Alteration of B cell function by hepatitis C virus

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A B S T R A C T

Hepatitis C Virus (HCV) is associated epidemiologically with B cell lymphoma. Recent reports have documented that these lymphomas can regress when patients are treated with antiviral therapy for their coexistent HCV. This exciting result implies that the continued presence of the virus is necessary for the survival of the lymphoma cells. HCV is also known to be the primary cause of mixed cryoglobulinemia (MC), a benign B cell proliferative disorder that can sometimes progresses to lymphoma. The connection between HCV infection and these B cell lymphoproliferative diseases may serve as an important model for how an immune response can go awry and lead to lymphoma. We hypothesize that rare B cells, which recognize the foreign viral antigen(s), could be diverted by the virus from its immune function to uncontrolled proliferation.

B cell proliferative diseases in Hepatitis C virus (HCV)-infected patients

HCV has infected over 170 million people worldwide. The virus cannot be reproducibly grown in tissue culture or in small animal models. The only known hosts for HCV are human and chimpanzee. Our current understanding of HCV pathogenesis is based mostly on epidemiological and molecular studies. The majority of HCV infected patients develop chronic hepatitis. Chronic HCV infection is the most prevalent cause of liver cirrhosis and is the major cause of mixed cryoglobulinemia (MC), a B cell proliferative disorder. It is estimated that 30-40% of patients chronically infected by HCV have MC.[1] Moreover, nearly all MC patients are infected by HCV, indicating that this virus plays a major role in the etiology of this B cell proliferative disease.[2] While MC is mostly an indolent disease, patients with MC can progress to overt B cell lymphomas.[3] MC patients responding to anti-viral therapy show regression of their expanded B cell populations. The disappearance of these B cell clones in response to anti- HCV treatment has been documented by tracking their unique clonal immunoglobulin (Ig) heavy chain rearrangements and/or their clonal Bcl-2 t(14;18) translocations.[4] These studies demonstrate that the expansion of clonal B cells is tightly linked to HCV infection.

HCV is associated with non Hodgkin lymphomas (NHL). In Italy 9-35% of patients with B cell NHL are chronically infected with HCV, compared to 2-13% of patients with other hematological malignancies and to 1-5% of healthy individuals.[5] In one US study (Southern CA) 22% of NHL patients were infected by HCV compared to 5%, of patients with non-malignant hematological conditions.[6] In contrast, a number of similar studies from other countries such as France, Scotland, Canada, Germany, and other U.S. regions have not found a high prevalence of HCV infection in lymphoma patients, reviewed in.[3] Currently, the reason for this geographically varied incidence of HCV association with lymphoma is not clear. Neither is the mechanism by which these two diseases could be associated. Nevertheless, Anti-viral treatment caused the regression of B cell lymphomas as documented for HCV-infected patients who also had marginal zone lymphoma and were treated with interferon-α +/- ribavirin.[7] Coincident with the clearance of viremia these patients showed regression of their splenic marginal zone lymphomas.

Viral- B cell interactions

The viral sequence predicts the presence of two envelope glycoproteins E1 and E2.[8] A soluble recombinant form of E2 was shown to interact with CD81 on the sur-

face of lymphocytes.[9] A large number of studies have confirmed the direct interaction between CD81 and HCV envelope proteins[10] and defined the critical residues of the CD81 involved in this interaction.[11] It is important to note that while CD81 is necessary, it is not sufficient for infectivity by the virus and that additional cell surface receptors, such as the scavenger receptor (SR-B1), the LDL receptor and DC-SIGN/L-SIGN have been implicated in the binding and internalization of viral pseudoparticles.[12,13]

Role of CD81 in B cells

We originally identified CD81 as the target of an anti-proliferative antibody (TAPA-1) in human B cells[14] CD81 is a member of the tetraspanin superfamily of cell-surface proteins. Tetraspanins play a role in cell adhesion, motility, proliferation, and differentiation[15] they share a common membrane topology, forming four transmembrane domains and two extracellular loops and a signature of conserved amino acid residues that distinguishes them from other molecules with four transmembrane domains.[16] Tetraspanins, in general and particularly CD81, tend to assemble multi-molecular complexes in the cell membrane. Components of these complexes differ in different cell types. Thus, triggering a specific tetraspanin molecule results in downstream signaling events that differ according to the cell type.[17] Because CD81 is a widely expressed molecule forming such varied molecular complexes, its engagement leads to different functional effects in cells of different origins. For example, engagement of CD81 by HCV E2 on T cells leads to their activation, while on NK cells it leads to their inhibition [18, 19]. CD81 is expressed in the liver, but its function in hepatocytes is still unknown.

