

## Review Article

# Cytokines and HCV-Related Disorders

**Poupak Fallahi,<sup>1</sup> Clodoveo Ferri,<sup>2</sup> Silvia Martina Ferrari,<sup>1</sup> Alda Corrado,<sup>1</sup>  
Domenico Sansonno,<sup>3</sup> and Alessandro Antonelli<sup>1</sup>**

<sup>1</sup> Department of Internal Medicine, School of Medicine, University of Pisa, Via Roma, 67, 56100 Pisa, Italy

<sup>2</sup> Department of Internal Medicine, Rheumatology Unit, School of Medicine, University of Modena and Reggio Emilia, Via del Pozzo, 71, 41100 Modena, Italy

<sup>3</sup> Department of Internal Medicine and Clinical Oncology, University of Bari, Piazza Giulio Cesare, 11, 70124 Bari, Italy

Correspondence should be addressed to Alessandro Antonelli, [alessandro.antonelli@med.unipi.it](mailto:alessandro.antonelli@med.unipi.it)

Received 5 January 2012; Accepted 17 February 2012

Academic Editor: Jürg Schifferli

Copyright © 2012 Poupak Fallahi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Cytokines are intercellular mediators involved in viral control and liver damage being induced by infection with hepatitis C virus (HCV). The complex cytokine network operating during initial infection allows a coordinated, effective development of both innate and adaptive immune responses. However, HCV interferes with cytokines at various levels and escapes immune response by inducing a T-helper (Th)2/T cytotoxic 2 cytokine profile. Inability to control infection leads to the recruitment of inflammatory infiltrates into the liver parenchyma by interferon (IFN)-gamma-inducible CXC chemokine ligand (CXCL)-9, -10, and -11 chemokines, which results in sustained liver damage and eventually in liver cirrhosis. The most important systemic HCV-related extrahepatic diseases—mixed cryoglobulinemia, lymphoproliferative disorders, thyroid autoimmune disorders, and type 2 diabetes—are associated with a complex dysregulation of the cytokine/chemokine network, involving proinflammatory and Th1 chemokines. The therapeutic administration of cytokines such as IFN-alpha may result in viral clearance during persistent infection and reverts this process.

## 1. Introduction

Cytokines are small soluble proteins secreted by immune system cells and other cells and are part of an intercellular communication system responsible for immune response [1]. These proteins play their role by binding specific cell receptors that either induce or inhibit cytokine-regulated genes. During viral infection, various cytokines play a role both in viral clearance and tissue damage [1].

## 2. Cytokines

Over 100 different cytokines have been reported, which are classified according to their primary role. In relation to their functions, cytokines can be classified in subgroups: (a) proinflammatory cytokines (interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-alpha); (b) T-helper (Th)1 cytokines, which are produced by Th1-activated lymphocytes (interferon (IFN)-gamma, IL-12, IL-18); (c) Th2-type cytokine which plays a role in the inhibition of cytokines derived from

Th1 cell which turns out to downregulate the function of Th1 immune responses, inhibiting antigen-presenting capacity of macrophage and promoting B-cell proliferation and therefore antibody production (IL-10, IL-4, IL-5, IL-13); (d) Th17 cytokines which are important for the differentiation of Th17 lymphocytes. IL-23, together with IL-6 and transforming growth factor (TGF)-beta, leads to the differentiation of Th0 to Th17 cells which carry out the function of secreting IL-17A, IL-17F, TNF-alpha, and IL-1 thus leading to proinflammatory reaction [1].

## 3. Chemokines

Chemokines are a large multifunctional family of cytokines (chemotactic cytokines) that induce the migration of cells to sites of infection or injury. Functionally chemokines fall into two main categories: homeostatic or proinflammatory. Homeostatic chemokines are produced constitutively; these are generally involved in lymphocyte trafficking, immune surveillance, and localization of lymphocytes with antigen

in the lymphatic system [2]. Other chemokines are only produced by cells during infection or following a proinflammatory stimulus and prompt the migration of leukocytes to an injured or infected site. Such inflammatory chemokines can also activate cells to raise an immune response.

Chemokines are structurally related, because most of them contain four invariant cysteine residues. Depending on the arrangement of the first two of these cysteines, chemokines are divided into four subfamilies: CXC (alpha), CC (beta), C (gamma), and CX3C (delta) [3]. Chemokines are produced as propeptides and are cleaved during secretion to produce an active mature protein [4] that functions by activating G-protein-coupled receptors. The receptors for these chemokines have been termed accordingly as CXCR, CCR, CR, and CX3CR [5].

#### 4. Hepatitis C Virus (HCV) and Immune Response

HCV is a hepatotropic, noncytopathic virus of the family Flaviviridae, which induces both acute and chronic necro-inflammatory liver disease [6, 7]. HCV escapes immune control in 60–85% of cases. When infecting the liver parenchyma, HCV continuously releases viral particles into the blood stream. The first line of defense that HCV will encounter includes natural killer (NK) cells and natural killer T (NKT) cells [8]. These cells are activated by type 1 IFN (alpha and beta) released by infected liver cells. NK and NKT cells constitute a relevant source of IFN-gamma and TNF-alpha [9]. These cytokines inhibit viral replication without destroying liver cells. NK cells are activated by IL-12 released from dendritic cells (DCs) and thus become empowered to eliminate infected cells [10]. NK cells may also induce partial or total DCs maturation [11].

DCs can process viral antigens and present them to specific immune system cells via class I and class II major histocompatibility complex (MHC) molecules. DCs capture viral particles through Toll-like receptors (TLRs). Upon activation, DCs secrete several types of cytokines (IL-12, TNF-alpha, IFN-alpha, IL-10) that will regulate and polarize the response of adjacent cells [12]. Mature DCs leave the liver after viral epitope collection and head for lymph nodes, where they will activate T cells in the specific immune system [13].

Cytokines released in the liver parenchyma induce chemokine release by liver cells, including IFN-gamma-inducible protein (IP-10/CXCL10), IFN-gamma-induced monokine (MIG/CXCL9), IFN-inducible T-cell alpha chemoattractant (I-TAC/CXCL11), macrophage inflammatory protein (MIP)-1alpha (MIP/CCL3), and MIP-1beta/CCL4, which recruit [14] specific cells capable of infection control.

Mature DCs and immature T cells, both of which express chemokine receptor CCR7, are recruited towards lymph nodes by secondary lymphoid-tissue cytokine (SLC/CCL21) [13]. In the lymph node, T cells expressing T-cell receptors (TCRs) appropriate for the recognition of epitopes presented by DCs in their MHC molecules are activated. The interaction between the TCR and MHC-viral epitope complex results in specific T-cell activation. Certain specific CD8

T cells, cytotoxic T lymphocytes (CTLs), become cytolytic, secrete type 1 cytokines, and travel to the infected liver [15–17]. Specific CD4+ T cells will regulate the adaptive response by secreting Th1 cytokines (IL-2, IFN-gamma, TNF-alpha) to facilitate a cell-mediated immune response and Th2 cytokines (IL-4, IL-10, IL-13) to regulate the humoral immunity [18]. It is widely accepted that adaptive immune response plays a key role in the control of HCV infection.

#### 5. Cytokines and HCV Chronic Infection

HCV manages to escape immune response. To this end they interfere with various immune mechanisms including cytokine activity modulation.

