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Primary, post-primary and non-specific immunoglobulin M responses in HCV infection

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Abstract

Delayed and variable antibody responses to hepatitis C virus (HCV) make it difficult to reliably diagnose acute HCV infection. IgM and IgG anti-HCV may be observed simultaneously as disease persists. IgM plays a key role in mixed cryoglobulinemia (MC), an immune complex disease strongly associated with persistent HCV infection. In MC, clonal or oligoclonal IgM rheumatoid factors facilitate the deposition of immune complexes in small blood vessels and tissue, leading to inflammation, complement activation, and tissue damage. Clonally expanded IgM⁺κ⁺ B cells expressing rheumatoid factor-like IgM are abundant in many HCV patients with MC. The observation that identical or similar IgM antibodies are expressed in different patients' clonally expanded B cells supports the conclusion that MC is driven by antigen-specific B cell activation, rather than polyclonal B cell activation or HCV replication in B cells. More study is required to identify the antigens that drive development of MC.

At least 130 million people worldwide are persistently infected with the hepatitis C virus (HCV) [1]. Infection is spread primarily through needles and blood products, but nosocomial and sexual transmission have also been reported [2]. It is estimated that more than half of those who contract HCV infection become chronically infected, but this is uncertain because acute HCV infection is frequently clinically silent [3]. It is believed that HCV establishes persistent infection by blunting both innate antiviral responses and HCV-specific adaptive immune responses early in the course of infection [4, 5]. Before the recent approval of directly acting antiviral drugs [6], treatment of HCV infection meant up to 48 weeks of pegylated IFNα and the nucleoside analog, ribavirin [7]. Antiviral therapy with pegylated IFNα and ribavirin is often poorly tolerated, is associated with significant morbidity, and is frequently ineffective in patients with chronic HCV infection [7]. In contrast, acute HCV infection may be readily cleared with an abbreviated course of IFNα (reviewed in [8]); this fact makes diagnosis of acute infection desirable. Because IgM is typically the first immunoglobulin isotype produced in an immune response, it was expected that acute HCV would be associated with high levels of HCV-specific IgM that then wane as IgG levels rise. In practice, however, IgM anti-HCV is not a useful marker of acute infection.

IgM responses in acute hepatitis C: limited utility for diagnosis

A number of groups have studied antibody responses over time after infection in subjects for whom the time of infection can be estimated [9-12]. Antibody responses are delayed in acute HCV infection; the minimum time from the onset of viremia to detectable seroconversion is estimated at 6-8 weeks [11], but seroconversion may take a year or longer [9, 13]. Levels of

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HCV-reactive IgM or IgG in the acute period do not foretell clearance or persistence of infection. IgM anti-HCV is readily detected in patients chronically infected with HCV [14-16], although levels are reportedly higher in acute than chronic infection [17]. Fluctuating levels of HCV core-specific IgM may distinguish acute HCV infection from an exacerbation of chronic HCV infection [12]. Even in acute HCV infection, nearly simultaneous detection of HCV-specific IgG and IgM has been reported [10, 18-20].

Pathogenic IgM in patients with chronic hepatitis C

Chronic HCV infection is associated with high rates of extrahepatic disease, notably including mixed cryoglobulinemia (MC) and an increased risk of B cell non-Hodgkin lymphoma [21-23]. MC may be a precursor to lymphoma [24]. While classic MC with the symptomatic triad of palpable purpura, weakness, and arthralgia is uncommon, some studies have observed that cryoglobulins can be detected in as many as one half of all patients with persistent HCV infection [25-28]. The fact that more than 90% of patients with symptomatic MC are indeed infected with HCV [29-31] is strongly suggestive of a causative relationship.

