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Interferon Therapy in Multiple Myeloma: Failure of Human Fibroblast Interferon Administration to Affect the Course of Light Chain Disease*

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Abstract—*Fibroblast interferon at a dosage of* 28×10^6 U/wk *failed to influence disease progression in a preterminal case of therapy-resistant light chain myeloma. In a second case, that had not previously been treated, a first course of fibroblast interferon* (30×10^6 U/wk) *associated with corticosteroids remained without effect. In this patient subsequent leukocyte interferon treatment was associated with a decrease in urinary light chain excretion and normalization of calcaemia, all other parameters remaining unaltered. A third patient with light chain disease was resistant to chemotherapy* ab origine. *None of the disease parameters responded to either fibroblast or leukocyte interferon therapy* (21×10^6 U/wk). *During therapy a downward trend occurred in the relative number of lymphocytes characterizable as B and T cells. Mitogenic reactivity remained unchanged except for a downward trend, during fibroblast interferon therapy, in reactivity to PHA and Con A after* 6 days *culturing. Spontaneous (background) mitogenesis upon culture showed an upward trend.*

INTRODUCTION

PREPARATIONS of interferon have been shown to inhibit outgrowth of virally induced or transplantable tumors in experimental animals. Multiple mechanisms (as reviewed [1–3]) have been considered to explain this effect. Most significantly, interferons inhibit cell division and enhance tumor cell killing activity of macrophages, sensitized T-cells and natural killer cells.

Interferon in sufficient amounts for clinical purposes can be obtained from cultured human cells of two types, leukocytes (or lymphoblastoid cells) and fibroblasts. These interferons differ in their chemical structure [4–6] and pharmacological properties [7, 8]. They also differ in at least some biological properties, for example in their relative effects on the growth of cultured human osteosarcoma cells and fibroblasts [9], and in their dose–response curves in certain assay systems [10].

Preparations of leukocyte interferon have already been used in clinical trials [11] on patients with various malignant diseases: osteosarcoma [12], lymphoma [13, 14], myeloma [13, 15–17], mammary carcinoma [13], laryngeal papilloma [18]. Fibroblast interferon has not yet been tested so extensively. A clear-cut beneficial effect was noted with laryngeal papilloma [19] and nasopharyngeal carcinoma [20] and some reports briefly mention osteosarcoma [7], neuroblastoma [7] and melanoma patients [21] treated with this type of interferon preparation.

In the present paper we describe our findings in three patients with myeloma, treated with fibroblast interferon. In two of these patients we were able to compare the effects of leukocyte and fibroblast interferon.

MATERIALS AND METHODS

Interferon preparations

Human fibroblast interferon was prepared from cultured diploid embryonic skin fibro-

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blasts or from the MG-63 cell line as described earlier [22, 23]. Leukocyte interferon was prepared according to the method described by Cantell *et al.* [24]. Part of the leukocyte interferon used in the present study (case 2) was a gift from Dr. K. Cantell (State Serum Institute, Helsinki, Finland). Doses are expressed in international units. The interferons were injected intramuscularly. The dose schedules are indicated in the case reports described below.

Analytical procedures

The main disease parameter followed was 24 hr urinary excretion of light chain protein. In cases 1 and 3 the excretion was sufficiently high for it to be measured as total urinary protein excretion using the biuret reaction after heat precipitation. In case 2 light chain excretion was low; therefore it was measured by single radial immunodiffusion (Mancini technique), using a rabbit antiserum against human κ-light chain (code A100, Dako, Copenhagen, Denmark).

Serum levels of immunoglobulins A, G and M (IgA, IgG, IgM) were also determined by radial immunodiffusion using commercially available plates.

Other laboratory tests (hemoglobin, serum calcium, serum creatinine, serum protein electrophoresis, B- and T-cell rosetting, mitogen stimulation tests) were done regularly by the hospital laboratory according to standard procedures.