*5.1. Innate Immunity.* A primary cell defense mechanism during initial infection is the synthesis of antiviral type 1 IFN-alpha/beta [19]. On binding its receptor, IFN-alpha/beta activates a number of intracellular mechanisms that can prevent viral replication and spread to other liver cells. HCV is a good inducer of IFN-alpha/beta expression [20]. However, HCV seems to be, at least in part, unresponsive to IFN-alpha/beta effects and may effectively replicate in the liver despite such gene induction. HCV can block type 1 IFN induction; this possibly results from the fact that nonstructural proteins (NS 3 and NS5A), and structural protein E2 may both potentially block the expression and transcription of IFN-alpha/beta-induced genes. HCV NS5A protein induces IL-8 expression, which is associated with IFN-alpha inhibition [21].

The outcome of a viral infection depends on the interplay between the host capacity to trigger potent antiviral responses and viral mechanisms that counteract them. Although Toll-like receptor (TLR)-3, which recognizes virally derived double-stranded (ds) RNA, transmits downstream antiviral signaling through the TIR adaptor Trif (TICAM-1), viral RNA-sensing RIG-like helicases (RLHs) use the mitochondrial-bound CARD protein Cardif (IPS-1/MAVS/ISA). The importance of these two antiviral signaling pathways is reflected by the fact that both adaptors are inhibited through specific cleavage triggered by the HCV serine protease NS3-4A [22, 23].

NK cells and NKT cells exert their antiviral action through direct, non-MHC-restricted cytotoxic mechanisms and IFN-gamma production [24]. In addition, they allow maturation for DCs favoring the development of Th1/Tcytotoxic (Tc)1 responses [10]. However, they do not seem to play a significant role in acute HCV infection [25]. It has been suggested that HCV can block NK cells and NKT cells functions thus preventing antiviral cytokines such as IFN-gamma from being produced, via an interaction between HCV E2 protein and NK-cell CD81 molecule [26].

During chronic infection with HCV, a decrease in IFN-alpha production by plasmacytoid DCs has been reported [27], such as a decrease in IL-12 production by myeloid DCs [28]. In fact, HCV structural proteins can interact with TLR-2 in monocytes and induce IL-10 production, which inhibits IL-12 and IFN-alpha production in DCs [29]. However, other studies reported an increased IFN-alpha production,

especially in patients who fail to respond to exogenous IFN-alpha, in whom IFN-stimulated genes (ISGs) are highly activated [30].

It has been also suggested that DCs cytokine profile cannot polarize T-cell responses towards a Th1/Tc1 response [31] and contributes to inadequate NK cells and NKT cells activation. However, other studies have shown that a progressive liver injury in chronic hepatitis C infection correlates with increased intrahepatic expression of Th1-associated cytokines [32, 33].

**5.2. Adaptive Response.** HCV CD4<sup>+</sup>-T cells play a key role in adaptive response in that they provide help in activating cytotoxic and humoral responses. They can secrete Th1-cytokines including IFN-gamma, which favors neutrophil and macrophage recruitment and leads to inflammatory response. They also may release Th2 cytokines such as IL-4 and IL-10, which limit Th1 cytokine-mediated response and favor the development of humoral response [34]. A multispecific, strong, sustained, CD4<sup>+</sup>-T-cell-specific Th1 response may be seen in infections with HCV infection evolving to resolution [35]. However, when infection becomes chronic, a weak CD4-T-specific response with few specificities and scarce type 1 cytokine production is observed [36].

CD8<sup>+</sup> CTLs can clear viruses using apoptosis-related cytolytic mechanisms and mechanisms mediated by type 1 cytokines (IFN-gamma, TNF-alpha). In chronic infection with hepatitis B virus or HCV, specific CTLs are few and engage few specific targets; they also display anergic characteristics with reduced type 1 cytokine secretion [37]. Another potential mechanism of blocked type 1 cytokine production results from regulatory T-cell activity. These cells can release IL-10 and TGF-beta and inhibit proliferation and cytokine synthesis in T cells, either directly or through other cytokines, in hepatitis C [38].

Cytokines produced by T cells play a role in the regulation of humoral responses; nevertheless, these responses cannot control chronic viral hepatitis, even though they play a role in the pathogenesis of extrahepatic manifestations [18].

## 6. Cytokines and Liver Damage

When specific immune response fails to control viral replication, the infected liver cells secrete IFN-gamma-induced chemokines such as CXC chemokine ligand CXCL9, CXCL10, and CXCL11, which result in the migration of nonspecific mononuclear cells into the liver [39], which are unable to control infection but result in sustained liver damage [40]. Inhibition of these chemokines limits nonspecific cell migration and hence reduces the inflammation [41]. The recruitment of persistent mononuclear infiltrates leads to the development of chronic inflammation, which results in sustained liver damage. Finally, chronic inflammation induces regenerating mechanisms in the liver parenchyma. Several factors influence this process, including cytokines such as IL-6, TNF-alpha, TGF-beta, hepatocyte growth factor, and epidermal growth factor. These and other factors activate transcription factors such as nuclear factor- $\kappa$ B, signal transducer, and activator of transcription 3 which initiate the

gene expression cascade leading to hepatocyte proliferation [42].

Persistent inflammation also activates hepatic stellate cells, myofibroblasts, and fibroblasts, which favors the development of liver fibrosis. The activation of these cells is regulated by pro-inflammatory cytokines such as TGF-beta, IL-6, TNF-alpha, CCL21, and platelet-derived growth factor, among other stimuli [43].

## 7. HCV-Related Extrahepatic Diseases (HCV-EHDs)

HCV is known to be responsible for both hepatic and HCV-EHDs. The most important systemic HCV-EHDs are HCV-related mixed cryoglobulinemia (MC) (MC+HCV) and lymphoproliferative disorders, while the most frequent and clinically important endocrine HCV-EHDs are autoimmune thyroid disorders (AITDs).

## 8. Cytokines, Cryoglobulinemia, and Lymphoproliferation

MC is a distinct syndrome clinically characterized by purpura, weakness, arthralgia, and involvement of one or more organ systems, including membranoproliferative glomerulonephritis, peripheral neuropathy, skin ulcers, liver damage, and diffuse vasculitis. Cryoprecipitable immunocomplexes, namely mixed (IgG-IgM) cryoglobulins, are the serological hallmark of the disease: IgG is the autoantigen and IgM, with rheumatoid factor (RF) activity, the autoantibody. MC is classified in type 2 and type 3 according to the presence of polyclonal or oligo-monoclonal IgMs. Because expansion of RF-producing B cells is the underlying disorder of MC, this condition is considered a "benign" B-cell lymphoproliferative disease [44, 45].

The mechanism(s) responsible for the lymphoproliferation surrounding MC remain unknown. Due to geographical heterogeneity in prevalence of MC+HCV, it is conceivable that unknown genetic and/or environmental factors may influence the development of this syndrome [46]. Several data are consistent with the possibility that chronic stimulation of B cells by viral epitopes could play an important role [47–49].

A wide body of evidence, in addition, strongly suggests that a key factor in the pathogenesis of MC+HCV is represented by the inhibition of the apoptosis of B cells, leading to their progressive accumulation. First, this is suggested by the histopathological characteristics of liver and/or bone marrow lymphocyte infiltrates in MC patients [50], as well as by the high prevalence of *bcl-2* rearrangement (t(14;18) translocation) in patients with MC, with regression of translocated B-cell clones after successful antiviral therapy [45, 51, 52].

