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Tumor necrosis factor α modifies resistance to interferon α in vivo: first clinical data

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Summary. Patients with Philadelphia-positive chronic-phase chronic myelogenous leukemia (CML) resistant to interferon (IFN) α were treated in a phase I/II study with recombinant human tumor necrosis factor α to overcome IFNα resistance. Doses of 40, 80, 120 or 160 μg/m² TNFα were given as 2-h infusions on 5 consecutive days every 3 weeks. IFNα (4×10^6 IU/m² s.c., daily) treatment was continued. Six patients were treated, completing 1–24 (median, 12) treatment cycles. Five of the six patients achieved partial hematological remission, while the remaining patient had to stop treatment because of WHO grade 4 thrombocytopenia following the first TNFα cycle. No complete hematologic remission or cytogenetic improvement was seen. Side-effects were similar to those described for both substances alone. Maximum tolerable TNF doses usually varied between 80 μg/m² and 160 μg/m². To examine possible pathways of TNF activity in these patients, interferon receptor status and (2′–5′)-oligoadenylate synthetase levels were examined in peripheral blood mononuclear cells. Both parameters remained unchanged during TNFα treatment. These preliminary data point to significant clinical efficacy of additionally applyed TNFα in IFNα-resistant CML patients.

Key words: Tumor necrosis factor – Interferon α

Introduction

Interferon (IFN) α has been shown to have significant efficacy in chronic-phase chronic myelogenous leukemia (CML) inducing frequent hematological responses and cytogenetic improvements [1, 20, 21, 27, 28]. In individual patients, even a complete disappearance of the Philadelphia chromosome and the CML-typical BCR/ABL rearrangement on Southern blotting has been observed [22, 28, 34]. However, primary or secondary resistance to IFN therapy is a major problem [11]. In some cases resistance is due to anti-IFNα antibodies and may be overcome by natural IFN preparations [33]. In the majority of patients, however, no anti-IFN antibodies are detectable [21].

Tumor necrosis factor (TNF) α has marked synergistic activities with interferons in many experimental settings [2, 31, 32] including antiproliferative effects on myeloid cell lines and CML cells [26, 29]. Recently, moreover, it was shown that TNFα is capable of overcoming IFNα resistance in HL 60 cells [12]. Therefore, TNFα is an interesting substance to be tested in patients with chronic-phase CML resistant to IFNα, though it is not active in this disease on its own [15].

Several clinical studies have already shown that combinations of TNFα and IFN can be administered safely [8, 23]. Following these data, we started a phase I/II study of IFNα and TNFα in chronic-phase CML patients resistant to IFNα alone. To detect possible pathways by which TNFα might influence IFNα responsiveness in CML, we measured interferon surface receptors and (2′–5′)oligoadenylate synthetase, an interferon-inducible enzyme, in patients' peripheral blood mononuclear cells (PBMC) during TNF application.

Materials and methods

Patient eligibility criteria. Patients included in the study had chronic-phase Philadelphia-positive (Ph⁺) CML with primary or secondary resistance ot IFNα. Primary resistance was defined as failure to induce either reduction of white blood cells (WBC) within 8 weeks or partial hematological remission (PHR) within 6 months after start of IFN therapy at maximum tolerable doses (at least 3×10^6 IU/m²) in the absence of overt infection or acute-phase disease. Secondary resistance was defined as progressive increase of WBC to more than 20000/μl for at least 4 weeks after previous HR or PHR in the absence of acute infection or acute-phase disease and despite maximum tolerable IFN doses (at least 3×10^6 IU/m²). IFNα resistance was verified in all patients immediately prior to study by failure of therapy with 4×10^6 IU/m² IFNα. Anti-IFNα antibodies had to be absent at the start of study. Functions of the liver and kidney as well as coagulation factors had to be near normal. All patients gave informed consent prior to entry.

