

Antibodies against double-stranded DNA and development of polymyositis during treatment with interferon

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Received 1 March 1997 and in revised form 2 March 1998

Summary

Alpha interferons have become effective palliative treatments for patients with neuroendocrine tumours such as carcinoids and endocrine pancreatic tumours. However, several reports indicate an increased incidence of both autoantibodies and autoimmune diseases in patients treated with interferon- α (IFN- α). We studied the development of antibodies against double-stranded DNA (dsDNA) and clinical signs of autoimmune disease in 214 patients with malignant carcinoids or endocrine pancreatic tumours consecutively admitted for treatment with IFN- α . Seventeen patients (8%)

developed antibodies against dsDNA, predominantly females (12 females and 5 males). One patient had clinical and laboratory signs of polymyositis. Among the other 16 patients, three developed hypothyroidism and in six patients the anti-dsDNA autoantibodies normalized despite continuing therapy. Although a significant number of patients developed autoantibodies against dsDNA, overt autoimmune disease related to these antibodies is a rare event and many patients spontaneously normalize these titres despite continuing IFN- α treatment.

Introduction

Alpha interferons have become effective palliative treatments for patients with neuroendocrine tumours such as carcinoids¹ and endocrine pancreatic tumours.² However, several reports indicate an increased incidence of both autoantibodies and autoimmune diseases in patients treated with IFN- α .^{3–10} Hypothyroidism, thyrotoxicosis and systemic lupus erythematosus (SLE) are examples of autoimmune disorders associated with IFN- α treatment.^{3,6–8}

SLE is characterized by a variety of antibodies. Among these, antibodies to dsDNA are highly specific for SLE and are thought to play a role in the pathogenesis of the disease.¹¹ Furthermore, considerable data have accumulated to suggest that endogen-

ous IFN- α may be involved in the pathogenesis of the disease.^{12,13} Recently, the appearance of antibodies against dsDNA was reported in patients with chronic lymphatic leukaemia receiving IFN- α therapy.¹⁴ None of these patients, however, were positive for dsDNA antibodies detected by *Crithidia* immunofluorescence, indicating that the antibodies were of relatively low affinity and consequently, no evidence of autoimmune disease was found. We have retrospectively studied a large number of patients treated with IFN- α , and observed that a substantial number of patients developed high-affinity antibodies to dsDNA on long-term therapy with IFN- α .

