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HCV Lymphotropism and Its Pathogenic Significance

Tomasz I. Michalak

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Abstract

Hepatitis C virus (HCV) is not only a hepatotropic but also a lymphotropic virus. Infection of the immune system appears to be a natural propensity of HCV and, as the accumulated data indicate, a common characteristic of both symptomatic and clinically silent but molecularly evident infection known as occult infection. The ability of HCV to infect cells of the immune system is consistent with a significantly greater prevalence of certain lymphoproliferative disorders in HCV-infected patients, such as mixed cryoglobulinemia and B-cell non-Hodgkin's lymphoma. This chapter recapitulates the approaches used to detect HCV and its replication within lymphoid cells, features of HCV compartmentalization in the lymphatic system and in different types of immune cells, and the cell culture models developed to study HCV lymphotropism. In addition, the characteristics of the molecules recently identified as those specifically mediating HCV entry leading to virus replication in B and T lymphocytes, which are distinct from those involved in virus entry to hepatocytes, are presented. Finally, the biological impact of HCV lymphotropism on the function of immune cells, virus persistence, and immune cell proliferation and lymphomagenesis is summarized.

Keywords: HCV immune cell tropism, T lymphocytes, B lymphocytes, monocytes, occult HCV infection, HCV persistence, immune cell functions, lymphomagenesis, cryoglobulinemia, B-cell non-Hodgkin's lymphoma

1. Introduction

The sequences of virological and immunological events leading to the pathological outcomes associated with hepatitis C virus (HCV) infection are not yet fully recognized. This is mainly because the onset of HCV infection rarely can be identified, the evolution to a symptomatic disease often takes decades, and the majority of predisposing factors and processes contributing to the development of the most important pathological consequences, such as cirrhosis,

hepatocellular carcinoma (HCC), and coinciding lymphoproliferative disorders, remain unknown. The fate of HCV during prodromal and convalescent phases of infection is also poorly recognized. Moreover, in addition to a symptomatic infection, which is normally accompanied by circulating HCV RNA and antibodies to HCV (anti-HCV), HCV can also persist as a clinically silent (occult) infection [1–3]. This infection is accompanied by very low levels of HCV RNA in serum (usually below 100–200 virus genome copies per mL), liver, and peripheral blood mononuclear cells (PBMCs), which are detectable with a significant difficulty, if at all, by clinical assays [4, 5]. This occult HCV infection (OCI) continues for decades after either spontaneous (self-limited) or antiviral therapy-induced resolution of hepatitis [1–4, 6–10]. OCI may have epidemiologic (e.g., contamination of blood and organ donations) and pathogenic (e.g., cryptogenic liver disease and oncogenicity) consequences which are not yet well recognized. Furthermore, experimental and clinical data indicate that HCV replicates not only in the liver but also in the lymphatic system, where it can modify development, proliferation, and function of immune cells [1, 2, 4, 5, 11–18]. It is also apparent that immune cells are reservoirs of persisting HCV where virus may hide from immune surveillance and elimination, similar to that in infections with other lymphotropic viruses [19–22]. The ability of HCV to infect cells of the immune system is consistent with a significantly greater prevalence of lymphoproliferative disorders in patients chronically infected with HCV, including mixed cryoglobulinemia (MC), B-cell non-Hodgkin's lymphoma (B-cell NHL), and marginal zone lymphoma [23–26]. Regression of these diseases in considerable numbers of patients treated with anti-HCV therapies is indicative of the direct role of HCV in the pathogenesis of those diseases [27, 28].

2. Identification of lymphotropic HCV replication

The current approach to the diagnosis of HCV infection, which typically includes testing of only serum samples but not samples from liver or otherwise easily accessible PBMC, using assays detecting only HCV RNA-positive (also termed as non-replicative, genomic, or vegetative) strand and anti-HCV, is an obstacle in the precise determination of HCV clearance, that is, cure. These limitations are also a source of controversies regarding the natural history and the longevity of infection, as well as the sites of virus persistence [29–33]. Consequently, patients who are considered free of infection may produce low levels of biologically competent virus for an extended time after vanishing of symptoms and biochemical normalization of liver function achieved due to either spontaneous resolution or clinically apparent sustained virologic response (SVR) to antiviral treatment [1, 4–6, 8, 9]. Testing of serial samples of serum or plasma and, in particular, PBMC collected a few months apart increases the detection of low levels of HCV RNA during follow-up, even when clinical assays of moderate or low sensitivity are applied. This is due to the fluctuating level of circulating virus during OCI that can temporarily increase to the levels detectable by these assays [2, 4, 5].

