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TOPIC HIGHLIGHT

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Immunological alterations in hepatitis C virus infection

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Abstract

A higher prevalence of immunological processes has recently been reported in patients with hepatitis C virus (HCV) infection, focusing the attention of physicians and researchers on the close association between HCV and immune disorders. HCV lymphotropism represents the most important step in the pathogenesis of virus-related immunological diseases and experimental, virologic, and clinical evidence has demonstrated a trigger role for HCV both in systemic autoimmune diseases, such as rheumatoid arthritis, Sjögren syndrome, hemolytic anemia and severe thrombocytopenia, and in organ-specific autoimmune diseases, such as autoimmune hepatitis, thyroid disorders and diabetes. This review will outline the principal aspects of such HCV-induced immunological alterations, focusing on the prevalence of these less characterized HCV extrahepatic manifestations.

Key words: Hepatitis C virus; Immune disorders; Cytopenia; Extrahepatic manifestation; Autoantibody

Core tip: Hepatitis C virus (HCV)-infected lymphoid tissue of the host represents a site for the persistence of HCV infection which exerts a chronic stimulus to the immune system, facilitating clonal B-lymphocyte expansion and consequent wide autoantibody production, including cryo- and non-cryo-precipitable immune complexes which may lead to organ- and non-organ-specific immunological alterations. This review outlines the principal aspects of such HCV-induced immunological alterations, focusing on the prevalence of these less characterized HCV extrahepatic manifestations.

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INTRODUCTION

Autoimmunity and viral infections are closely related, and the hepatitis C virus (HCV), is recognized as one of the viruses most often associated with autoimmune features. For this reason HCV is not only associated with chronic hepatic inflammation but also an array of extrahepatic complications. In the majority of these associated extrahepatic manifestations, the pathogenic mechanism appears to be immunologically driven, with many having features of autoimmunity. HCV infection has been associated with both organ-specific [thyroiditis, diabetes, autoimmune hepatitis (AIH)] and systemic autoimmune diseases and this association has generated growing interest in recent years since it is often observed in patients with chronic HCV infection.

PATHOGENESIS OF HCV RELATED IMMUNE DISORDERS

HCV lymphotropism represents the most relevant step in the pathogenesis of virus-related immunological dis-

orders[1]. Indeed, infected lymphoid tissue of the host represents a site for the persistence of the HCV infection[2-6]. HCV exerts a chronic stimulus to the immune system, facilitating the clonal B-lymphocyte expansion and consequent wide autoantibody production, including cryo- and non-cryoprecipitable immune complexes[3,7-9] which may lead to organ- and non-organ-specific immunological alterations[3,7,8,10]. The first step is translocation, demonstrated in a high percentage of HCV-infected patients, with consequent Bcl-2 proto-oncogene activation, antiapoptotic activity and prolonged survival of lymphocytes[3,7,9,10]. Besides, the identification of HCV envelope protein E2 able to bind the CD81 molecule expressed on both hepatocytes and B-lymphocytes seems to be crucial for HCV-driven autoimmunity[3,7,9,10].

Dysregulation of cytokine networks skewing regulatory T-cells to a Th2 phenotype, which may be associated with enhanced humoral immune responses and autoantibody production has also been related to the expansion of autoantibody-producing B-cells and chronic lymphoproliferation in HCV infection[11]. HCV infections induce a massive chemokine and cytokine burst and therefore recruit leukocytes to the site of infection with the goal to stop viral spread. This excitation of the human defense system could stimulate a potentially self-reactive lymphocytes inducing autoimmunity in susceptible individuals[11].

Many studies have linked Th1 immune response with HCV infection[12], mixed cryoglobulinemia (MC)[13] and organ specific autoimmune disorders[14]. These findings suggest that a possible common immunological Th1 pattern could be the pathophysiological base of the association of autoimmunity related HCV infections.

