

Autoimmunity Against Pancreatic Islets and Other Tissues Before and After Interferon- α Therapy in Patients With Hepatitis C Virus Chronic Infection

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apy may be useful in assessing the risk of patients developing autoimmune disease.

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OBJECTIVE — The aim of the study was to investigate the prevalence of clinical and latent autoimmune diseases in Italian patients with hepatitis C virus (HCV) chronic infection before and after treatment with interferon- α (IFN- α).

RESEARCH DESIGN AND METHODS — The evidence of clinical autoimmune disease and the presence of autoantibodies were assessed in 70 patients with HCV chronic infection. Autoantibodies to islet cell (ICA), glucagon-producing cells (GCA), parietal cell (PCA), adrenal cortex (ACA), adrenal medulla (AdMA), nuclei (ANA), liver-kidney microsomal (LKM-Ab), mitochondrial, and smooth muscle (SMA) were tested using the classic indirect immunofluorescence technique. Autoantibodies to GAD (GADAb), second islet cell autoantigen (IA2-Ab), and insulin (IAA) were tested by radioimmunoassay, and thyroid microsomal autoantibodies (TMHA) and thyroglobulin autoantibodies (TGHA) were assessed by hemoagglutination test.

RESULTS — None of the 70 patients studied showed evidence of clinical disease before treatment with IFN- α . However, 1 (1.4%) patient was positive for ICA, 2 (2.8%) were positive for GCA, 2 (2.8%) for GADAb, 5 (7.1%) for PCA, 2 (2.8%) for ANA, 3 (3.7%) for SMA, 4 (5.7%) for TMHA, and 2 (2.8%) for TGHA. These frequencies were not significantly different when compared with healthy control subjects. There were 29 (41%) patients who were positive for IAA at low titers compared with 2% of the control subjects (significantly different $P < 0.0001$). ICA titers of one patient positive for ICA/GADAb increased during the IFN- α therapy, and the patient developed type 1 diabetes 5 months after the beginning of treatment. IAA levels did not change during the course of treatment, and none of the IAA⁺ patients developed diabetes. Thyroid autoantibody titers increased in 3 of the 4 initially positive patients, with 1 patient becoming positive and 2 thyroid antibody-positive patients developing overt hypothyroidism during IFN- α treatment. PCA titers increased in 1 of 5 positive patients. Antibodies to other autoantigens did not change during the course of treatment.

CONCLUSIONS — We have not found an increased frequency of clinical or latent autoimmune diseases in patients with chronic HCV infection. However, this study suggests that screening patients for autoantibodies (in particular, thyroid and pancreas) before and during IFN- α ther-

Hepatitis C virus (HCV) infection may affect not only the liver but also various nonhepatic tissues (1). An increased rate of diabetes in patients with HCV-related hepatic cirrhosis compared with other etiologies has been reported, but in many cases, diabetes has not been clearly classified because of the lack of genetic and immunological data (1,2). There are conflicting reports of prevalences of thyroid autoimmune disease (clinical or latent) in patients with HCV chronic infection compared with HCV⁻ subjects (3). These discrepancies may be related to ethnic differences in the populations studied, selection of the patients, diagnostic criteria, and the techniques used for the immunological assessment. The mechanism for the extrahepatic manifestations associated with HCV infections is unclear, but it could be related to a direct pathological effect on the cells or autoimmune processes against target antigens (1).

As a drug, interferon- α (IFN- α) has been shown to have different biologic effects (antiviral, antiproliferative, and immunomodulatory), and it is used for treatment of chronic viral and neoplastic diseases (4). IFN- α acts on many target cells and organs (4). Different side effects have been reported in patients treated with IFN- α , but their incidence and prognosis in the case of adverse reactions remain largely unknown (4). In particular, an increase in the production of thyroid autoantibodies with subsequent development of clinical thyroid diseases has been described in patients undergoing IFN- α therapy for chronic hepatitis caused by HCV (5,6). Furthermore, a high prevalence of diabetes as well as other autoimmune diseases was found after IFN- α therapy (5,6). Among a number of patients who developed type 1 diabetes, so far only 8 have been accurately studied for immunological and genetic markers before and at the time of the development of clinical disease (7). All