Identification of HCV infection in immune cells is not just about the mere detection of virus RNA-positive strand, since the occurrence of this strand alone may reflect incidental cell surface attachment or cellular uptake of virus or its genomic material. A combination of a few

approaches has been applied to credibly detect replicating HCV in immune cells [4]. They involve the documentation of (1) HCV RNA-negative (also termed as replicative or anti-genomic) strand [1, 6, 9, 34]; (2) viral structural and/or, preferentially, nonstructural proteins within the cytoplasm of infected cells [15, 34]; (3) distinctive HCV variants in total PBMC or their cell subsets when compared to those in plasma or in liver of infected patients, or emergence of HCV variants in cultured cells which are distinct from those occurring in inocula used to infect them [15, 34]; (4) susceptibility of infected cells to *ex vivo* treatment with interferon alpha (IFN-α) [6, 35] or direct acting antivirals (DAAs) [9, 34]; (5) transmission of infection to virus-naïve cells by cell-free supernatants from primary-cultured cells or *de novo*-infected cultures [34, 35]; (6) display of biophysical properties of virions by viral particles released during culture of infected cells [35], and (7) visualization of HCV virions released from infected cells by immunogold electron microscopy (IEM) with virus envelope-specific antibody [6, 35]. However, although the immune cells can support the complete cycle of HCV replication and production of biologically competent virion particles, the virus load per cell and the level of virus replication in either *in vivo-* or *in vitro*-infected immune cells are much lower than those seen in the HCV JFH-1 (Japanese fulminant hepatitis-1)-Huh7.5 cell infection system. Also, for comparison, the loads of HCV RNA per cell in total PBMC were found to be lower than those per hepatocyte in chronic hepatitis C (CHC), but comparable to each other during OCI [7]. Overall, investigations of HCV lymphotropism remain challenging and require highly sensitive detection techniques and meticulous approaches.

3. HCV compartmentalization in the immune system

HCV displays a remarkable genetic variability and typically exists in an infected host as a heterologous population of closely related subpopulations of viral particles carrying slightly different genomic sequences, called collectively as quasispecies. The 5′-untranslated region (5′-UTR) of virus genome contains an internal ribosome entry site (IRES) essential for viral RNA translation. This sequence is highly conserved among different HCV genomes, and arise of variants within this region is usually an indicator of a sustained virus change. HCV derived from extrahepatic locations tends to display variations in the IRES sequence when compared to the genomes from plasma and liver [5, 36]. Some of these substitutions are located at particular nucleotide positions, suggesting that they may reflect virus adaptation to replication in a non-hepatic milieu. In addition, variants within the hypervariable region-1 (HVR1) of the virus E2 protein, although much more common than those in the 5′-UTR, may also reflect the site-restricted replication of HCV variants. Overall, compartmentalization of HCV variants in immune cells is considered to be a reliable indicator of hepatocyte-independent virus replication [36].

The existence of HCV quasispecies with affinity to immune cells has been suggested shortly after HCV discovery [37–39]. Evidence for lymphotropic HCV variants was found in patients with CHC, acute hepatitis C, as well as in asymptomatic individuals with persistent OCI [4, 14, 15, 40–47]. Analyses of HCV variants residing in PBMC by clonal sequencing and single-stranded conformational polymorphism (SSCP) revealed features which argued against the possibility of carry-over of variants from plasma-derived HCV RNA or from