Figure 1: Direct engagement of HCV E2 by the BCR and CD81 on the surface of a B cell leading to dual stimulation.

In B cells CD81 associates with the CD19/CD21 co-receptor complex (Figure 1). This signaling complex serves as a link between the innate and the acquired immune systems. The dual binding of an antigen opsonized by complement to both the complement receptor (CD21) and to a specific B cell receptor (BCR) triggers synergistic signaling pathways. Indeed, it was demonstrated that co-engagement of the BCR and the CD19/CD21/CD81 complexes lowers the threshold of B cell activation.[20] HCV, by virtue of binding directly to CD81 could react with a specific BCR and deliver a dual activation signal to specific B cells.

The B cell immune response to HCV infection

Support for the dual receptor hypothesis comes from our published studies in which we analyzed the immune response of patients to HCV infection. In these studies we tested the ability of the immunoglobulin, secreted by B cells from an HCV-infected patient, to block interactions between cells and viral envelope proteins. We characterized a panel of human hybridomas derived from peripheral B cells of an asymptomatic HCV-infected individual. These hybridomas were selected because of their reactivity with the HCV envelope glycoprotein E2. The panel included 10 hybridomas, 9 of which produced mAbs that recognized conformational epitopes within the viral envelope protein. Six of the 9 conformational mAbs inhibited binding of E2 to cells in a CD81-dependent manner. This study demonstrated that HCV-infected patients produce antibodies that may neutralize the binding of the virus to the CD81 receptor. Interestingly, five of the six neutralizing mAbs were broadly reactive and bound E2 from several HCV genotypes, indicating an immune response to common epitopes that may play a role in viral-cell interaction.

Earlier studies of HCV-infected patients with cryoglobulinemia have demonstrated that these immunoglobulins were derived from B cells with a biased V gene repertoire.[21] We therefore wondered whether the immune response against HCV is also restricted. We analyzed the V gene usage of the panel of human anti-HCV E2 mAbs. Sequences of the V region genes expressed by the 10 hybridomas demonstrated that each hybridoma expressed unique VH and VL genes. Remarkably, 7/10 of the anti-E2 hybridomas used the VH1-69 gene or the closely related VH1-e gene. Analysis of replacement to silent mutation ratios indicated that these V genes had undergone somatic mutation and antigenic selection. The preferential usage of the VH1-69 gene was known to occur in HCV-associated type II MC.[21] Moreover, the same biased use of the VH1-69 gene was found in BCRs from HCV-associated lymphomas.[22] Taken together, these studies implicate a biased immune response to HCV E2, which is
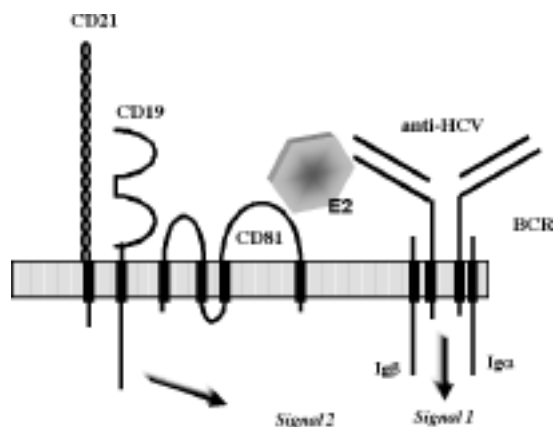
reproduced in pathogenic B cell proliferative disorders associated with this viral infection.