Several studies have shown an increased expression of interferon-gamma (IFN-γ), and IFN-γ inducible chemokines (C-X-C motif chemokine 10 - CXCL10), in hepatocytes and in lymphocytes of HCV-infected patients[12,15,16], which are directly related to the degree of inflammation and an increase in circulating levels of IFN-γ and CXCL10[17,18].

Furthermore, it has been shown that NS5A and core proteins, alone or by a synergistic effect with Th1 cytokines [IFN-γ and tumor necrosis factor-α (TNF-α)], are capable of upregulating CXCL10 and monokine induced by gamma interferon (MIG) gene expression and secretion in cultured human hepatocyte derived cells. These data suggest that CXCL10 produced by HCV-infected hepatocytes could play a key role regulating T-cell trafficking into a Th1-type inflammatory site by recruiting Th1 lymphocytes, that secrete IFN-γ and TNF-α, with a synergistic effect on CXCL10 secretion by hepatocytes, thus perpetuating the immune cascade[19].

HCV AND SYSTEMIC AUTOIMMUNE DISEASES

Mixed cryoglobulinemia

MC is the most well documented extrahepatic manifestation of HCV infection[2,20]. MC, which is defined by documenting cryoprecipitates in serum (Ig precipitates from serum at temperatures under 37 ℃ and dissolves upon re-warming), is characterized by the presence of circulating immunocomplexes produced by a benign proliferation of B-cells. MC represents the link between HCV and various autoimmune and lymphoproliferative disorders. Although serum cryoglobulins (CGs) are frequently present in patients with chronic HCV[3-5,21,22], in many of them CGs are present at low levels and symptoms are often absent or very mild. Only about 5% of HCV-infected subjects have clinically overt MC syndrome.

HCV-related arthritis

Chronic oligo-polyarthritis during chronic HCV infection is often associated with MC but can also represent an independent entity. Indeed, it is not rare to observe a simple association between HCV infection and classical rheumatoid arthritis (RA) that can co-exist by chance or can be related to the ability of HCV to act as a trigger of the immune disease in individuals genetically predisposed to RA.

A polyarthritis, which is often non-erosive and rarely progressive, and involves small joints is the most common kind of arthritis associated with HCV chronic infection without the coexistence of cryoglobulinemia. Instead, 40%-80% of HCV-infected patients with MC[23] are reported to have a bilateral and symmetric arthralgia, which is non-deforming and includes mainly the knees and hands, and, more seldom, the elbows and ankles. Rheumatoid factor (RF) activity is found in 70%-80% of MC patients but is not correlated with the presence of articular disease, as patients chronically infected with HCV in the absence of HCV-MC or RF may have prominent articular symptoms. Usually there is no evidence of joint destruction, and antibodies to cyclic citrullinated peptide, which are highly specific to rheumatoid arthritis, are absent[24]. These evidences suggest that HCV infection should be considered in the differential diagnosis of patients with atypical arthritis.

Sjögren syndrome

Another autoimmune condition associated with HCV is a chronic lymphocytic sialoadenitis similar to sialoadenitis associated with idiopathic Sjögren syndrome (SS), which has been reported in approximately 50% of patients with HCV infection[25].

Some authors have distinguished the HCV-related sicca syndrome from Sjogren's syndrome based on several differences, including absence of anti-SSA and anti-SSB antibodies, pericapillary and non pericanalary lymphocytic infiltration, lack of glandular canal damage, high prevalence of mixed cryoglobulinemia (50%), hypocomplementemia (51%), and systemic vasculitic manifestations (58%)[25-28]. Moreover, the lymphocytic type of the infiltrate in the minor salivary gland shows a predominance of CD8 lymphocytes which is not observed in primary SS[29]. Although the possible etiopathogenetic

role of HCV in SS remains a controversial issue[27], the explanation for this extrahepatic manifestation could be a cross reactivity between the HCV envelope and host salivary tissue which lead to an immune reaction directed against salivary glands[26]. The correct classification of patients with sialoadenitis related to HCV chronic infection have important clinical, prognostic and therapeutic implications since it may evolve into a B cell malignant lymphoma, especially in the presence of MC[10,30].