virus nonspecifically attached to the cell surface [5–9, 13, 15, 42, 48, 49]. Furthermore, HCV variants from lymphoid cells were genetically related, but distinct from those occurring in serum or liver. In some instances, HCV quasispecies identified in lymphoid cells were detectable in serum but not in liver tissue, indicating that the majority of circulating virus could be of extrahepatic origin. In this regard, the analysis of the HVR1 from liver, PBMC, and serum showed that certain virus variants occurring in serum resided only in PBMC but not in the liver [42, 44]. Moreover, HCV quasispecies from one cell type, for example, CD8+ T lymphocytes, were statistically more genetically like one another when compared to variants from other immune cell subsets, such as CD4+ T cells. In one pertinent study, HCV RNA sequences carried by CD8+ T lymphocytes were phylogenetically clustered close to one another, but not to those detected in CD4+ T cells or CD19+ B cells [48]. It has been found that certain sequence polymorphisms within the IRES of the 5′-UTR of the HCV genomes originating from lymphoid cells coincided with a different IRES translational activity which could promote HCV replication in cells carrying those variations [36, 50]. Similarly, it was demonstrated using liver-derived hepatoma Huh7 cells that HCV IRES variants originating from plasma displayed a significantly higher translational activity than those from HCV residing in B cells [51]. On the other hand, the IRES variants of virus replicating in B cells displayed a similarly low translational efficiency in Raji and Daudi B-cell lines as well as in hepatoma Huh7 cells, suggesting not only their extrahepatic origin but also a low capacity to replicate in B cells [51].

As already alluded to, the studies on HCV compartmentalization in the immune system demonstrated the existence of replicating virus in all the main subsets of circulating lymphomononuclear cells, including B cells, T lymphocytes, and monocytes [1, 13, 15, 42, 52]. There is also evidence for HCV replication in other immune cell types, such as dendritic cells (DCs) [53, 54]. In one of our studies, the virus load and the level of HCV replication were quantified in total PBMC as well as in affinity-purified cell subsets from these PBMCs, including CD4+ and CD8+ T lymphocytes, B cells, and monocytes from patients with CHC or OCI [15]. This investigation showed significant differences in the level of immune cell subset infection between patients with CHC and OCI with overall greater HCV loads in immune cells in CHC compared to those with OCI. In addition, monocytes carried the greatest HCV amounts in CHC, while B cells tended to contain the highest virus loads, and monocytes were the least frequently infected in OCI. Interestingly, while the total PBMCs were HCV RNA nonreactive in some individuals, the immune cell subsets isolated from these PBMCs clearly displayed virus RNA and its replicative strand, suggesting preferential or exclusive infection of the particular immune cell subset. This also indicated that the testing of total PBMC may not always identify residing virus and, thus, the analysis of individual immune cell types should be considered. In this study, HCV replication in immune cells was ascertained by the detection of (1) HCV RNA replicative (negative) strand, (2) HCV nonstructural 5a protein (NS5A), and (3) HCV variants distinct from those found in plasma of the same patients. In addition, immune cells were exposed *ex vivo* to a T-cell-stimulating mitogen in the presence of human recombinant interleukin-2 (IL-2) to augment HCV replication. We previously showed that such a treatment increases HCV replication in immune cells and improves detection of virus [15, 55]. Overall,

the study identified the main immune cell types involved in HCV infection in CHC and OCI and demonstrated that the immune system supports HCV replication regardless of the clinical appearance of infection.

It should be mentioned that the identification of HCV in the lymphatic system is not limited only to PBMC. HCV genomes and its proteins were also demonstrated in lymph nodes and bone marrow [47, 56]. In regard to lymph nodes, replicating HCV genomes and virus core and NS3 proteins were detected within biopsied B-cell-rich lymphoid follicles from patients with CHC [47]. In one study, not only did B cells appear to be the primary site of HCV infection in this secondary lymphoid tissue, but clonal sequencing analyses also indicated that in certain patients, HCV residing in lymph node-derived B cells could contribute up to 40% of the total level of viremia [47]. Furthermore, it is of note that HCV RNA sequences found in cerebro-spinal fluid of patients co-infected with human immunodeficiency virus type 1(HIV-1) were identified to be more similar to virus sequences in PBMC and lymph nodes than to those in plasma. This raised a possibility that cells of the monocyte/macrophage lineage may carry HCV into the brain, and that resident microglial cells maintain its replication independently of the liver [56, 57]. In addition, HCV RNA-positive and -negative strands, as well as HCV structural and nonstructural proteins, were readily detectable in CD34+ hematopoietic progenitor cells in the bone marrow of patients with CHC [58], reinforcing the notion of extrahepatic HCV replication. However, there was no evidence of primary CD34+ cells from healthy individuals supporting *de novo* HCV infection. The same study showed that CD34+ cells from CHC patients could release HCV RNA into culture supernatant, linking the development of CD34+ cells to their susceptibility to HCV.

