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Interferon γ as Immunomodulator in a Patient with Multiple Myeloma

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Abstract. We describe here a patient with multiple myeloma, who, while in remission after chemotherapy, received 100 μg of rIFN-γ (Imukin, Boehringer, Ingelheim) subcutaneously 3 times a week for 4 weeks as supportive therapy before autologous peripheral blood stem cell transplantation (PBSCT). The patient was monitored for serum IFN, TNF, IL-2 activities and for the ability of peripheral blood leukocytes (PBL) to produce IFN-α/β, IFN-γ, IL-2 and TNF-α after *in vitro* induction. Changes in the percent of plasma cells in the bone marrow, in the total and differential white blood cell counts, in T cell subsets and NK cells were also monitored. IFN-γ yielded no clinical antitumor activity. The number of bone marrow plasma cells increased, however, the percentage of blood and bone marrow NK cells and the CD4/CD8 T cell subset ratio decreased. Monitoring the cytokine production ability of PBL during IFN-γ therapy revealed an increase in IL-2, IFN-γ and TNF-α titers produced upon *in vitro* induction after 2 weeks of treatment (6 injections of rIFN-γ). However, after 9 injections there was a significant decrease in IFN-γ and IL-2 production in the PBL, and at the end of therapy (12 injections) the decrease not only in IL-2 and in IFN-γ but also in IFN-α production was observed. In contrast to these changes, TNF production was strongly enhanced and reached the level observed before the therapy. These data suggest that the schedule of IFN-γ therapy in multiple myeloma should perhaps be adapted to become more effective, taking advantage from the immunomodulating activity of IFN-γ.

Key words: multiple myeloma, rIFN-γ therapy; TNF.

Introduction

Human multiple myeloma (MM) is a B cell neoplasia, characterized by the accumulation of malignant plasma cells primarily in bone marrow. Several groups have shown that interleukin 6 (IL-6) is an essential myeloma-cell growth factor *in vitro*[10, 23] and that proliferative response of myeloma cells to IL-6 *in vitro* is correlated with their proliferative capacity *in vivo*. Moreover, IL-6 is overproduced in patients with MM correlating with disease severity and patients survival[15, 22].

Interferon γ is a secretory product of activated T cells which can inhibit IL-6-related biological processes, the IL-6-dependent synthesis of acute-phase proteins[16], IL-6-dependent growth of human myeloma cell lines or freshly isolated myeloma cells from patients[12, 22] and also the cytokine-mediated bone marrow resorption, which is a major clinical feature of MM[26].

The therapeutic potential of IFN-γ for several infec-

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tions and malignant diseases has led to its development as a pharmacological agent. IFN-γ has been described as an effective drug in the treatment of patients with chronic granulomatous disease, reducing the severity and frequency of infective episodes[3, 8, 27], and also in several hematological malignancies such as chronic myelogenous leukemia, acute lymphoblastic leukemia and myelodysplastic syndromes, used as a single antineoplastic agent or in combination with other cytokines[1, 19, 25]. In addition, patients with multiple myeloma have been treated with high doses of rIFN-γ, however, with a minimal or no effect[21, 24]. Clinical trials of IFN-γ at the maximal tolerated or very high doses may have pushed the drug to concentrations beyond its physiological level of activity. Therefore, some clinical trials have been conducted in order to define a more biologically relevant dose and schedule. In patients with metastatic renal cell carcinoma receiving a low dose (100 μg) of IFN-γ subcutaneously, weekly, this cytokine was more effective with minimal toxicity than in patients receiving high doses of IFN-γ[6, 14, 17, 20].

Here we report a patient with MM, who while in partial remission after chemotherapy received low-dose IFN-γ treatment as supportive therapy before autologous peripheral blood stem cell transplantation (PBSCT). We were interested in how IFN-γ therapy may regulate other cytokine (IFNs and TNF) production, if it had any effect on the imunophenotype and distribution of peripheral blood leukocytes and if this low-dose IFN-γ therapy can be used in other patients with MM.

Materials and Methods

Patient. This 41-year-old man has had lambda chain multiple myeloma since November 1995. He has received 10 courses of standard chemotherapy (VMBCP – vincristine, melphalan, BCNU, cyclophosphamide, prednisone). In June 1996 there was a significant decrease in plasma cells level to 8% and monoclonal protein excretion in urine also decreased to 0.256 g/l for 24 h. In June 1996 he received IFN-γ treatment for 4 weeks and thereafter the bone marrow examination revealed an increase of plasma cells content to 18%, but protein excretion still remained on a low level (0.612 g/l/24 h). In October 1996 he received cytostatic treatment (VAD – vincristine, adriamycine, dexamethasone), because of disease progression and an increase of plasma cells in the bone marrow to 50%. After cytostatic treatment a significant regression of the disease was achieved. At present he is being prepared for autologous peripheral blood stem cells transplantation (PBSCT). Patient's permission was obtained for all studies relating to therapy with IFN-γ.