Analysis of the B cell receptor (BCR) of HCV-associated B cell lymphomas: evidence for antigen-driven lymphomagenesis

The monoclonal nature of B cell lymphomas provides a unique opportunity to investigate the BCR expressed on the surface of an expanded B cell population. We determined the V gene repertoire used by HCV-associated lymphomas in our patient population. By producing the BCR proteins we were able to test them for binding to HCV antigens. Intact tumor cells cannot be used for this purpose because E2 also binds CD81.[11,23] We therefore molecularly rescued the V_H and V_L region genes from 7 independent lymphoma biopsies, obtained from patients who were also infected by HCV. The molecularly rescued V genes were then inserted into an expression vector for the production of soluble immunoglobulins.[24] The rescued soluble immunoglobulins were then tested for their binding to the HCV E2 protein. One of the 7 rescued lymphoma immunoglobulins reacted with the HCV E2 antigen. This binding was similar in every respect to that of a well-characterized human anti-E2 mAb. In contrast, none of 23 rescued immunoglobulins from non-HCV associated lymphoma cases bound E2. The HCV-associated NHL immunoglobulin precipitated E2 molecules with a size distribution that was identical to that precipitated by the known anti-E2 mAb. Both immunoglobulins bound multiple E2 glycoforms. The lymphoma-derived immunoglobulin bound E2 from all tested genotypes, implying reactivity with a conserved E2 epitope. The lymphoma in this patient evidently arose from a B cell that was part of an immune response to the HCV-E2 protein. We believe this to be the first identification of a cognate antigen for a human lymphoma BCR.

The seven cases analyzed show a limited V region gene repertoire with a high frequency of the V_H 1-69

and VK3-A27 genes (Table 1). These isolated V region genes were unique to each patient, as they had accumulated unique somatic mutation patterns, indicating antigenic selection. This same bias in V gene repertoire was reported in a study of Italian HCV-associated lymphomas[22] and in the normal B cells that respond to the HCV envelope protein E2[24] Taken together these findings support the hypothesis that lymphomas can arise from B cells that are part of an immune response against an HCV antigen.

Role of CD81 in the dual activation of B cells

Co-engagement of the BCR and the CD19/CD21 complex results in the prolonged association of the BCR with lipid rafts and prolonged signaling from the rafts as compared to that achieved by BCR cross-linking alone.[25] The ability of the CD19/CD21/CD81 complex to enhance BCR signaling by stabilizing its association with rafts appears to be a novel mechanism by which co-receptors can function. To determine the role of CD81 in the enhancement of BCR signaling by the CD19/CD21 co-receptor complex we employed our CD81-deficient mice.[26] Our studies provide evidence that CD81 is critical for the function of the CD19/CD21 co-receptor complex in B cells. In wild-type B cells, CD81 associates with lipid rafts upon co-engagement of the BCR and the CD19/CD21 complex. In contrast, B cells from our CD81-deficient mice were impaired in their ability to partition the co-ligated BCR and CD19/CD21 complexes into lipid rafts, which resulted in reduced signaling.

B cell lymphomagenesis

There are at least two possible mechanisms by which the immune response of normal B cells to HCV infection could go awry:

1. Dual stimulation, whereby B cells responding to the virus receive a chronic activating signal through both their BCR and through the CD19/CD21/CD81 complex placing them at risk for malignant transformation.

2. Facilitated viral entry, whereby B cells that bind the virus through both receptors efficiently internalize the virus, which then could cause genomic instability.

Experimental systems in which to test the dual receptor hypothesis in both its cell signaling and viral entry aspects will be discussed.

Table 1. V gene usage of HCV-associated lymphomas identified to-date.

Patient	V_H	VL
1	V_H 1-69	V_K3-A27
2	V_H 4-59	V_K3-A27
3	V_H 3-48	V_K1-012
4	V_H 4-34	V_K3-A27
5	V_H 3-15	V_K1-012
6	V_H 1-e *	V_K3-A27
7	V_H 1-69	V_K1-L15

**Closely related to V_H 1-69.

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