4. Replication of HCV in primary immune cells and cultured immune cell lines

Direct support for the inherent propensity of HCV to enter and propagate in cells of the immune system stems from *in vitro* studies where HCV-susceptible stable human lymphocytic cell lines, normal human PBMC, or primary immune cell subsets isolated from PBMC were exposed to HCV. In this regard, it is important to note that only authentic, patient-derived virus, but not laboratory-constructed recombinant HCV clones, including HCV JFH-1, infect either primary immune cells or susceptible immune cell lines [59, 60]. Hence, it was reported that HCV carried in the serum or plasma of HCV-positive patients was infectious to Raji and Daudi B-cell lines [18, 61] and to T-cell lines, such as Molt-4 [62–64], HPB [65] and Jurkat (all derived from patients with acute T-lymphoblastic leukemia) [63, 64], and pre-stimulated PM1 (originated from acute cutaneous T-cell lymphoma) [63, 64]. It was also shown that HCV released from SB, a B-cell line established from the splenocytes of an HCV-positive patient with type II MC and monocytoid lymphoma [66], was infectious to human primary CD4+ T cells [67], and Molt-4 and Jurkat T cells [68]. Others have demonstrated the ability of primary lymphoid cells from healthy individuals, including total PBMC [11, 43, 52], T lymphocytes [6, 15, 35], B cells [8, 15, 51, 69, 70], and monocytes/macrophages [15, 71] to support HCV infection.

The studies from our laboratory showed that authentic HCV of different genotypes can infect total T cells enriched in culture from PBMC of healthy donors by their intermittent stimulation with phytohemagglutinin (PHA) in the presence of human recombinant IL-2 [6, 34, 35, 63, 64]. Replication and secretion of infectious HCV virions in this system were ascertained by the detection of (1) HCV RNA replicative strand and NS5a and/or core protein in infected cells [6, 34, 35, 63], (2) the emergence of HCV variants not existing in inocula used to infect the cells [6, 34], (3) the release of HCV RNA-reactive particles with buoyant density and ultra-structural properties of virions [34, 35], (4) virions released by infected cells via IEM [6, 34], and (5) the serial passage of HCV produced by the *de novo*-infected cells in virus-naïve primary T cells [35]. Moreover, the *de novo* HCV infection was inhibited by IFN-α- [35] and HCV-specific protease inhibitor Telaprevir [34, 63]. Furthermore, the system was adapted to include readily available Molt-4 and Jurkat T cells as infection targets, making it independent from freshly isolated human PBMC [63, 64]. In this model, the infection of essentially intact cells with unmodified, naturally occurring HCV allowed for the determination of (1) infectivity of low levels of HCV persisting in the course of OCI [6], (2) CD5 as the T-cell-specific receptor that mediates infection of the cells with patient-derived HCV [63], (3) differential expression of candidate HCV receptors in human T cells prone or resistant to infection with authentic HCV [64], and (4) an impact of infection with HCV on the suppression of CD4$^+$ T lymphocyte proliferation [72]. In the most recent study [34], the same infection system was also applied to recognize quantitative differences between CD4$^+$ and CD8$^+$ T-cell subsets in the level of HCV replication and to define properties of the virus produced by these cells. These investigations showed that although HCV replicates in both cell subtypes, the level of HCV replication in CD4$^+$ T cells was significantly higher than that in CD8$^+$ T cells. Intracellular HCV NS5a and core proteins were displayed at a similar frequency in both subtypes, that is, in 0.9 and 1.2% of CD4$^+$ and CD8$^+$ cells, respectively. Double staining for HCV NS5a protein and CD4 or CD8 T-cell differentiation markers provided conclusive evidence that virus replicated in both cell types. In addition, virus produced by CD4$^+$ and CD8$^+$ cells displayed different biophysical properties than those characterizing viral particles occurring in plasma used to infect the cells, confirming that new virus was produced, and the same virus was infectious to naïve CD4$^+$ of CD8$^+$ T cells isolated from a healthy donor. Remarkably, the data obtained from the *in vitro* infection of CD4$^+$ and CD8$^+$ T cells were comparable to those characterizing the infection of primary CD4$^+$ and CD8$^+$ T cells isolated from HCV-infected patients [15], although the level of HCV replication tended to be higher in *in vitro* than in *in vivo* conditions. Since HCV-specific T-cell effector activity is considered to be a principal factor underlying the pathogenesis of CHC as well as the resolution of hepatitis [73, 74], HCV infection of the T cells may have a direct impact on the virus-specific immune T-cell-depended responses and, in consequence, advance both virus infection and disease process.

