

Review article

# Autoimmune reactions in the course of the hepatitis C virus (HCV) infection

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## Abstract

The immune response to the presence of the virus, both specific and non-specific, plays a decisive role in the natural history of the infection, and influences the intensity of lesions in the liver. Despite the great progress which we were able to observe over the last several years, many issues still require clarification.

The problem of autoimmune reactions during hepatitis C virus (HCV) infection includes at least two issues. First, the risk of exacerbating reactions against the organism's own tissues that existed before the treatment. There is also an increased risk of the development of *de novo* autoimmune reactions, triggered mostly by interferon  $\alpha$ . Hepatitis C virus infection predisposes to the development of diseases characterised as being certainly or probably immune-mediated.

Currently the situation has changed due to introducing non-interferon therapies for HCV treatment, which eliminate the risk associated with immunotherapy in patients with autoimmune diseases, yet the therapies are not widely available.

**Key words:** HCV, autoimmune reactions, pegylated interferon.

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Infection with the hepatitis C virus (HCV) is currently one of the most common problems of infectious hepatology. Assuming that anti-HCV antibodies are found in approx. 2% of our country's population, and in up to 4% of the global population, it is one of the greatest threats to public health at both the global and domestic levels. The issue of the percentage of seropositive individuals in whom it is associated with an active infection remains debatable. Most epidemiological analyses, especially those from the early stages of HCV research, included a statement that the chronicisation of the infection process can apply to as many as 80% of anti-HCV-positive individuals [1]. Recent studies, including the largest epidemiological study in Poland so far, which encompassed more than 26 thousand people, however, shed a different light on this matter. It was demonstrated that among 496 seropositive individuals (which accounted for 1.9% of the study

subjects), HCV-RNA (Ribonucleic acid) was present in "only" 31%. This is important, as PCR (polymerase chain reaction) examination is becoming the essential test for determining the patient's status [2]. The issue of the presence of the virus's genetic material, i.a. an active HCV infection, is connected with the problem of immunisation caused by HCV. The immune response to the presence of the virus, both specific and non-specific, plays a decisive role in the natural history of the infection, and influences the intensity of lesions in the liver [3]. Despite the great progress which we were able to observe over the last several years, many issues still require clarification.

The problem of autoimmune reactions during HCV infection includes at least two issues: 1) the risk of exacerbating reactions against the organism's own tissues that existed before the treatment; and 2) the development of *de novo* autoimmune reactions, triggered

mostly by interferon  $\alpha$ . Hepatitis C virus infection predisposes to the development of diseases characterised as being certainly or probably immune-mediated, such as autoimmune hepatitis, Guillain-Barré syndrome, glomerulonephritis, cryoglobulinemia, vasculitis, Behçet's disease, and thyroid gland diseases. In the last case, the evident factor is antibodies targeting thyroglobulin (anti-TG) and thyroperoxidase (anti-TPO). According to some researchers, the percentage of people infected with HCV with thyroid-gland disorders amounts to 42% [4-6]. The list of disease entities, which are only probably immune-mediated or connected with HCV, is even longer. Such diseases as idiopathic pulmonary fibrosis, myasthenia gravis, peripheral neuropathy, coeliac disease, and autoimmune gastritis, are being more and more categorised as examples of possible etiopathogenetic interrelations with HCV infection [6].

Urticarial vasculitis can serve here as an example of complex interrelations. This disease entity is often identified in association with mixed cryoglobulinemia in the course of HCV infection; however, there have been virtually no cases of its association with HCV infection with no cryoglobulinemia. What is therefore the conditioning factor behind the autoimmune nature of urticarial vasculitis – the virus or cryoglobulins, whose creation it influences? In addition, in this case, as well as in other cases, there are causative factors of autoimmune processes other than HCV [6].

