggested that there might be a progression from a polyclonal stimulation to slow malignant transformations of B cells, insufficient patients have been followed to date to confirm such a progression.

The nature of the IgM RF has been analysed extensively, in particular the monoclonal IgM of type II cryoglobulins in HCV. Most of these monoclonal IgM RF express an unusual idiotype (Wa) corresponding to selected germline V genes [5] and the B cells clones producing these monoclonal IgM show evidence of ongoing somatic hypermutation. A similar phenotype has been observed in a number of B cell malignancies, such as follicular lymphoma, MALT lymphoma and monoclonal gammopathy of unknown significance (MGUS) [10]. However, the pattern of somatic mutations in the HCV-associated B cell clones was not suggestive of antigen driven affinity maturation; rather, selection was against mutations which could generate antibodies of high affinity antibodies. Consistently, monoclonal RF of patients with HCV are of low affinity compared to those found in rheumatoid arthritis. These findings suggest that in hepatitis C the driving force behind the production of RF is not simply equivalent to a strong immune response. Rather, it is a unique process during which B cells expand, with a preferential expansion of B cells producing RF, followed or accompanied directly by an environment favourable for the immortalization of one specific clone.

These observations return us to knowledge gained in basic immunology, as IgM RF of cryoglobulins correspond to natural antibodies found in normal individuals [11]. Low affinity IgM RF are natural antibodies present in human plasma that have a specificity for IgG-Fc determinants, but are in fact polyreactive as they cross-react with other autoantigens. They contain most often the kappa light chain and use the same selected germline V genes for heavy and light chains than RF of cryoglobulins. These characteristics are found in human fetal B cells expressing RF and in B cells following polyclonal stimulation induced by lipopolysaccharide (LPS) or Epstein–Barr virus (EBV). The evident advantage of these low-affinity RF is that they will recognize and bind to microrganisms covered with specific IgG antibodies, producing agglutination and complement activation. The resulting enhancement of inflammation might be essential for the host to get rid of infectious agents. Interestingly, $CD5^+$ B cells (also named B1 cells), which are the major source of natural IgM RF, are increased in the peripheral blood in HCV-infected individuals.

In hepatitis C RF-producing B cells might be stimulated directly by the virus itself. On B cells CD81 is a member of a signalling complex that includes CD19 and CD21 [12]. Cross-linking of these complexes lowers the threshold for B cell activation and proliferation. Recently CD81 was identified as one of the candidate receptors for HCV on B cells [13]. Thus, the binding of HCV to CD81 might explain the continuous polyclonal B cell activation observed in hepatitis C and the increase of $CD5^+$ cells in the periphery with production of many autoantibodies (RF, ANA, antismooth muscle antibodies, etc.).

A second step towards malignant transformation (emergence of one dominant clone producing one monoclonal IgM RF, i.e. a cryoglobulin type II) may follow this initial activation. The B cells in the portal tracts of the liver of hepatitis C patients have monoclonal or oligoclonal patterns of expansion [14]. Kitay-Cohen *et al*. have found that 13 of 15 patients with HCV and cryoglobulinaemia type II had a Bcl-2 rearrangement in their peripheral blood leucocytes [15]. A successful antiviral treatment led to the disappearance of this translocation from peripheral blood cells in the majority of patients [16,17]. Why the translocation appears so frequently in HCV with cryoglobulins remains undefined, but indicates that the viral infection produces an environment favourable for the emergence of transforming events. The lack of HCV infection of malignant cells suggests that the oncogenic role of HCV in B cell lymphomatogenesis is probably an external trigger [18].

The IgM RF of type II cryoglobulins are present in high concentration in the plasma of hepatitis C patients (in g/l) similar to paraproteins found in MGUS. Therefore, it should be of no surprise that, in the presence of such a high concentration of IgM

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RF, immune complexes (IC) consisting of IgG-IgM RF are formed. From the known data about the affinity of monoclonal IgM RF it can be calculated that in plasma a measurable fraction of the IgG is complexed by IgM, despite the low affinity of the RF for IgG. The formation of cryoglobulins requires two reactions: first, binding of the IgM RF to IgG, then precipitation of this complex at less than 37°C [19]. The fraction of IgM RF and IgG being complexed increases slightly at 4°C but from the fraction of IgG and IgM in the cryoprecipitate *versus* supernatant one has a crude estimate of the affinity of the IgM RF. The free IgM RF in the supernatant does not represent a different population of IgM RF but is the result of equilibrium between free and bound IgM. Clinical observations have confirmed that *in vivo* the concentration of IgM and IgG are in some kind of equilibrium allowing IgM, IgG and the complexes formed to circulate. Indeed, in two case reports in which high quantities of polyclonal intravenous immunoglobulins were infused in patients with type II cryoglobulins, the addition of Ag favoured the formation of IC and their precipitation in many organs including the skin and kidney, resulting ultimately in acute renal failure [20,21]. Immediate plasmapheresis reversed the renal failure in both cases. Thus, in a given patient, one may suggest that clinical signs appear when the concentrations of the reagents (IgM and/or IgG) reach a critical level.