For immunofluorescence studies on lymphocytes, human peripheral bloodmononuclear cells were isolated by Ficoll/Hypaque (Nyegaard, Oslo, Norway) density centrifugation (30 min, 400 *g*) and washed twice with Hanks balanced salt solution. The cells were fixed with 0.04% formaldehyde solution in phosphate-buffered saline, pH 7.4 (PBS) for 5 min, centrifuged for 15 min at 400 *g* at 4°C and washed twice with 1% bovine serum albumin (BSA) and 0.02% ethylenediaminotetraacetate (EDTA) in PBS at pH 6.8. They were suspended in this medium at a concentration of 20×10^6 cells/ml. Aliquots of 50 μl were reacted for 30 min with 50 μl of an appropriate dilution of antiserum FGAHu Fab-TRITC (Nordic, Tilburg, The Netherlands); a tetramethylrhodamineisothiocyanate-labeled $(Fab')_2$-fragment of a goat antiserum against human Fab-fragments was used as a reagent to identify B-cells. Antisera known as OKT3, OKT4 and OKT8 were used to identify all mature T-cells, helper cells, and cytotoxic suppressor cells respectively [2, 4–6]. These sera were kindly provided to us by Ortho Pharmaceuticals, Beerse, Belgium. Cells were washed twice with BSA/0.02% EDTA. The cells that had been treated with the OKT antisera were then reacted for 30 min with GAM-IgG-FITC (Nordic, Tilburg, The Netherlands), a fluoresceineisothiocyanate-labeled goat antiserum against mouse IgG, and washed twice with 1% BSA/0.02% EDTA. The stained cells were transferred to a microscope slide in a drop of glycerine/PBS, 9/1, vol/vol. The preparation was sealed with paraffine and the slides were examined in a fluorescence microscope. First the morphology of each cell was judged by phase contrast microscopy. Subsequently, fluorescence under ultraviolet light was observed.

RESULTS

Case reports

Case 1 (code No. 051) was a 69-yr old man suffering from a λ-type light chain disease. For 3 yr he had been treated with multiple cytostatic drugs and irradiation, to which he had eventually become resistant. At the time interferon therapy was initiated the patient's condition was rapidly deteriorating. He had multiple osteolytic lesions, high plasmocyte counts in the bone marrow ranging from 2 to 6% and high urinary excretion of light chains (3–5 g/24 hr). Hemoglobin and calcium levels were within normal range. Fibroblast interferon (8×10^6 U) was administered as the only drug on alternate days. Except for mild pyrexia, no side effects of the interferon treatment were noted. However, no benefit was apparent after one month of therapy, as light chains continued to be excreted at an unchanged rate (Fig. 1). Shortly after interruption of the treatment, the patient died of septicaemia.

Case 2 (code No. 052) was a 59-yr old woman with a previously untreated κ-type light chain disease. She complained of diffuse bond pain with multiple costal fractures. Mild anaemia, low serum immunoglobulin levels, high excretion of light chain and high serum calcium levels were present (Table 1). The bone marrow contained an average of 28% plasmocytes. Intramuscular injections of fibroblast interferon (10×10^6 U) were given three times a week during 3 months. Mild pyrexia (~38°C) was the only side effect. After 1 wk, a marked hypercalcaemia occurred, which was successfully treated by supplementing the interferon treatment with prednisone (Fig. 2a) and salt infusions. Subjective improvement, disappearance of bone pain and a marked decrease in light chain excretion were noted under this combined treatment. These improvements