The identification of HCV in 1989, and the subsequent identification of the virus's presence in a series of diseases of unknown aetiology, as well as in the mechanism often classified as "possible autoimmune background", resulted in HCV being cited as the most important factor immunising the human body. Over time, the scope of HCV-dependent diseases has become more and more specific, largely thanks to the implementation of modern diagnostic methods [7,8]. These, of course, did not resolve all the doubts. Is the fact of HCV replication, which can be proven by the presence of antigenomic and genomic HCV-RN, being present in coincidence with cutaneous lesions in the course of lichen planus, proof of a pathogenetic relationship? And what about other patients, with no such sequences? Can the presence of the viral genome fragments in the brains of people with HCV explain their depressive behaviour? How can we therefore explain that such fragments were also found (during autopsies) in individuals in which HCV had been eradicated? And finally, does the idiopathic and interferon- or ribavirin-based elimination of HCV equal the breaking of the chain of autoimmune phenomena? These questions have bothered HCV researchers for years,

and unfortunately, there are no explicit answers to many of them [9].

When it comes to extrahepatic immune-mediated processes, B lymphocytes are of special importance. An increase in the concentration of the B-lymphocyte activating factor (BAFF) is associated with autoimmune phenomena, which is proven by clinical symptoms, as well as by laboratory-test results. There have been suggestions that it may be BAFF that plays the decisive role in the development of HCV-dependent autoimmune reactions [10]. The lymphotropic nature of HCV is another factor conducive to the development of autoimmune processes, especially to the production of organs' non-specific antibodies. Interaction between the virus and B lymphocytes lowers their activity threshold, facilitating the production of antibodies [11,12].

We should also mention here the importance of genetic factors. For example, Vento *et al.* suggest that the presence of HLA (human leukocyte antigen) haplotype A1-B8-DR3 and DR4 is associated with the presence of autoimmune reactions and the possibility of developing hepatitis fulminans [13,14]. Recently, researchers have demonstrated a relationship between the development of mixed cryoglobulinemia and the presence of HLA haplotype HLA-B6-DR3. On the other hand, HLA-DR4 antigen can be found in HCV-positive patients with rheumatoid arthritis. Autoimmune thyroid diseases affect women with haplotype HLA-DR3. Genetic factors can explain why the utilisation of the IFN- $\alpha$  therapy in some patients results in the loss of tolerance for the organism's own cells and the occurrence of severe autoimmune reactions [15].

As observed by some authors, the apoptosis process involving the Fas-L/Fas system in chronic hepatitis C affects mainly immune-system cells present in the liver parenchyma. Of course, any such conclusion has to be made very carefully, as the complexity of the mechanisms of this process requires the utilisation of sophisticated methods in respect of peripheral blood cells [16]. On the other hand, Israeli researchers, when confirming the lack of differences in bcl-2 expression in peripheral blood cells of individuals with chronic hepatitis C, simultaneously demonstrated a decrease in nuclear-factor kappa-B (NFkappaB) expression, which, according to them, was an important reason for the increased apoptosis of peripheral blood lymphocytes [17]. This situation applies only to CD5-cells, as CD5+ cells seem to be somewhat resistant to apoptosis, which can be of importance in the development of autoimmune processes [18]. However, a substantially lower level of NFkappaB in patients infected with HCV with indicators of autoimmune reactions, such as cryoglobulins

and rheumatoid factors, is puzzling [19]. An additional element is the excessive expression of CD81, regarded as an HCV receptor, on the surface of CD5 lymphocytes. What is however most important in the evidence of the relevance of these immunological parameters in the development of HCV pathologies is their reversibility after successful antiviral treatment [20].

Another factor conducive to immunisation is the molecular-mimicry mechanism. In the case of HCV infection, like in other infectious-disease processes, the similarity between some particles and antigens of the organism facilitates the stimulating of the production of antibodies against the organism's own cells. It is known that such a mechanism takes place in the case of antinuclear antibodies (ANA) and anti-smooth muscle antibodies (ASMA). What is more, a homology between amino-acid sequences in HCV polyprotein and some myosin (282ATQQEL) and actin (313AMQQKL) sequences was demonstrated [21]. However, despite this homology, no double reactivity of antibodies was established, which shows the complexity of the process of immunological tolerance towards one's own antigens.

There is also a hypothesis that the damaging impact of the virus on the cells can trigger the release of antigens, which are normally protected from identification by the immune system. This can be associated with their splitting, and a change in structure or expression on the cell surface. One can venture a statement that such effects of inflammation are possible irrespective of the causal factor, yet we can discuss auto-immune processes only in the case of selected pathogens. Perhaps the aforementioned apoptosis is of key importance here, as it safeguards the organism's integrity, and is responsible for the elimination of "hazardous" cells [22].