MC is a lymphoproliferative disorder frequently involving IgM-producing B cells. In MC, immune complexes containing HCV RNA, polyclonal IgG, and rheumatoid factor accumulate in small vessels and in tissues, activating complement and causing a diverse array of symptoms and pathology [31, 32]. A high percentage of symptomatic patients report a rash (palpable purpura) in the lower extremities, weakness, and joint pain [31]. Peripheral neuropathy, sicca syndrome, vasculitis, membranoproliferative glomerulonephritis, and skin ulcerations may occur, depending on the deposition of immune complexes [31, 32]. MC is difficult to diagnose because the cryoglobulins—by definition cold-precipitable—can be lost due to their tendency to precipitate during the interval between blood collection and sample processing. Blood to be tested for a cryoprecipitate must be held at 37°C until after serum separation, and the isolated serum then maintained at 4°C for up to seven days to allow visualization of the precipitate (reviewed in [32]). Cryoglobulins can be classified as type I (monoclonal Ig only, typically associated with a malignancy), type II (a mixture of monoclonal and polyclonal Igs) and type III (polyclonal Igs) [32]. HCV patients are at risk of type II or, to a lesser degree, type III MC [32]. The rheumatoid factor in HCV patients with MC is frequently a clonal or oligoclonal IgM with a κ light chain. We [33, 34] and others [24, 35-40] have observed the presence of clonally expanded B cells, which express IgM κ antibody at the protein and RNA levels, in the blood and tissues of HCV patients with MC or with non-Hodgkins lymphoma. Strikingly, the clonally expanded B cells in many patients express rearranged immunoglobulin genes encoded by V_H1-69 (also known as V_H 51p1) and V κ 3-20 (also known as kv325 and V κ A27) [33-35, 37-40]. These B cells express an IgM κ antibody with rheumatoid factor activity [41].

The pathogenesis of HCV-related MC is puzzling. How does HCV, a hepatotropic virus, promote B cell clonal expansion, autoantibody production, and lymphoma? A number of hypotheses have been put forth (reviewed in [4]).

Polyclonal activation?

It has been noted that CD81, one of several factors required for HCV entry, is expressed on B cells as part of the B cell co-receptor complex. One possibility is that HCV binds to CD81 and promotes B cell activation. This model is inconsistent with the very limited clonal diversity observed in abnormally activated B cells in HCV-associated MC [33-35, 37-39]. Activation of B cells through a shared receptor such as CD81 would be expected to drive polyclonal B cell proliferation, which is not seen in HCV patients with or without MC.

HCV infection in B cells?

Direct HCV infection of B cells, leading to altered B cell proliferation or activation, has also been proposed. In support of this hypothesis, some groups have reported the detection of a variable level of HCV RNA and protein in HCV patients' B cells [42-46]. We have found that B cells do not support replication of laboratory-derived HCV, and that they do not express the full set of entry factors required for HCV infection of susceptible cell types [47, 48]. Peripheral blood B cells in HCV patients are associated with a very low level of HCV RNA, far below one copy per cell ([42-46] and our own unpublished data). If B cells were dysregulated as a result of being infected with HCV, we would expect that the abnormal B cell subset would contain at least one HCV genome per cell, but this has not proven to be the case. Indeed, highly purified rheumatoid factor-producing B cells from HCV MC patients do not contain significant levels of HCV RNA (E. D. Charles, C. Brunetti, S. Marukian, and L. B. Dustin, unpublished data).

Antigen-driven activation?

Our data support a model in which antigen-driven B cell activation plays a key role in the clonal expansion of a very characteristic subset of B cells. Similar or identical immunoglobulin gene rearrangements are seen in the clonally expanded B cells in different HCV patients with MC, in multiple studies from different countries [33-39]. What is the antigen? A hint may come from the observation that the V_H1-69 immunoglobulin gene segment is present in a number of broadly neutralizing antibodies active against an array of viruses, including HCV [37, 49, 50], HIV [51-53] and influenza [54-56]. Work is ongoing to determine whether the rheumatoid factors overproduced in HCV patients with MC have reactivity with HCV envelope antigens. We speculate that HCV-associated MC represents an exuberant antiviral antibody response gone awry.

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