5. Molecules mediating entry leading to HCV replication in immune cells

The HCV envelope is composed by two glycoproteins, E1 and E2. These proteins are primarily responsible for the virion attachment to the cell surface molecules serving as receptors and for the subsequent steps of viral entry [75]. The ability of HCV to infect human cells has

been interpreted almost exclusively in the context of the interactions between HCV JFH-1 strain, related strains, or pseudoparticles and hepatocyte-like Huh7 cells or their subclone Huh7.5. Based on these studies, tetraspanin CD81 [76], glycosaminoglycans [77], low-density lipoprotein receptor (LDL-R) [77], scavenger receptor class B-type 1 (SR-B1) [76, 78], the tight junction protein claudin-1 (CLDN-1) [79], occludin (OCLN) [80–82], and co-factors, such as epidermal growth factor receptor (EGFR) and ephrin receptor A2 (EphA2) [83], have been proposed to contribute either directly or indirectly to HCV entry into hepatocytes. In addition, the Niemann-Pick C1-like 1 (NPC1L1) cholesterol absorption receptor and transferrin receptor 1 (TfR1) have been implicated in HCV entry [84, 85]. However, the degree to which these individual molecules participate in HCV infection of normal human hepatocytes by naturally occurring virus requires validation, particularly since the majority of these molecules are ubiquitously displayed on many cell types. On the other hand, molecules determining HCV lymphotropism remained entirely unknown until recently.

An important finding toward the recognition of virological determinants mediating HCV lymphotropism was recently provided which showed that a distinct virus subpopulation capable of encoding particular E1E2 (envelope) epitopes might be responsible for the infection of B lymphocytes [86]. Isolated HCV E1E2 glycoproteins from patient B cells were able to confer the ability to enter and replicate in B cells to a non-lymphotropic HCV JFH-1 strain, as demonstrated by the detection of viral RNA and proteins within those cells. Interestingly, the B-cell tropism coincided with a loss of the JFH-1 strain ability to infect liver cancer-derived, hepatocyte-like Huh7 cells, implying that a lymphotropic variant constituted a separate population of viral particles displaying unique E1E2 envelope specificity. These results also supported a notion that a receptor for HCV on B cells is distinct from that on hepatocytes.

The recent finding that a co-stimulatory receptor B7.2 (CD86) is involved in the infection of human memory B cells by the abovementioned HCV SB strain [66] substantiated the involvement of a hepatocyte-distinct receptor in HCV lymphotropism [87] (**Table 1**). The study showed that the virus E1E2 envelope and 5′-UTR sequences determine lymphotropism of the SB strain and that silencing of the virus sensor retinoic acid-inducible gene I (RIGI) or overexpression of micrcoRNA-122 permitted the persistence of viral replication in B cells. Furthermore, the interaction of the SB virus E2 protein with the cell B7.2 protein reduced the surface display of B7.2 on memory B cells and inhibited their function. Interestingly, it was also found that memory B cells in HCV-infected patients expressed significantly lower levels of surface B7.2 when compared to those in HCV-negative individuals, but they carried significantly higher levels of HCV RNA than naïve B cells derived from HCV-positive patients. This comprehensive study provided important data on several aspects of HCV B-cell tropism and its potential functional and pathological consequences.

By applying the HCV-human T-cell infection system established in our laboratory [35], it was uncovered that CD5, a lymphocyte-specific 67-kDa glycoprotein belonging to the scavenger receptor cysteine-rich family, is essential for the infection of human T cells by authentic, patient-derived HCV [63] (**Table 1**). This work also demonstrated that CD81 likely contributes as a co-receptor, since both anti-CD5 and anti-CD81 monoclonal antibodies inhibited HCV infection. However, only CD5-positive T cells were susceptible to infection [63]. Thus, it appears that while CD81 contributes to the broad recognition of cells by HCV, CD5 facilitates HCV tropism toward T lymphocytes. In this context, primary human hepatocytes and hepatoma cell lines

