Autoimmune phenomena are frequent in chronic hepatitis C virus (HCV) infection, and treatment of patients with chronic viral hepatitis creates an essential turning point for therapy of these diseases. The presence of these auto-reactivities in HCV generates clinical overlaps and dilemmas in the correct classification and treatment of such patients. A variety of autoantibodies are often observed in patients with HCV: rheumatoid factor, antinuclear antibodies, antibodies to double stranded deoxyribonucleic acid, antineutrophil cytoplasmic antibody, anti-smooth muscle antibodies, antiliver kidney microsomal 1 antibodies, antigastric parietal cell antibodies, antimitochondrial antibodies, anticardiolipin antibodies, cryoglobulins, and a high incidence of thyroid antibodies. Association between HCV and autoimmune phenomena, also in relation to interferon-alpha therapy has been reported the most frequent being autoimmune thyroid disease. These adverse effects are attributed to the immunomodulatory properties of type I interferon, and should be distinguished from autoimmunity associated with chronic viral hepatitis in which interferon treatment may indeed be beneficial. This review describes the immune response in HCV and effects of interferon-alpha in exacerbation of pre-existing autoimmune disorders or the de novo induction of autoimmunity during interferon therapy in patients with HCV.

Key words: Immune response, hepatitis C, autoimmunity.
pré-existentes ou induzidas durante a terapêutica com interferon em pacientes com HCV.

**Palavras-chave:** Resposta imune, hepatite C, autoimunidade.

**Introduction**

Autoimmune phenomena are frequent in chronic hepatitis C virus (HCV) infection resulting in production of autoantibodies. A variety of autoantibodies are often observed in patients with HCV: rheumatoid factor (RF), antinuclear antibodies (ANA), antibodies to double stranded deoxyribonucleic acid (ds-DNA), antineutrophil cytoplasmic antibody (ANCA), anti-smooth muscle antibodies (ASMA), antiliver kidney microsomal 1 antibodies (anti-LKM1), antigastric parietal cell antibodies (anti-GPCA), antimitochondrial antibodies (AMA), anticardiolipin antibodies (ACL), cryoglobulins, and a high incidence of thyroid antibodies (anti-TPO). (1,2)

The development of autoimmune disease in patients with HCV infection depends on the interaction of multiple factors. Various mechanisms were reported to be responsible for the association of HCV and autoimmunity. Of these, enhanced T cell apoptosis was reported to contribute to viral persistency and disease severity. The issue of HCV-related autoimmunity has partly been shown to be related to the resistance of CD5+ B cell subpopulation to apoptosis. (3)

HCV infection is associated with immune-complex mediated disorder this and condition provides clues to the possible role of viruses as triggers of autoimmunity. The interaction between hepatitis C virus and its receptor on B lymphocytes is the likely trigger of a polyclonal activation leading to the production of autoantibodies. These appear not to be an epiphenomenon but to be markers of hepatocyte damage. (4)

Association between HCV infection and autoimmune phenomena, also in relation to interferon-alpha (IFN-α) therapy, has been reported with many extrahepatic manifestations, the most frequent being autoimmune thyroid disease. (5) Thyroid dysfunction has been described among the side-effects during IFN-α therapy for chronic hepatitis C, and autoimmune thyroid disorders are among the well-known adverse effects, occurring at any time after the start of treatment with a median of 17 weeks. (6,7)

This review summarizes the immune response in HCV infection and effects of interferon-alpha in exacerbation of pre-existing autoimmune disorders or the de novo induction of autoimmunity during interferon therapy in patients with HCV.

**Hepatitis C virus infection**

HCV infection was first suspected in the 1970's, when most blood transfusion infections were associated with neither hepatitis A nor hepatitis B virus. This new type of hepatitis transmitted by blood was then called “non-A, non-B” hepatitis. The genome of hepatitis C virus was identified in 1989, and the name hepatitis C was subsequently applied to the human infection caused by this single-strand ribonucleic acid (RNA) virus of positive polarity. (6) Hepatitis C virus belongs to the Hepacivirus genus, Flaviviridae family, and has six major genotypes, and more than 70 subtypes. (5)

Hepatitis C virus infects around 170 million people in the world, with an estimated global incidence of 3-4 million new infections per year. (10) HCV infection may be unresolved in approximately 85% of infected individuals, representing an important cause of liver cirrhosis and hepatocellular carcinoma.

