Induction of autoantibodies to the adrenal cortex and pancreatic islet cells by interferon alpha therapy for chronic hepatitis C

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Abstract

Background/aims—Interferon alpha (IFN-α) therapy for chronic hepatitis C may trigger induction of autoimmunity against several organs. Immune reactions against distinct adrenocortical protein antigens involved in adrenal autoimmune disease have not been reported to date. Therefore, we investigated the development of highly sensitive and specific adrenal autoantibodies in patients with chronic hepatitis C in response to IFN-α treatment. In addition, we studied induction of pancreatic islet and thyroid autoantibodies.

Patients/methods—Sera of 75 patients (42 males, 33 females; mean age 47 (13) years) were analysed before, during, and after IFN-α therapy (9–18×10⁶ IE/week; mean duration 8.3 (3.5) months). Autoantibodies (Abs) to adrenal 21-hydroxylase (21OH-Abs), and to islet glutamic acid decarboxylase (GAD65-Abs) and protein tyrosine phosphatase (IA2-Abs) were determined by a radiobinding assay using [35S]labelled protein generated by an in vitro translation system. Thyroid antibodies were measured by a commercially available ELISA.

Results—Thirteen of 75 patients were initially positive for some of the autoantibodies. During or after IFN-α therapy, 3/62 initially negative patients (4.8%) developed 21OH-Abs, GAD65-Abs or IA2-Abs appeared in 5/62 and 1/62 patients, respectively (9.7% in total). In 12/62 patients (19.4%), thyroid specific antibodies appeared. In none of the 21OH-Ab positive subjects was adrenal dysfunction observed, and no patient with islet autoantibodies developed diabetes mellitus or impaired glucose tolerance.

Conclusions—IFN-α induces 21OH-Abs in some cases, while islet and thyroid specific autoantibodies are more frequently found. However, our results indicate for the first time that the adrenal cortex also has to be considered as a potential target of IFN-α related autoimmunity.

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Keywords: hepatitis C; interferon alpha; autoantibodies; adrenal cortex; pancreatic islet cells

Autoimmune phenomena occurring during treatment with interferon alpha (IFN-α) for chronic hepatitis C virus (HCV) infection have often been observed. In particular, the thyroid gland has been shown to be a target of IFN-α associated autoantibodies, with frequent development of thyroid dysfunction.

Apart from this well known example of IFN-α related autoimmunity, other endocrine organs are possibly targets. Recently, smaller studies showed the development of insulin autoantibodies (IAA) and sporadic manifestations or deterioration of diabetes mellitus is associated with IFN-α therapy. Considering that type 1 (insulin dependent) diabetes mellitus (IDDM) only becomes manifest after destruction of the majority of pancreatic β cells, it seems likely that autoimmune reactions against the islets are more frequent after IFN-α treatment than clinical symptoms.

Previous studies clearly indicated the high diagnostic sensitivity of antibodies (Abs) to glutamic acid decarboxylase (GAD65-Abs) and protein tyrosine phosphatase (IA2-Abs) as predictive markers of IDDM. Particularly in adulthood, common markers such as islet cell antibodies and IAA have a lower prevalence than GAD65-Abs in patients with IDDM. Furthermore, GAD65 has been found to be the major autoantigen in the nonobese diabetic mouse model which may be the triggering antigen for subsequent epitope spreading. GAD65-Abs might therefore represent the most important autoantibodies for disease prediction.

Concerning the adrenal cortex, there are no data on IFN-α induced immune responses against distinct antigens involved in adrenal autoimmune disease. In view of the occurrence of adrenal autoimmunity in organ specific autoimmune diseases, for example autoimmune thyroid disease, IDDM, and Addison’s disease in type II autoimmune polyglandular syndrome, it seems possible that the adrenal cortex may also be susceptible to an IFN-α induced autoimmune response. No induction of adrenal cortex antibodies (ACA) was found in a previous study investigating a small cohort of

Abbreviations used in this paper: Abs, antibodies; ACA, adrenal cortex antibodies; ACTH, corticotrophin; ANA, antinuclear antibodies; GAD65, glutamic acid decarboxylase; HCV, hepatitis C virus; IA2, protein tyrosine phosphatase; IAA, insulin autoantibodies; IDDM, insulin dependent diabetes mellitus; IGt, impaired glucose tolerance; IFN-α, interferon alpha; LKM, liver-kidney microsomal antibodies; NIDDM, non-insulin dependent diabetes mellitus; 21OH, 21-hydroxylase; TG, thyroglobulin; TPO, thyroid peroxidase.
patients with chronic hepatitis B. However, in contrast with the relatively insensitive immunofluorescence test used, measurement of antibodies to 21-hydroxylase (21OH-Abs) by radiobinding assay represents a much more sensitive tool to detect autoimmune related mechanisms in the diagnosis of Addison’s disease.28 29