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Methods

Patients

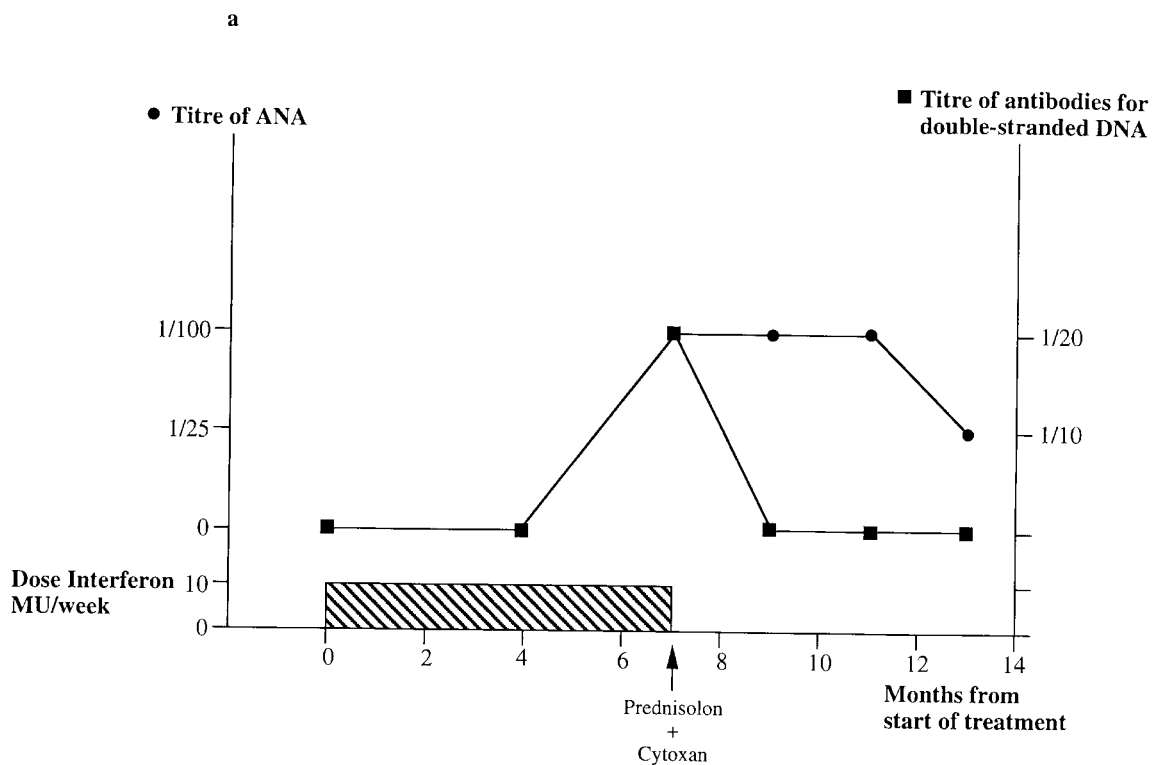
The study was done in 214 patients with histologically-verified malignant carcinoids or endocrine pancreatic tumours, admitted to our department for treatment with IFN- α . During IFN- α therapy, serum from the patients were analysed every three to four months for the development of antinuclear antibodies (ANA). When positive, we investigated the serum for antibodies against dsDNA by a method described below. The records from patients with recognized development of antibodies against dsDNA were evaluated for clinical signs and symptoms of autoimmune disease. Living patients were particularly asked about such signs and symptoms in retrospect. Liver and kidney functions as well as haematological parameters were recorded and correlated to the IFN- α dose given. The patients were treated subcutaneously with IFN- α , either human leukocyte interferon, recombinant interferon- α -2a (Roferon, Hoffman-La Roche AG) or interferon- α -2b (Intron-A, Schering-Plough). The administered dose ranged from 9 MU weekly to 36 MU weekly, with median dose of 15 MU weekly. In five cases, human interferon- γ (Finnish Red Cross) was added at a dose of 1 MU weekly. The control group consisted of 13 patients with carcinoid tumours who received an alternative treatment with a somatostatin analogue (octreotide) 100 μ g twice/day subcutaneously.

Assays

The occurrence of ANA was screened by routine immunofluorescence-technique using HEp-2 cells as ANA substrate. Samples with ANA-titres $\geq 1/100$ were considered positive, and those demonstrating a homogenous staining pattern were further analysed for antibodies against dsDNA by *Crithidia luciliae* immunofluorescence. Samples demonstrating an anti-dsDNA titre $\geq 1/10$ were considered positive. FITC-conjugated goat antibodies to human IgG (heavy and light chain) were used as secondary antibodies for ANA as well for anti-dsDNA antibody determination. The FITC-conjugated secondary goat anti-human sera, *Crithidia luciliae* preparations and HEp-2 cell slides were all from Imunoconcept.

Results

None of the patients had positive ANA with titres above 1/100 before start of α -IFN therapy. Forty-eight of 214 patients (22%) were found to have a positive ANA with titres $\geq 1/100$. In 32, the ANA titres were homogenous; 12 showed a speckled pattern, two patients developed nuclear patterns and in another two, centromere staining patterns. Among the 32 patients developing positive ANA titres with homogenous patterns, 17 had antibodies against dsDNA on at least one occasion. Median time from start of treatment to detection of anti-dsDNA antibody



