A progress report of a Phase I study of interferon-gamma and interleukin-2 and some comments on the mechanism of the toxicity due to interleukin-2

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Introduction

The proliferation of T-lymphocytes both *in vitro* and *in vivo* is dependent not only on the presence of the T-cell growth factor, interleukin-2 (IL-2), but also on the expression of IL-2 receptors (IL-2R) on the surface of responding cells. Following antigenic stimulation the generation of IL-2R is a complex process in which the cytokine interferon-gamma (IFN-G) plays a key role (4). It has also been demonstrated that T-cells activated by IFN-G develop a maximal proliferative response to IL-2, after 4 days exposure to IFN-G (4–6, 9). The aims of this study are, therefore, to induce IL-2R expression by the use of IFN-G in order to optimize the proliferative and immunomodulatory effect of IL-2. The toxicity of IL-2 results from damage to endothelial cells. This damage produces leakage of plasma proteins and consequently fluid from the vascular compartment into the interstitium. The fall in intravascular volume results in tachycardia and hypotension and the accumulation of fluid in the interstitial space causes oedema, weight gain and even effusions, ascites and lung oedema. This syndrome is very similar to that observed in overwhelming infections and septic shock. In sepsis, activation of the complement system occurs and the activation products of this system are involved in the pathogenesis of the shock syndrome and its complications (1, 2, 7, 8, 10). Amongst these activation products are C5a, C3a and C4a, which because of their biological effects are also known as anaphylotoxins. We were therefore prompted to study activation of the complement system in patients receiving high-dose IL-2. These data will be published in detail elsewhere but a summary of the results will also be presented here.

IFN-G and IL-2 study

Patients eligible for this study included those with a histologically proven solid tumor, who were between the ages of 18 and 70 years, had a good performance status (KI $\geq$ 60%) and a life expectancy of at least 3 months. A further requirement was that they should

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Table 1. Characteristics of patients treated with interferon-gamma plus interleukin-2

IFN-G dose IL-2 dose	10 $\mu\text{g}/\text{m}^2$ Bolus	100 $\mu\text{g}/\text{m}^2$ Bolus	100 $\mu\text{g}/\text{m}^2$ Continuous
Number	13	18	10
Median age	51 (27–68)	52 (23–72)	49 (36–68)
Median KP	90 (60–90)	80 (50–90)	90 (70–90)
Prior chemotherapy	6	5	2
Prior radiotherapy	3	3	3
Melanoma	5	5	4
Renal adenocarcinoma	3	6	3
Soft tissue sarcoma	2	0	1
Colorectal	1	4	0
Mesothelioma	1	1	1
Non-small cell lung	1	2	0
Unknown primary	0	0	0

Table 2. Changes in some immune parameters consequent upon treatment with interferon-gamma (mean values)

	IFN-G 10 $\mu\text{g}/\text{m}^2/\text{day}$		IFN-G 100 $\mu\text{g}/\text{m}^2/\text{day}$	
	Before	After	Before	After
Total lymphocytes ($\times 10^9/\text{l}$)	1.1	1.0	1.2	1.8
IL2-R-positive lymphocytes ($\times 10^6/\text{l}$)	0	4	15	9
MHC class II positive cells ($\times 10^6/\text{l}$)	54	68	123	248
Serum β_2 microglobulin levels (ng/ml normal ≤ 1870)	2054	3063	2133	5693

have preserved hematological function and no serious major organ dysfunction. The protocol was approved by the ethical and scientific research committees of the Free University Hospital.

Patients were admitted to hospital and received five daily intramuscular (i.m.) injections of IFN-G. Two dose levels of IFN-G were employed, namely 10 $\mu\text{g}/\text{m}^2/\text{day}$ and 100 $\mu\text{g}/\text{m}^2$. The maximum tolerated dose (MTD) of IL-2 was determined for each dose level of IFN-G. Three patients were to be entered at each IL-2 dose level. If WHO grade 3 or 4 toxicity was encountered in any one of these three patients two further patients were to be treated at that IL-2 dose level. If three of the five patients experienced WHO grade 3 or 4 toxicity then the MTD for IL-2 was said to have been reached. The starting dose of IL-2 was 0.5 mU/ m^2/day given by 15 min infusion. In the initial protocol the IL-2 was administered as a 15-min infusion once per day. At the end of the study further patients were treated with an IFN-G dose of 100 $\mu\text{g}/\text{m}^2/\text{day}$ but with the IL-2 given by continuous infusion over 120 h (5 days). Cycles of therapy were repeated every 3 weeks until progressive disease was documented.

