Editorial

Hepatitis C and B Cells: Induction of Autoimmunity and Lymphoproliferation May Reflect Chronic Stimulation Through Cell-Surface Receptors

Hepatitis C virus (HCV), an RNA flavivirus, causes a chronic infection in over 85% of exposed individuals. In addition to causing chronic hepatitis, HCV causes chronic stimulation of B cells, resulting in production of rheumatoid factors (RF) derived from a highly restricted gene set, and mixed cryoglobulinemia type II (MC II). As an indirect consequence, HCV induces various autoimmune disorders and in rare cases, non-Hodgkin's B cell lymphomas (NHL). Illustrating these points in the current issue of *The Journal of Rheumatology*, Ramos-Casals, *et al* describe 6 patients who developed the triple association of HCV, autoimmune disorders, particularly Sjögren's syndrome (SS), and NHL[1].

Recent studies may help to understand the pathways whereby HCV induces its varied extrahepatic syndromes. It appears that chronic stimulation of B cells by HCV leads to a potent but restricted antibody response, with generation of viral antigen-antibody complexes and RF secretion. In some cases, likely influenced by genetic or viral factors, the restricted B cell response undergoes additional oncogenic events and gives rise to a monoclonal B cell expansion that sometimes becomes a lymphoma. Of interest, successful treatment of HCV in some such patients results in remission of the lymphoma.

It is now clear that several human infectious agents can induce B or T cell lymphomas, including the Epstein-Barr virus, human T lymphotrophic virus type I, *Helicobacter pylori*, and HCV. In general, the mechanism whereby the first 2 agents induce lymphomas are through intracellular actions in infected lymphocytes, whereas the latter 2 agents appear to act on B cells through antigen or other cell-surface receptors[2].

The process whereby HCV infection leads to the development of lymphoma is unknown, but growing evidence indicates that HCV may directly stimulate and possibly infect B cells. Receptors for HCV on B cells include the low density lipoprotein receptor[3] and CD81, which is a component of the complement receptor 2[4] (see below). In addition, some HCV proteins can modulate transcription or cellular growth via intracellular actions[5].

HCV induces a strong antibody response to its envelope glycoprotein E2. Since E2 binds to B cells via CD81, antibodies to E2 may block binding of HCV to cells and be protective against HCV infection in some situations[4]. When E2 binds to B cells via CD81, it then associates with CD19 and CD21, forming a complex that lowers the activation threshold[6]. Therefore, HCV can bind to B cells via 2 receptors: cell-surface immunoglobulin (Ig) specific for E2 and CD81. The result of such dual engagement may be crosslinking of surface Ig and CD2, which markedly reduces the threshold of B cell activation[7]. This increased B cell reactivity can enhance the effect of chronic stimulation by HCV and may play a role in the frequent emergence of autoantibodies such as antinuclear antibodies and RF, and lymphoproliferative disorders.

Additional steps are probably needed for transformation to lymphoma. One such step may be activation of proto-oncogenes by chromosomal translocation or mutations. An increased prevalence of the t(14;18) translocation, which results in deregulated overexpression of the Bcl-2 anti-apoptotic protein, occurs in HCV-positive patients, with and without MC II. Treating HCV resulted in loss of the t(14;18)+ B cell clones in 6 of 7 treated patients compared with one of 6 nontreated patients[8].

Studies of the antibody response to HCV-E2 illuminate the potential link between MC II and development of lymphoma in some patients. In asymptomatic patients infected with HCV, antibodies to E2 are derived from a set of restricted Ig heavy and light chain V genes (51p1 and

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kv325, also known as V1-69 and Vκ3-A27, respectively)[9]. This VH/VL gene pair is also utilized in synthesis of monoclonal RF in patients with MC II (see below). Although reactivity of an antibody cannot be inferred from analysis of the coding V genes, when Quinn, *et al* analyzed antibodies cloned from HCV-associated lymphomas, they found that the anti-E2 antibodies were derived from the 51p1 and kv325 Ig V genes[10]. These findings support the hypotheses that induction of RF secretion in these B cells may in part be occurring as an antibody response to E2, and some HCV-associated lymphomas derive from B cells that were initially activated by the HCV-E2 protein.