HCV related cardiac disorders

Several observations suggest that HCV infection is an important cause of a variety of otherwise unexplained heart diseases. Indeed, it was reported that (+) or (-) chain HCV-RNAs can be detected in the biopsied myocardial tissue or in the autopsied heart suggesting that HCV might proliferate in the myocardium[31], resulting in induction of cardiomyopathy. Frustaci *et al*[32] have shown that HCV replicates in myocardial tissue of patients with myocarditis, and that HCV infection may contribute to the development of an autoimmune myocarditis, frequently associated with myocardial antibodies and responsive to immunosuppressive therapy. In 2000, Matsumori suggested that some specific HCV clones with high affinity for the heart can develop and cause cardiomyopathy[33] and in 2006, in a large study involving more than 1000 patients, the same group identified anti-HCV antibodies, HCV RNA, NT-proBNP, and cardiac troponin I and T in sera stored for up to 17 years, and found the anti-HCV antibodies were more prevalent in patients with myocarditis than in the general US population[34]. These results suggest that in regions where its prevalence is high, HCV infection may be an important cause of myocarditis and heart failure. Moreover, the same authors concluded that NT-proBNP is a more sensitive marker of myocardial injury than cardiac troponins in patients with heart failure from HCV myocarditis. More recently, other studies confirmed that NT-proBNP is a sensitive biomarker for identifying patients with heart failure caused by HCV-related myocarditis[35,36]. Antonelli *et al*[36] assessed serum NTproBNP in 50 HCV-positive patients and in 50 sex- and age-matched controls. HCV patients showed significantly higher mean NT-proBNP level than controls[35]. This result was confirmed by the same group in another study where TNF-α was also found to be higher in HCV+ patients with respect to controls, suggesting the presence of subclinical cardiac dysfunction[36].

AUTOIMMUNE CYTOPENIAS IN PATIENT WITH HCV INFECTION

Hemolytic anemia and severe thrombocytopenia were the most frequent cytopenias observed in patients with HCV infection. The different types of immune-mediated cytopenias may be severe and clinically significant.

Hemolytic anemia

Although autoimmune hemolytic anemia (AHA) has frequently been reported in association with HCV in the setting of interferon (IFN) treatment[37,38], it has also been observed as an isolated extrahepatic manifestation. The existence of AHA in patients with chronic hepatitis was first described in 1951, when Hyman *et al*[39] described AHA in 3 patients with chronic liver involvement. In 1973, Panush *et al*[40] described a patient with chronic active hepatitis who presented with AHA with a positive Coombs test, who responded to treatment with steroids. In 1982, Portell *et al*[41] reported 5 patients with chronic hepatopathy (3 with active chronic hepatitis and 2 with cirrhosis) and a positive Coombs AHA, with positive ANA in 4 and sicca syndrome in 1. In 2001, 2 cases of HCV infection associated with Coombs-positive AHA, in the absence of treatment with IFN, were reported by Srinivasan[42] and Chao *et al*[43], respectively. In 2003, Ramos-Casals *et al*[44] presented the largest series of cases of HCV-related AHA not treated with antiviral therapy. Seventeen HCV patients, mostly women with a mean age of 56 years, presented a high level of association with autoimmune diseases, with cryoglobulinemia as the most frequent immunologic marker. Most patients had a history of liver cirrhosis and even if they had a good response to corticosteroids, the prognosis was poor (56% mortality).

HCV-associated immune thrombocytopenic purpura

Although thrombocytopenia during the course of chronic liver disease is usually attributed to hypersplenism, an autoimmune mechanism has been suggested as playing a role in some patients with HCV infection. This hypothesis is based on the observation of a greater prevalence of thrombocytopenia and antiplatelet antibodies in HCV patients compared with HBV patients[45], and of the frequency of HCV infection seen among patients initially diagnosed with idiopathic thrombocytopenic purpura (ITP)[46-48]. The pathophysiology of infection-related ITP involves diverse immunologic pathways as well as non-immune mechanisms that accelerate platelet destruction and/or decrease platelet production.