The aim of the present study was to investigate patients undergoing IFN-α treatment for chronic HCV infection for induction of autoimmune responses to the adrenal cortex and pancreatic β cells. As a marker of adrenal autoimmunity, we studied the occurrence of 21OH-Abs prior to, during, and following IFN-α therapy. In parallel, we measured GAD65-Abs and IA2-Abs as markers of islet autoimmune reactions. To compare these events with known IFN-α triggered forms of autoimmunity, we determined the development of antithyroid autoantibodies in these patients. Additionally, in subjects who tested positive for any of the autoantibodies, we evaluated the clinical relevance of these findings by functional tests.

METHODS

SUBJECTS

Seventy five of 288 naive patients (42 males, 33 females; mean age 47 (13) years) treated with IFN-α for chronic HCV infection at Hannover Medical School, Germany, between 1991 and 1998 were included in the study (baseline characteristics are shown in table 1). Chronic HCV infection was diagnosed by detection of anti-HCV antibodies (EIA third generation, Abbott Laboratories, Chicago, Illinois, USA) and HCV-RNA by polymerase chain reaction (PCR) (Perkin-Elmer, Norwalk, Connecticut, USA). All patients were analysed for presence of translational grade35S methionine labelling of recombinant IFN-α2b (Intron A, Essex Pharma, Munich, Germany) at a dose of 9–15×106 IE/week. Among the 75 patients enrolled in the study, 55 (73.3%) were treated with recombinant IFN-α-2b (Intron A, Essex Pharma, Munich, Germany) at a dose of 9–15×106 IE/week recombinant IFN-α-2a (Roferon-A, Hoffmann-La Roche, Grenzach-Wyhlen, Germany), and 13 (17.3%) received lymphoblastoid IFN-α (Wellferon, Glaxo Wellcome, Hamburg, Germany) at a dose of 9×106 IE/week. The mean duration of therapy was 8.3 (3.5) months.

<table>
<thead>
<tr>
<th>Autoantibody markers</th>
<th>All patients (n=75)</th>
<th>Autoantibody positive patients (n=32)</th>
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<tr>
<td>Age (y)</td>
<td>47 (13)</td>
<td>48 (15)</td>
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<tr>
<td>Sex (M:F)</td>
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<td>42:33</td>
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<td>Body mass index (kg/m²)</td>
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<td>HCV genotype (1a:1b:3a:4c)</td>
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<td>3:15:4:1</td>
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<td>Therapy duration (months)</td>
<td>8.3 (3.5)</td>
<td>8.1 (3.7)</td>
</tr>
<tr>
<td>IFN-α type</td>
<td>7:55:13</td>
<td>4:20:8</td>
</tr>
</tbody>
</table>

*Analysed only in 57/75 patients. HCV, hepatitis C virus; IFN-α, interferon alpha.

Detection of Autoantibodies

Antibodies to 21OH, GAD65, and IA2 were detected using radiobinding assays previously described.30 31 Briefly, plasmid cDNAs encoding for full length human GAD65 (kind gift from Dr Å Lernmark, University of Washington, Seattle, Washington, USA), IA2, ICA512bdc (kind gift from Dr GS Eisenbarth, Barbara Davis Center for Childhood Diabetes, Denver, Colorado, USA), and 21OH (kind gift from Dr Bon-Chu Chung, Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan) cloned into the pcDNAII vector (Invitrogen, CH Groningen, Netherlands) were amplified in Escherichia coli XL1-blue. After purification by Jetstar Plasmid Maxiprep (Genomed, Bad Oeynhausen, Germany) cDNA was expressed in vitro by coupled transcription and translation using a SP6 coupled rabbit reticulocyte lysate (Promega, Mannheim, Germany) in the presence of translational grade 35S methionine (1000 Ci/mmol; NEN Life Science Products, Cologne, Germany). The efficiency of the reaction was evaluated by measurement of radioactivity in trichloroacetic acid precipitated samples.32 33 34 35S labelled protein (20 000 cpm) was incubated with 5 µl of serum in immunoprecipitation buffer overnight at 8°C. Using Multiscreen-DV Filtration Plates (0.65 µm pore size; Millipore, Eschborn, Germany) antibody bound antigen was separated from free antigen by incubation of each sample in duplicate with protein A-Sepharose (Pharmacia, Freiburg, Germany) and washing with immunoprecipitation buffer. After resuspension of the filters in scintillation fluid, immunoprecipitated radioactivity was evaluated in a beta counter. Antibody levels were expressed as relative indices (GAD65 index, IA2 index, and 21OH index) using one positive and two negative standard sera in each assay. The upper limit of normal was determined using the mean +3 SD of antibody levels of more than 200 healthy subjects. It was calculated to be 0.06 for 21OH-Abs, 0.035 for GAD65-Abs, and 0.04 for IA2-Abs. In the 1995 Combinatorial Islet Autoantibody Workshop,36 the diagnostic sensitivity and specificity of our islet autoanti-
body assays were 85% and 99% for GAD65-Abs and 64% and 99% for IA2-Abs, respectively.