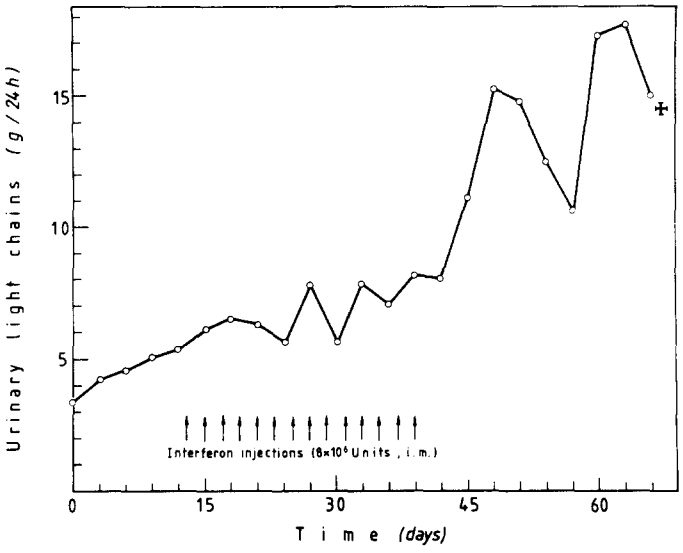

Fig. 1. Treatment schedule, urinary light chain excretion, and serum calcium in myeloma case 1 (Interferon given by intramuscular injections at times indicated by arrows.)

persisted during reduction and after interruption of the prednisone treatment, but no significant modification in haemoglobin or immunoglobin levels occurred. For 14 wk after all treatment was discontinued, the condition of the patient remained stable (Table 1). On the 28th week of observation light chain excretion and serum calcium levels increased (Fig. 2b). Therefore, fibroblast interferon treatment was re-instated for 6 wk. As neither parameter responded, it was decided to attempt a 5-wk course of leukocyte interferon treatment according to the schedule of Mellstedt *et al.* [17] (daily injections of 3×10^6 U). During the 4 first weeks both light chain excretion and calcaemia remained high, but a progressive decrease in both parameters occurred in the 4th and 5th week. This apparent improvement persisted for at least 4 wk after discontinuation of treatment. Other disease parameters remained unaltered (Table 1). The patient was subsequently treated with conventional chemotherapy.

Case 3 (code No. 058) was a 42-yr old man. His κ-light chain disease was discovered following pathological fracture of two ribs. Over a period of 6 months the patient received 7 courses of melphalan/cyclophosphamide/vincristine/prednisone therapy, No improvement in disease parameters was observed over this period: protein excretion remained high; high plasmocyte counts were recorded in the bone marrow, and serum immunoglobulin levels remained low (Table 2). Furthermore, radiological examination revealed progressive involvement of dorsal vertebrae, humeri and femora. The patient then received a combined treatment of pred-

Table 1. Hemoglobin, serum immunoglobulins and bone marrow plasmocyte counts in myeloma case 2

Disease parameter	Before interferon treatment*	Between 1st and 2nd treatment course	After 2nd treatment course
Hemoglobin (g/l)	107	127	95
IgA (g/l)	0.36	0.40	0.40
IgG (g/l)	6.36	8.10	6.30
IgM (g/l)	0.19	0.20	0.10
Bone marrow plasmocyte count(%)	28	10–20	34

*Two courses of interferon treatment with an interval of 14 wk. First treatment course: intramuscular injections of fibroblast interferon (10×10^6 U per injection, 3 times weekly for 13 wk). Second course: fibroblast interferon (10×10^6 U, 3 times weekly, 6 wk) followed by leukocyte interferon (3×10^6 U, 6 times weekly, 5 wk).