The extensive research on determining which antigens in the course of HCV infection are the most important target of antibodies failed to specify those "important and less-important". It was highlighted that e.g. anti-neutrophil cytoplasmic antibodies (ANCA), which are present, i.a. in the course of systemic vasculitis, are specific to proteinase 3. In turn, anti-dihydro-lipoamide dehydrogenase antibodies (anti-E3) are more frequently present in HCV patients than in PBC (primary biliary cirrhosis) patients, yet in both these cases their significance has not been fully explained [16,23]. However, it is known that they are virtually absent in HBV (hepatitis B virus) infection.

In turn, analyses of the prevalence of auto-immune processes in patients infected with various genotypes indicate no interrelation with this factor. Autoimmune reactions are not restricted to the HCV-induced pro-

duction of antibodies, but apply to a much broader range of phenomena [8].

For infectious-disease specialists, the topic of phenomena of an autoimmune nature is associated with problems with making the right diagnosis and the necessity to make a decision on qualifying the patient for (or disqualifying from) antiviral therapy, which is no less important. Giving the final answer to the question which reactions are a result of HCV infection, and which a mere coincidence, is not an easy task. One has to be aware of the fact that, as mentioned in the introduction to this text, in Poland alone, anti-HCV can be found in more than 700 thousand individuals [2]. Therefore, there is a real chance of the concomitance of two medical problems. In addition, the probability of coincidence is increased by the fact that many infections are of an iatrogenic nature, and in principle apply to people with other health-related problems.

The immunological response to HCV infection can include the production of cryoglobulins, rheumatoid factor (RF), ANA, anti-cardiolipin antibodies (aCL), anti-liver kidney microsomal antibodies (anti-LKM), and the creation and deposition of HCV/anti-HCV complexes. Among the above, a special role is attributed to cryoglobulins, whose presence in HCV patients is commonly regarded as an indicator of mixed cryoglobulinemia [24]. The etiopathogenetic interrelation, initially suggested in epidemiological data, was eventually confirmed using the polymerase chain reaction (PCR). HCV-RNA sequences were identified in cryoprecipitates and cutaneous lesions, while in the inflammatory foci of capillaries the presence of HCV complexes and IgM and IgG antibodies was demonstrated using immuno-histochemical methods and in-situ hybridisation techniques. Immunological complexes, by activating the endothelium cells of vessels, cause an increase in vascular permeability and damage to the vessel wall. Additional evidence is the regression of cutaneous lesions, following successful treatment with interferon alpha and ribavirin. Interestingly enough, in most cases cryoglobulinemia is of type II and III; however, in individuals infected with HCV genotype 2, type I cryoglobulinemia can also be observed [9,24].

Mixed cryoglobulinemia can be diagnosed in association with membranous glomerulonephritis, membrano-proliferative glomerulonephritis, vasculitis, diabetes, coeliac disease and myasthenia gravis. It should be explicitly highlighted that membrano-proliferative glomerulonephritis can appear in HCV patients without the associated cryoglobulinemia [9].

The relationship between HCV infection and systemic lupus erythematosus (SLE) still has not been explained. It is a fact that in some geographical regions

the prevalence of anti-HCV antibodies is higher in SLE patients than among blood donors. On the other hand, the clinical symptomatology of SLE in HCV-infected people is slightly different from the remaining cases. Therefore, it has been suggested that SLE patients be divided into three groups: 1) patients with a false-positive result of antibody tests; 2) HCV-infected individuals with “real” SLE; and 3) patients with SLE-like syndromes probably induced by HCV infection [25]. “All” that is required is to correctly classify each patient, which of course appears to be the most difficult task.

Big controversies are sparked by the relationship between HCV infection and anti-phospholipid syndrome. In the case of most HCV patients with aCL, there are no clinical manifestations of this syndrome, which indicates the lack of significance of aCL, especially if lupus anticoagulant testing yields a negative result. Doubts as to the pathogenetic relationship resulted in guidelines of scientific associations lacking a provision on the necessity of anti-HCV testing in patients with anti-phospholipid syndrome [26].