For additional detection of GAD65-Abs, a commercially available radioimmunoassay with 125I labelled recombinant human antigen (Medipan Diagnostica, Selchow, Germany) was used following the manufacturer’s instructions.

Autoimmune response to thyroid tissue was evaluated by measuring antibodies to thyroglobulin (TG-Abs) and thyroid peroxidase (TPO-Abs) (normal values <60 IU/ml (male) and <100 IU/ml (female)) by synchon ELISA assays (Elias Medizintechnik, Freiburg, Germany).

Evaluation of β cell function was performed by an oral glucose tolerance test. Glucose and insulin plasma levels were measured prior to and 120 minutes after ingestion of 75 g of glucose. Insulin was evaluated by a radioimmunoassay (Pharmacia and Upjohn Diagnostics, Freiburg, Germany).

Thyrotropin and total thyroxine, as markers of thyroid function, were measured by chemiluminescence assays (Chiron Diagnostics, Fernwald, Germany). Thyroxine binding globulin was determined using a radioimmunoassay (CIS Diagnostik, Dreieich, Germany).

STATISTICAL ANALYSIS
Baseline data were descriptively summarised, and assessment of differences in antibody frequencies was made using χ² methods. p<0.05 was considered to indicate statistical significance. Mean (SD) values were computed for all continuous data.

Results
PREVALENCE OF AUTOANTIBODIES IN PATIENTS WITH CHRONIC HCV INFECTION PRIOR TO IFN-α THERAPY
A total of 13/75 patients (17.3%) with chronic HCV infection showed some of the autoantibodies prior to IFN-α therapy (table 2); mainly thyroid (TG-Abs, n=6; TPO-Abs, n=4) but also islet cell (GAD65-Abs, n=3) and adrenal cortex autoantibodies (21OH-Abs, n=1). One subject initially showed thyroid antibodies was positive for both TG-Abs and TPO-Abs. For islet cell specific autoantibodies, only one of three patients with pre-existing GAD65-Abs tested positive with both the 35S and 125I assays. No subject initially showed IA2-Abs.

In the control group, autoantibodies to 21OH, GAD65, and IA2 were found in 0/75, 1/75, and 2/75 subjects, respectively. The difference in antibody prevalence in patients prior to IFN-α therapy was not significant (p>0.05).

Excluding the 13 initially positive patients, 62 of 75 subjects enrolled in this study were investigated for the development of autoantibodies in response to IFN-α.

IFN-α TREATMENT INDUCES ADRENAL CORTEX AUTOANTIBODIES
Two patients developed 21OH-Abs within 3–4 months of therapy. During further treatment and follow up, 21OH-Abs were induced in one additional subject (table 3). Altogether, 3/62 initially negative patients (4.8%), two male and one female, developed IFN-α related 21OH-Abs.

None of the positive patients showed clinical signs of adrenal dysfunction prior to, during, or at follow up (three months after IFN-α therapy). Cortisol response to a 250 µg ACTH challenge performed 2–4 years after IFN-α treatment was normal in three subjects, one of whom was initially positive for 21OH-Abs (table 4).

DEVELOPMENT OF ISLET SPECIFIC AUTOANTIBODIES DURING IFN-α THERAPY
During the first 3–4 months of therapy, IA2-Abs appeared in one patient, and a further subject developed GAD65-Abs (positive by the
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In four additional patients, GAD65-Abs were induced during the following treatment and follow up (table 3). IFN-α related islet specific autoantibodies thus appeared in 6/62 initially negative patients (9.7%, three males and three females).

In none of the antibody positive patients were symptoms of diabetes mellitus observed throughout the observation period. Oral glucose tolerance to 75 g of glucose performed 1–4 years after IFN-α therapy was normal in seven subjects, including the three initially positive patients (table 4).

