Induction of antinuclear antibodies in patients with rheumatoid arthritis receiving treatment with human recombinant interferon gamma

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SUMMARY Of six patients with rheumatoid arthritis (RA) treated with human recombinant interferon gamma for two to eight months, three developed antinuclear antibodies (ANAs). This was accompanied by a simultaneous clinical exacerbation of the disease activity. In this study both anti-inflammatory and immunostimulatory effects of human recombinant interferon gamma in patients with RA were observed.

There is increasing application of different interferon preparations in the treatment of patients with malignant disorders, especially hairy cell leukaemia, chronic myeloid leukaemia, and different solid tumours, such as renal cell carcinoma, osteosarcoma, and nasopharyngeal carcinoma. Interferons are also used in the experimental treatment of different autoimmune disorders, including Behçet's disease¹ and rheumatoid arthritis.² As interferon gamma has potent immunomodulatory effects and is antiviral, this agent may be important at the effector or target cell level, or both, and thus may participate in the development of human autoimmune disease.

This assumption is supported by the data of Hooks *et al*, who detected interferon in the serum of patients with systemic lupus erythematosus and Sjögren's syndrome.³ Another potential role of interferon gamma in the induction of autoimmunity is its known stimulation of major histocompatibility (MHC) antigens, such as the human class II antigens HLA-DR, DP, and DQ.⁴ The enhanced expression of MHC class II antigens on antigen presenting cells is probably a crucial step in the initiation of an aberrant immune response by T helper/inducer cells and in the generation of autoreactive T cell clones.

On the basis of the effects of human interferon gamma on the course of rheumatoid arthritis in several clinical pilot studies⁵⁻⁶ we treated 13 patients with human recombinant interferon gamma.² In this

paper we report the induction of antinuclear antibodies in three of six patients treated for two to eight months.

Patients and methods

PATIENT POPULATION

Thirteen patients with definite or classical rheumatoid arthritis according to the American Rheumatism Association criteria entered the clinical study. The disease duration was from six months to 31 years and age range was 23-65 years. Eleven patients were seropositive by latex fixation and the Waaler-Rose test for rheumatoid factor. Two patients were seronegative. Before entering the study all patients had been treated with non-steroidal anti-inflammatory drugs, and 12 patients had received different remission inducing drugs two months before. Six patients additionally received low dose steroids (5-15 mg prednisone equivalent daily).

SOURCE AND ADMINISTRATION OF INTERFERON GAMMA

Recombinant interferon gamma (a gift of Bioferon, Laupheim, FRG) was used in a clinical phase II trial in patients with RA unresponsive to conventional remission inducing treatments. Before entering the study informed consent was obtained from all patients. The aim of the study was to examine the clinical effects and to record side effects under a dosage schedule which had been established by the previous clinical pilot studies.

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All patients received 50 µg (1.2×10^6 units) interferon gamma subcutaneously on day 1, 100 µg/day from day 2 to 10, and from day 11 to 20 100 µg/day if there had been a clinical response (at least a 15% improvement of the Ritchie index). If the improvement was less than 15% the dosage was increased to 200 µg/day. If good clinical response occurred a maintenance dosage of 3×100 µg/week subcutaneously followed the initial phase of the study. In three cases maintenance dosage was lowered to 3×50 µg/week because antinuclear antibodies were detected in patients' sera. After two, four, six, and eight months of interferon gamma treatment patients were re-evaluated clinically and by several laboratory parameters.

MONITORING OF CLINICAL AND LABORATORY PARAMETERS

The following were tested on day 0, 10, and 20 in the initial phase and after two, four, six, and eight months in the maintenance phase: (a) clinical parameters: pain index according to Ritchie, grip strength, morning stiffness, steroid requirement; (b) laboratory parameters: erythrocyte sedimentation rate (ESR), α_2 acid glycoprotein, blood counts, serum aspartate transaminase, serum alanine transaminase, IgM rheumatoid factor by nephelometric determination, antinuclear antibodies (ANAs) by indirect immunofluorescence technique using sections of rat kidney, liver, and stomach, antibodies to native double stranded DNA by an indirect immunofluorescence test with *Crithidia luciliae* as substrate.

Results

Thirteen patients with RA received subcutaneous injections of recombinant human interferon gamma for 20 days. Six of these patients showed a good clinical response and received maintenance treatment for three, four, six, and eight months. Treatment was stopped in four patients after two, four, or six months because of acute exacerbation of the disease. Two patients were treated successfully with interferon gamma for six and eight months.

The results of clinical and laboratory monitoring of these patients have been published previously.² Briefly, only two patients out of 13 showed a long lasting improvement of joint pains and joint motility with interferon gamma treatment. In four patients the initial good therapeutic effect gradually decreased after two to four months of maintenance treatment. Parallel to the clinical improvement only a partial improvement of grip strength and morning stiffness occurred. Only two patients showed a decrease of the ESR in the initial phase, and in four

patients a decrease of acute phase proteins and thrombocyte counts paralleled clinical improvement.