HCV	HCV genotype	Immune cell target	Receptor molecule	Receptor properties	Receptor physiological function	Expected consequences of HCV-receptor interaction
Authentic (wild-type), patient plasma-derived (Ref. [63])	1–4	T cell	CD5	67-kDa glycoprotein, cysteine-rich scavenger receptor superfamily	Co-stimulatory molecule modulating positively or negatively intracellular signaling pathways induced by the antigen-specific T and B cell receptors	Unknown
SB variant, B-cell lymphoma-derived (Ref. [87])	2b	B cell	B7.2 (CD86)	60–100-kDa glycoprotein, immunoglobulin superfamily	Co-stimulatory molecule interacting with CD28 for T-cell activation and CTLA4 for T-cell immune regulation	Reduction of B7.2 on memory B cells and inhibition of the cells function in HCV-positive patients

Table 1. Receptor molecules mediating HCV entry and replication in human lymphocytes.

were found to express trace amounts of CD5 mRNA but not protein [63, 64], clearly indicating that HCV utilizes different receptors to enter different cell targets. This was confirmed in a subsequent study that investigated the expression of hepatocyte HCV candidate receptors on human T lymphocytes prone or resistant to HCV infection with authentic virus [64]. The expression of SR-B1, occludin, CLDN-1 and -6, CD5, and CD81 was determined by real-time polymerase chain reaction (RT-PCR), and their proteins quantified by immunoblotting in T-cell lines found to be prone or resistant to HCV infection, PBMC, primary T cells and CD4^{+} and CD8^{+} T-cell subsets, and compared to hepatoma-derived, well-differentiated Huh7.5 and HepG2 cells. SR-B1 protein was found in T and hepatoma cell lines but not on PBMC or primary T lymphocytes, while CLDN-1 was detected only in HCV-resistant (when unstimulated) PM1 cell line and hepatoma cell lines, and CLDN-6 was equally expressed across all cells investigated. OCLN protein occurred in HCV-susceptible Molt-4 and Jurkat T cells and in trace amounts in primary T cells, but not in PBMC. CD5 was expressed by HCV-prone T-cell lines, primary T cells, and PBMC, but not by non-susceptible T and hepatoma cell lines, while CD81 was detected in all cell types except HepG2. Furthermore, knocking down OCLN in a virus-prone T-cell line inhibited HCV infection, while *de novo* infection downregulated both OCLN and CD81 and upregulated CD5 without modifying SR-B1 expression. Overall, while no association between SR-B1, CLDN-1, or CLDN-6 and the susceptibility to HCV was found, CD5 and CD81 expression coincided with virus lymphotropism and that of OCLN with permissiveness of T-cell lines, but seemingly not primary T cells. This study narrowed the range of candidate entry receptors utilized by HCV to infect T lymphocytes

among those already uncovered using laboratory-grown HCV and Huh7.5 cells and confirmed that authentic HCV utilizes different receptors to enter hepatocytes and lymphocytes. The use of different receptors to infect multiple cell types is not uncommon among viruses. For example, HIV-1 uses CD4 to infect T cells but predominantly CCR5 to infect macrophages [88, 89]. The measles virus utilizes SLAM to infect lymphocytes, macrophages, and DC, but also infects SLAM-negative epithelial and endothelial cells [90, 91]. Also, EBV uses CD21 to infect B cells and DC, but this virus also replicates in epithelial cells which do not transcribe CD21 [92, 93]. In summary, CD5 was the first identified cell surface receptor that governs human cell permissiveness to HCV in a cell-type-specific manner. Since CD5 is also expressed on a minor subset of human pro-B lymphocytes [94], this molecule may potentially contribute to HCV entry to these B cells. Taken together, the results from several studies imply that cell-type-specific surface molecules, rather than a combination of molecules naturally displayed on many diverse cell types, mediate HCV tropism toward lymphocytes.