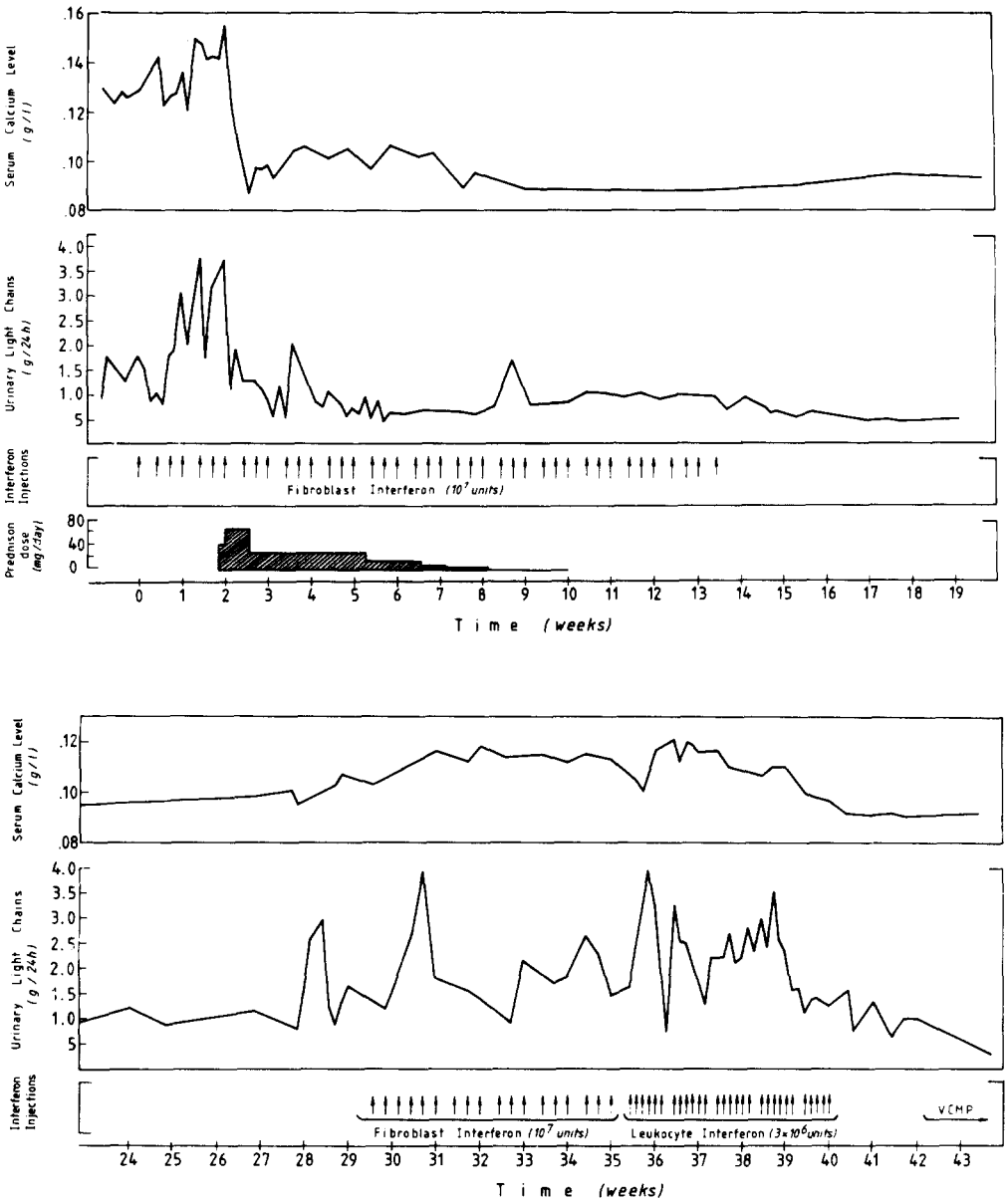

Fig. 2. Treatment schedule and urinary light chain excretion in myeloma case 2 (Interferon given by intramuscular injection at times indicated by arrows; VCMP = therapy with vincristine, cyclophosphamide, melphalan, prednisone).

Table 2. Hemoglobin, serum immunoglobulins and bone marrow plasmocyte counts in myeloma case 3

Disease parameter	Before therapy	During therapy with: VCMP†	fibroblast interferon‡	leukocyte interferon‡
Hemoglobin (g/l)	108	97	83	72
IgA (g/l)	0.27	0.11	0.09	0.1
IgG (g/l)	4.72	3.10	2.93	2.50
IgM (g/l)	0.15	0.13	<0.09	<0.09
Bone marrow plasmocyte count %	95	23	92	90