IFN-γ treatment. Recombinant, human IFN-γ (Imukin, Boehringer, Ingelheim) was administered subcutaneously 3 times a week for 4 weeks (totally 12 injections) at a fixed dose of 100 μg (about 10^6 IU). Blood samples were taken before IFN-γ therapy, every 2–4 during 24 h after the first injection and also on 2, 7, 14, 21 and 28 day of the therapy, but always before an IFN-γ injection was given and blood cells counts were performed. Bone marrow was taken before (day 0) and after IFN-γ therapy (day 28).

Blood was also taken from 10 healthy midle-aged persons, and served as controls in the experiments with cytokine induction.

Cytokine monitoring. Heparinized blood sample were diluted with Eagle's MEM to obtain 1×10^6/ml density of leukocytes, which was followed by adding cytokine inducers: Newcastle disease virus (NDV) 5 $TCID_{50}$/leukocyte, phytohemagglutinin (PHA, Sigma), 50 μg/ml or LPS from *E. coli* 0111: B4 (Sigma) 10 μg/ml, and then blood cell cultures were incubated at 37°C (5% CO_2) for 4 h (with LPS), 24 h (with NDV) or 72 h (with mitogen). The cultures were centrifuged and IFN and TNF activity was measured in the supernatants.

Cytokine assay. The presence of IFN-γ in the serum during 24 h after the first injection (IFN-γ monitoring in the blood), the presence of IL-2 in the serum as well as in supernatants of blood cell cultures after induction with PHA were measured by the immunoenzymatic method (ELISA) using IFN-γ or IL-2 kits (Predicta, Genzyme).

IFN-α/β activity present in supernatants of blood cell cultures and serum was measured by using the biological method based on inhibition of the cytopathic effect (CPE) of virus in A549 cell line as described[4] with one modification; encephalomyocarditis virus (EMC) instead of vesicular stomatitis virus (VSV) as a challenge was used. IFN was characterized by neutralization with specific anti-IFN antibodies as desribed[4].

TNF activity was measured by the biological method based on its cytotoxic action on L929 cell line in the presence of actinomycin D and characterized by neutralization with anti-TNF-α antibodies (Genzyme) as described in detail[5].

Immunophenotyping of peripheral blood leukocytes (*PBL*) *and bone marrow leukocytes* (*BML*). Unstimulated PBL and BML were examined using the following monoclonal antibodies (mAbs): CD4, CD8, CD45RA and combination CD3-/16+/56+ (Dakopatts, Copenhagen, Denmark). Double color immunofluores-

cence studies were performed using a combination of phycoerythrin (PE) or fluorescein isothiocyanate (FITC) conjugated mAbs. Cells (10^6) were incubated with antibodies for 30 min at 4°C, washed twice with PBS and incubated with a lysing reagent at room temperature until lysis of erthrocytes was complete (10–15 min). Cells were analyzed on Cytoron flow cytometer (Ortho Diagnostic Systems). In each experiment 10^4 cells were scored and expressed as the percent of total cell counts.

Results and Discussion

The concentrations of IFN-γ in serum following the first subcutaneous injection of 100 μg of IFN-γ were examined. Peak concentration was reached by 6 h (500 pg of IFN-γ/ml of serum) and IFN disappeared from blood after 26 h (data not presented). This pharmacokinetics was typical for subcutaneous injections of IFN-γ[28].

Analysis of white blood cell (WBC) counts and absolute number of circulating granulocytes and lymphocytes before and during IFN-γ therapy revealed that 48 h after the first dose of IFN-γ a slight decrease in WBC number was seen and the greatest changes were observed when the patient received totally 3 injections of IFN-γ (7 day). A low WBC count was mostly caused by significant drop in absolute number of granulocytes. Leukopenia and granulocytopenia lasted for 2 weeks when the patient received totally 6 injections of IFN-γ. Three weeks after the therapy onset (9 injections) the lymphocyte number was higher, but the granulocyte number was still lower in comparison to initial values (Fig. 1).