**THYROID SPECIFIC AUTOANTIBODIES ARE MOST FREQUENTLY INDUCED BY IFN-α TREATMENT**

Within 3–4 months of treatment, TG-Abs appeared in three subjects. During further therapy and follow up, nine additional patients showed TG-Abs, four of whom also developed TPO-Abs (table 3). Altogether, antithyroid autoantibodies were induced in 12/62 initially negative patients (19.4%, seven males and five females).

Thyroid dysfunction was observed in 9/21 patients with thyroid antibodies. One patient showed hypothyroidism before IFN-α treatment. In eight patients thyroid dysfunction developed after IFN-α therapy (table 4).

**COEXISTENCE OF AUTOANTIBODIES**

Autoimmune response to several organs was observed in one patient, a 14 year old female with both chronic HCV infection and probable autoimmune hepatitis type II. She developed 21OH-Abs, GAD65-Abs, and TG-Abs within the first three months of IFN-α treatment.

**NO CORRELATION BETWEEN AUTOANTIBODIES AND CLINICAL DATA**

A past history of autoimmune diseases was found in 3/75 patients. One suffered from autoimmune hypothyroidism and another from rheumatoid arthritis (negative for rheumatoid factor). The third patient showed LKM-1 positive autoimmune hepatitis in addition to chronic HCV infection. Autoantibodies without clinical symptoms were observed in 14/75 patients, including 2/75 subjects showing ANA with a maximum titre of 1:160, and 12/75 subjects with some of the antibodies analysed in this study (table 2).

Analysis of the relationship between the occurrence of autoantibodies and patient age, sex, past history of autoimmune diseases, therapy duration, response to IFN-α treatment, and type of IFN-α was not significant (p>0.05).

**Discussion**

In the present study, 21OH-Abs were induced by IFN-α therapy in 3/62 initially negative patients (4.8%) with chronic HCV infection. Adrenal dysfunction was not detectable in any subject with 21OH-Abs. Islet specific autoantibodies appeared in 6/62 patients (9.7%), and thyroid autoantibodies were induced in 12/62 subjects (19.4%). None of the patients with islet autoantibodies developed diabetes mellitus or impaired glucose tolerance (IGT). Eight of 20 thyroid antibody positive subjects without initial organ dysfunction developed thyroid dysfunction after IFN-α therapy.

The high rate of induction of thyroid autoantibodies in response to IFN-α (19.4%) is comparable with findings by other groups (6–38.8%). Development of islet cell specific autoantibodies after IFN-α therapy has been reported only in smaller studies, whereas this is the first report demonstrating a direct effect of IFN-α treatment on the appearance of 21OH-Abs, thereby defining the antigen involved by use of a highly sensitive and specific test for adrenocortical autoimmunity.

Chronic HCV infection has previously been thought to increase the prevalence of thyroid autoantibodies independent of IFN-α therapy. However, recent studies involving larger numbers of patients showed no higher prevalence of thyroid antibodies in subjects with chronic hepatitis C compared with healthy controls, suggesting a direct effect of IFN-α on induction of autoantibodies. Similarly, no increase in GAD65-Abs in naive patients suffering from chronic HCV infection was recently reported, while Mason et al suggested an increased risk for the development of diabetes mellitus in chronic HCV infection. The prevalence of IA2-Abs and 21OH-Abs in these patients has not been studied. In the present investigation, the prevalences of GAD65-Abs, IA2-Abs, and 21OH-Abs in untreated patients with chronic hepatitis C was comparable with the control group (p>0.05). However, we demonstrated a higher prevalence of all of these autoantibodies during and following IFN-α therapy caused by an increase in antibody titres in a number of initially negative individuals. We therefore suggest that IFN-α plays an important role in the development of islet and adrenal specific autoantibodies in these patients. Fattovich et al recently found no induction of islet, adrenocortical, or thyroid autoactivity in IFN-α treated patients with chronic hepatitis B and thus it seems possible that IFN-α therapy for chronic HCV infection in particular, and not IFN-α per se, triggers endocrine autoimmune reactions.

There are only few data available on induction of GAD65-Abs in response to IFN-α. Imagawa and colleagues recently investigated 40 patients undergoing IFN-α therapy for chronic viral hepatitis and detected the development of GAD65-Abs in one subject (2.5%). The lower incidence of autoantibody induction compared with our findings (9.7%) may have resulted from different measurement methods, as they used a radioimmunoassay kit with antigen purified from porcine brain. In our study, we included two different assays to detect GAD65-Abs. The 35S assay identified a higher number of patients with GAD65-Abs than the 125I assay (7/75 and 3/75, respectively). This is best explained by the higher sensitivity of the 35S assay. Sensitivities of 75–85% and 71% have been reported for the 35S assay and the 125I assay, respectively.

