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ORIGINAL ARTICLE

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IFN-α regulates IL 10 production by CML cells in vitro

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Abstract High levels of spontaneous in vitro IL 10 secretion by a subset of untreated chronic phase CML patients' cells are shown to be decreased in the presence of IFN-α. However, the lower level of spontaneous IL 10 secretion by healthy control cells are was not depressed by IFN-α. In contrast to its effects on IL 10 production, IFN-α increased the low spontaneous secretion of IL 1β by patients' cells, bud did not further increase the higher levels of spontaneous IL 1β secretion by normal cells. It had no effect on secretion of TNF-α by patients or normals. Spontaneous secretion of IL-1α (or IFN-γ) by patients' cells was not observed whether or not IFN-α was present. Therefore, one mechanism of action of IFN-α in vivo may involve decreasing endogenous IL 10 secretion (thereby reducing suppressive effects on T cell reactivity) and increasing IL 1β secretion (thereby enhancing antigen presentation).

Key words IFN-α · CML cells

Introduction

Interferon-α (IFN-α) has been used successfully for many years in the treatment of chronic phase CML. Recent meta-analyses comparing the use of IFN-α with chemotherapy demonstrate a significant improvement in survival with regimens employing IFN-α [4]. Moreover, accumulating evidence suggests that IFN-α may be able to reduce residual disease to such an extent that tumour cells are no longer detectable by PCR in very long-term follow-up [11]. The mechanism of this action of IFN-α is not clear. One possibility is that IFN-α has a direct anti-proliferative effect on the tumour cells. Alternatively, IFN-α may facilitate effective immunity against the tumour cells by enhancing natural killer activity. For example, we have shown a correlation between length of time on IFN-α therapy up to one year, and the gradually increasing capacity of the patients to generate LAK cells capable of killing autologous CML cells in vitro [16]. However, there is also evidence that IFN-α treatment may enhance specific T cell immunity directed to bcr/abl sequences, a molecular marker for CML [15]. Consistent with a role for adaptive immunity in maintaining IFN-α-induced remission is the finding that there is an association between the patient's HLA type and their therapeutic response to IFN-α [3].

That human T cells are able to recognise at least the b3/a2 fusion products of the bcr/abl translocation has been shown by several groups both in the context of MHC class I and class II-restricted immunity [2, 5, 12, 17, 24–26]. Although most studies were limited to showing that T cells from normal healthy donors recognized bcr/abl synthetic peptide, in some cases recognition of leukaemic cells in the absence of added peptide was demonstrated, suggesting that the tumour cells themselves can process and present immunogenic bcr/abl sequences [24]. Moreover, in one report, it was demonstrated that patients' cells could be sensitized to synthetic peptides, and that the sensitized cells were able to recognize and kill HLA- and bcr/abl-matched tumour cells thereafter [26]. Other, as yet unidentified, targets on CML cells may also be recognizable in a tumour-specific fashion by T cells sensitized against autologous tumour cells in a modified mixed lymphocyte/tumour cell (MLTC) culture system [19]. In that study, we demonstrated that untreated chronic phase CML patients fell into two groups; a minority of patients where prolifer-

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ation in an autologous MLTC was easily measurable, and a majority where this was not the case. In the latter, we considered that one reason for lack of responses was the high levels of spontaneous IL 10 production observed in the non-responders but not responders in this system. Accordingly, non-responders could be converted into responders in vitro by neutralising endogenous IL 10 [19]. In the present communication, we have investigated the effects of IFN-α on cytokine secretion as an in vitro model for the mechanism of action of IFN-α in vivo.

Materials and methods

CML patients' cells

Patients' cells were obtained after informed consent as previously described [20]. Briefly, PBMC were isolated from untreated chronic phase CML patients (mean leukocytes 130,000) and normal healthy donors by centrifugation over Ficoll/Hypaque. Myeloid cells predominated in these CML patients' preparations (mean 75 ± 21% CD14, 87 ± 21% CD33, 33 ± 11% CD34). Cells were cryopreserved so that large numbers of patients could be tested at the same time. CML patients were selected for use in this study on the basis of their T cells' inability to respond to autologous tumour cells, which is partially linked to high levels of spontaneous IL 10 production in vitro [19].