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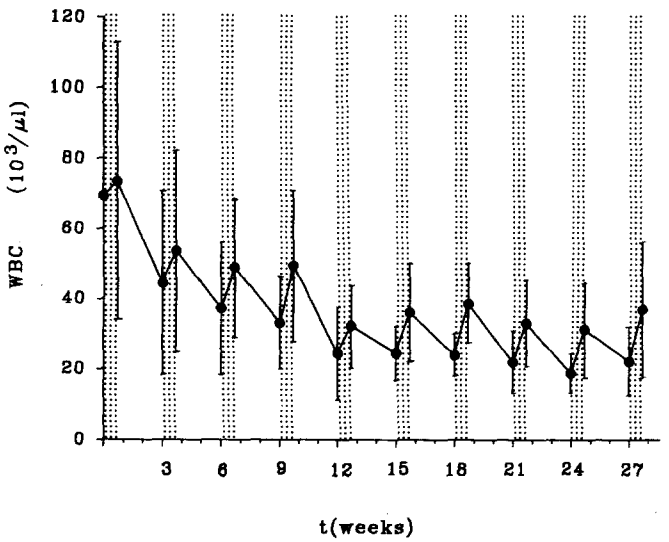

Fig. 1. White blood counts (*WBC*) before and on day 5 of tumor necrosis factor treatment. Mean values and standard deviations of four patients during the first 10 treatment cycles are given

Material and treatment modalities. Recombinant human (rhu) IFNα-2b was supplied by Schering Plough (Kenilworth, N. J., USA); the specific activity was $(1-2)\times 10^8$ IU/mg protein and a dose of 4×10^6 IU/m^2 was given once daily s.c. RhuTNF was provided by Knoll AG (Ludwigshafen, FRG); the specific activity was 8.2×10^6 units/mg protein as measured in the L929 cytotoxicity assay without adding actinomycin D. It was administered as an i.v. infusion dissolved in 50 ml 5% human albumin over 2 h. Five-day cycles consisting of one i.v. infusion daily were administered every 3 weeks. Patients were randomized to starting doses of either 40 µg/m^2 TNF (escalated via 80 µg/m^2 to 160 µg/m^2 in cases of no response) or 160 µg/m^2. Dosage was reduced if intolerable side-effects occurred. To reduce side-effects, 1 g paracetamol was routinely administered 30 min prior to TNF and 1000 ml saline was given concomitantly with TNF. Severe side-effects were treated with indomethacin or diclofenac.

Evaluation. The hematological and cytogenetic response to treatment was assessed according to standard criteria [28]: complete remission (CR), normalization of peripheral blood WBC count and differential count as well as absence of the Philadelphia chromosome in bone marrow on cytogenetic examination; hematological remission (HR), normalization of peripheral blood WBC count and differential count; partial hematological remission (PHR), 50% reduction of peripheral blood WBC count but at least below 20 000/µml; failure (F), any other outcome. Ultrasound measurement of splenic size was done prior to all treatment cycles and bone marrow biopsies including cytogenetic studies were performed after three cycles. Patients were hospitalized and examined before, during and after each TNF cycle. Evaluation included medical history, physical examination, complete blood cell counts, bilirubin, liver enzymes, creatinine, electrolytes, blood urea nitrogen and coagulation studies. Systemic side-effects were evaluated by patients' questionnaires. Side-effects were graded according to WHO. Acute effects (occurring and subsiding within 24 h of TNF application) and chronic effects (continuing or occurring more than 24 h after last TNF application) were analysed separately.

Analysis of interferon receptors. IFNα-2b was ^{125}I-labeled by the method of Bolton and Hunter [3] to a specific activity of 140–180 MBq/µmol. Labeled IFN was purified by gel filtration on Sephadex G-25. It retained 70%–80% of its antiviral activity assayed by inhibition of the cytopathic effect of encephalomyocarditis virus on WISH cells using an unlabeled IFNα-2b standard for reference. Labeled IFNα was stored in aliquots at −80°C and used within 3 weeks. For binding assays, PBMC were incubated with increasing concentrations of ^{125}I-IFNα in 1.5-ml conical tubes for 90 min at 23°C in RPMI medium containing 10% fetal calf serum.

Table 1. Response to tumor necrosis factor (TNF) interferon (IFN) treatment

Response	No. patients
Partial hematological remission	5/6
Substantial reduction of splenomegaly	1/4
Hematological remission	0/6
Cytogenetic response	0/6

Table 2. Response to TNF/IFN treatment

Parameter	Pretherapy	TNF/IFN
$10^{-3}\times$WBC[a] (µl^{-1})	91.5± 92.2	13.8± 4.5
$10^{-3}\times$Platelets (µl^{-1})	484 ±321	282 ±165
LDH[b] (U/l)	435 ±205	227 ± 14

[a] WBC, total peripheral blood white cells
[b] LDH, lactate dehydrogenase serum titers

Nonspecific binding was determined by co-incubation with a 500-fold excess of unlabeled IFNα. Cell-bound ^{125}I-IFNα was determined according to the method of Langer and Pestka [13]. Scatchard analysis of binding data and data reduction were carried out with a personal computer version of the program LIGAND [17]. All determinations were done as triplicates.