Dose-dependent, reversible granulocytopenia is the most common hematological abnormality following IFN-γ therapy[11], and can be explained by inhibition of human hemopoietic progenitor proliferation, especially dependent on stimulation with G-CSF. However, the increase in the number of lymphocytes at the end of IFN-γ therapy was not a typical feature.

Immunophenotyping of PBL revealed a decrease in CD4+ and increase in CD8+ cells percentage and in the consequence a decrease in CD4/CD8 ratio from 1.78 before to 1.29 after therapy. It was accompanied by CD45RA+ cells increase indicating that the increase in the total lymphocyte number was mainly caused by virgin, naive, non-primed CD8+ lymphocytes. Additionaly, the percentage of CD3-/16+/56+ (mainly NK cells) also sligtly decreased after IFN-γ therapy. Immunophenotyping of BML revealed the same tendency: decreased CD4+/CD8+ ratio from 1.54 before to 0.95 after therapy and a slight decrease in the percentage of NK cells (Fig. 2).

Together with the observations that in bone marrow after IFN-γ therapy the number of plasma cells increased from 8 to 18%, these data indicate that there were no benefitial effects of IFN-γ therapy.

These results are in agreement with clinical observations of other authors that peripheral blood of patients treated with IFN-γ exhibited a very low enhancement of NK and lack of enhancement of LAK cell activity. However, addition *in vitro* of IL-2 led to therapy-dependent increase of cytotoxicity indicating that IFN-γ low-dose therapy, combined with IL-2, may be more effective[1, 24, 29].

Monitoring PBL ability to produce some cytokines upon *in vitro* induction, which has been described to act in synergy with IFN-γ as IFN-α/β, IL-2 and TNF,

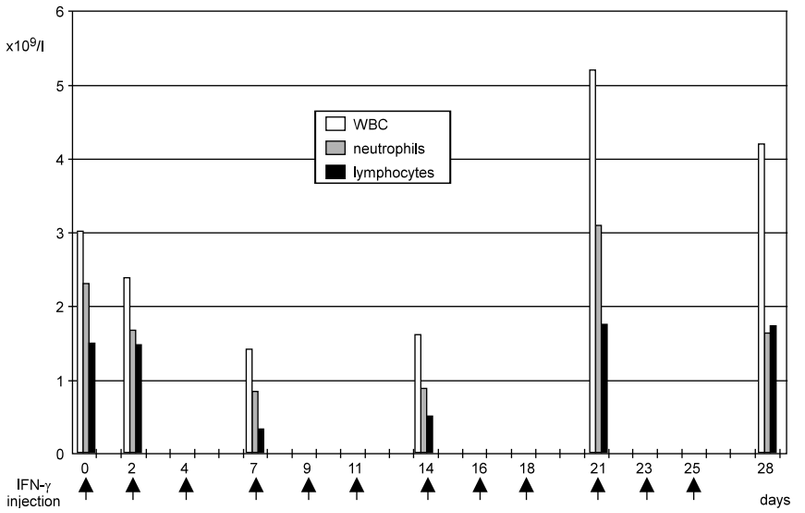

Fig. 1. White blood cells (WBC), neutrophils or lymphocyte absolute counts during IFN-γ therapy