However, the cryoprecipitation reaction is not that simple. In chronic HCV many viral elements, including soluble viral proteins, are complexed in plasma by viral-specific antibodies such as those described by Sansonno *et al*. in this issue [22]. These IC may or may not accelerate the clearance of the antigen involved depending on their size and the number of antibodies molecules bound per IC. Large IC containing many IgG are able to activate complement, react with Fc receptors and will have the property to be bound by RF with high avidity. The binding of RF occurs preferentially to IC than to monomeric IgG (multivalent *versus* monovalent binding). Thus, in patients with hepatitis C, it was no surprise that in the cryoprecipitate HCV RNA could be found, and specific anti-HCV antibodies enriched compared to the supernatant. However, next to these specific viral and antiviral components there was an overwhelming quantity of polyclonal IgG, which were not related to HCV, as well as IgM RF. In the work published in this issue, Sansonno expands on these data and adds new information [22]. He demonstrates definitively that the IgM RF does not react with HCV, but is responsible for the secondary reaction inducing cryoprecipitation of the viral RNA or soluble HCV proteins found in plasma, which have formed IC with specific antiHCV antibodies. Interestingly, non-enveloped HCV core protein circulates in plasma bound mainly to specific antibodies, as suggested by their almost complete precipitation by the IgM RF. Why are such complexes not cleared rapidly by the fixed macrophages in liver and spleen? The production of such antigen might be very high, but it is possible that in the presence of IgM RF the physiological clearance of those IC is impaired. Madi *et al*. [23] have shown that in the presence of IgM RF-soluble IC could not bind efficiently to Fc receptors, could not fix complement correctly, and even when opsonized with C3 had only a reduced capacity to bind to C3 receptors. The occupancy of the Fc portion of IgG by IgM RF was probably the main factor involved. Such large complexes (IC coated with IgM RF) might rather activate and deplete complement than fix it, because IgM is a poor acceptor for C4/C3 [24]. This is in concordance with the findings described by Sansonno.

Whether the presence of HCV or proteins thereof are involved directly in the local deposition of cryoglobulins in tissues such as the kidney and skin remains unresolved, despite their presence in immune deposits [25,26]. Indeed, any antigen trapped in the cryoglobulin will be found at the site of immune aggregation. Furthermore, tissue deposition of type II cryoglobulins in Sjögren's syndrome without HCV involvement occurs in the same organs and is indistinguishable from that of HCV associated cryoglobulinaemia. The physicochemical properties of cryoglobulins might play a major role here. The temperature-dependent conformational changes that occur after the IgM RF has bound to its antigen are not yet understood. Related properties of cryoglobulins might lead to their deposition in the kidney (concentration of the proteins in the glomerulus, pressure, specific interactions with the endothelial cells, etc.). Izui's group suggested that the combination of both RF and cryoprecipitability is responsible for the vasculitis in their mouse model. Interestingly, the temperature at which the mice are kept determined the presence of vasculitis and glomerulonephritis [27]. Whereas mice kept at room temperature developed glomerular depositions of cryoglobulins, there was no glomerulonephritis in mice living in a warm environment (37°C). The most likely hypothesis to explain this finding is that the large cryoglobulinemic aggregates formed in superficial blood vessels might not dissociate fast enough before arriving in the kidney.

That modifications of the concentration of the components making up peripheral ICs involved (viral antigen, specific antibodies and IgM RF) define the presence of clinical disease in cryoglobulinaemia has been confirmed by Sansonno *et al*. [28]. Patients with HCV-associated type II/III cryoglobulinaemia resistant to interferon therapy were treated with anti-CD20 monoclonal antibodies. The level of IgM RF and specific anti-HCV antibodies diminished, whereas the viral load increased. Despite this increase in viral load, the clinical signs and symptoms abated in most patients and the vasculitis resolved. These data remind us that manipulating the immune reactants correctly still represents a powerful mean to diminish the severe vasculitis seen in many patients, and to this avail plasmapheresis remains an appropriate procedure to treat the acute vasculitis of cryoglobulinaemia.

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