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Abbreviations: ACA, adrenal cortex autoantibodies; AdMA, adrenal medulla autoantibodies; AMA, mitochondrial autoantibodies; ANA, nuclear autoantibodies; GADAb, GAD autoantibodies; GCA, glucagon-producing cells autoantibodies; HCV, hepatitis C virus; IA2-Ab, second islet cell autoantigen; IAA, insulin autoantibodies; ICA, islet cell autoantibodies; IFN- α , interferon- α ; JDF U, Juvenile Diabetes Foundation units; LKM-Ab, liver-kidney microsomal autoantibodies; MHC, major histocompatibility complex; PCA, parietal cell autoantibodies; PCR, polymerase chain reaction; RIA, radioimmunoassay; SMA, smooth muscle autoantibodies; TGHA, thyroglobulin autoantibodies; TMHA, thyroid microsomal autoantibodies.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—Clinical, histological, and virological features of the study group

Clinical profiles and laboratory data	Patients (n = 70)	%
Sex (M/F)	60/10	—
Mean age (years)	36.2 \pm 12 (range 22–65)	—
Duration of disease (years)*	8.5 (range 2–21)	—
HCV genotypes		
1b	23	32.8
1a	6	8.6
2c	8	11.4
3a	29	41.4
4	4	5.7
Chronic hepatitis + cirrhosis	3	4.3
Chronic hepatitis	67	95.7
HAI score (mean \pm SD)	6.7 \pm 1.3	—

*Duration of the disease has been evaluated considering either the first detection of anti-HCV antibody and/or the finding of abnormal liver function test or the onset of acute hepatitis C. HAI, hepatitis activity index.

were positive for one or more markers of pancreatic autoimmunity before treatment and all had HLA-DR3 and/or HLA-DR4 genetic markers of autoimmune type 1 diabetes. These observations suggested that in predisposed individuals, IFN- α therapy could amplify an already present autoimmune aggression against β -cells, which can result in the onset of clinical disease (7). However, it is unclear whether IFN- α alone may be a sufficient triggering factor to induce seroconversion in genetically predisposed individuals.

The aim of this study was to investigate the prevalence of clinical and latent autoimmune diseases (organ- and non-organ-specific) and the change over time of specific antibodies in Italian patients with chronic hepatitis caused by HCV, before and after treatment with IFN- α .

RESEARCH DESIGN AND METHODS

Patients

Seventy patients (60 men and 10 women) mean age 36.2 years (range 22–65) (Table 1) with chronic hepatitis C were recruited from those treated with recombinant IFN- α between 1990 and 1997 at the Infectious Diseases Department (S. Bortolo Hospital, Vicenza, Italy). Nine patients had a family history of diabetes (7 for type 2 diabetes and 2 for type 1 diabetes). One patient had a family history of nonspecified thyroid disease.

All patients were enrolled according to the following criteria: 1) treatment by IFN- α with the same protocol for 6 months; 2) histologic demonstration of

chronic hepatitis; and 3) availability of serum samples from each patient before and after at least 1 year from IFN- α therapy suspension. Furthermore, patients were followed after the suspension of the IFN- α therapy for at least 4 years.

All patients were positive in the screening test for anti-HCV and confirmed by recombinant immunoblotting assay version 2 (RIBA 2; Ortho Diagnostic Systems, Raritan, NJ) but were negative for either hepatitis B surface antigen or human immunodeficiency virus (Abbott Laboratories, Chicago). HCV-RNA tests using nested polymerase chain reaction (PCR) with primers in the 5' noncoding region were positive in all patients before commencement of IFN- α therapy (8). HCV genotypes were analyzed by nested PCR using genotype-specific primers of the core region and by DNA enzyme immune assay DEIA (Sorin Biomedica, Saluggia, Italy) hybridization with subtype-specific probes according to the classification of Viazov et al. (9) and Simmonds et al. (10). None of the patients enrolled in the present study had a personal history of or declared habitual alcohol abuse. Of the 70 subjects studied, 52 (74%) had a history of previous intravenous drug use, 5 (7%) had received blood transfusions, and the remaining 13 (19%) had no apparent risk for blood-borne infection. Histologic examination of percutaneous liver biopsy specimens obtained before beginning treatment with IFN- α confirmed chronic hepatitis in each patient. Hepatic necroinflammatory activity and fibrosis were scored according to the system of Knodell et al. (11). The evaluation of liver

histology was carried out by the same pathologist in a blinded fashion. All patients were given recombinant IFN- α 2b (Intron A; Shering Plough, Milan, Italy) 5 mU 3 times/week for 3 months followed by 3 mU 3 times/week for another 3 months. The main characteristics of the patients studied are shown in Table 1.