(2′–5′)oligoadenylate synthetase. (2′–5′)Oligoadenylate synthase levels were measured in Ficoll-separated PBMC. Cells were lysed and the intracellular synthase activity was determined according to the method of Merlin et al. [14]. All data were calculated as median values of duplicate probes with a maximal difference of 20% between the two probes. A (2′–5′)oligoadenylate synthase standard, prepared from IFN-treated K562 cells, was run with every experiment and results were expressed in laboratory units as multiples of the standard. An activity of 1 laboratory unit was approximately 0.7 nmol ATP converted to (2′–5′)oligoadenylate mg protein^{-1} h^{-1}.

Results

Patients' characteristics

Six patients, four male and two female, have been treated. Their age ranged from 28 to 66 years (median, 47); the time from diagnosis was 7–72 months (median, 22). Performance status according to the WHO scale was 0 (one patient), 1 (four patients), and 2 (one patient). Patients were pretreated with IFNα plus IFNγ (5), hydroxyurea (4), IFNα alone (3), busulfan (3) and TNFα alone (1). Two patient showed primary resistance, four patients secondary resistance to IFNα.

Response to therapy

In five out of six patients a partial hematological remission (PHR) could be induced (Table 1). In the sixth patient, therapy had to be stopped after the first treatment cycle because of severe thrombocytopenia. PHR was achieved after 2–8 treatment cycles (median, five). Not only WBC (Fig. 1) but also platelets and lactate dehydrogenase decreased substantially with TNF/IFN treatment (Table 2). Four patients presented with marked splenomegaly and in

Table 3. Acute TNF/IFN side-effects

Side-effect	No. patients	WHO scale			
		I	II	III	IV
Chills	6/6	–	2	4	–
Fever	6/6	–	3	3	–
Fatigue + malaise	6/6	2	3	1	–
Myalgias	5/6	1	1	3	–
Headache	5/6	2	3	–	–
Neuropathy (legs+back)	4/6	–	2	–	2
Stiff neck	3/6	2	1	–	–
Dry mouth	3/6	2	1	–	–
Confusion	2/6	–	1	–	1
Arthralgia	2/6	–	2	–	–
Thirst	2/6	1	1	–	–
Nausea	1/6	–	–	1	–
Itching	1/6	1	–	–	–
Diarrhoea	1/6	1	–	–	–
Paresthesia	1/6	1	–	–	–
Testicular cramp	1/6	1	–	–	–

Table 4. Chronic TNF/IFN side-effects

Side-effect	No. patients	WHO scale			
		I	II	III	IV
Weakness+fatigue	5/6	–	3	1	1
Anemia	5/6	3	1	1	–
Neuropathy (legs+back)	3/6	–	1	2	–
Thrombocytopenia	2/6	–	–	1	1
Myalgia	2/6	–	2	–	–
Arthralgia	2/6	1	1	–	–
Lack of concentration	2/6	1	1	–	–
Hair loss	1/6	1	–	–	–

Table 5. Hepatic toxicity of TNF/IFN treatment

Parameter	No. patients	WHO scale			
		I	II	III	IV
AST[a]	5/6	1	4	–	–
ALT	5/6	1	1	3	–
AP	4/6	3	1	–	–
GGT	4/6	1	1	2	–

[a] Serum titers of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP) and γ-glutamyltransferase (GGT) were measured

one of them a substantial decrease in splenic volume (>50%) was detected during treatment. However, no complete hematological remission (HR) and no cytogenetic response was achieved.