Thirty-one patients have been entered into this study and their characteristics are described in Table 1. The IFN-G was well tolerated by the patients. Although there were more influenza-like side-effects with the higher dose of IFN-G (WHO grade II pyrexia: 10 $\mu\text{g}/\text{m}^2/\text{day}$ = 18% and 100 $\mu\text{g}/\text{m}^2/\text{day}$ = 29%) these were always mild and the IFN-G was not stopped in any patient because of toxicity. An increase in the percentage of peripheral blood lymphocytes expressing the IL-2R could not be demonstrated but induction of MHC class II and increases in serum β_2 microglobulin levels were greater with 100 $\mu\text{g}/\text{m}^2/\text{day}$ of IFN-G (Table 2).

The MTD for IL-2 was 3.0 mU/m²/day in combination with both the 10 µg/m²/day and 100 µg/m²/day doses of IFN-G. However, the dose-limiting toxicities were different for two doses of IFN-G, suggesting that IFN-G modifies IL-2 toxicity. The mean change in systolic blood pressure was -22.5 mm Hg for 3.0 U/m² IL-2 following 10 µg/m²/day IFN-G but +3.5 mm Hg for the same IL-2 dose after 100 µg/m²/day. For the higher dose of IFN-G the dose limiting, toxicity, was elevation of hepatic enzymes. Of four patients given 100 µg/m²/day IFN-G in combination with 3.0 mU/m²/day IL-2 two had WHO grade IV rises in gamma GT and one a grade III elevation. All three patients given 3.0 mU/m²/day of IL-2 but with the lower dose of IFN-G had grade II elevations of gamma GT. Other liver transaminases were elevated but not to the same degree as the gamma GT.

A further 10 patients have been treated with IFN-G 100 µg/m²/day in combination with 1.0 or 2.0 mU/m²/day of IL-2 but given as a continuous infusion over 5 days. Subjective toxicity consisting of malaise, lethargy, anorexia and pruritis were worse when IL-2 was administered continuously. Hepatic toxicity was evident at a dose of 2.0 mU/m²/day IL-2 with three of six patients given this dose having WHO grade III rises in gamma GT. The capillary leak syndrome, as measured by weight gain and fall in serum albumin, was also worse with continuously infused IL-2 (mean % weight gain: +1.7 (+3.3/-0.3) for bolus IL-2 at 2.0 mU/m²/day vs. +4.9 (+5.1/-3.4) for continuous IL-2).

In conclusion, a dose of 2.0 mU/m²/day of IL-2 can be safely administered in combination with 100 µg/m²/day of IFN-G. With an IL-2 dose of 3.0 mU/m²/day liver enzyme changes are marked but rapidly reversible upon cessation of IL-2. This dose of IL-2 could therefore be employed in Phase II studies in combination with 100 µg/m²/day of IFN-G but the long-term effects of such hepatic toxicity remain to be determined.

Complement activation due to IL-2 therapy

In this study the patients received 30-min infusions of IL-2 daily for 5 days. They then had 2 days rest and subsequently received a further 5 days of IL-2. A second cycle of IL-2 was to be given 1 month after the first. The starting dose of IL-2 was 3.0 mU/m²/day. This was increased to 6 mU/m²/day after 2 days and, provided tolerance was acceptable, subsequently to 9 and then 12 mU/m²/day. Haemodynamic parameters including pulmonary artery wedge pressure and cardiac output were measured at regular intervals. The following variables were measured daily: C3, C4, Factor B, Cl-esterase inhibitor and pre-albumin. C3a was measured at several intervals during the day before and after administration of IL-2. Levels of C3, C4, factor B and pre-albumin were determined with a nephelometer (Behringwerke AG, Marburg, FRG) using monospecific antisera. C3a desarg was measured with a radioimmunoassay (3), and expressed as nanomoles per liter (nmol/l).

Based on estimations in normal donors, levels of C3a exceeding 5 nmol/l were considered elevated.

C3a levels increased markedly during IL-2 therapy from 4 nmol/l (mean) before therapy to 23 nmol/l at the end of the cycle (Fig. 1). Activation of C3 occurred via the classical pathway since C4a levels also increased during therapy. An inverse relationship (not statistically significant) was observed between C3a levels and systemic vascular resistance. A positive correlation ($P = < 0.001$) was found between mean daily C3a levels and daily weight gain (Fig. 2). In addition, C3a levels were inversely correlated with serum albumin

Figure 1. Serum C3a complement levels in patients receiving interleukin-2 on days 1–5 and 8–10.

Figure 2. Correlation between the daily weight gain and serum C3a complement levels in patients receiving interleukin-2 on days 1–5 and 8–10.

levels ($P = < 0.001$). Thus administration of high doses of IL-2 induces activation of the complement system mainly via the classical pathway. We suggest that this activation may contribute to the pathogenesis of the vascular leakage syndrome and hemodynamic changes that occur during IL-2 therapy. A more complete understanding of the pathogenesis of IL-2 toxicity will undoubtedly lead to means by which this toxicity can be minimized thus improving the therapeutic index of IL-2.

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