Furthermore, B-lymphocyte stimulator (BLyS) serum levels are significantly correlated with B-cell proliferation during chronic HCV infection. These results strongly suggest a role for BLyS in the induction and expression of HCV- B-cell proliferation [53–55]. Chemokine CXCL13, also known

as BCA-1 (B cell-attracting chemokine-1) or BLC (B-lymphocyte chemoattractant), is a major regulator of B-cell trafficking. HCV infection may be associated with B-cell dysfunction and lymphoproliferative disorders, including MC+HCV. The results by Sansonno et al. [56] indicate that upregulation of CXCL13 gene expression is a distinctive feature of HCV-infected patients. Higher levels of this chemokine in the liver as well as in the skin of patients with active MC+HCV vasculitis suggest a possible interrelation between these biologic compartments.

Recently, Saadoun et al. [57] studied the local immune response in the liver, which is considered the principal site for immune reactions involved in MC pathogenesis. In that study, the cytokine profile of liver-infiltrating T lymphocytes from MC+HCV patients and without MC (of type 2) were compared. They showed that, although no differences were found in the proportion of CD4+, CD8+ liver T cells, the ability of freshly isolated liver T cells to produce type 1 cytokines in response to stimulation with phorbol myristate acetate and ionomycin for 6 hours was significantly higher in MC+HCV patients than in HCV-infected controls without MC, whereas production of type 2 cytokines by these cells was similar (IL-4) or reduced (IL-10).

This agrees with previous data obtained in peripheral blood mononuclear cells [58], ruling out the possibility of a discrepancy between the response of peripheral and liver T cells. Interestingly, in both studies by Saadoun et al. and Loffreda et al. [57, 58], a reduced expression of IL-10 (a strong inhibitor of IFN-gamma production) is demonstrated regardless of the different sources. These observations suggest that the evolution of HCV infection toward MC is characterized by a strong Th1 response.

Several studies have shown an increased expression of IFN-gamma [59] and IFN-gamma-inducible chemokines [60], in particular CXCL10, in hepatocytes and in lymphocytes of HCV-infected patients [61, 62], directly related with the degree of inflammation and with an increase of circulating levels of IFN-gamma and CXCL10 [14, 63–66].

Furthermore, it has been shown that NS5A and core proteins, alone or by the synergistic effect of cytokines, such as IFN-gamma and TNF-alpha, are capable of upregulating CXCL10 and CXCL9 gene expression and secretion in cultured human hepatocyte-derived cells [67], suggesting that CXCL10 produced by HCV-infected hepatocytes could play a key role regulating T-cell trafficking into a Th1-type inflammatory site as the liver tissue during chronic HCV infection, by recruiting Th1 lymphocytes that secrete IFN-gamma and TNF-alpha, which induce CXCL10 secretion by hepatocytes, thus perpetuating the immune cascade [68].

Furthermore, we have recently shown that circulating CXCL10, CXCL11, IFN-gamma-inducible (Th1) chemokines are higher in patients with MC+HCV than in chronic hepatitis C (CHC) patients. Moreover, our studies demonstrate markedly high serum levels of CXCL10 and CXCL11 in patients with MC+HCV compared to healthy controls in particular in the presence of active vasculitis. A strong relationship between circulating IFN-gamma and CXCL11 was shown, strongly supporting the role of a Th1 immune response in the pathogenesis of MC+HCV patients [69–74].

For comparison the prototype Th2 chemokine (C-C motif) ligand 2 (CCL2) was not significantly different in patients with MC+HCV and active vasculitis than in MC patients, and it suggests that the Th1 CXCL10 chemokine is specifically involved in the appearance of vasculitis in these patients [74].

The pro-inflammatory cytokines IL-1beta, IL-6, and TNF-alpha have also been evaluated in MC+HCV patients. In fact, MC+HCV patients show significantly higher mean IL-1beta, IL-6, and TNF-alpha levels than the controls or the HCV patients. If the importance of IL-1beta and IL-6 in the pathogenesis of MC is confirmed, these results will open the way for the evaluation of new therapies for MC [75].

On the whole the above-mentioned data underline the importance of the activation of the Th1 immunity in the immunopathogenesis of MC+HCV, but suggest a complex dysfunction of the cytokine/chemokine network in these patients, involving also pro-inflammatory cytokines.

## 9. Cytokines and AITDs Associated with HCV and MC

The pattern of thyroid disorders observed in HCV infection is characterized by the presence of increased circulating levels of antithyroid peroxidase antibody (AbTPO) and increased risk of hypothyroidism in AbTPO positive subjects [76–80].

This pattern is similar to that observed in IFN-alpha-treated patients, too [81].

Differences in geographical distribution [82], genetic variability in the populations studied [83], and environmental cofactors, such as iodine intake or other infectious agents [84, 85], could play an important role in the development of AITD.

Recently it has been shown that high levels of CXCL10 are present in patients with autoimmune thyroiditis (AT), in particular in the presence of hypothyroidism [68], and an involvement of Th1 immune response in the induction of AT [86], Graves' disease, and Graves' ophthalmopathy [87] has been shown. Furthermore, the presence of HCV in the thyroid of chronically infected patients has been recently demonstrated [88, 89]; however, other studies are needed to further confirm this point.

On the above-mentioned bases, it has been speculated that HCV thyroid infection may act by upregulating CXCL10 gene expression and secretion in thyrocytes (as previously shown in human hepatocytes [67]) recruiting Th1 lymphocytes that secrete IFN-gamma and TNF-alpha, which induce CXCL10 secretion by thyrocytes, thus perpetuating the immune cascade, that may lead to the appearance of AITDs in genetically predisposed subjects.

This hypothesis has been recently confirmed by two studies that demonstrated high serum levels of CXCL10 in MC+HCV patients and showed that CXCL10 is significantly higher in the presence of AT compared to MC+HCV patients without thyroiditis [90, 91]. For comparison the prototype Th2 chemokine CCL2 was not significantly different in patients with MC+HCV in the presence of AT than in MC+HCV patients, and it suggests that the Th1 CXCL10

chemokine is specifically involved in the appearance of AT in these patients [91].

Among the pro-inflammatory cytokines, IL-1beta and TNF-alpha were not associated with the presence of AT in MC+HCV patients, while IL-6 was modestly but significantly increased in patients with AT [71, 92].

On the whole the above-mentioned data underline the importance of the activation of the Th1 immunity in the immunopathogenesis of AT in patients with MC+HCV.

## 10. Cytokines and Type 2 Diabetes Mellitus (T2DM) Associated with HCV and MC

Several clinical epidemiological studies since 1994 have reported that HCV infection is linked to diabetes [93]. The association between HCV infection, in patients without cirrhosis (a well-known risk factor for T2DM), and T2DM has been first studied in two of our studies, in patients with chronic HCV infection (HCV+) associated with MC (MC+HCV) [94] and in patients with HCV-related chronic liver disease [95].

There is one population study (National Health and Nutrition Examination Survey-NHANES III 1988–1994) that showed an adjusted odds ratio of 3.8 for T2DM for those who were aged >40 years and HCV+ [96] and increased incidence of T2DM [97].

There have been a few reports, too, that IFN treatment of HCV infection improves glucose tolerance [94, 98] when HCV infection is eradicated; however, another study did not confirm these results [99].

Altogether the above-mentioned data indicate that HCV chronic infection is a risk factor for developing T2DM.

### 10.1. Mechanism

**10.1.1. Insulin Resistance and Steatosis.** It is speculated that insulin resistance (as a consequence of hepatic steatosis (i.e., present in about 50% of the subjects with HCV infection) [93] and/or elevated expression of TNF-alpha (strongly correlated with the degree of liver diseases and the level of insulin resistance) [89]) may lead to the development of T2DM [93].