High affinity binding of HCV to the platelet membrane with subsequent binding of anti-HCV antibody might lead to phagocytosis of platelets[49]. Dysregulation of the host immune system triggering the production of autoantibodies against platelet glycoproteins has also been postulated[45,50]. However there have been conflicting data on the presence of specific antibodies in platelets in patients with HCV-related ITP[45,50-52].

Thrombocytopenia in HCV patients may be present even in the absence of clinically evident liver disease or splenomegaly and may be mistakenly diagnosed as primary chronic immune thrombocytopenic purpura (CITP)[48,53]. Six cross-sectional studies have reported serologic evidence of HCV infection in 20% of patients with a clinical diagnosis of CITP[48,53-57], and in the largest series published to date, HCV antibodies were identified in 30% of

250 patients fulfilling the American Society of Hematology criteria for CITP[54]. There were significant differences in the demographic characteristics of patients with HCV-positive compared with patients with HCV-negative CITP. Patients positive for HCV were older and the incidence was distributed equally between the sexes compared with the female predominance in HCV-negative CITP.

ORGAN-SPECIFIC AUTOIMMUNE DISEASES

Thyroid disorders and HCV

Autoimmune thyroid involvement and hypothyroidism were significantly more frequent in patients with chronic hepatitis C (CHC) than in comparison groups such as patients with viral hepatitis B or D[58-60] or normal subjects[61,62]. The most frequent thyroid disorder in this setting is the presence of circulating anti-thyroid antibodies which is more commonly reported in female subjects[58]. The prevalence of abnormally high levels of anti-thyroid antibodies observed in these patients ranges from 2% to 48%[58,61,63,64], with heterogeneous geographic distribution[65]. These discrepancies are related to variable genetic predisposition and environmental co-factors, such as iodine intake or other infectious agents[66]. The evidence of a subclinical hypothyroidism was observed in 2%-9% of patients with chronic HCV infection, particularly in those patients with MC[59,60,62,63,67], and these patients seem to be susceptible to Hashimoto's autoimmune thyroiditis and Grave's disease when treated with interferon.

Antonelli *et al*[21] in 2004 analyzed 630 consecutive patients affected by CHC compared with a large control group of subjects from iodine-deficient and sufficient areas and with 86 patients with chronic hepatitis B. They demonstrated that patients with CHC were more likely to have hypothyroidism, anti-thyroglobulin and anti-thyroid peroxidase antibodies than any of the other groups. The same group evaluated thyroid function, the presence of thyroid autoantibodies, thyroid nodules and thyroid cancer, in 93 HCV + MC consecutive patients matched by sex and age to 93 patients with CHC without MC and 93 healthy (HCV-negative) controls. Subclinical hypothyroidism and thyroid autoimmunity were significantly more frequent in HCV + MC patients than in HCV-negative controls. Moreover, serum thyroid peroxidase antibodies were also significantly more frequent in HCV + MC patients than in CHC patients. Finally, the prevalence of thyroid nodules was not significantly different in the three groups[68]. In conclusion, pooling all data about HCV-positive patients (with CHC or HCVAb positivity) and using as control healthy subjects and HBV-infected patients, there was a significant increase in the prevalence of both thyroid autoimmune disorders (OR = 1.6; 95%CI: 1.4-1.9] and hypothyroidism (OR = 2.9; 95%CI: 2.0-4.1)[69].

Some authors have reported that patients with chronic HCV have a higher prevalence of papillary thyroid carcinoma[70,71]. In 2002, the prevalence of thyroid cancer in a series of 94 HCV-related mixed cryoglobulinemic patients was investigated[70]. A control group was obtained from a sample of the general population (2401 subjects) who had undergone thyroid ultrasonography. The prevalence of thyroid nodules was higher, although not significantly so, in control subjects than in MC patients but 2 patients with papillary thyroid cancer were found in the MC series, while no case was observed among controls.