Serum samples collected before and after therapy suspension were tested for organ- and non-organ-specific autoantibodies. As the control, we tested serum samples collected from 100 healthy subjects (without any autoimmune disease in their personal and/or family history) matched for sex and age with the study group.

Organ-specific autoantibodies

The following organ- and non-organ-specific autoantibodies were tested:

Pancreatic autoantibodies

- *Islet cell autoantibodies.* Islet cell autoantibodies (ICA)-IgG were detected by the classic indirect immunofluorescence technique on cryostatic sections of normal human pancreas, as previously described (12). Titers of reactivity were expressed in Juvenile Diabetes Foundation units (JDF U), considering a normal titer to be <5 JDF U. Sensitivity and specificity of ICA results from our laboratory were validated in the international ICA proficiency test.
- *GAD autoantibodies and autoantibodies to the second islet cell autoantigen.* GAD autoantibodies (GAD-Ab) and second islet cell autoantigen (IA2-Ab) were detected by radiobinding assay with in vitro translated [³⁵S]methionine-labeled GAD65 or IA2-Ab, as previously described (13). Results were converted into arbitrary units by extrapolation from a standard curve with a local standard, designated as 100 U (undiluted) and diluted 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64 in negative serum. The thresholds for positivity were determined from the 99th percentile of the control subjects and corresponded to 3 U for GAD-Ab and 1.5 U for IA2-Ab.
- *Insulin autoantibodies.* Insulin autoantibodies (IAA) were detected by radioimmunoassay (RIA) using a commercial kit (CIS Bio-international) and calculating the percent binding. The positive sera were absorbed by cold insulin and retested calculating the residual activity that was determined subtracting percent binding of set B (after absorption) from the percent binding of set A (before absorption) to give the $\Delta\%$. The threshold established based on control subjects was 7 \pm 3% (mean \pm SD). The sensitivity and specificity of IAA results from our laboratory were defined in the international IAA proficiency test (14).

Thyroid autoantibodies. Microsomal antibodies (TMHA) and thyroglobulin autoantibodies (TGHA) were detected, using commercial kits (Thymume M and T; Abbott-Murex, U.K.) by a passive hemoagglutination technique as previously described (15). Titer 1/100 was considered a normal value in both assays.

Gastric autoantibodies. Parietal cell autoantibodies (PCA) were detected by the classic indirect immunofluorescence technique on normal human gastric cryosections as previously described (15). The threshold established for positive samples was $<1/3$.

Adrenal autoantibodies. Adrenal cortex autoantibodies (ACA) and adrenal medulla autoantibodies (AdMA) were detected by the classic indirect immunofluorescence technique on normal human adrenal cortex cryosections as previously described (15). Nonreactivity was considered a normal result.

Non-organ-specific autoantibodies

Nuclear (ANA), mitochondrial (AMA), liver-kidney microsomal (LKM-Ab), and smooth muscle (SMA) autoantibodies were detected by the classic indirect immunofluorescence technique on cryostatic sections of rat kidney and liver as previously described (15). The threshold established as a normal value was $<1/10$.

RESULTS

Clinical study

None of the 70 patients studied had clinical thyroid diseases, type 1 diabetes, pernicious anemia, Addison's disease, or non-organ-specific autoimmune diseases at the beginning of IFN- α therapy.

Organ-specific autoantibodies

Pancreatic autoantibodies. Before IFN- α treatment, one patient (1.4%) had a low titer (1:5 JDF U) of the classic ICA pattern and high levels of GADAb (>100 U). Another patient had high levels (92 U) of GADAb associated with low levels (7.9%) of IAA, whereas 2 other patients (2.8%) were positive for glucagon-producing cells (GCA). All 4 patients were negative for IA2-Ab.