As we are the first to evaluate the prevalence of 21OH-Abs and IA2-Abs after IFN-α
therapy, no data are currently available for direct comparison with our results. The only other study on adrenocortical autoimmunity showed no ACA development in 32 IFN-α treated patients with chronic hepatitis B. The higher incidence of adrenal specific antibodies in our study may be due to the higher sensitivity of 21OH-Abs analysed by radiobinding assay (86%, 24/28 Addison patients) compared with ACA measured using an immunofluorescence technique (43%, 12/28 Addison patients), or may depend on disease specific factors as we investigated patients suffering from chronic HCV in contrast with the hepatitis B study previously reported.

The pathogenesis of endocrine autoimmunity in response to IFN-α treatment is still unclear. IFN-α is known to increase MHC class I antigen expression on cell membranes but further mechanisms leading to autoantibody production have not been sufficiently elucidated. Recent reports suggest an effect of type I interferons in inhibiting B cell apoptosis by upregulation of the antiapoptotic survival factors Bcl-2 and Bcl-xL. This may be further influenced by a permissive effect of HCV attachment to human CD81 on B cell activation, as recently suggested. Although very few autoantibodies exhibit a direct effect on target tissues, a higher incidence of activated B cells may lead to priming of autoreactive T cells. As an additional effect, IFN-α directly impairs glucose metabolism. However, this effect was suggested to be based on non-immune mediated mechanisms.

Coexistence of autoantibodies after IFN-α treatment was observed in only one young female who developed 21OH-Abs, GAD65-Abs, and TG-Abs. This 14 year old girl suffered from chronic HCV infection in addition to probable LKM-1 positive autoimmune hepatitis which is frequently associated with autoimmune endocrinopathies. As Choudhuri et al. recently showed immunological cross reactivity between a major epitope of LKM-1 and the homologous regions of 21OH and carboxyptidase H, an autoantigen in IDDM, it seems possible that in this LKM-1 positive patient IFN-α treatment triggered an autoimmune response to at least one cross reacting autoantigen.

The correlation of autoantibody reactivity with clinical signs of islet and adrenal dysfunction has not been tested previously. In our functional tests performed after IFN-α therapy, no signs of adrenal insufficiency were detected in any antibody positive patient. However, during IFN-α treatment it may be difficult to distinguish between clinical signs related to common side effects of IFN-α and Addison’s disease (for example, anorexia, weight loss, fatigue, mental depression, and gastrointestinal disorders). Adrenal autoantibodies and/or adrenal dysfunction may thus be considered in patients with more persistent symptoms after IFN-α therapy, especially in individuals with a history of autoimmune diseases.

In none of our patients did diabetes mellitus or IGT develop in response to IFN-α treatment. However, there is evidence that patients with pre-existing or IFN-α associated islet autoantibodies may develop clinical alterations after IFN-α therapy, as suggested by reports on sporadic manifestation of IFN-α related diabetes mellitus. Furthermore, previous studies showed early progression to insulin dependency in patients suffering from type 2 (non-insulin dependent) diabetes mellitus (NIDDM) who tested positive for islet autoantibodies. As we frequently detected islet autoantibodies after IFN-α therapy (9.7%), patients at risk (islet autoantibodies, HLA-DR3/DR4, IGT, and positive family history) of diabetes mellitus or patients with pre-existing NIDDM should be investigated for islet autoantibodies and/or islet dysfunction during IFN-α treatment.

In spite of the lack of IFN-α related manifestations of adrenal and islet autoimmune diseases in our study, it is possible that autoantibody positive patients may develop organ dysfunction in the long term. Furthermore, higher dose and longer term IFN-α therapy as therapeutic options for chronic HCV infection may lead to an increased frequency of endocrine autoimmune diseases. Thus the development of islet specific autoantibodies in particular, in almost 10% of cases, may lead to an increased incidence or earlier occurrence of IDDM with these therapeutic regimens.

In summary, IFN-α induces autoantibodies to the adrenal cortex in some cases while development of islet specific and thyroid specific autoantibodies is a more frequent event. Although in our study no manifestations of adrenal or islet dysfunction were observed, the thyroid as well as the adrenal cortex and pancreatic islet cells should be considered as potential targets of IFN-α induced autoimmunity.

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