A more recent study[71] prospectively investigated the prevalence and features of thyroid cancer in 308 patients with CHC in comparison with 2 large sex- and age-matched control groups from the general population with different iodine intake. Thyroid status was assessed by measurement of circulating thyroid hormones and autoantibodies, thyroid ultrasonography, and, when indicated, fine-needle aspiration cytology. The authors have found that circulating thyrotropin, anti-thyroglobulin, and anti-thyroperoxidase antibodies levels, and the prevalence of hypothyroidism were significantly higher in HCV patients and 6 cases of papillary thyroid cancer were detected among HCV patients, whereas only 1 case was observed in controls, suggesting a high prevalence of thyroid papillary cancer in HCV patients. Because of this high prevalence of thyroid disorders, the guidelines on management of CHC recommend investigation of thyroid function, including free T4 and TSH in all patients, and since interferon-based therapy could exacerbate thyroid dysfunction, thyroid function tests should be fully evaluated prior to initiating HCV treatment.

Diabetes mellitus and HCV

Data from the literature have shown a higher incidence of type 2 diabetes mellitus with chronic HCV when compared with patients with other liver disorders[72-74]. In a large study[75] involving 229 consecutively recruited MC-HCV patients compared with 217 sex- and age-matched controls without HCV infection, the prevalence of type 2 diabetes was significantly higher in MC-HCV patients than in controls. Moreover, MC-HCV diabetic patients more often had non-organ-specific autoantibodies than non-diabetic MC-HCV patients.

Another study conducted in 2005 by the same group[22], established the prevalence and clinical phenotype of type 2 diabetes in a large series of non-cirrhotic HCV patients. The prevalence of type 2 diabetes was significantly higher in HCV patients compared with control subjects or non-cirrhotic HBV patients. Moreover, type 2 diabetic HCV patients had a significantly lower BMI than type 2 diabetic control subjects and significantly higher BMI than non-diabetic HCV patients. In contrast, no association with diabetes mellitus type 1 has been identified[22,72,73,76-78]. The association between chronic HCV and diabetes mellitus seems to be independent of the severity of the liver disease and is associated with insulin-resistance, but not with the presence of pancreatic anti-insulin antibodies[79]. In contrast, interferon treatment of HCV has been associated with the appearance of diabetes mellitus type 1 and development of anti-

pancreas autoimmunity[80-82].

AIH and HCV infection

Finally, an intriguing, still controversial aspect is the possible etiopathogenetic role of HCV in AIH[3,6,8,9,65]. Patients with AIH may present with mixed cryoglobulins, HCV infection, and extrahepatic manifestations such as thyroiditis, sicca syndrome, and arthritis[6], while patients with HCV infection show one or more non-organ-specific auto-antibodies. The antigenic target specificity of HCV-related autoantibodies shows only quantitative differences compared with those associated with "primary" AIH[8].

In clinical practice, the search for serum autoantibodies should be limited to cases for whom treatment with IFN is planned. An exception may be cases where clinical data (female gender, young age), high biochemical activity (transaminase-globulins) and histological aspects (interfaces hepatitis) of liver disease may suggest the presence of AIH with superimposed HCV infection.

The heterogeneous geographical distribution of HCV-associated AIH[65] suggests a possible involvement of various pathogenetic co-factors; among these, HCV might trigger a particular AIH clinico-serological subset, which is prevalent in specific geographical areas.

CONCLUSION

In the case of patients with chronic HCV infection, the possible existence of extrahepatic manifestations should be taken into account and an accurate analysis of clinical and anamnestic data is recommended. Some patients may display the entire complex spectrum of HCV-related disorders which could be mild for many years and progress, generally during a long follow-up, to more severe systemic manifestations. In the last few years, very consistent data have been accumulated through different *in vivo* and *in vitro* models, suggesting that a more accurate characterization of the modalities and consequences at the molecular level of HCV infection of lymphatic cells may be of great importance in the future for the clarification of the pathogenesis of several pathological manifestations of HCV.

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