The knowledge of the natural history of HCV infection is still incomplete, because the acute infection is often asymptomatic in many individuals, as demonstrated in epidemiological studies involving HCV and hemotherapy centers. (11) Moreover, this clinical form of HCV infection may present different geographical characteristics, which may be associated with ethnic/race and environmental factors such as hepatitis C virus genotype and co-infection with other pathogenic agents. (12) The estimated mortality due to acute HCV infection is very low (≤ 0.1%), contrasting with that verified for chronic HCV.

The transmission of HCV is mainly caused by infected blood or its products. However, other risks have been demonstrated for HCV infection, which are mainly represented by the intravenous use of illicit drugs; transplantation of HCV-infected organ, and haemodialysis. Less frequent HCV infection has been documented due to occupational exposure to contaminated blood. Sporadic reports of HCV due to household exposure, vertical transmission, unsafe sex and intranasal cocaine use, have been also published. (13)

However, the use of both third generation anti-HCV immunoassays and polymerase chain reaction to detect HCV-RNA in blood donors provoked an important fall in HCV infection, which has today an estimated incidence ratio of 1: 500,000-2,000,000 transfusions. (14)

HCV is the most common cause of chronic liver disease and cirrhosis in the world, and represents the main cause of liver transplantation in the United States of America, Australia, and Europe. Chronic infection is evidenced by the demonstration of HCV-RNA in the blood for at least 6 months after virus contamination. (13)

Patients that exhibit clinical or laboratory signs of chronic liver disease must be diagnosed for chronic HCV infection through the demonstration of both anti-HCV antibodies and HCV-RNA. (15)

**Immune response in hepatitis C**

The innate immune response to HCV is responsible for the activation of cytokines such as interferon (IFN) which activate antiviral proteins that inhibit the replication of the virus while the adaptive immune
Andrade LJO, et al • Immune response in hepatitis C

Response to HCV neutralizes viral particles and destroys infected cells.\(^{11}\)

After HCV infection, there is expression of the hypervariable NS1/E2 region on the surface of the virus, which stimulates B cells to produce high antibody titers of antibodies with the objective of destroying the permanence of the virus.\(^{17}\)

Since there is a weak humoral immune response to HCV, it is believed that the reactivity of cytotoxic T-lymphocytes or CD8+ T cells is fundamental to viral elimination,\(^{18}\) and that impairment of this reactivity is one of the factors responsible for the chronicity of the infection (figure 1).\(^{19}\)

The different components of the immune system play important roles in the outcome of HCV infection. In only 20% of HCV-infected individuals these immune responses successfully clear the virus, while 50-80% of infected individuals experience viral persistence. The inefficiency of the immune system in eliminating the virus is not well understood as humoral and cellular immune responses are induced.\(^{20}\)

Hepatitis C virus belongs to the Hepacivirus genus, *Flaviviridae* family, and has six major genotypes, and more than 70 subtypes.\(^{9}\) The chronic infection is evidenced by the demonstration of HCV-RNA in the blood for at least 6 months after virus contamination.\(^{13}\)

Recent studies suggest that liver inflammation in HCV infection is controlled by several mechanisms, including host regulatory immune responses and viral polypeptides interacting with cells involved in innate and adaptive immunity, in particular revealing the central role of T cells in viral control and clearance.\(^{21,22}\)

Besides hepatocytes, HCV infects different cells, including leukocytes and epithelial cells of different organs. However, it does not cause cytotoxicity, suggesting that both the hepatic injury and the extra-hepatic clinical manifestations caused by HCV are probably mediated by immune events of cryoglobulinemia, immune complex persistence and autoimmune recognition.\(^{23}\) Thus, the pathogenesis of HCV infection involves a complex virus/host interaction.