6. Functional and pathological consequences of HCV lymphotropism

The replication of HCV in immune cells, even at low levels, has a potential to affect their function, proliferation kinetics, and yield pathological outcomes, similar to infections with other lymphotropic viruses. Although the data remain overall sparse, there is a meaningful progress in some aspects. In particular, the study of a lymphotropic HCV SB strain brought the recognition of various specific mechanisms by which HCV may modify immune cell proliferation and function [95]. Among others, it has been shown that the transient infection of primary CD4^{+} T cells and selected T-cell lines with the SB strain distorted the IFN-γ/STAT-1/T-bet signaling leading to the inhibition of IFN-γ production [67, 96]. It was also reported that infection with this strain suppressed the proliferation of primary CD4^{+} T cells and their differentiation toward the Th1 lineage, as well as inhibited Molt-4 T-cell proliferation while enhancing their CD95 (Fas)-mediated apoptosis [68]. A study from our group showed that naturally occurring, patient-derived HCV inhibited the proliferation of primary CD4^{+}, but not CD8^{+} T cells, without augmenting cell death [72]. Interestingly, the results also suggested that just an exposure to authentic HCV in the absence of molecularly evident viral replication might be sufficient to inhibit CD4^{+} T-cell proliferation. It has also been shown that HCV core protein is capable of the transcriptional activation of the IL-2 promoter in T cells [97] and could modulate T-cell responses by inducing spontaneous and T-cell receptor (TCR)-mediated oscillations of calcium ions [98]. Others demonstrated that HCV core protein upregulated the expression of anergy-related genes in Jurkat T cells stably expressing this protein, and this coincided with the activation of nuclear factor of activated T cells (NFAT) and suppression of the IL-2 promoter [99]. In addition, it was reported that the direct binding of HCV core protein to complement receptor, gC1qR, on CD4^{+} and CD8^{+} T cells upregulated the expression of programmed death-1 (PD-1). This was accompanied by the dysregulation of T-cell activation, proliferation, and apoptosis [100]. These alterations were restored by blocking the engagement of the PD-1 and programmed death ligand-1 (PDL-1) pathway.

Interesting findings have been recently reported regarding the effect of exposure of primary human T cells to authentic, plasma-occurring HCV and to virus E2 protein or E2 encoding RNA on the TCR-signaling pathways [101]. It is of note that TCR signaling is critical for the normal functioning of CD4^{+} and CD8^{+} T cells, including their differentiation, activation, proliferation, and effector functions. The study showed that HCV interferes with TCR signaling and impairs T-cell activation *via* two distinct mechanisms. The first included intracellular processing of virus E2 coding RNA into a shorter, 51 nucleotide-long RNA fragment, predicted to be a dicer substrate. This virus-derived sequence targets a regulatory phosphatase involved in Src kinase signaling (abbreviated as PTPRE), subsequently inhibiting TCR signaling. In the second mechanism, the lymphocyte-specific Src kinase (Lck) phosphorylated HCV E2 protein, resulting in the inhibition of nuclear transportation of activated NFAT and in turn reducing TCR activation. It was concluded that HCV particles deliver viral RNA and E2 protein to T cells and that the highly conserved motifs of both RNA and protein inhibit TCR signaling, contributing to T-cell dysfunction and virus persistence. A second study from the same group showed that PTPRE levels are significantly reduced in liver tissue and PBMC of HCV-infected patients compared to those of uninfected controls [102]. It was demonstrated that a deficiency in PTPRE expression impaired antigen-specific TCR signaling, while antiviral therapy rescued the enzyme expression in PBMC and restored antigen-specific TCR signaling. Overall, the data indicated that HCV infection of T cells hinders TCR signaling and that short, regulatory HCV RNA sequences intracellularly derived from HCV genomic RNA are crucial in this process. The data from our studies showing that the authentic, patient-derived HCV can productively infect CD4^{+} and CD8^{+} T cells both *in vivo* [15] and *in vitro* [34] provide an indispensable link between HCV infection and the findings reported in the above studies.

The propensity of HCV to infect B cells is consistent with a significantly greater prevalence of certain B-cell proliferative disorders, particularly B-cell NHL, in HCV-infected individuals. Several lines of evidence support a link between HCV infection and B-cell NHL. These include (1) a strong epidemiological association of B-cell NHL with persistent HCV infection [25], (2) clinical data demonstrating that successful anti-HCV therapy often results in the remission of B-cell NHL [27, 28], (3) experimental data showing that transgenic mice expressing the full-length HCV genome specifically in B cells spontaneously develop B-cell NHL [103], and (4) both *in vivo* and *in vitro* data indicating that B lymphocytes are susceptible to infection and capable of supporting HCV replication [15, 18].