†7 courses of vincristine/cyclophosphamide/melphalan/prednisone over a 6 month period.
‡3.5×10^6 U/day, 6 times weekly, 6 wk; prednisone 40 mg daily.

nisone (60 mg per day for 2 wk, and 40 mg/day subsequently) and fibroblast interferon (3.5×10^6 U/day, 6 times weekly, 6 wk). During this period no improvement in disease parameters occurred. Daily light chain excretion remained high (Fig. 3); haemoglobin and serum IgG decreased; other parameters remained unchanged (Table 2). In view of this apparent failure of fibroblast interferon to exert a favorable effect on the disease, it was decided to attempt a 6 wk treatment consisting of continued daily prednsione doses of 40 mg of leukocyte interferon (3.5×10^6 U daily, 6 days/wk). In contrast to what had been seen in myeloma case No. 2, no improvement in disease parameters became evident. Light chain excretion remained high (Fig. 3); haemoglobin and IgG levels in the serum continued to decrease; plasmocyte counts in the bone marrow remained high (Table 2). Furthermore, the patient suffered from increasing bone pain. In view of this continued unfavorable evolution, interferon therapy was arrested.

During interferon treatment various immunological parameters were followed. This was done in order to test whether changes in these parameters would occur as an early indication of a later beneficial effect on the disease. Specifically, the distribution of various categories of lymphocyte populations was determined using rosetting as well as immunofluorescence techniques (see Materials and Methods). In Fig. 4 it can be seen that the relative number of B-cells, as measured by rosette assay, remained unchanged during the treatment; the number of B-cells carrying Fab-

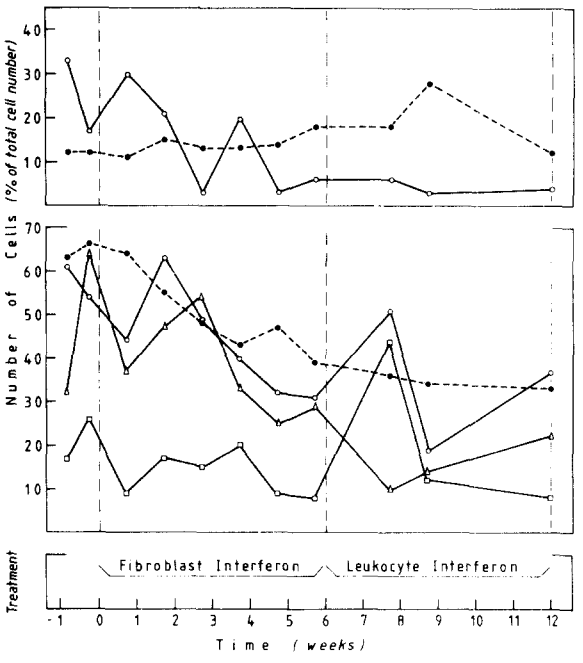

Fig. 4. Distribution of lymphocyte populations during interferon therapy in myeloma case 3. Upper panel: B-cells (O—O: staining with Fab-antiserum; ●--●: B-rosette counts). Lower panel: T-cells (O—O: staining with pan-T-antibody; □—□: staining with T-helper-antibody; △—△: staining with T-suppressor antibody; ●--●: T-rosette counts).

receptors decreased from 20 to 30% to less than 10%. In the T-cell lineage the general trend was also a decrease as measured by T-rosette assay as well as by immunofluorescence using a pan-T antibody. The population characterized by an antibody quoted to be specific for helper cells also followed this downward trend. The