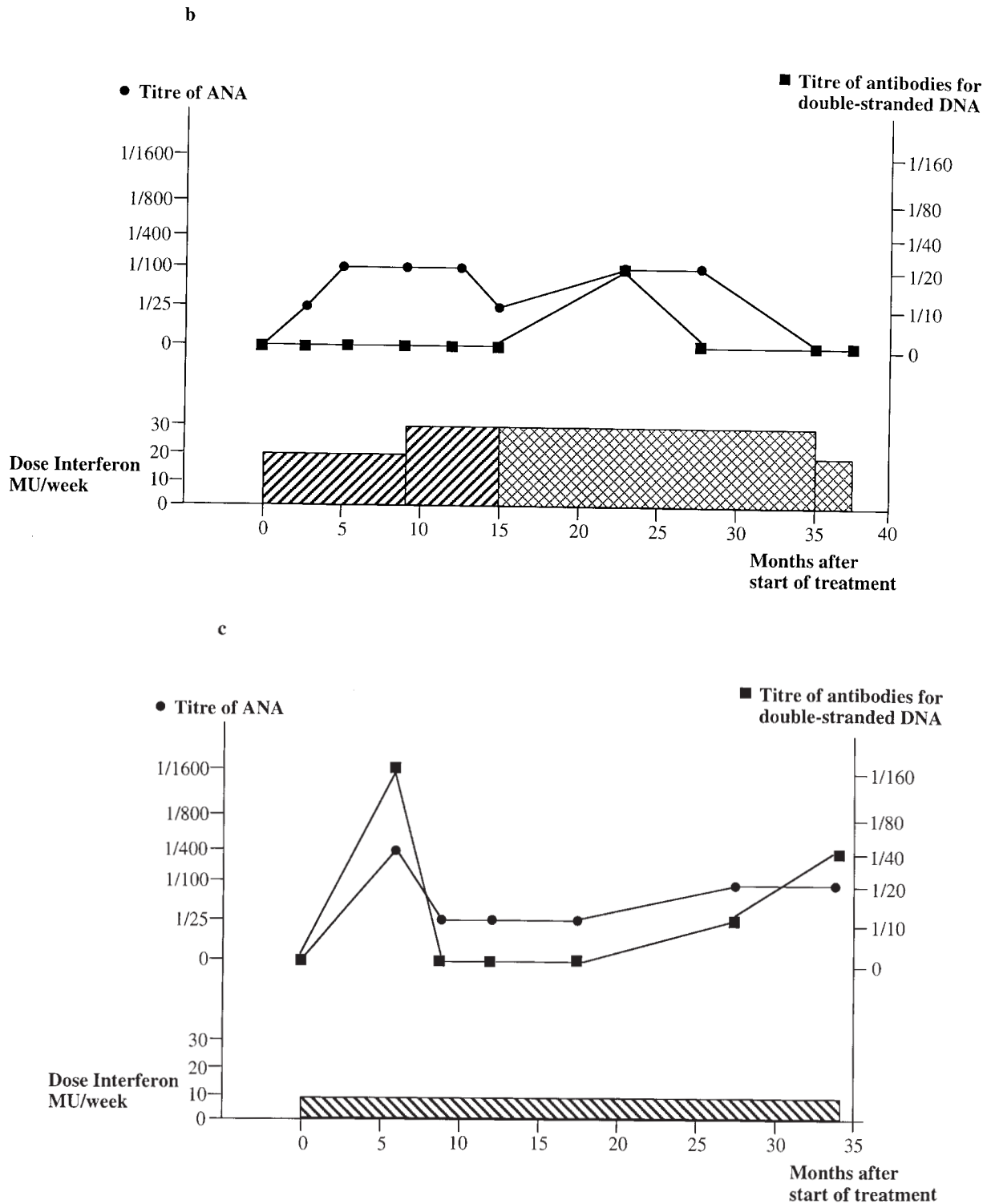


Figure 1. **a** Titre of antibodies to antinuclear antibodies (ANA) and double-stranded DNA according to treatment and time in a 69-year-old female. MU = 10^6 units. **b** Titre of antibodies to ANA and double-stranded DNA according to treatment and time in a 57-year-old female. **c** Titer of antibodies to ANA and double-stranded DNA according to treatment and time in a 79-year-old female.

ies was 35 months (range 6–96 months). The 17 patients with dsDNA antibodies included 12 females and five males with a median age of 58 years at start of treatment (range 42–79 years).

Among the patients who developed antibodies against dsDNA, one patient had an atrophic gastritis and three patients had hypothyroidism diagnosed before start of IFN- α treatment. Another three patients

developed hypothyroidism after 7, 8 and 18 months of treatment, respectively. All these patients had thyroglobulin antibodies and/or antibodies against microsomal thyroid antigens in their sera. Sixteen patients did not present any symptoms or signs of SLE, mixed connective tissue disease or Sjögren's syndrome, when antibodies against dsDNA were observed. One patient (case report) developed severe acute polymyositis after 6 months of treatment with interferon- α -2b. The drug was withdrawn, and she had to be treated with steroids and cyclophosphamide.