The mechanisms of lymphomagenesis associated with HCV infection were investigated by several groups, and a few concepts have been proposed (reviewed in [17]). However, it should be taken into consideration the fact that MC frequently precedes the development of B-cell NHL in HCV-infected individuals, indicating that MC might be a transitional step in the progression to lymphoma [104]. The proposed mechanisms of the pathogenesis of HCV-associated of lymphoma (i.e., lymphomagenesis) can be divided into two main categories. One includes the protracted stimulation of B cells by HCV antigens leading to pathologically augmented proliferation of the cells; a process that may involve different intracellular mechanisms. Another category relates to direct HCV infection and replication within B cells causing alterations in B-cell receptor (BCR) signaling or mutagenic changes in the cellular DNA

leading to oncogenic transformation of the infected B cells. It should be noted that BCR signaling is essential for the development and activation of normal B cells and is recognized as a critical pathway in the pathogenesis of several B-cell malignancies [105]. The expected role of HCV antigens in the stimulation of B cells is exemplified by the findings from the *in vitro* studies demonstrating that the engagement of CD81 on B cells by HCV E2 protein, which binds with a high affinity to the large extracellular loop of CD81, resulted in cell activation without BCR involvement [106]. This study also reported that the majority of HCV-infected patients display circulating B cells with an activated phenotype that disappears following treatment with HCV antivirals. Another relevant study showed that the exposure of CD14+ cells to recombinant HCV core protein augmented the production of IL-6 through Toll-like receptor-2 pathway which coincided with an increased B-cell proliferation [107]. With regard to the second category of the mechanisms, a recent study confirmed the expression of HCV proteins in B cells from infected patients as well as in isolated B cells exposed *in vitro* to authentic patient-derived HCV and established that these cells have upregulated BCR signaling [18]. The study demonstrated a hierarchy of molecular events in which the overexpression of HCV nonstructural protein NS3/4a interferes with checkpoint kinase 2 (CHK2), which in turn phosphorylates Hu-antigen R (HuR) that regulates its target mRNAs that encode proteins of stress response, cell proliferation, and tumorigenesis [108]. The BCR-signaling pathway was found to be the top-ranked pathway showing an increased association with activated HuR and being upregulated by NS3/4a overexpression. This study revealed an important biological role of NS3/4a in the regulation of BCR signaling and advanced understanding of the molecular processes underlying HCV-associated B-cell proliferation. In a study from another group, it has also demonstrated that HCV NS3/4a protein also interacts with ATM (ataxia telangiectasia mutated) and inhibits DNA repair in non-lymphoid cells [109]. It is known that the prior mentioned CHK2 is the key downstream molecule of ATM and is a key sensor of DNA damage. Therefore, it is possible that the augmented BCR signaling and enhanced mutagenesis may cooperate in the induction of pro-oncogenic changes in HCV-infected B cells. In addition to the studies acknowledged earlier, the mechanisms of HCV infection-associated lymphoproliferation and lymphomagenesis have been, and continue to be, the subjects of several other studies (reviewed in [17, 110, 111]).

7. Conclusions

The authenticity of HCV lymphotropism is now evident, and sizable progress was made in deciphering this event. The accumulated experimental, clinical, and, to some degree, epidemiological data indicate that HCV not only propagates in the liver but also within the immune system, where the virus can modify the proliferation and function of affected cells and induce lymphoproliferative disease. It also became apparent that immune cells constitute a site of long-term persistence of HCV, where virus hides from immune surveillance and elimination, similar to other lymphotropic viruses. Accumulated evidence indicates that HCV replication in immune cells is a constant feature of both symptomatic and occult infections, although the degree to which individual immune cell types support infection significantly varies between patients. Despite recent progress, there remains a substantial void in the data

on several fundamental aspects of HCV lymphotropism and the biological effects of lymphotropic variants. In addition, the range of immunological and pathological implications of HCV lymphotropism, in particular, the contribution to aiding virus persistence and protraction of liver disease, and the mechanisms of virus-triggered lymphoproliferation, required further studies. The progress achieved to date provides strong justification for the need to intensify research in this field.

Acknowledgements

Conflict of interest

Nothing to declare.

Author details

Tomasz I. Michalak

Address all correspondence to: timich@mun.ca

Molecular Virology and Hepatology Research Group, Division of BioMedical Science, Faculty of Medicine, Health Sciences Center, Memorial University, St. John's, NL, Canada

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