**10.1.2. Direct Islet Cell Destruction by HCV.** Masini et al. [100] recently demonstrated a direct cytopathic effect of HCV at the islet cell level.

**10.1.3. Possible Autoimmune Induction.** The type of diabetes manifested by patients with HCV chronic infection is not the classical T2DM. The labelling of HCV+ patients as T2DM is purely conventional and possibly inaccurate: the lines separating type 1 diabetes from latent autoimmune diabetes in adults (LADA) and from T2DM are fading away as new pathogenetic information is obtained [101].

Three studies have previously reported [94, 95, 102] that HCV+ patients T2DM were leaner than T2DM controls and showed significantly lower LDL-cholesterol and systolic and diastolic blood pressure. Furthermore, MC-HCV+ patients

with T2DM had non-organ-specific autoantibodies more frequently (34% versus 18%) than nondiabetic MC-HCV+ patients [94].

An immune-mediated mechanism for MC-HCV+ associated diabetes has been postulated [94], and a similar pathogenesis might be involved in the diabetes of HCV+ patients. This hypothesis is strengthened by the finding that autoimmune phenomena in T2DM patients are more common than previously thought [103]. Since the prevalence of classic beta-cell autoimmune markers in HCV+ patients has not been found to be increased [89], other immune phenomena might be involved [104].

On the above-mentioned bases, it could be interesting to speculate that HCV infection of beta cells [100] may act by upregulating CXCL10 gene expression and secretion (as previously shown in human hepatocytes) recruiting Th1 lymphocytes that secrete IFN-gamma and TNF-alpha, which induce CXCL10 secretion by beta cells, thus perpetuating the immune cascade that may lead to the appearance of beta cells dysfunction in genetically predisposed subjects.

This hypothesis has recently been confirmed by a study that demonstrates higher serum levels of CXCL10 in HCV+ patients with T2DM with respect to those without [64, 105].

## 11. Therapeutic Role of Cytokines in Chronic Viral Hepatitis

IFN-alpha is the only cytokine currently used in the treatment of chronic viral hepatitis. In CHC, pegylated IFN-alpha combined with ribavirin leads to sustained viral clearance in 50% of patients [106]. The most important effect of IFN-alpha is directly antiviral; however, it has also immunomodulating actions that favor Th1/Tc1 response restoration [107–109]. On the other hand, ribavirin, a wide-spectrum antiviral agent used in combination therapy for hepatitis C, has immunomodulating effects that induce type 1 cytokine production [110]. Sustained viral load reduction with antiviral agents has also been seen to facilitate specific T response recovery with type 1 cytokine production in hepatitis C [111].

An exogenous administration of Th1-inducing cytokines such as IL-12 [112] or anti-inflammatory cytokines such as IL-10 has also been attempted to reduce intrahepatic inflammation severity [113]. However, such therapies remain experimental, and their effectiveness is unclear.

From a theoretical standpoint Tc1-associated chemokine receptors may represent an interesting therapeutic target in the development of drugs for patients with chronic hepatitis unresponsive to antiviral agents, their aim being a reduction of liver inflammation and progression to fibrosis by blocking inflammatory cell migration into the liver [39, 41].

Treatment-induced and spontaneous clearance of HCV infection are affected by various host factors. Polymorphisms in the region of the gene *IL-28B* are associated with HCV clearance, implicating the gene product, IFN-lambda3, in the immune response to HCV. Although it is not clear how the *IL-28B* haplotype affects HCV clearance, IFN-lambda3 upregulates IFN-stimulated genes, similar to IFN-alpha and -beta, but via a different receptor. There is also evidence that IFN-lambda3 affects the adaptive immune response.

It is known that IL-28B may establish a robust T-cell adaptive immune response [114, 115]. This effect may explain the relationship between single-nucleotide polymorphism (SNPs) near IL-28B, adaptive response, and viral clearance [116].

The IL-28B genotype can be considered, along with other factors, in predicting patient responses to therapy with pegylated IFN-alpha and ribavirin [117, 118].

Clinical studies assessing safety and efficacy in the treatment of HCV with exogenous IFN-lambda3 are currently underway. Early results suggest that IFN-lambda3 treatment inhibits HCV replication and is associated with a limited side effect profile. However, hepatotoxicity in both healthy volunteers and HCV-infected patients has been described [119].

## 12. Conclusion

Cytokines are intercellular mediators involved in viral control and liver damage as induced by infection with HCV. The complex cytokine network operating during initial infection allows a coordinated, effective development of both innate and adaptive immune responses. However, HCV interferes with cytokines at various levels and escape immune response by inducing a Th2/Tc2 cytokine profile. Inability to control infection leads to the recruitment of inflammatory infiltrates into the liver parenchyma by IFN-gamma-inducible CXCL9, -10 and -11 chemokines, which results in sustained liver damage and eventually in liver cirrhosis; however, fibrogenesis may also follow distinct paths. The most important systemic HCV-EHDs—MC, lymphoproliferative disorders, and AITDs—are associated with a complex dysregulation of the cytokine/chemokine network, involving pro-inflammatory and Th1 chemokines. The therapeutic administration of cytokines such as IFN-alpha may result in viral clearance during persistent infection and reverts this process.

## Conflict of Interests

The authors have no conflict of interests to declare.