In the control group, only one patient was found to be positive for ICA (1%), whereas none showed positivity for GAD-Ab or IA2-Ab. The prevalence of these autoantibodies in patients was increased but not significantly different from normal control subjects.

There were 29 (41%) patients positive for IAA at low titers. In the control group, IAA were present in 2 (2%) patients. The positivity for IAA in HCV+ patients was significantly higher than that in the normal control group ($P < 0.0001$).

During treatment, the patient positive for both ICA and GADAb showed an increase of the ICA titers (from 5 to 80 JDF U) and the persistence of high titers of GADAb (>100 U). He developed type 1 diabetes 5 months after the start of therapy and is on insulin therapy 4 years after the suspension of IFN- α therapy. This case subject had a family history of type 1 diabetes, as previously described (7).

After IFN- α therapy, the patient positive for GAD of GADAb and IAA maintained high levels (48 U) and low levels (8.2%) of IAA; 2 other patients remained positive for GCA, without any changes in the positivity for GADAb, IA2-Ab, and IAA; and none developed type 1 diabetes.

Thyroid autoantibodies. Before treatment, TMHA were positive in 4 patients (5.7%) (3 women and 1 man) at titers of 1:100, 1:400, 1:1,600, and 1:6,400, respectively. Two of these were also positive for TGHA (1:100 and 1:320, respectively). In the control group, 3 subjects (3%) were positive for thyroid autoantibodies; thus, the prevalence of these autoantibodies in patients was increased but not significantly different from that of normal control subjects. After treatment, in 3 of 4 initially positive patients, TMHA titers increased from 1:100 to 1:1,600, from 1:400 to 1:1,600, and from 1:6,400 to 1:25,600, respectively; in the fourth patient, titers remained unchanged. A fifth patient (a woman who was initially negative) seroconverted for TMHA (from 0 to 1:400). Among the 2 initially TGHA+ patients, 1 showed an increase of the titers from 1:100 to 1:640, whereas no change was observed in the other. The 2 patients initially positive for thyroid autoantibodies developed clinical hypothyroidism, and they are still on L-thyroxine therapy 6 months after the beginning of IFN- α therapy; one of these patients had a family history of thyroid disease.

Gastric autoantibodies. Before IFN- α therapy, PCA were found positive in 5 patients (7.2%) (4 men and 1 woman) with titers of 1:10, 1:10, 1:10, and 1:80, respectively. In the control group, 2 (2%) subjects were found positive for PCA. The prevalence of PCA in patients was increased but not significantly different than that in the normal control group. After therapy, 1 PCA+

patient increased in titer from 1:10 to 1:80, whereas in the other 4, the levels of seropositivity remained unchanged.

Adrenal autoantibodies. Before and after IFN- α treatment, all patients were negative for ACA and AdMA, and none developed clinical adrenal insufficiency. All of the normal control subjects were negative for ACA and AdMA.

Non-organ-specific autoantibodies

Before IFN- α treatment, the screening for non-organ-specific autoantibodies revealed the presence of ANA in 2 patients (2.9%) at titers of 1:80 and 1:160 and the presence of SMA in 3 other patients (4%). After therapy, no variation in the titers of non-organ-specific autoantibodies was found, and no collagen diseases were observed. In the normal control group, ANA were present in 2 (2%) and SMA in 2%. The incidence of ANA and SMA in patients was not significantly different from that in the normal control subjects. The other autoantibodies tested were negative in all of the control subjects' sera.

CONCLUSIONS — A significant increase in autoimmune phenomena, particularly an increased frequency of diabetes (1,2), has been described in patients treated with IFN- α for infections or neoplasms, but the pathogenesis or mechanisms of this increase is still unknown (16). Furthermore, a review of a large cohort of Italian patients with HCV chronic hepatitis reported that in these patients, diabetes that requires insulin therapy was more frequent than that in the control population (6). In these studies, no immunological and genetic data were reported. Recently, we described both a patient with chronic HCV hepatitis treated with IFN- α who developed type 1 diabetes and at the same time we reviewed 7 other similar cases (7). This study showed that patients who developed type 1 diabetes were positive for 1 or more pancreatic autoantibodies before IFN- α therapy, and all were positive for HLA-DR3 and/or HLA-DR4 (7). These observations suggested that treatment with IFN- α might amplify an already existing autoimmune response against β -cells. However, it is under discussion if in these patients, when compared with healthy control subjects, the HCV infection can induce an increase in the frequency of pancreatic autoimmunity. Furthermore, the IFN- α therapy induces a seroconversion as occurs in the thyroid autoimmunity.