Cytokines and sera

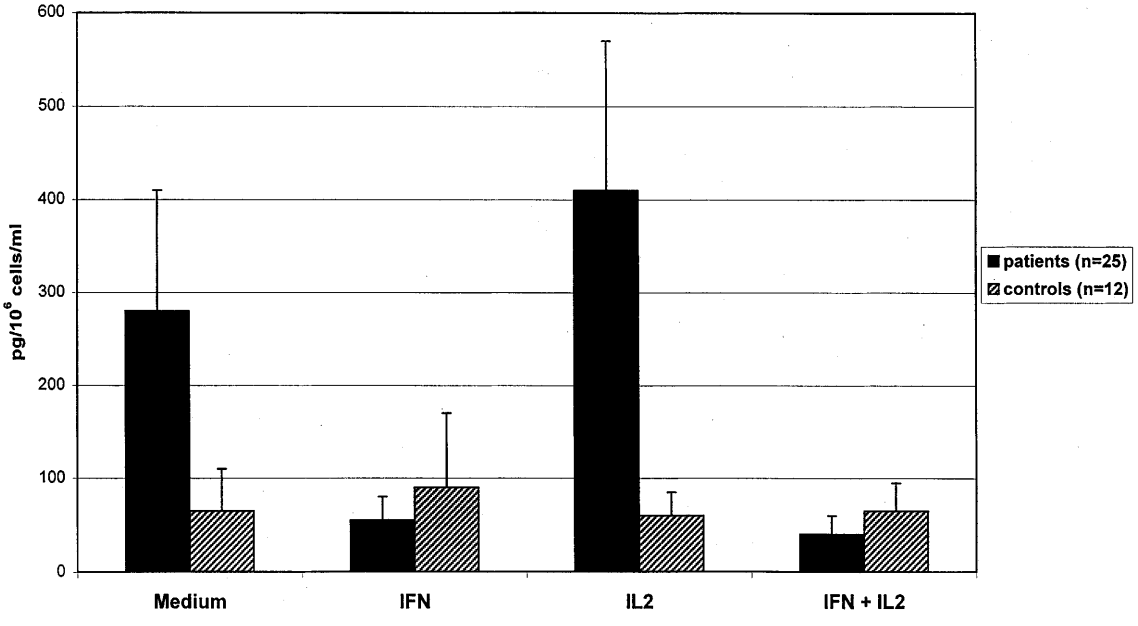
ELISA tests were used for assessing concentrations of cytokines in culture media. Cells were cultured at 2.5×10^5/ml in RPMI 1640 supplemented with 10% FCS (medium control) or with 100 U/ml IL 2, 100 U/ml IFN-α or both. Cell-free supernatants were assayed in ELISA with anti-cytokine antibody pairs purchased from PharMingen (Hamburg) and used according to the manufacturer's recommendations. Purified natural IL 2 (Lymphocult T-HP) was a gift of the Biotest Company, Frankfurt, as described [21]. Purified recombinant IFN-α was obtained from Endogen.

Results

Influence of IFN-α and IL 2 on IL 10 release in vitro

Previous studies had identified a group of CML patients' the PMBC of which spontaneously secreted high levels of IL 10 when cultured for 48 h in vitro [19]. Normal donors' cells, in contrast, secreted very little IL 10 under the same culture conditions [19]. These results are confirmed in Fig. 1, which shows the mean amount of IL 10 produced per 10^6 cells per ml in 48 h when cultured in medium alone (column labelled "medium"). The patients' PBMC produce on average around four times as much IL 10 as the healthy controls. The effect on IL 10 production of including 100 U/ml of IFN-α, 100 U/ml of IL 2 or 100 U/ml of both in the culture medium is also shown in Fig. 1. Addition of IFN-α alone greatly reduced the spontaneous secretion of IL 10 by patients' cells, but had little or no effect on controls' cells. IL2, on the other hand, seemed if anything to enhance IL 10 production by patients' cells, again with little or no effect on controls. Both IFN-α and IL 2 together had a similar effect to IFN-α alone, rather than IL 2, and again the spontaneous secretion of IL 10 by the patients' cells was