Case report

Female (KF), born in 1922 (Figure 1a). This 69-year-old lady was referred to the Department of Internal Medicine, University Hospital, Uppsala in October 1991, because of a pancreatic tumour and liver metastases diagnosed one month earlier at a county hospital. Biopsies from the tumour showed neuroendocrine tumours on histopathology. A prolactinoma was also diagnosed, and the patient suffered from multiple endocrine neoplasia type 1 (MEN 1). She was treated with IFN- α (3 MU) three times per week and was feeling well except for low-grade muscle pain after the injections. Six months after start of IFN- α treatment, the patient had severe muscle pain in the legs, the arms and also in the back. The patient also suffered from muscle weakness and fatigue. At that time the sedimentation rate increased from 42 mm/h before start of interferon to 100 mm/hour. Before IFN- α therapy, the liver enzymes were normal including lactate dehydrogenase 5.2 μ kat/l (ref. <6.0 μ kat/l). No autoantibodies could be detected. After 6 months serum, lactate dehydrogenase was 8.8 μ kat/l, and serum myoglobin and serum creatinin kinase became elevated, maximum level 740 μ g/l (ref. <50 μ g/l) and 5.0 μ kat/l (ref. <2.5 μ kat/l), respectively, whereas antibodies against SS-A, SS-B, Scl-70 and Jo-1 were all negative. At that time, ANA was positive at a titre of 1/100 and antibodies against dsDNA could be detected at a titre of 1/20. Electromyography showed signs of polymyositis but also signs of low-grade polyneuropathy. A biopsy from an affected muscle revealed necrotic muscle fibres, some basophilic regenerating fibres and also infiltrating inflammatory cells but no vasculitis (Figure 2 a,b). A diagnosis of acute polymyositis was made. The patient was evaluated for HLA-haplotype and was DR15 (2) and DR7 positive. The patient started on treatment with prednisolone 30 mg/day and cyclophosphamide 50 mg twice per day. She gradually improved with less muscle pain, return of muscle strength, and gradual normalization of muscle enzymes. Cyclophosphamide treatment was continued for two

years; prednisolone was tapered during six months after the cyclophosphamide treatment was stopped. Four years after the trial with interferon, the patient is in good clinical condition. The only sequel is numbness in the feet.

Other patients

In the other 16 patients, erythrocyte sedimentation rate (ESR), liver-aminotransferases, serum creatinine, urinary albumin, and haemoglobin did not change significantly from start of therapy until development of antibodies against dsDNA. Platelet and leukocyte counts showed a slight reduction in relation to IFN- α dose. Two patients developed neutralizing IFN- α -2a antibodies after 12 and 22 months of treatment, and one patient developed neutralizing IFN- α -2b antibodies after 38 months of treatment. These patients were switched to human leukocyte IFN.

Figure 1b illustrates one patient in whom spontaneous disappearance of antibodies against dsDNA (defined as at least two negative analyses) occurred, despite continuing treatment with IFN- α . This phenomenon occurred in a further five patients, and declining titres (more than two titre steps) were observed in another three. In two patients, after spontaneous normalization of anti-dsDNA during ongoing treatment, a second peak was observed, illustrated in Figure 1c. The addition of interferon- γ in one patient on IFN- α treatment, elicited increased titres of antibodies to both ANA and dsDNA. Another four patients were treated with a combination of IFN- γ and IFN- α without developing any anti-dsDNA titres. Neither the dose nor the type of IFN- α correlated with development of anti-dsDNA antibodies.

Out of 13 patients with carcinoid tumours that were treated with the somatostatin analogue octreotide, one patient developed positive ANA titre 1/100 but no anti-dsDNA autoantibodies, nor any symptoms of autoimmune disease.