In the five responding patients, 5–24 (median, 12) TNF cycles were administered. After PHR was achieved no further significant treatment results were noted. The lowest white blood counts were achieved after 3–12 (median, 5) TNF cycles and WBC tended to increase slightly during the following cycles. Two patients ceased to participate while in PHR for 11 and 12 treatment cycles, but in both patients the WBC counts increased above 20000/μl within 2 months of cessation of treatment. In two other patients, PHR status was maintained only for 1 or 2 cycles, and was not regained thereafter, despite administration of increased TNF doses for at least 5 further cycles. One patient prolonged the treatment interval at his own request to 5 weeks after achieving PHR within 3 cycles. During 2 further cycles PHR status was lost.

All five patients who received multiple cycles of TNF are still in chronic-phase CML on various alternative treatments 15–22 months after cessation of study. The sixth patient was lost for follow-up in the chronic phase of the disease after 8 months.

No TNF dose dependence concerning treatment results could be proven.

Toxicity

Clinical. Side-effects of the combination were similar to the side-effects seen with both substances alone (Tables 3, 4). Acute side-effects were dependent on the TNF dose. Maximum tolerable TNF doses varied between 80 μg/m^2 and 160 μg/m^2 in the five patients treated with TNF for longer periods. Dose-limiting toxicities in these patients were severe myalgias and skeletal pains (two patients), confusion and temporary aphasia (one patient), and thrombocytopenia (one patient), while in one patient the maximum tolerable TNF dose was not reached at 160 μg/m^2. One patient experienced dose-limiting WHO grade 4 thrombocytopenia after his first treatment cycle of 40 μg/m^2 and was taken off study. Four treatment cycles had to be interrupted. This was due to confusion (one patient), severe myalgias and skeletal pains (two patients), and hepatic toxicity (one patient).

Chronic weakness and fatigue, not reversible during TNF-free periods, were observed in one patient. These side-effects responded readily to reduction of IFN dose from 7×10^6 to 5×10^6 IU.

Hepatic. Reversible hepatotoxicity during TNF cycles was observed in all six patients. Toxicities ranged between WHO grades 1 and 3 and transaminases as well as cholestatic enzymes were elevated (Table 5). Maximum enzyme serum levels were measured between days 3 and 5 of TNF application. In all patients, hepatotoxicity was reversible, usually completely, during TNF-free periods.

Hematological. As already described, WHO grade 4 thrombocytopenia required cessation of study in one patient at 40 μg/m^2 TNF. In another patient (TNFα dose 160 μg/m^2), grade 3 thrombocytopenia developed during the first 4 treatment cycles. Thereafter, platelets remained stable in the range of 30000–40000/μl without need for TNF dose reduction. Anemia of WHO grade 1–3 was seen in all five patients treated with multiple cycles of TNF.

Interferon receptors and (2′–5′)oligoadenylate synthetase

Numbers of interferon receptors per cell were low in all patients, as expected with chronic IFNα application, and did not change substantially during TNF cycles (Table 6).

Table 6. IFN class I receptors[a] and (2′-5′)oligoadenylate synthase[b] levels in peripheral blood mononuclear cells during TNF/IFN[c] treatment

Patients	IFN receptors				(2′-5′)Oligo-adenylate synthase (LU)	
	No./cell		K_d (nM)			
	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
1	565	365	0.41	0.49	2.71	2.50
2	284	392	0.42	1.70	3.44	3.56
3	236	249	0.35	0.37	2.07	2.33
4	194	247	0.98	0.95	3.69	3.37
5	–	–	–	–	0.82	1.05

[a] Interferon class I receptors (no./cell, number of receptors per cell; K_d, dissociation constant for receptors). Mean values of two cycles are given for each patient

[b] Activity of (2′-5′)oligoadenylate synthetase given in laboratory units (LU). Mean values of three cycles are given for each patient

[c] Probes were taken on the first (day 1) and last (day 5) day of TNF treatment prior to administration of TNF or IFN

Mean values per cell were 319 ± 167 on day 1 and 313 ± 76 on day 5 of TNF application. Also, the affinity of interferon receptors remained fairly constant except in one patient, in whom K_d increased substantially during 2 consecutive cycles (0.62 nM to 2.10 nM and 0.22 nM to 1.40 nM).

(2′–5′)Oligoadenylate synthase levels in PBMC remained constant during TNF treatment. Mean values were 2.55 ± 1.16 LU on day 1 and 2.56 ± 1.00 LU on day 5 of the TNF cycles.