## References

- [1] J. W. Steinke and L. Borish, "Cytokines and chemokines," *Journal of Allergy and Clinical Immunology*, vol. 117, no. 2, supplement, pp. S441–S445, 2006.
- [2] E. J. Fernandez and E. Lolis, "Structure, function, and inhibition of chemokines," *Annual Review of Pharmacology and Toxicology*, vol. 42, pp. 469–499, 2002.
- [3] p. M. Murphy, M. Baggiolini, I. F. Charo et al., "International union of pharmacology. XXII. Nomenclature for chemokine receptors," *Pharmacological Reviews*, vol. 52, no. 1, pp. 145–176, 2000.
- [4] A. Zlotnik and O. Yoshie, "Chemokines: a new classification system and their role in immunity," *Immunity*, vol. 12, no. 2, pp. 121–127, 2000.
- [5] R. Horuk, "Chemokine receptors," *Cytokine and Growth Factor Reviews*, vol. 12, no. 4, pp. 313–335, 2001.
- [6] G. M. Lauer and B. D. Walker, "Hepatitis C virus infection," *The New England Journal of Medicine*, vol. 345, no. 1, pp. 41–52, 2001.
- [7] D. Ganem and A. M. Prince, "Hepatitis B virus infection—natural history and clinical consequences," *The New England Journal of Medicine*, vol. 350, no. 11, pp. 1118–1129, 2004.
- [8] A. I. Su, J. p. Pezacki, L. Wodicka et al., "Genomic analysis of the host response to hepatitis C virus infection," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 24, pp. 15669–15674, 2002.
- [9] L. G. Guidotti and F. V. Chisari, "Noncytolytic control of viral infections by the innate and adaptive immune response," *Annual Review of Immunology*, vol. 19, pp. 65–91, 2001.
- [10] A. Moretta, "Natural killer cells and dendritic cells: rendezvous in abused tissues," *Nature Reviews Immunology*, vol. 2, no. 12, pp. 957–964, 2002.
- [11] E. Marcenaro, M. Della Chiesa, F. Bellora et al., "IL-12 or IL-4 prime human NK cells to mediate functionally divergent interactions with dendritic cells or tumors," *Journal of Immunology*, vol. 174, no. 7, pp. 3992–3998, 2005.
- [12] J. Banachereau, F. Briere, C. Caux et al., "Immunobiology of dendritic cells," *Annual Review of Immunology*, vol. 18, pp. 767–811, 2000.
- [13] I. F. Charo and R. M. Ransohoff, "Mechanisms of disease: the many roles of chemokines and chemokine receptors in inflammation," *The New England Journal of Medicine*, vol. 354, no. 6, pp. 610–621, 2006.
- [14] p. L. Shields, C. M. Morland, M. Salmon, S. Qin, S. G. Hubscher, and D. H. Adams, "Chemokine and chemokine receptor interactions provide a mechanism for selective T cell recruitment to specific liver compartments within hepatitis C-infected liver," *Journal of Immunology*, vol. 163, no. 11, pp. 6236–6243, 1999.
- [15] M. K. Maini, C. Boni, G. S. Ogg et al., "Direct ex vivo analysis of hepatitis B virus-specific CD8<sup>+</sup> T cells associated with the control of infection," *Gastroenterology*, vol. 117, no. 6, pp. 1386–1396, 1999.
- [16] G. M. Lauer, E. Barnes, M. Lucas et al., "High resolution analysis of cellular immune responses in resolved and persistent hepatitis C virus infection," *Gastroenterology*, vol. 127, no. 3, pp. 924–936, 2004.
- [17] M. K. Maini, C. Boni, C. K. Lee et al., "The role of virus-specific CD8<sup>+</sup> cells in liver damage and viral control during persistent hepatitis B virus infection," *Journal of Experimental Medicine*, vol. 191, no. 8, pp. 1269–1280, 2000.
- [18] L. G. Guidotti and F. V. Chisari, "Immunobiology and pathogenesis of viral hepatitis," *Annual Review of Pathology*, vol. 1, pp. 23–61, 2006.
- [19] C. E. Samuel, "Antiviral actions of interferons," *Clinical Microbiology Reviews*, vol. 14, no. 4, pp. 778–809, 2001.
- [20] C. B. Bigger, B. Guerra, K. M. Brasky et al., "Intrahepatic gene expression during chronic hepatitis C virus infection in chimpanzees," *Journal of Virology*, vol. 78, no. 24, pp. 13779–13792, 2004.
- [21] S. J. Polyak, K. S. A. Khabar, D. M. Paschal et al., "Hepatitis C virus nonstructural 5A protein induces interleukin-8, leading to partial inhibition of the interferon-induced antiviral response," *Journal of Virology*, vol. 75, no. 13, pp. 6095–6106, 2001.
- [22] M. Yoneyama, M. Kikuchi, T. Natsukawa et al., "The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses," *Nature Immunology*, vol. 5, no. 7, pp. 730–737, 2004.
- [23] M. Rebsamen, E. Meylan, J. Curran, and J. Tschoopp, "The antiviral adaptor proteins Cardif and Trif are processed and inactivated by caspases," *Cell Death and Differentiation*, vol. 15, no. 11, pp. 1804–1811, 2008.

- [24] C. A. Biron, K. B. Nguyen, G. C. Pien, L. p. Cousens, and T. p. Salazar-Mather, "Natural killer cells in antiviral defense: function and regulation by innate cytokines," *Annual Review of Immunology*, vol. 17, pp. 189–220, 1999.
- [25] R. Thimme, S. Wieland, C. Steiger et al., "CD8<sup>+</sup> T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection," *Journal of Virology*, vol. 77, no. 1, pp. 68–76, 2003.
- [26] C. T. K. Tseng and G. R. Klimpel, "Binding of the hepatitis C virus envelope protein E2 to CD81 inhibits natural killer cell functions," *Journal of Experimental Medicine*, vol. 195, no. 1, pp. 43–49, 2002.
- [27] A. Ulsenheimer, J. T. Gerlach, M. C. Jung et al., "Plasmacytoid dendritic cells in acute and chronic hepatitis C virus infection," *Hepatology*, vol. 41, no. 3, pp. 643–651, 2005.
- [28] D. D. Anthony, N. L. Yonkers, A. B. Post et al., "Selective impairments in dendritic cell-associated function distinguish hepatitis C virus and HIV infection," *Journal of Immunology*, vol. 172, no. 8, pp. 4907–4916, 2004.
- [29] G. Szabo and A. Dolganiuc, "Subversion of plasmacytoid and myeloid dendritic cell functions in chronic HCV infection," *Immunobiology*, vol. 210, no. 2–4, pp. 237–247, 2005.
- [30] p. Bellecave and D. Moradpour, "A fresh look at interferon-alpha signaling and treatment outcomes in chronic hepatitis C," *Hepatology*, vol. 48, no. 4, pp. 1330–1333, 2008.