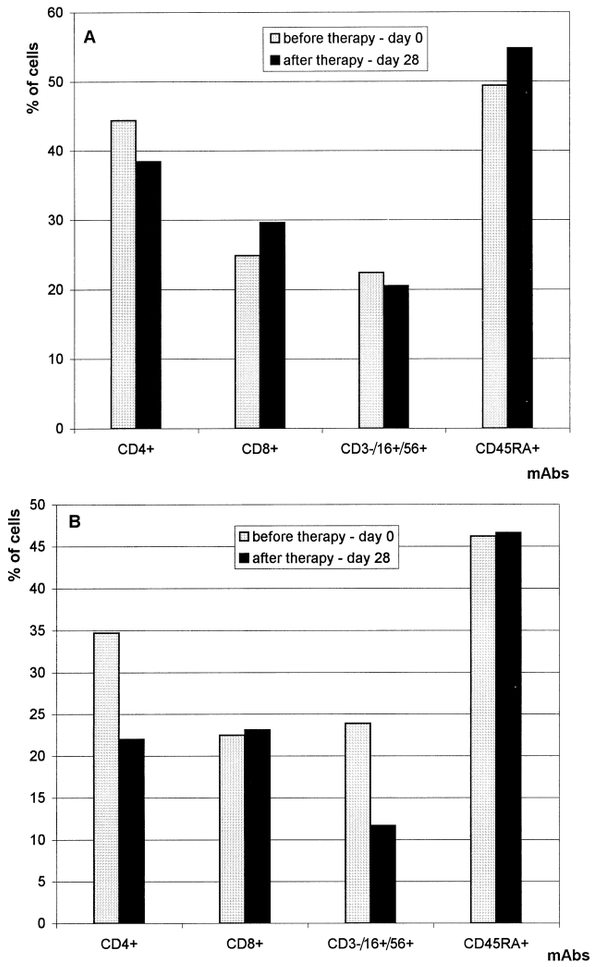

Fig. 2. Percentage of blood (A) and bone marrow (B) leukocyte reactivity with monoclonal antibodies (mAbs) measured by flow cytometry

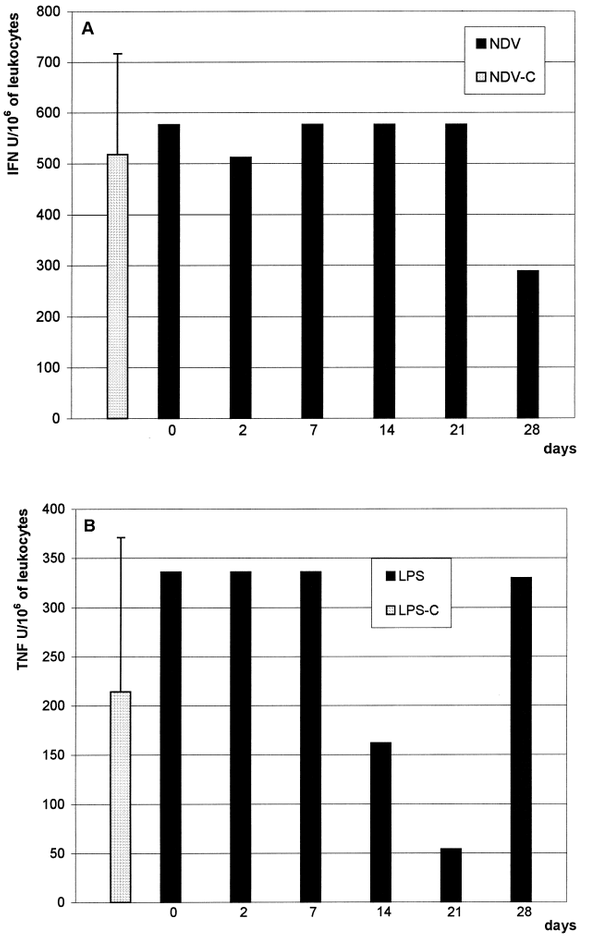

Fig. 3. Influence of IFN-γ therapy on IFN-α/β (A) and TNF (B) production ability of PBL. INF-α/β inducer *in vitro*: Newcastle disease virus (NDV) 5 $TCID_{50}$/leukocyte. TNF inducer: LPS 10 μg/ml. NDV-C – IFN production ability of control PBL. LPS-C – TNF production ability of control PBL. Spontaneous production of IFN and TNF in cultures of blood cells *in vivo* was lower than 6 U/ml

revealed that no increase of IFN-α/β production was seen during IFN-γ therapy, and at the end a significant decrease in IFN production was observed (Fig. 3). About 70–80% of this IFN activity was neutralized by polyclonal anti-IFN-α antibodies.

In contrast to decreased IFN α/β production, PBL induced by PHA produced more IFN-γ *in vitro*, when blood was taken from the patient on days 2 and 7 of therapy (after 3 injections of IFN-γ). However, it should be noticed that the ability of the patient's PBL to produce IFN-γ was at the beginning of therapy significantly lower than in PBL of healthy controls. A high response of PBL from the patient to PHA decreased slowly thereafter and returned to the some values as at the beginning of therapy (Fig. 4).

IL-2 production by the patient's PBL after PHA induction *in vitro* increased slightly after 1 injection of IFN-γ to the level observed in control PBL, but dropped to low values after 9 injections (Fig. 4).

A high ability of blood leukocytes to produce endogenous IFN-γ is very important because of several activities of this cytokine as inhibition of IL-6-dependent growth of myeloma cells[26] and stimulation of cytotoxic activity of macrophages and monocytes[3]. Our results indicating that IFN-γ therapy enhanced the relative ability of PBL of patient with MM to produce IFN-γ are in agreement with observations of other authors that IFN-γ can enhance *in vitro* and *in vivo* its own m-RNA expression[2, 9].