In this report we concentrate on six patients with RA receiving maintenance treatment, three of whom developed increased titres of ANAs coinciding with clinical exacerbation of RA in two cases (patients 1 and 4). In these two patients interferon gamma dosage was lowered to 3×50 µg/week after four months; ANA titres fell again and clinical improvement occurred. Table 1 shows the results of the laboratory monitoring parameters. The data show at least a partial coincidence between the increase of ANAs and the increase of IgM rheuma-

Table 1 Laboratory parameters during interferon gamma treatment

Patient No. time	ESR* (mm/h)	α_2 GP* (g/l)	RF* (IU/ml)	ANAs*
<i>Patient 1</i>				
Day 0	72	281	1890	1:20
Day 10	74	285	NT	NT
Day 20	67	213	2406	1:20
Month 2	58	NT*	1454	1:40
Month 4	67	NT	1560	1:320
Month 6	60	264	948	1:80
Month 8	65	264	1448	1:80
<i>Patient 2</i>				
Day 0	13	149	0	0
Day 10	14	NT	0	0
Day 20	15	NT	0	0
Month 2	20	NT	0	0
Month 4	18	170	0	0
Month 6	16	NT	0	0
<i>Patient 3</i>				
Day 0	47	192	54	0
Day 10	30	143	55	0
Day 20	28	129	53	0
Month 2	53	NT	62	1:40
Month 4	80	NT	58	1:40
<i>Patient 4</i>				
Day 0	124	342	7260	1:40
Day 10	125	NT	521	1:80
Day 20	125	266	573	1:20
Month 2	129	NT	NT	1:320
Month 4	126	NT	NT	1:160
Month 6	133	347	12963	1:160
<i>Patient 5</i>				
Day 0	24	176	173	0
Day 10	30	NT	178	0
Day 20	28	152	165	0
<i>Patient 6</i>				
Day 0	56	224	326	1:80
Day 10	76	NT	784	1:40
Day 20	82	NT	NT	NT
Month 2	67	286	912	1:40

*ESR=erythrocyte sedimentation rate; α_2 GP= α_2 glycoprotein; RF=rheumatoid factor; ANAs=antinuclear antibodies; NT=not tested.

toid factors and α_1 acid glycoprotein. Only in patient 1 was the fall of ANA titres after dose reduction of interferon gamma paralleled by a slight fall of rheumatoid factors.

In all cases which had ANA titres during interferon gamma treatment tests for antibodies to native double stranded DNA with *Crithidia luciliae* as substrate were negative.

Discussion

It is well known that interferons have not only antiviral but also immunomodulatory properties. That is especially true for interferon gamma, as recently reviewed by Kirchner.⁷

Owing to the favourable results of clinical pilot studies with enriched lymphokine preparations in patients with RA, we performed a clinical phase II trial with human recombinant interferon gamma. We were aware of the theoretical problems of using a putative amplifier of the immune response in the treatment of an autoimmune disorder. This study seemed to be justified, however, by the results of the pilot trials.

Our recent study did not confirm the favourable results of previous trials with interferon gamma in RA.² In only two patients out of 13 did we observe a remission of more than six months associated with treatment. Moreover, the clinical improvement was only partially reflected by improvements in humoral inflammatory parameters such as reduction of the ESR and the acute phase proteins.

When laboratory parameters were monitored, however, we found increased or newly developed ANAs after two months' treatment with recombinant interferon gamma in three patients. This is to our knowledge the first time that the appearance of ANAs has been described during treatment with interferon gamma in patients with RA. Interestingly, the increase of serum ANA titres was accompanied by a simultaneous clinical exacerbation of RA. Conversely, after interferon gamma dose reduction ANAs decreased again and clinical improvement occurred. Thus interferon gamma dosage seems to be important whether ANAs do develop during treatment or not. From the study we obtained some evidence that recombinant interferon gamma may induce autoimmune responses in RA, but may also reduce clinical and humoral inflammatory activity in some patients. These findings are similar to previous observations describing the induction of endocrine

autoimmunity in patients receiving long term treatment with human leucocyte derived interferon. Burman *et al* found that interferon gamma and aberrant HLA-DR expression on thyroid cells was associated with thyroid autoimmunity presenting as hypothyroidism or thyrotoxicosis.⁹

We do not know the reasons for the development of ANAs during interferon gamma treatment in some patients with RA. Probably a dose dependent mechanism is important. It is also unclear why some patients respond to treatment and others do not respond at all. To clarify these questions a further multicentre study with interferon gamma in much lower doses was initiated as a placebo controlled trial in patients with RA. Definite results of this German study will be published soon.

It remains of great importance to minimise the potential risk of enhancing autoimmune mechanisms by lowering interferon gamma doses. In addition, it is of interest to examine any possible relation between induction of autoimmunity of therapeutic response with MHC class II expression on antigen presenting cells of patients receiving interferon gamma and to test immunogenetic influences on the immunostimulatory activity of interferon gamma.

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