Additional evidence that HCV can induce B cell lymphomas are observations by Hermine, *et al*, who reported outcomes for HCV-infected patients having splenic lymphoma with villous lymphocytes, a generally indolent lymphoma affecting the elderly[11]. After treatment with interferon-α2b and ribavirin, the lymphomas of all 9 HCV-infected patients responded, and 7 had a complete remission with loss of detectable HCV RNA. In contrast, none of 6 HCV-negative patients with the same type of lymphoma responded to treatment using the same regimen. These findings indicate that treating HCV in patients with certain NHL can lead to regression of the lymphoma.

Three of the 6 patients described by Ramos-Casals, *et al* had MC II, which is a distinctive manifestation of HCV infection. MC II is defined by the presence of cryoprecipitable immune complexes that contain monoclonal IgM RF, polyclonal IgG (which includes anti-HCV antibody), and HCV virions. The monoclonal IgM RF in MC II is derived from a highly restricted set of both heavy and light chain Ig V genes[12,13]. These IgM RF typically have the Wa cross-reactive idiotype[12]. The property of cryoprecipitation appears to be conferred mainly by the IgM RF molecule.

As discussed above, the V gene usage in cryoglobulin RF is not random, as about half of IgM RF in MC II are encoded by a single pair of Ig heavy and light chain V genes, V 1-69 and kv325, respectively. The narrowness of this restriction is further highlighted by the fact that only certain alleles of V1-69, known as the 51p1-related genes[14], are actually seen in cryoglobulin RF[15]. Analysis of the Ig V region gene coding sequences suggests that HCV-induced IgM RF are antigen-driven because they contain some somatic mutations, although they do not appear to undergo affinity maturation, which is a characteristic of many RF in rheumatoid arthritis[13]. MC II are virtually always associated with monoloclonal B cell expansions present in the liver, bone marrow, and/or blood[16] and appear to have this same V gene bias, favoring the 51p1/kv325 combination, that is found in HCV-associated lymphomas[17]. These findings suggest that the lymphomas were derived from RF-secreting B cells in type II MC. As noted above, finding the same combination of restricted Ig genes in anti-E2 responses suggests involvement of E2 in driving RF secretion.

Ramos-Casals, *et al* found that 4 of 6 HCV-infected patients with NHL also had SS. Growing evidence indicates that HCV may be a cause of SS[18]: up to 80% of HCV-infected individuals have some salivary or lachrymal abnormality and mild sialadenitis[18]; HCV has been detected in saliva of HCV-infected patients with SS; positive- and negative-strand HCV-RNA has been detected by both polymerase chain reaction and in situ hybridization in minor salivary glands of patients with sialadenitis and chronic hepatitis C, indicating active viral replication[19]; and transgenic mice carrying the HCV envelope genes E1 and E2 develop Sjögren-like sialadenitis[20].

Patients with primary SS have an increased risk of developing NHL[21]. Lymphomas complicating SS arise frequently in extranodal mucosal sites, including the salivary glands, the stomach, and the lung. Most of these lymphomas are low-grade, and of various histological types. An important predisposing factor for the occurrence of NHL in patients with SS is the presence of serum MC II[22].

Mariette has suggested that in patients with SS, with or without HCV infection, polyclonal B cell activation is present in extranodal sites, leading to secretion of RF[23]. The mechanism for production of RF may involve stimulation by antigen-antibody complexes, or cross-reactivity of a RF to a viral antigen, as mentioned above. Lymphomagenesis in such patients is likely a multi-step process, perhaps beginning with chronic stimulation of polyclonal RF-secreting B cells via their B cell receptors. Supporting this notion, Martin, *et al* found that the Ig product of malignant B cells from NHL from 2 HCV-negative patients with primary SS had RF activity and both used the kv325 gene[24]. Such RF-secreting B cells appear to be undergoing continual cycling. A lack of normal T cell control in the microenvironment of salivary or mucosal germinal centers may increase their chance of malignant transformation.

Since V gene usage of anti-E2 antibodies has not been broadly studied, it is not known how prevalent the 51p1/kv325 combination is in the overall anti-HCV response. Nevertheless, HCV appears capable of chronically stimulating some B cells to induce a restricted antibody response with significant likelihood of producing monoclonal IgM RF and MC II. Similar to the situation seen in HCV-negative SS, such RF-positive B cells appear to have a heightened reactivity and a propensity to undergo neoplastic transformation.

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