Viral clearance following the cure of acute hepatitis C, spontaneously or after IFN-\(\alpha\) therapy, is usually associated with strong cellular immune response in the infected individuals, which are mediated by both CD4 and CD8 T cell, and represented by a Helper T cells (Th) Th1 cytokine profile in some studies. In contrast, the persistence of HCV infection has been characterized by a Th2 cytokine profile, which has been associated with high titer of neutralizing antibodies and low cellular immune response.\(^{24}\) The immune mechanisms involved in viral clearance have been intensely investigated to provide new subsidies for the development of HCV vaccine, and to understand the pathogenesis of the HCV infection.

Some data suggest that cytokines involved in the regulation of the immune response may display a key role in HCV infection, contributing for HCV persistence, hepatic injury and autoimmunity.\(^{25,26}\) The mRNA analysis of intra-hepatic cytokines of HCV carriers showed that the progressive hepatic lesion, demonstrated by fibrosis and portal inflammation, was correlated with the increased expression of Th1 cytokines (IFN-\(\alpha\) and interleukin [IL]-2), and low expression of Th2 cytokines IL-4 and IL-10. A correlation between alanine aminotransferase (ALT) levels and IFN-\(\alpha\) mRNA was observed in these studies. However, an expression of Th2 cytokines (IL-4, IL-6 and IL-10) was also reported in the peripheral blood lymphocytes of HCV carriers, which was comparable to that observed in healthy controls. More patients expressed these cytokines, and also tumor necrosis factor-alpha (TNF-\(\alpha\)) and Transforming growth factor beta (TGF-\(\alpha\)), after IFN-\(\alpha\) treatment.\(^{27,28}\)

An investigation carried out with the study of 74 HCV carriers demonstrated elevated serum levels of IL-4 and IL-10 in 49% and 31% of these individuals, which did not show any correlation with epidemiologic, clinic or virologic data. In contrast, other study observed an increase in both Th1 and Th2 cytokines in HCV carriers and a drop in both IL-4 and IL-10 after IFN-\(\alpha\) treatment.\(^{29}\)

The involvement of Th1 cytokines in the immunopathogenesis of

---

**Figure 1. Immune response mechanism in hepatitis C.**

- **Primary exposure to HCV**
  - **Humoral Immune Response**
    - Phagocytosis by macrophages
    - Antigen
    - Activation Helper T cells
    - B cells
    - Cytokines and CD8+
    - Viral elimination
    - Killing of infected cell
  - **Cellular Immune Response**
    - Activated Helper T cells
    - Activated cytotoxic T cells

- **Th1 cytokine profile**
  - IFN-\(\alpha\)
  - IL-2

- **Th2 cytokine profile**
  - IL-4
  - IL-10

---
chronic HCV infection has been evidenced by the demonstration of a high number of CD4+ T cells producers of IFN-α in both asymptomatic HCV carriers presenting low ALT and low viral load, as well as in HCV carriers exhibiting high viral load that have cirrhosis or without this pathology. The number of lymphocytes expressing IL-4 was diminished in these patients, while they had high serum levels of both soluble IL-2 receptor and IFN-α.\(^{32}\)

Liver CD4+ T cells from HCV carriers seem to exhibit a predominant Th1 cytokine pattern, which was documented only in CD8+ lymphocytes from peripheral blood.\(^{31}\) The inexistence of an increased Th2 immune response in HCV carriers was also demonstrated in cell culture of peripheral mononuclear blood cells either stimulated with mitogens or HCV antigens, which was documented by no difference in the percentage of CD4+ T cells producing either IL-4 or IL-10. Such a finding contrasted with the observation of increase in the percentage of cells producing IFN-α and IL-2, which were identified as CD4+, CD8+, naïve-CD45RA+ and memory CD45RO+ T cells.\(^{32}\)