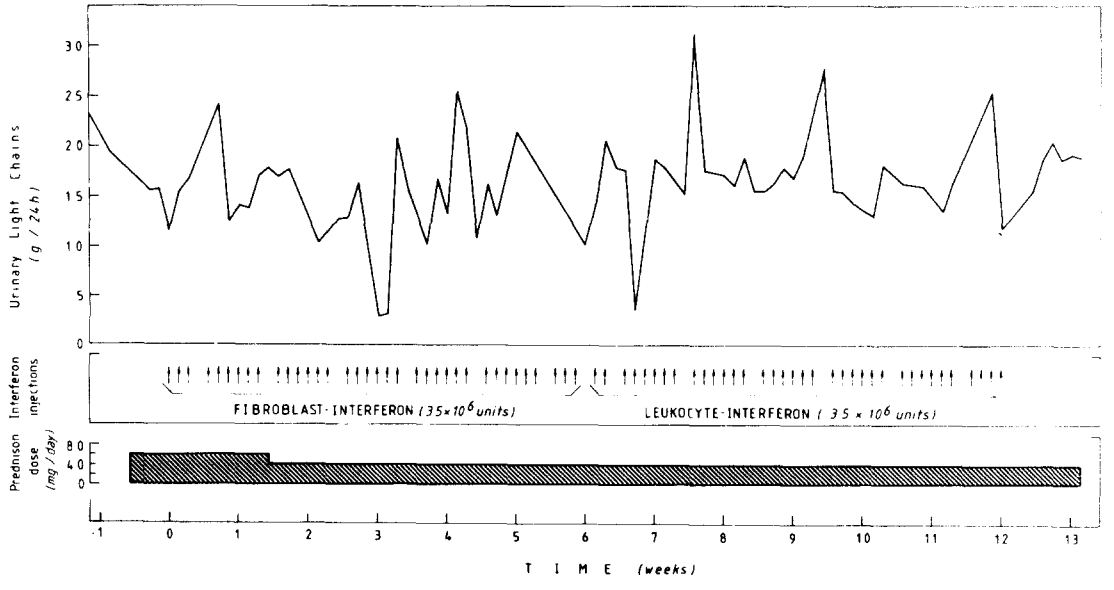

Fig. 3. Treatment schedule and urinary light chain excretion in myeloma case 3.

population labeled by an antibody quoted as suppressor cell-specific remained constant.

The response of the total lymphocyte population towards various mitogenic stimuli was also followed. The results of these determinations are summarized in Fig. 5. Spontaneous mitogenesis upon culture increased during interferon treatment, as evident from the lowest panel of Fig. 5. The response to ConA and PHA tested after one day in culture remained unaltered. In contrast, the response tested after 6 days in culture showed a downward trend during treatment with fibroblast interferon. During subsequent treatment with leukocyte interferon, the 6-day reactivity slowly restored to its initial value. Finally, mitogenesis as elicited by MLC remained constant throughout the treatment with both interferons.