Discussion

The present investigation demonstrates that by the application of TNFα clinical IFNα resistance in chronic-phase CML can be overcome. As depicted in Fig. 1. WBC tended to decline steadily during the initial courses of TNF treatment. Thereafter, this effect gradually disappeared. The intermittent increases of WBC during the days of TNF treatment were most likely caused by transient stimulation of cortisone production [18]. The clinical efficacy of TNFα, however, is limited as no complete hematological remissions and no cytogenetic improvements were achieved.

Toxicities of this therapy were substantial. Acute side-effects of TNF application (chills, fever, myalgias) did not significantly differ from the toxicities of treatment described for TNF alone. The maximum tolerable dose of TNF was in the same range as described for single-agent treatment with TNF [4, 6, 15]. These acute toxicities had to be differentiated from more chronic side-effects (fatigue, alopecia, neuropathy) similar to those experienced with long-term IFN treatment [21]. The impression that chronic effects tend to be IFN-related is strengthened by the observation in one patient with intolerable chronic weakness and fatigue who readily responded to reduction of IFNα from 7×10^6 to 5×10^6 IU. A constant finding was a marked though reversible hepatotoxicity. This confirms observations of a phase I study showing significant elevations of hepatic enzymes after TNF application especially in patients suffering from chronic myelogenous leukemia [15]. Thrombocytopenia developed only in two patients, but when it occurred its severity was substantial (WHO grade 3–4). Cessation of therapy was required in one of these patients. Thrombocytopenia was not related to dose or duration of treatment, as grade 4 toxicity developed in one patient treated with the lowest TNF dose of 40 μg/m^2 for just one cycle. Some degree of anemia was seen in all patients treated for longer periods. However, this was no clinical problem, as the severity was usually mild to moderate and patients responded well to erythrocyte transfusions, if required.

The mechanisms by which TNF did overcome resistance to IFNα in the patients treated remain unknown. The preliminary data of this study suggest that the effect is not related to actions on the receptor level. With one exception, the number of IFN receptors per cell as well as IFN binding affinities remained fairly constant during TNF treatment. This fits in well with data showing no significant differences on the interferon receptor level between IFNα-sensitive and -resistant CML patients [25]. Activation of the (2′–5′)oligoadenylate synthetase system, a well-examined interferon-induced enzyme system, has been postulated to correlate with interferon responsiveness in CML in the past [7, 25]. Recent data [5], however, as well as our own observations in CML patients treated with IFN alone [16] do not support this hypothesis. The present study detected activation of the (2′–5′)oligoadenylate synthetase system, as expected with long-term IFN application in all patients, but the enzyme titers were not influenced by TNF application. Therefore, a role of the synthetase system in the TNF-induced modulation of IFN resistance seems unlikely.

An important question remains the optimal dosage of TNF. The 5-day cycles were chosen in this study, since safe applications of TNF alone or in combination with other cytokines have been demonstrated using this schedule [15, 23]. The fact that WBC increased again during the third TNF-free week in one patient with prolonged treatment intervals suggests that longer TNF-free intervals are not desirable. To test, whether more frequent applications of TNF are more efficient we treated one of our patients after cessation of regular study with one or two TNF infusions per week in combination with IFNα. This schedule appeared not to be effective, as WBC increased substantially during treatment.

Continuous infusions of TNF have been safely given to patients for up to 5 days [30]. However, in the setting of IFN-resistant CML much longer treatment periods are required. As local injection of TNF is associated with marked inflammation [9, 24], the only feasible way for continuous exogenous administration of TNF seems to be infusion via infusion pumps. Recent observations, indicating that interleukin-2 (IL-2) even at comparatively low doses induces TNF in peripheral blood mononuclear cells [10, 19], may, however, offer a more elegant way to generate continuous TNF levels by daily s.c. injections of IL-2. Therefore, the combination of subcutaneous IL-2 and IFNα is presently being investigated in a phase I/II study.

Concerning the clinical relevance of the data, it must be said that the results achieved do not justify the exposure of

larger numbers of patients to the marked, although tolerable, side-effects of this protocol. On the other hand, the finding, that resistance to IFNα can be overcome by TNF is an interesting example of a successful combination of cytokines in a clinical setting. Furthermore, these results may help in the search for drugs used in combination with IFNα to improve treatment results in chronic myelogenous leukemia.

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