- [31] T. Kanto, M. Inoue, M. Miyazaki et al., "Impaired function of dendritic cells circulating in patients infected with hepatitis C virus who have persistently normal alanine aminotransferase levels," *Intervirology*, vol. 49, no. 1–2, pp. 58–63, 2006.
- [32] J. Napoli, G. A. Bishop, p. H. Mcguinness, D. M. Painter, and G. W. Mccaughan, "Progressive liver injury in chronic hepatitis C infection correlates with increased intrahepatic expression of Th1-associated cytokines," *Hepatology*, vol. 24, no. 4, pp. 759–765, 1996.
- [33] A. Bertoletti, M. M. D'Elis, C. Boni et al., "Different cytokine profiles of intrahepatic T cells in chronic hepatitis B and hepatitis C virus infections," *Gastroenterology*, vol. 112, no. 1, pp. 193–199, 1997.
- [34] M. Moser and K. M. Murphy, "Dendritic cell regulation of TH1-TH2 development," *Nature Immunology*, vol. 1, no. 3, pp. 199–205, 2000.
- [35] C. L. Day, G. M. Lauer, G. K. Robbins et al., "Broad specificity of virus-specific CD4<sup>+</sup> T-helper-cell responses in resolved hepatitis C virus infection," *Journal of Virology*, vol. 76, no. 24, pp. 12584–12595, 2002.
- [36] H. R. Rosen, C. Miner, A. W. Sasaki et al., "Frequencies of HCV-specific effector CD4<sup>+</sup> T cells by flow cytometry: correlation with clinical disease stages," *Hepatology*, vol. 35, no. 1, pp. 190–198, 2002.
- [37] R. Thimme, D. Oldach, K. M. Chang, C. Steiger, S. C. Ray, and F. V. Chisari, "Determinants of viral clearance and persistence during acute hepatitis C virus infection," *Journal of Experimental Medicine*, vol. 194, no. 10, pp. 1395–1406, 2001.
- [38] O. Franzese, p. T. F. Kennedy, A. J. Gehring et al., "Modulation of the CD8<sup>+</sup>-T-cell response by CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells in patients with hepatitis B virus infection," *Journal of Virology*, vol. 79, no. 6, pp. 3322–3328, 2005.
- [39] J. R. Larrubia, S. Benito-Martinez, M. Calvino, E. Sanz-de-Villalobos, and T. Parra-Cid, "Role of chemokines and their receptors in viral persistence and liver damage during chronic hepatitis C virus infection," *World Journal of Gastroenterology*, vol. 14, no. 47, pp. 7149–7159, 2008.
- [40] J. R. Larrubia, M. Calvino, S. Benito et al., "The role of CCR5/CXCR3 expressing CD8<sup>+</sup> cells in liver damage and viral control during persistent hepatitis C virus infection," *Journal of Hepatology*, vol. 47, no. 5, pp. 632–641, 2007.
- [41] K. Kakimi, T. E. Lane, S. Wieland et al., "Blocking chemokine responsive to  $\gamma$ -2/interferon (IFN)- $\gamma$  inducible protein and monokine induced by IFN- $\gamma$  activity in vivo reduces the pathogenetic but not the antiviral potential of hepatitis B virus-specific cytotoxic T lymphocytes," *Journal of Experimental Medicine*, vol. 194, no. 12, pp. 1755–1766, 2001.
- [42] R. Taub, L. E. Greenbaum, and Y. Peng, "Transcriptional regulatory signals define cytokine-dependent and—independent pathways in liver regeneration," *Seminars in Liver Disease*, vol. 19, no. 2, pp. 117–128, 1999.
- [43] G. Ramadori and B. Saile, "Inflammation, damage repair, immune cells, and liver fibrosis: specific or nonspecific, this is the question," *Gastroenterology*, vol. 127, no. 3, pp. 997–1000, 2004.
- [44] C. Ferri and A. L. Zignego, "Relation between infection and autoimmunity in mixed cryoglobulinemia," *Current Opinion in Rheumatology*, vol. 12, no. 1, pp. 53–60, 2000.
- [45] A. L. Zignego, C. Ferri, F. Giannelli et al., "Prevalence of bcl-2 rearrangement in patients with hepatitis C virus-related mixed cryoglobulinemia with or without B-cell lymphomas," *Annals of Internal Medicine*, vol. 137, no. 7, pp. 571–580, 2002.
- [46] V. S. Wong, W. Egner, T. Elsey, D. Brown, and G. J. M. Alexander, "Incidence, character and clinical relevance of mixed cryoglobulinaemia in patients with chronic hepatitis C virus infection," *Clinical and Experimental Immunology*, vol. 104, no. 1, pp. 25–31, 1996.
- [47] p. Pileri, Y. Uematsu, S. Campagnoli et al., "Binding of hepatitis C virus to CD81," *Science*, vol. 282, no. 5390, pp. 938–941, 1998.
- [48] V. De Re, S. De Vita, A. Marzotto et al., "Pre-malignant and malignant lymphoproliferations in an HCV-infected type II mixed cryoglobulinemic patient are sequential phases of an antigen-driven pathological process," *International Journal of Cancer*, vol. 87, no. 2, pp. 211–216, 2000.
- [49] D. Sansonno, G. Lauletta, L. Nisi et al., "Non-enveloped HCV core protein as constitutive antigen of cold-precipitable immune complexes in type II mixed cryoglobulinaemia," *Clinical and Experimental Immunology*, vol. 133, no. 2, pp. 275–282, 2003.
- [50] C. Ferri, S. Pileri, and AL. Zignego, "Hepatitis C virus infection and non-Hodgkin's lymphoma," in *Infectious Causes of Cancer. Targets for Intervention*, J. Goedert, Ed., pp. 349–368, The Human Press, Totowa, NJ, USA, 2000.
- [51] A. L. Zignego, F. Giannelli, M. E. Marrochi et al., "T(14;18) translocation in chronic hepatitis C virus infection," *Hepatology*, vol. 31, no. 2, pp. 474–479, 2000.
- [52] F. Giannelli, S. Moscarella, C. Giannini et al., "Effect of antiviral treatment in patients with chronic HCV infection and t(14;18) translocation," *Blood*, vol. 102, no. 4, pp. 1196–1201, 2003.
- [53] D. Sansonno, A. Carbone, V. De Re, and F. Dammacco, "Hepatitis C virus infection, cryoglobulinaemia, and beyond," *Rheumatology*, vol. 46, no. 4, pp. 572–578, 2007.
- [54] D. Sène, N. Limal, p. Ghillani-Dalbin, D. Saadoun, J. C. Piette, and p. Cacoub, "Hepatitis C virus-associated B-cell proliferation—the role of serum B lymphocyte stimulator (BlyS/BAFF)," *Rheumatology*, vol. 46, no. 1, pp. 65–69, 2007.
- [55] M. Fabris, L. Quartuccio, S. Sacco et al., "B-Lymphocyte stimulator (BlyS) up-regulation in mixed cryoglobulinaemia