Discussion

The main finding in the present study was an increased frequency of both ANA and antibodies against dsDNA in patients treated with IFN- α . The occurrence of ANA during IFN- α therapy has previously been described in several reports,^{5-7,9,10} but antibodies against dsDNA have been reported only occasionally.¹⁴ However, in the small study by Ehrenstein *et al.* antibodies against dsDNA were only detected using an ELISA assay, and not by *Crithidia luciliae* immunofluorescence. This would indicate that the antibodies were of low affinity without

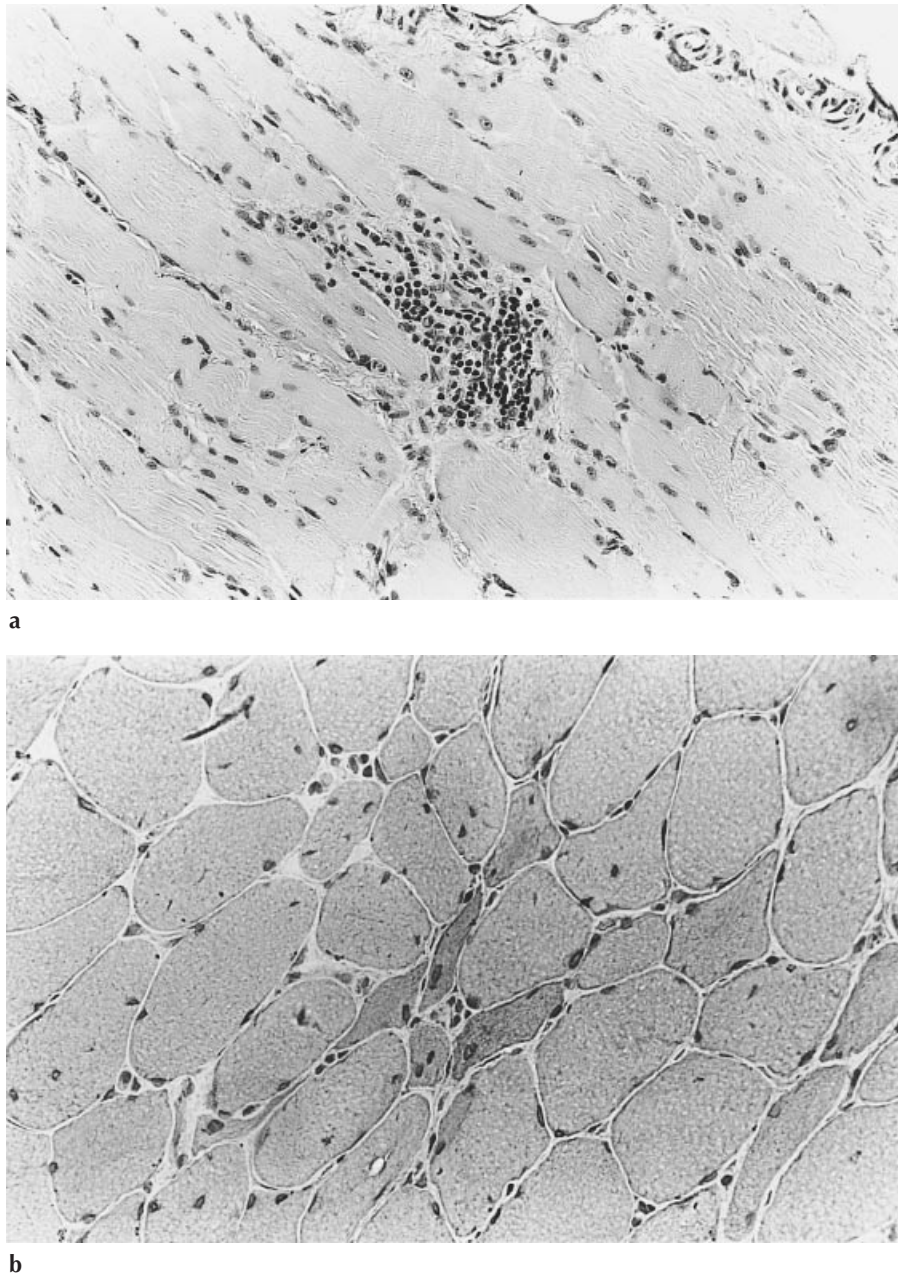


Figure 2. **a** Biopsy from the right quadriceps muscle with signs of inflammatory myopathy, with a large cluster of mononuclear inflammatory cells within the fascicle. Paraffin section. Htx.eo. **b** Cryostat section from the same biopsy as in Figure 1. In the centre there is a group of regenerating muscle fibres with enlarged and somewhat more centrally placed cell nuclei, i.e. signs of myopathy. Htx.

pathogenic significance. Consequently, no patient developed autoimmune disease in their investigation.