The problem of the concomitance of HCV infection and sialadenitis, whose course is virtually identical to that of idiopathic Sjögren’s syndrome, is strikingly different. Both epidemiological data and the results of immunological studies suggest its pathogenetic connection with the infection. It is known that sialadenitis and the sensation of dryness are common with chronic HCV infection. The analysis of 137 HCV-positive patients with Sjögren’s syndrome symptoms, conducted by Spanish authors, demonstrated joint involvement in 44% of patients, vasculitis-type lesions in 20%, and neuropathy in 16%. There is a fairly broad panel of immunological indicators associated with Sjögren’s syndrome, and it is subject to substantial individual variations. Antinuclear antibodies are present in 65% of cases, while the presence of cryoglobulins, as well as complement deficiency applied to 50% of patients, and anti-Ro/SS-A or anti-La/SS-B antibodies are found in fewer patients, especially in women [27]. The correct diagnosis is important insofar that, according to Ramos-Casals *et al.*, HCV can be the most crucial etiopathogenetic factor in Sjögren’s syndrome. HCV-dependence can be indicated by the presence of IgM monoclonal immunoglobulins with a more restrictive expression and HLA-DQB1\*02 [28].

Another problem is related to the concomitance of coeliac disease and HCV infection. As observed by Germenis *et al.*, coeliac disease with anti-transglutaminase antibodies is conditional on the degree of liver damage, irrespective of its aetiology (the comparison included patients with various stages of liver disease,

caused by chronic viral infections, alcohol, and other toxic factors, non-alcoholic fatty-liver disease, and autoimmune hepatitis) [11].

HCV infection can stimulate the creation of different non-organ-specific autoantibodies, often with a low titre. The clinical significance of this phenomenon remains to be explained [8]. It appears that it goes beyond the simplified view that it is a result of the non-specific polyclonal stimulation of B cells.

Diseases with a proven or probable autoimmune origin for years have been a counter-indication of antiviral therapy, which has resulted from the structure of clinical studies. Currently increasingly more often the therapy indications are extended, and in the case of evident etiopathogenetic links with HCV (e.g. cryobulinemia) alpha interferon and ribavirin therapy with or without the addition of new HCV inhibitors is widely chosen, however interferon-free regimens will replace this procedure soon.

However, developing an autoimmune reaction is the largest risk associated with HCV. The most frequent risks include (1) thyroid-gland disorders, among which it is possible to distinguish: (a) autoimmune thyroiditis; Hashimoto’s thyroiditis; (b) Graves’ disease. There is also the possibility of non-autoimmune diseases such as (c) destructive thyroiditis and (d) non-autoimmune hypothyroidism. Moreover, the following can be observed: (2) autoimmune thrombocytopenia, (3) autoimmune anaemia, (4) psoriasis, (5) rheumatoid arthritis, (6) disorders resembling systemic lupus, (7) autoimmune gastritis, (8) sarcoidosis; and liver diseases with a defined autoimmune origin, such as (9) primary biliary cirrhosis and (10) autoimmune hepatitis (AIH).

The disturbed balance of the Th-1 response seems to be the cause of interferon-therapy-induced AIH and Graves’ disease. The two adverse events of the therapy appear to be the most significant, as they can lead to life-threatening thyroid crises and hyper-acute hepatitis [29].

The presented data on the coexistence of autoimmune reactions in patients with chronic HCV do not point to a single procedure to be applied. It seems that the general recommendations are still valid: 1) a final (differential) diagnosis should be aimed at; 2) it is necessary to individualise the procedure (an assessment of the HCV infection status); 3) it is necessary to evaluate the risk and the potential benefits (it should be borne in mind that unjustified inaction is unacceptable); and 4) careful monitoring of the patient and cooperation with specialists from various medical disciplines.

The subject matter of immunisation and immunology in the course of HCV infection still remains

not fully explored, posing a challenge to researchers of the correlations between the infection and immunological reactivity. The important practical implications of the described phenomena mean the results of research studies on the issue are awaited with high expectations. On the other hand, before implementing conclusions from experiments to everyday clinical practice, a careful assessment of the new findings is indispensable, as therapeutic errors can have grave consequences for the patient. Currently the situation has changed due to introducing interferon-free therapies for HCV treatment, which eliminate the risk associated with immunotherapy in patients with autoimmune diseases, yet the therapies are not widely available and the aforementioned issues are still relevant.

## Disclosure

Authors report no conflict of interest.

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