- syndrome and hepatitis-C virus infection," *Rheumatology*, vol.46, no. 1, pp. 37–43, 2007.
- [56] D. Sansonno, F. A. Tucci, L. Troiani et al., "Increased serum levels of the chemokine CXCL13 and up-regulation of its gene expression are distinctive features of HCV-related cryoglobulinemia and correlate with active cutaneous vasculitis," *Blood*, vol. 112, no. 5, pp. 1620–1627, 2008.
- [57] D. Saadoun, O. Boyer, H. Trébeden-Nègre et al., "Predominance of type 1 (Th1) cytokine production in the liver of patients with HCV-associated mixed cryoglobulinemia vasculitis," *Journal of Hepatology*, vol. 41, no. 6, pp. 1031–1037, 2004.
- [58] S. Loffreda, p. Muratori, L. Muratori, L. Mele, F. B. Bianchi, and M. Lenzi, "Enhanced monocyte Th1 cytokine production in HCV-infected cryoglobulinemic patients," *Journal of Hepatology*, vol. 38, no. 2, pp. 230–236, 2003.
- [59] R. Patzwahl, V. Meire, G. Ramadori, and S. Mihm, "Enhanced expression of interferon-regulated genes in the liver of patients with chronic hepatitis C virus infection: detection by suppression-subtractive hybridization," *Journal of Virology*, vol. 75, no. 3, pp. 1332–1338, 2001.
- [60] S. Mihm, S. Schweyer, and G. Ramadori, "Expression of the chemokine IP-10 correlates with the accumulation of hepatic IFN- $\gamma$  and IL-18 mRNA in chronic hepatitis C but not in hepatitis B," *Journal of Medical Virology*, vol. 70, no. 4, pp. 562–570, 2003.
- [61] A. A. Matskevich and D. S. Strayer, "Exploiting hepatitis C virus activation of NF $\kappa$ B to deliver HCV-responsive expression of interferons alpha and gamma," *Gene Therapy*, vol. 10, no. 22, pp. 1861–1873, 2003.
- [62] M. Murata, S. Nabeshima, N. Maeda, H. Nakashima, S. Kashiwagi, and J. Hayashi, "Increased frequency of IFN- $\gamma$ -producing peripheral CD8<sup>+</sup> T cells with memory-phenotype in patients with chronic hepatitis C," *Journal of Medical Virology*, vol. 67, no. 2, pp. 162–170, 2002.
- [63] Y. Itoh, A. Morita, K. Nishioji et al., "Clinical significance of elevated serum interferon-inducible protein-10 levels in hepatitis C virus carriers with persistently normal serum transaminase levels," *Journal of Viral Hepatitis*, vol. 8, no. 5, pp. 341–348, 2001.
- [64] A. Antonelli, C. Ferri, S. M. Ferrari, M. Colaci, and p. Fallahi, "Immunopathogenesis of HCV-related endocrine manifestations in chronic hepatitis and mixed cryoglobulinemia," *Autoimmunity Reviews*, vol. 8, no. 1, pp. 18–23, 2008.
- [65] A. Antonelli, C. Ferri, M. Galeazzi et al., "HCV infection: pathogenesis, clinical manifestations and therapy," *Clinical and Experimental Rheumatology*, vol. 26, no. 1, supplement, pp. S39–S47, 2008.
- [66] A. Antonelli, C. Ferri, and p. Fallahi, "Hepatitis C: thyroid dysfunction in patients with hepatitis C on IFN- $\alpha$  therapy," *Nature Reviews Gastroenterology and Hepatology*, vol. 6, no. 11, pp. 633–635, 2009.
- [67] A. Apolinario, p. L. Majano, R. Lorente, O. Núñez, G. Clemente, and C. García-Monzón, "Gene expression profile of T-cell-specific chemokines in human hepatocyte-derived cells: evidence for a synergistic inducer effect of cytokines and hepatitis C virus proteins," *Journal of Viral Hepatitis*, vol. 12, no. 1, pp. 27–37, 2005.
- [68] A. Antonelli, M. Rotondi, p. Fallahi et al., "High levels of circulating CXC chemokine ligand 10 are associated with chronic autoimmune thyroiditis and hypothyroidism," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 11, pp. 5496–5499, 2004.
- [69] A. Antonelli, p. Fallahi, S. M. Ferrari et al., "Circulating CXCL11 and CXCL10 are increased in hepatitis C-associated cryoglobulinemia in the presence of autoimmune thyroiditis," *Modern Rheumatology*. In press.
- [70] A. Antonelli, C. Ferri, S. M. Ferrari et al., "High serum levels of CXCL11 in mixed cryoglobulinemia are associated with increased circulating levels of interferon- $\gamma$ ," *The Journal of Rheumatology*, vol. 38, no. 9, pp. 1947–1952, 2011.
- [71] A. Antonelli, C. Ferri, S. M. Ferrari et al., "Interleukin-1 $\beta$ , C-X-C motif ligand 10, and interferon-gamma serum levels in mixed cryoglobulinemia with or without autoimmune thyroiditis," *Journal of Interferon and Cytokine Research*, vol. 30, no. 11, pp. 835–842, 2010.
- [72] A. Antonelli, C. Ferri, S. M. Ferrari et al., "Serum concentrations of interleukin 1 $\beta$ , CXCL10, and interferon- $\gamma$  in mixed cryoglobulinemia associated with hepatitis C infection," *Journal of Rheumatology*, vol. 37, no. 1, pp. 91–97, 2010.
- [73] A. Antonelli, C. Ferri, p. Fallahi et al., "High values of CXCL10 serum levels in mixed cryoglobulinemia associated with hepatitis C infection," *American Journal of Gastroenterology*, vol. 103, no. 10, pp. 2488–2494, 2008.
- [74] A. Antonelli, C. Ferri, p. Fallahi et al., "CXCL10 and CCL2 serum levels in patients with mixed cryoglobulinaemia and hepatitis C," *Digestive and Liver Disease*, vol. 41, no. 1, pp. 42–48, 2009.
- [75] A. Antonelli, C. Ferri, S. M. Ferrari et al., "Serum levels of proinflammatory cytokines interleukin-1 $\beta$ , interleukin-6, and tumor necrosis factor  $\alpha$  in mixed cryoglobulinemia," *Arthritis and Rheumatism*, vol. 60, no. 12, pp. 3841–3847, 2009.
- [76] A. Antonelli, C. Ferri, p. Fallahi et al., "Thyroid disorders in chronic hepatitis C virus infection," *Thyroid*, vol. 16, no. 6, pp. 563–572, 2006.
- [77] A. Antonelli, C. Ferri, p. Fallahi et al., "Thyroid involvement in patients with overt HCV-related mixed cryoglobulinaemia," *QJM: An International Journal of Medicine*, vol. 97, no. 8, pp. 499–506, 2004.
- [78] A. Antonelli, C. Ferri, A. Pampana et al., "Thyroid disorders in chronic hepatitis C," *American Journal of Medicine*, vol. 117, no. 1, pp. 10–13, 2004.
- [79] A. Antonelli, C. Ferri, and p. Fallahi, "Thyroid cancer in patients with hepatitis C infection," *Journal of the American Medical Association*, vol. 281, no. 17, p. 1588, 1999.
- [80] A. Antonelli, C. Ferri, p. Fallahi et al., "Thyroid cancer in HCV-related chronic hepatitis patients: a case-control study," *Thyroid*, vol. 17, no. 5, pp. 447–451, 2007.
- [81] M. F. Prummel and p. Laurberg, "Interferon- $\alpha$  and autoimmune thyroid disease," *Thyroid*, vol. 13, no. 6, pp. 547–551, 2003.
- [82] M. Lenzi, p. J. Johnson, I. G. McFarlane et al., "Antibodies to hepatitis C virus in autoimmune liver disease: evidence for geographical heterogeneity," *The Lancet*, vol. 338, no. 8762, pp. 277–280, 1991.
- [83] L. M. Prentice, D. I. W. Phillips, D. Sarsero, K. Beever, S. M. McLachlan, and B. R. Smith, "Geographical distribution of subclinical autoimmune thyroid disease in Britain: a study using highly sensitive direct assays for autoantibodies to thyroglobulin and thyroid peroxidase," *Acta Endocrinologica*, vol. 123, no. 5, pp. 493–498, 1990.
- [84] I. G. McFarlane, "Autoimmunity and hepatotropic viruses," *Seminars in Liver Disease*, vol. 11, no. 3, pp. 223–233, 1991.
- [85] R. Minelli, L. E. Braverman, T. Giuberti et al., "Effects of excess iodine administration on thyroid function in euthyroid patients with a previous episode of thyroid dysfunction