Recent studies on have showed that the efficient T cell response against the virus, which may be demonstrated in subjects spontaneously cured of HCV infection, is mainly caused by a low mutagenesis of HCV proteins, and is associated with moderate titers of neutralizing antibodies. In contrast, the evasion of HCV from carrier immune response is mainly associated with a high viral epitope diversity, inefficient T cell immunity, and production of high titers of neutralizing antibodies against epitopes of highly mutated HCV proteins. As a consequence, while only 30% of infected people spontaneously cure their HCV infection, 55%-85% of infected people become HCV carriers.\(^{14,23}\) Over a period of 20-30 years, 10%-20% of the HCV carriers may develop cirrhosis, while hepatocellular carcinoma may be demonstrated in 1%-20% of the HCV carriers.\(^{14,23}\) The inexistence of an increased Th2 immune response in HCV carriers was also demonstrated in cell culture of peripheral mononuclear blood cells either stimulated with mitogens or HCV antigens, which was documented by no difference in the percentage of CD4+ T cells producing either IL-4 or IL-10. Such a finding contrasted with the observation of increase in the percentage of cells producing IFN-α and IL-2, which were identified as CD4+, CD8+, naïve-CD45RA+ and memory CD45RO+ T cells.\(^{32}\)

Recent studies on have showed that the efficient T cell response against the virus, which may be demonstrated in subjects spontaneously cured of HCV infection, is mainly caused by a low mutagenesis of HCV proteins, and is associated with moderate titers of neutralizing antibodies. In contrast, the evasion of HCV from carrier immune response is mainly associated with a high viral epitope diversity, inefficient T cell immunity, and production of high titers of neutralizing antibodies against epitopes of highly mutated HCV proteins. As a consequence, while only 30% of infected people spontaneously cure their HCV infection, 55%-85% of infected people become HCV carriers.\(^{14,23}\) Over a period of 20-30 years, 10%-20% of the HCV carriers may develop cirrhosis, while hepatocellular carcinoma may be demonstrated in 1%-7% of the HCV persons specially those with cirrhosis.\(^{33}\)

HCV infection is an insidious liver disease, which is often associated with extra-hepatic manifestations, documented by laboratory findings of autoimmunity and clinical manifestations of autoimmune disorders. Different immune events, which include autoantibody production and cryoglobulinemia, may be detected in HCV carriers, whose prevalence seems to be correlated with ethnic/ race and environmental factors.

Various immunological phenomena have been described in patients being exposed to HCV.\(^{34}\) Sjögren syndrome has been well documented as an extra-hepatic manifestation of HCV infection, and there is enough evidence to associate this autoimmune syndrome with non-Hodgkin B-lymphoma. Besides, most HCV carriers present high prevalence of mixed cryoglobulinemia, which may provoke vasculitis, leg ulcers and nephritis.\(^{23}\) Mixed cryoglobulinemia, a small-vessel systemic vasculitis, is characterized by the coexistence of autoimmune and lymphoproliferative alterations; therefore, it represents the prototype of HCV-associated disorders.\(^{35}\)

Many organ- and non-organ-specific autoantibodies are commonly found in the sera of HCV infected patients. The nature of the IgM RF has been analysed extensively in HCV infection, in particular the monoclonal IgM of type II cryoglobulins. Although there is speculation that HCV can induce the RF response, there are no reports of a specific HCV protein that is capable of inducing RF. Indeed 70% of patients chronically infected with HCV do not mount a sustained RF response.\(^{36}\) Rheumatoid factor B cells are part of the normal repertoire and significant titers of RF are induced during normal antiviral or antibacterial immune responses. Under the pressure of chronic antigen stimulation, RF repertoire is remodelled, and progressively includes monospecific, somatically mutated RF similar to those observed in chronic autoimmune diseases such as rheumatoid arthritis.\(^{37}\) These findings suggest that in HCV infection the driving force behind the production of RF is not simply equivalent to a strong immune response.