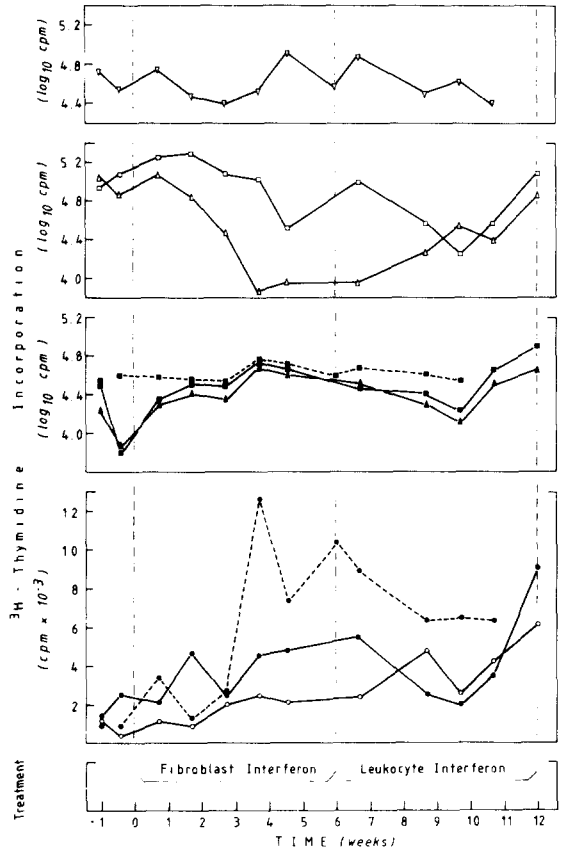

Fig. 5. Blastogenic response (^3H-thym idine incorporation) after in vitro *stimulation of lymphocytes of myeloma case* 3, *during interferon therapy. Panel A*: ▽—▽: *mixed lymphocyte reaction. Panel B: mitogren-stimulation after* 6 *days culturing* (□—□: *pokeweed mitogen*; △—△: *conA*). *Panel C: mitogen-stimulation after* 1 day *culturing*, (■—■: *PHA, lab J.B.*; ■—■: *PHA, lab M.W.*; ▲—▲: *ConA*). *Panel D: spontaneous mitogens* (●—●: 1 day *culture, lab J.B.*; ○—○: 6 day *culture*).

DISCUSSION

Mellstedt *et al.* [17] showed that injections of interferon prepared from human leukocytes had marked effects in two patients with multiple myeloma in terms of clinical, haematological and biochemical markers. Less definite effects were seen in the two other patients studied. Idestrom *et al.* [16] described initial improvement followed by progression under leukocyte interferon therapy in a single patient with myeloma (IgG-λ type). Recently Gutterman *et al.* [13] reported complete or partial remissions in 4 out of 10 cases of multiple myeloma treated with leukocyte interferon. In the present study fibroblast interferon essentially failed to induce remissions in three cases of light chain disease. Similar failure of fibroblast interferon therapy to affect myeloma was reported by McPherson and Tan [25] who treated 2 patient for 6 wk with doses increasing from 3 to 30×10^6 U/wk. Two of our patients also received leukocyte interferon. An apparent partial remission occurred subsequent to this treatment in one patient.

The number of myeloma cases treated with interferon is still too small to allow conclusions. However, the results obtained so far may provide some suggestions as to which type of clinical experiment may yield the most useful results. From the results obtained in case 2 it would seem that fibroblast interferon by itself is less effective in influencing the course of multiple myeloma than leukocyte interferon given in comparable doses. It is clear, however, that leukocyte interferon therapy in its present form had an effect in only a minority of the patients [13]. From our data as well as those of the literature it seems that cases that have not yet become resistant to chemotherapy are most likely to respond to leukocyte interferon.

In two of the patients described in the present paper, therapy with fibroblast interferon was supplemented with corticotherapy. In one patient (case 2) this was necessitated by the development of high serum calcium levels: the combined treatment with fibroblast interferon and prednisone was followed by clinical improvement. Since a second course of treatment, consisting only of fibroblast interferon, remained without effect in the same patient, we chose to use combination therapy in case 3: no improvement was noted.

Corticosteroids have been shown to reversibly inhibit the synthesis and excretion of Bence Jones protein in the majority of myeloma patients [26]. Corticosteroids are also part of certain generally accepted regimens of

chemotherapy for myeloma. One might therefore anticipate that corticotherapy would be useful as an adjuvant for interferon therapy in myeloma. The results obtained in our study do not seem to support this view. Therefore, it may not seem advisable to include corticotherapy in future clinical trials. In view of its immunosuppressive effect, it might as well be considered to counteract the effect of interferon.

The antitumor potential of interferon is largely attributed to immunomodulatory effects [1, 3]. In one of the three patients (case 3) the evolution of various immunological parameters was followed during treatment with interferon. The purpose of this investigation was to see whether the occurrence of a particular change in immunological reactivity could allow to predict the effect on the clinical course of the disease. The changes that were recorded (upward trend in spontaneous lymphocyte mitogenesis upon culture; downward trend in the relative numbers of B- or T-lymphocyte subpopulations; downward trend, with fibroblast interferon therapy, in response to mitogens after 6 day culture) are difficult to interpret since they may be unrelated to the therapy, or related to corticotherapy. However, it may be useful to do similar measurements in future recipients of interferon therapy in order to see which changes are a constant feature in responding and in non-responding patients.

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