- induced by interferon-alpha treatment," *Clinical Endocrinology*, vol. 47, no. 3, pp. 357–361, 1997.
- [86] A. Antonelli, M. Rotondi, p. Fallahi et al., "Increase of interferon- $\gamma$  inducible  $\alpha$  chemokine CXCL10 but not  $\beta$  chemokine CCL2 serum levels in chronic autoimmune thyroiditis," *European Journal of Endocrinology*, vol. 152, no. 2, pp. 171–177, 2005.
- [87] A. Antonelli, M. Rotondi, S. M. Ferrari et al., "Interferon- $\gamma$ -inducible  $\alpha$ -chemokine CXCL10 involvement in Graves' ophthalmopathy: modulation by peroxisome proliferator-activated receptor- $\gamma$  agonists," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 2, pp. 614–620, 2006.
- [88] E. J. Gowans, "Distribution of markers of hepatitis C virus infection throughout the body," *Seminars in Liver Disease*, vol. 20, no. 1, pp. 85–102, 2000.
- [89] J. Bartolomé, E. Rodríguez-Iñigo, p. Quadros et al., "Detection of hepatitis C virus in thyroid tissue from patients with chronic HCV infection," *Journal of Medical Virology*, vol. 80, no. 9, pp. 1588–1594, 2008.
- [90] A. Antonelli, C. Ferri, p. Fallahi et al., "High values of CXCL10 serum levels in patients with hepatitis C associated mixed cryoglobulinemia in presence or absence of autoimmune thyroiditis," *Cytokine*, vol. 42, no. 1, pp. 137–143, 2008.
- [91] A. Antonelli, C. Ferri, p. Fallahi et al., "Alpha-chemokine CXCL10 and beta-chemokine CCL2 serum levels in patients with hepatitis C-associated cryoglobulinemia in the presence or absence of autoimmune thyroiditis," *Metabolism*, vol. 57, no. 9, pp. 1270–1277, 2008.
- [92] A. Antonelli, C. Ferri, S. M. Ferrari et al., "The presence of autoimmune thyroiditis in mixed cryoglobulinemia patients is associated with high levels of circulating interleukin-6, but not of tumor necrosis factor-alpha," *Clinical and Experimental Rheumatology*, vol. 29, no. 1, supplement 64, pp. S17–S22, 2011.
- [93] H. Noto and p. Raskin, "Hepatitis C infection and diabetes," *Journal of Diabetes and its Complications*, vol. 20, no. 2, pp. 113–120, 2006.
- [94] A. Antonelli, C. Ferri, p. Fallahi et al., "Type 2 diabetes in hepatitis C-related mixed cryoglobulinaemia patients," *Rheumatology*, vol. 43, no. 2, pp. 238–240, 2004.
- [95] A. Antonelli, C. Ferri, p. Fallahi et al., "Hepatitis C virus infection: evidence for an association with type 2 diabetes," *Diabetes Care*, vol. 28, no. 10, pp. 2548–2550, 2005.
- [96] S. H. Mehta, F. L. Brancati, M. S. Sulkowski, S. A. Strathdee, M. Szklo, and D. L. Thomas, "Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States," *Annals of Internal Medicine*, vol. 133, no. 8, pp. 592–599, 2000.
- [97] S. H. Mehta, F. L. Brancati, S. A. Strathdee et al., "Hepatitis C virus infection and incident type 2 diabetes," *Hepatology*, vol. 38, no. 1, pp. 50–56, 2003.
- [98] H. Tanaka, G. Shiota, and H. Kawasaki, "Changes in glucose tolerance after interferon- $\alpha$  therapy in patients with chronic hepatitis C," *Journal of Medicine*, vol. 28, no. 5–6, pp. 335–346, 1997.
- [99] C. Giordanino, E. Bugianesi, A. Smedile et al., "Incidence of type 2 diabetes mellitus and glucose abnormalities in patients with chronic hepatitis C infection by response to treatment: results of a cohort study," *American Journal of Gastroenterology*, vol. 103, no. 10, pp. 2481–2487, 2008.
- [100] M. Masini, D. Campani, U. Boggi et al., "Hepatitis C virus infection and human pancreatic beta-cell dysfunction," *Diabetes Care*, vol. 28, no. 4, pp. 940–941, 2005.
- [101] E. A. M. Gale, "Latent autoimmune diabetes in adults: a guide for the perplexed," *Diabetologia*, vol. 48, no. 11, pp. 2195–2199, 2005.
- [102] M. Skowroński, D. Zozulińska, J. Juszczyk, and B. Wierusz-Wysocka, "Hepatitis C virus infection: evidence for an association with type 2 diabetes: response to Antonelli et al," *Diabetes Care*, vol. 29, no. 3, p. 750, 2006.
- [103] A. Antonelli, T. Tuomi, M. Nannipieri et al., "Autoimmunity to CD38 and GAD in type I and type II diabetes: CD38 and HLA genotypes and clinical phenotypes," *Diabetologia*, vol. 45, no. 9, pp. 1298–1306, 2002.
- [104] A. Antonelli, C. Ferri, p. Fallahi et al., "Hepatitis C virus infection: evidence for an association with type 2 diabetes: response to Skowronski et al," *Diabetes Care*, vol. 29, no. 3, p. 751, 2006.
- [105] A. Antonelli, C. Ferri, S. M. Ferrari, M. Colaci, D. Sansonno, and p. Fallahi, "Endocrine manifestations of hepatitis C virus infection," *Nature Clinical Practice Endocrinology and Metabolism*, vol. 5, no. 1, pp. 26–34, 2009.
- [106] M. p. Manns, J. G. McHutchison, S. C. Gordon et al., "Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial," *The Lancet*, vol. 358, no. 9286, pp. 958–965, 2001.
- [107] S. M. Alavian, B. Behnava, and S. V. Tabatabaei, "Comparative efficacy and overall safety of different doses of consensus interferon for treatment of chronic HCV infection: a systematic review and meta-analysis," *European Journal of Clinical Pharmacology*, vol. 66, no. 11, pp. 1071–1079, 2010.
- [108] S. M. Kamal, J. Fehr, B. Roesler, T. Peters, and J. W. Rasenack, "Peginterferon alone or with ribavirin enhances HCV-specific CD4<sup>+</sup> T-helper 1 responses in patients with chronic hepatitis C," *Gastroenterology*, vol. 123, no. 4, pp. 1070–1083, 2002.
- [109] Y. F. Yang, M. Tomura, M. Iwasaki et al., "IFN $\alpha$  acts on T-cell receptor-triggered human peripheral leukocytes to up-regulate CCR5 expression on CD4<sup>+</sup> and CD8<sup>+</sup> T cells," *Journal of Clinical Immunology*, vol. 21, no. 6, pp. 402–409, 2001.
- [110] Q. Ning, D. Brown, J. Parodo et al., "Ribavirin inhibits viral-induced macrophage production of TNF, IL-1, the procoagulant fg12 prothrombinase and preserves Th1 cytokine production but inhibits Th2 cytokine response," *Journal of Immunology*, vol. 160, no. 7, pp. 3487–3493, 1998.
- [111] C. Boni, A. Penna, G. S. Ogg et al., "Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: new perspectives for immune therapy," *Hepatology*, vol. 33, no. 4, pp. 963–971, 2001.
- [112] E. I. Rigopoulou, D. Suri, S. Chokshi et al., "Lamivudine plus interleukin-12 combination therapy in chronic hepatitis B: antiviral and immunological activity," *Hepatology*, vol. 42, no. 5, pp. 1028–1036, 2005.
- [113] D. R. Nelson, G. Y. Lauwers, J. Y. N. Lau, and G. L. Davis, "Interleukin 10 treatment reduces fibrosis in patients with chronic hepatitis C: a pilot trial of interferon nonresponders," *Gastroenterology*, vol. 118, no. 4, pp. 655–660, 2000.
- [114] M. p. Morrow, p. Pankhong, D. J. Laddy et al., "Comparative ability of IL-12 and IL-28B to regulate Treg populations and enhance adaptive cellular immunity," *Blood*, vol. 113, no. 23, pp. 5868–5877, 2009.
- [115] M. p. Morrow, J. Yan, p. Pankhong et al., "IL-28B/IFN $\lambda$ -3 drives granzyme B loading and significantly increases CTL killing activity in macaques," *Molecular Therapy*, vol. 18, no. 9, pp. 1714–1723, 2010.
- [116] M. Pilli, A. Zerbini, A. Penna et al., "HCV-specific T-cell response in relation to viral kinetics and treatment outcome

- (DITTO-HCV project),” *Gastroenterology*, vol. 133, no. 4, pp. 1132–1143, 2007.
- [117] H. Abe, C. N. Hayes, H. Ochi et al., “IL28 variation affects expression of interferon stimulated genes and peg-interferon and ribavirin therapy,” *Journal of Hepatology*, vol. 54, no. 6, pp. 1094–1101, 2011.
- [118] T. Fukuhara, A. Taketomi, T. Motomura et al., “Variants in IL28B in liver recipients and donors correlate with response to peg-interferon and ribavirin therapy for recurrent hepatitis C,” *Gastroenterology*, vol. 139, no. 5, pp. 1577–1585, 2010.
- [119] R. p. Donnelly, H. Dickensheets, and T. R. O’Brien, “Interferon-lambda and therapy for chronic hepatitis C virus infection,” *Trends in Immunology*, vol. 32, no. 9, pp. 443–4450, 2011.