Many instances have been reported in which IFN-γ synergizes with TNF-α both *in vitro* and *in vivo*: in

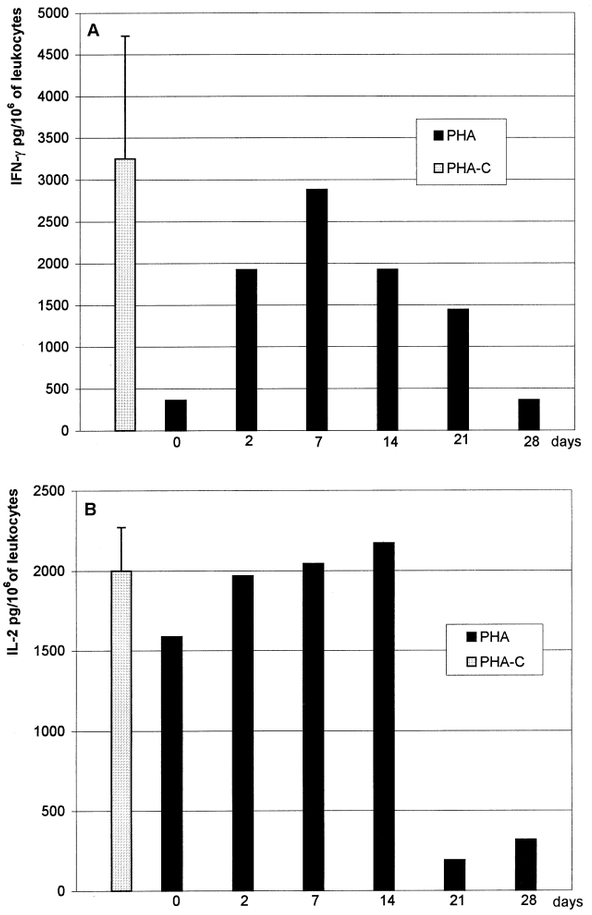

Fig. 4. Influence of IFN-γ therapy on IFN-γ (A) and IL-2 (B) production ability of PBL. IFN-γ and IL-2 inducer *in vitro*: PHA 50 μg/ml. PHA-C – IFN-γ and IL-2 production ability of control PBL. Spontaneous production of IFN-γ in cultures of blood cells *in vitro* was lower than 36 pg/10^6 of leukocytes and IL-2 lower than 90 pg/10^6 of leukocytes

cytotoxicity for tumor cells, induction of microbicidal activity in macrophages, induction of NO release and expression of surface adhesion molecules[30]. On the other hand, TNF can be responsible for inhibition of normal hematopoiesis and has been described as a positive growth regulator of B cell malignancies[7]. In our observations the PBL of the patient with multiple myeloma, taken before IFN-γ therapy, exhibited a high ability to produce TNF-α in comparison to controls after stimulation *in vitro* with LPS, and IFN-γ therapy did not enhance this ability. However, at the end of the therapy TNF titers produced by PBL dropped to low values (Fig. 3). It is very difficult to judge if this change was benefitial or not for the patient. It should be noticed that no IFN, IL-2 and TNF activity was detected in serum of patient during IFN-γ therapy.

IL-2 is the cytokine which can function as IFN-γ inducer, especially in large granular lymphocytes (LGL)[13]. On the other hand, IL-2 and IFN-γ are necessary in the development of cytotoxic T cells[18]. A slight stimulation of IL-2 production by IFN-γ therapy may be considered as beneficial for the patient with malignancy.

The disappointing results of IFN-γ therapy in the patient with MM and apparent lack of significant therapeutic efficiency of this cytokine strongly suggest that the therapy schedule should be changed to a more biologically relevant. It can be seen from the figures that during 2 weeks of therapy (6 injections of IFN-γ) the enhancing effect of IFN-γ and IL-2 production ability of PBL was seen. However, additional 6 injections of IFN-γ caused disturbances in the production of the cytokines examined. We suggest that in MM IFN-γ should be administered 3 times a week, for 2 weeks with a 2 week brake, thus permitting the immune system to regain homeostasis and avoid the development of hyporeactivity of immunocompetent cells. Low-dose therapy should be used because it permits co-administration of other antineoplastic agents as cytostatics or other cytokines such as IL-2. We suppose that administration of IL-2, together with IFN-γ, may result in substantial improvement in therapy, because of non-overlapping immunological actions of both cytokines.

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