To investigate this question, we studied the prevalence of the serological markers of

pancreatic autoimmunity in HCV⁺ patients before and after IFN- α therapy. Our study shows that the prevalence of ICA, GADAb, and/or IA2-Ab in patients with HCV chronic infection is not different from the prevalence of these autoantibodies in normal control subjects. Although GCA have been detected in 2 HCV⁺ patients, these autoantibodies are not considered clinically significant markers of pancreatic autoimmunity (17). A relatively high prevalence (42%) of low levels of IAA was found among our patients. IAA levels did not change during the IFN- α therapy and none of the IAA⁺ patients developed type 1 diabetes. The frequent finding of IAA not associated with ICA in sera from patients with common viral infections has been reported previously (18). It has been postulated that viruses may trigger the production of IAA through polyclonal immunocyte activation (18). In another study, an increase in IAA levels not associated with ICA was observed after IFN- α therapy, but none of the positive patients developed type 1 diabetes (19). Consequently, our findings of an increased prevalence of low levels of IAA in patients with HCV infection is in agreement with the previous observations that the production of IAA might be secondary to the viral infection itself (18). In contrast, high levels of IAA in association with other markers of pancreatic autoimmunity are currently well-recognized diagnostic and predictive markers of type 1 diabetes in children and in the first-degree relatives (20,21). We suggest that IFN- α therapy might only amplify the pancreatic autoimmune phenomena in these patients.

The prevalence of clinical thyroid autoimmune diseases and the prevalence of thyroid autoantibodies in patients with HCV chronic infection, before IFN- α therapy are not clearly defined because discrepant results have been reported in different studies (3). This may be due to the different characteristics of the populations studied, to the different diagnostic criteria, and/or to the differences in the methods used for autoantibody detection (1). However, our study does not confirm the increased prevalence of thyroid autoantibodies in patients with HCV chronic infection before IFN- α therapy. In agreement with previous reports (3,5,6), our study shows that after IFN- α treatment, the titer of thyroid autoantibodies tends to increase or a seroconversion can be observed in initially negative patients; in both situations, a high risk of developing clinical thyroid disease is evident.

In these patients, the IFN- α therapy may both amplify and induce new phenomena of thyroid autoimmunity.

The primary mechanism by which the IFN- α therapy begins and amplifies chronic thyroiditis, Graves' disease, and type 1 diabetes is still under discussion. Actual concepts concerning the pathogenesis of IFN-associated autoimmunity include induction of major histocompatibility complex (MHC) and other molecules as well as the modulation of lymphocyte functions (22,23). However, in experimental models of autoimmune thyroid disease, a dissociation was found between the MHC class I and II expression versus the production of thyroid autoantibodies (24). Another possible explanation is that cytokines might act as mediators of immune-endocrine regulation circuits that can interfere with the endocrine system on all levels of the hypothalamic-pituitary-adrenal axis (25).

Finally, among our patients with HCV chronic hepatitis before IFN- α therapy, we have not found evidence of other organ and non-organ autoimmune diseases or an increased prevalence of organ- and/or non-organ-specific autoantibodies compared with healthy control subjects, and this remained unchanged after the treatment. This cannot be easily explained, but it shows that non-organ-specific autoimmune diseases might have both different immunological mechanisms and different genetic susceptibility with respect to organ-specific autoimmunity.

In conclusion, our study shows that in patients with HCV chronic hepatitis, the prevalence of clinical or latent autoimmune diseases is not higher than that in apparently healthy normal control subjects. However, our results indicate that patients who are initially positive for organ-specific autoantibodies (in particular, thyroid and pancreas) and those who seroconvert are at high risk of developing clinical autoimmune disease after treatment with IFN- α .

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