In contrast, antibodies of high affinity against dsDNA during IFN- α treatment were detected in 8% of our patients. These antibodies appeared after a much longer period (median 35 months) than previously described organ-specific autoantibodies (median 5 months) and low-affinity dsDNA antibodies (after 6–12 months).^{6,14} High-affinity antibodies against dsDNA generally reflect disease activity in SLE, and are associated with nephritis.¹⁵ Furthermore,

such antibodies can also bind to cell surface proteins and might therefore have a role in tissue injury and inflammation.¹⁶ One of our patients also developed a clinical and histological picture of acute polymyositis which had to be treated with steroids and a cytotoxic drug. We have earlier described another patient who developed antibodies against dsDNA and a SLE syndrome during IFN- α treatment.⁷ Therefore, we suggest that patients who develop high-affinity antibodies against dsDNA are at increased risk of autoimmune disease.

A role for IFN- α in autoimmune disorders has been suggested previously, based on its detection in the sera of patients with various autoimmune diseases.^{12,13} For example, in untreated SLE patients, circulating IFN- α can be detected in 90% of the patients and the level correlates positively with disease activity.¹³ Furthermore, many of the side-effects associated with IFN- α treatment resemble symptoms in SLE and other autoimmune diseases.

The exact mechanism whereby IFN- α contributes to the development of both autoantibodies and autoimmune disease are at the moment unclear. IFN- α may promote the production of pathogenic antibodies, and one study actually found that IFN- α induced production *in vitro* of antibodies with a lupus-associated idiotype using pokeweed-mitogen-stimulated lymphoid cells.¹⁷ IFN- α can also induce transcription from many genes in cells, for example bcl-2 oncoprotein which is involved in translocation and protects cells against apoptosis.^{14,18} Since bcl-2 is overexpressed in lymphocytes from patients with SLE,¹⁹ and defect apoptosis may be of importance in this disease, IFN- α might act via induction of this gene.²⁰

In addition, IFN- α induces the expression on cells of MHC class I molecules; these enhance presentation of endogenous antigens and consequently promote the T-cell immune response. The HLA haplotype may also be relevant in this context, because most autoimmune conditions are associated with particular HLA profiles.²¹ Our patient presented the HLA-haplotype DR 15 (2) and DR 7, as did another four patients with antibodies to dsDNA. The importance of female sex-hormones in autoimmune diseases²² is stressed by the striking predominance of females among our patients who developed ANA. We had some problem in obtaining a representative control group, since most patients receive IFN- α alone or in combination with somatostatin analogue, octreotide. However of our 13 control patients, none developed antibodies to ds-DNA. Therefore we believe that this phenomenon is related to IFN- α treatment *per se*.

Since SLE and other autoimmune disorders are a consequence of many different factors, both genetic and exogenous, the finding that only a minority of IFN- α -treated patients develop autoantibodies and, particularly, obtain an autoimmune disease seems logical. Furthermore, the IFN- α treatment *per se* may be immunosuppressive and thus protecting the patient against autoimmune disease. This possibility is supported by reports that IFN- α/β administered late during immunization of mice can suppress a cellular immune reaction such as delayed-type hypersensitivity.²³ This may also explain the fact that the anti-dsDNA antibodies disappeared in most patients despite continuing IFN- α treatment.

In summary, this study shows that high-affinity antibodies against dsDNA and associated autoimmune disease may develop because of IFN- α treatment. Although women are at higher risk of developing autoimmune disease, both men and women treated with IFN- α should be monitored carefully for signs of autoimmunity. We have also focused on the possible role of IFN- α in the initiation and evolution of autoimmune disorders. Such role deserves further investigations both *in vitro* and more directly in such patients, and we have now initiated such studies.

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