Positive serum ANA has been documented in patients with HCV infection. The prevalence of antinuclear antibodies (ANA) ranges between 6% and 22%, and they are usually presented at allow titer (1:40-1:80).\(^{38}\)

HCV and the expression of the HCV NS3 and core protein have been proposed as factors involved in the induction of double-stranded DNA breaks and enhancement of the mutation frequency of cellular genes.\(^{39}\) Anti-dsDNA antibodies are strongly suggestive of systemic lupus erythematosus although they are present in only 40-60% of patients with this disease. Serological presence of anti-dsDNA antibodies is a common phenomenon in HCV patients. Pathogenic anti-DNA antibodies typically have an IgG isotype and demonstrate high avidity for ds-DNA.\(^{40}\) One study involving 518 patients with HCV showed anti-dsDNA positive in 8.5% of them.\(^{41}\)

ANCA are autoantibodies directed against antigens found in cytoplasmic granules of neutrophiles and monocytes and has been found in 10% patients with HCV associated mixed cryoglobulinemia\(^{42}\) being generally strongly associated with primary systemic vasculitides.

ASMA are IgG or IgM antibodies that are primarily directed against filamentous actin (F-actin). Since F-actin is present in all smooth muscle fibers, these antibodies are not organ specific. Approximately 10-66% of patients with HCV have a positive ASMA, usually in low titer.\(^{43}\) The association between HCV and ASMA does not appear to be significantly different from that in other hepatic disorders, particularly hepatitis B.\(^{44}\)

Anti-LKM-1 the serological marker of a subset of autoimmune hepatitis, is also detected in up to 11%
of the HCV-infected subjects. Anti-LKM I is reactive against a 50KD antigen in the liver and kidney, which has been identified as the cytochrome monooxygenase P-450IId6. The observation that autoantibodies anti-LKM is commonly detected in patients with chronic HCV suggest that a B cell response to HCV may contribute to extrahepatic and autoimmune immunopathology.

Anti-GPCA are less frequently encountered in patients with HCV. The measuring of anti-GPCA levels in chronic HCV patients treated with IFN is very important, especially in those who develop thyroid dysfunction during treatment.

AMA has been recognized as a heterogeneous group of autoantibodies classified according to their mitochondrial antigen specificity and disease association. In acute liver failure AMA are found against all major liver antigens. Approximately 1.5-8% of patients with HCV have a positive AMA. One study suggests that AMA present in patients with HCV do not always recognize the same epitopes as in primary biliary cirrhosis, and that these antibodies may disappear after eradication of HCV, indicating that the production of AMA is linked to the presence of the virus. ACL are frequently found in patients with HCV infection and in these patients they may be implicated in the occurrence of thrombosis and in the development of thrombocytopenia when directed against the cofactor of thrombocytopenia. In chronic viral hepatitis, ACL are frequently cofactor independent. Studies of ACL in connection with chronic HCV infection have been conducted and a study conducted with patients with asymptomatic chronic HCV infection associated acute ischemic stroke showed a high prevalence of IgG ACL (46%).

HCV appears to be frequently involved in inducing Hashimoto’s thyroiditis. It has been postulated that the pathogenesis of these diseases involve genetic and environmental factors. Recent studies have shown a high prevalence of anti-thyroid antibodies in patients with HCV, also before IFN treatment, suggesting that autoimmune thyroid disease could be induced by HCV infection. Anti-TPO are present in 5-28% of patients with HCV infection and thyroid dysfunction occurs in 2-31% of patients.

Therefore, the immune responses against HCV play a crucial role in the pathogenesis of chronic disease, and the host response to HCV can be divided into innate and adaptive subcomponents. The innate response components are first line of defense, and the adaptive immune system including the spleen and lymph nodes is influenced by properties of both the organism and the antigen. Hepatitis C virus displays numerous mechanisms to evade the host defense, and an efficient T-cell response at the early stage of infection is critical to clear the virus.

References


41. Wu YY, Hsu TC, Chen TY, Liu TC, Liu GY, Lee YJ et al. Protei-


Correspondence:
Dr. Luis Jesuino de Oliveira Andrade
R. São Marcelo, 246 - Zildolândia
Zip Code: 45.600-700 - Itabuna, Bahia, Brazil.
e-mail: luisjesuino@yahoo.com.br