Fig. 1 Influence of IL 2 and IFN-α on IL 10 release in vitro. Patients' (*filled bars*, $n = 25$) and normal healthy controls' (*hatched bars*, $n = 12$) PBMC were cultured at 2.5×10^5/ml at a density of 5×10^5/16 mm-diameter culture well for 48 h in medium with 10% FCS ("Medium") with 100 U/ml IL 2 ("IL 2"), 100 U/ml IFN-α ("IFN") or 100 U/ml of both ("IFN + IL 2"). After this period, cell-free supernatants were collected and stored at −70 °C until assayed by ELISA for content of IL 10. Results are given as mean titer ± SDM (which is very high because of inter-individual variation in both patient and control groups) expressed as pg produced per 10^6 cells per ml in 48 h



suppressed. These results indicate potent regulatory effects of IFN-α on IL 10 production by CML cells.

Influence of IFN-α and IL 2 on IL 1β release in vitro

Previous studies had indicated a deficit in IL 1 secretion by patients' cells [19]. These previous results were obtained for IL 1α. Because the primary secreted form of IL 1 is IL 1β, spontaneous release of this cytokine is presented here. Figure 2 shows that whereas control cells produced over one ng of IL 1β per 10^6 cells per ml in 48 h, as with IL 1α, patients' cells produced much less than controls. Addition of IFN-α to the culture medium resulted in an improvement of IL 1β production by patients' cells, but not to the level of control cells. IL 2 had no effect on IL 1β release by either patients' or controls' cells, and a mixture of IFN-α and IL 2 seemed somewhat less effective than IFN-α alone.

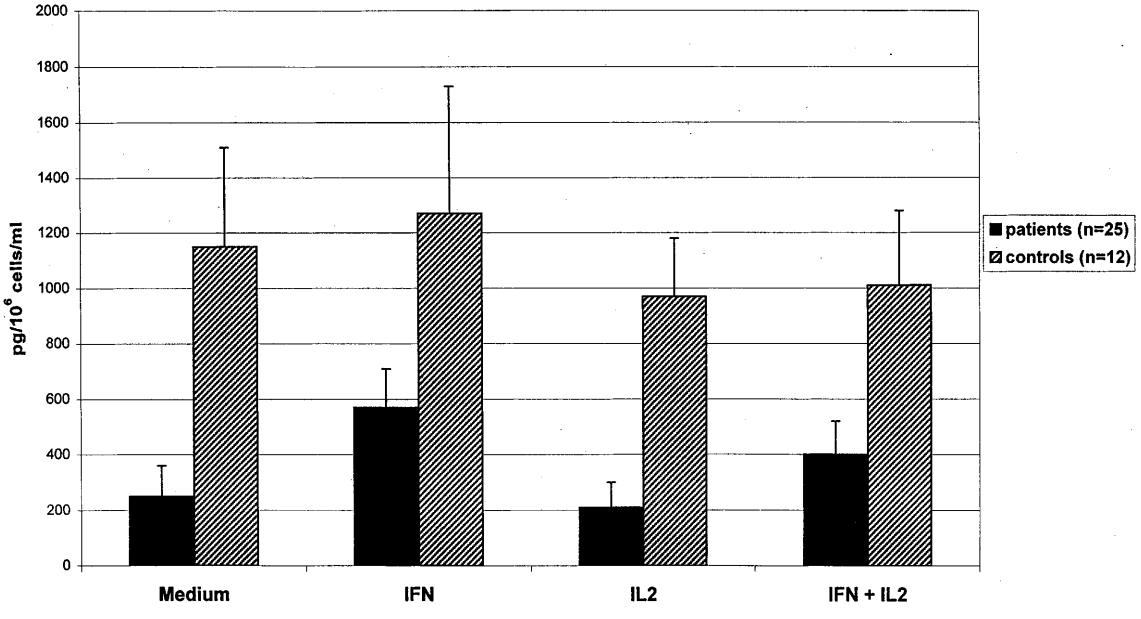
Influence of IFN-α and IL 2 on TNF-α release in vitro

Spontaneous TNF-α production by control cells was high, with a mean of >1 ng/ml/10^6 cells in 48 h, whereas production by patients' cells was around half of this (Fig. 3). The addition of IFN-α, IL 2 or both together had no effect on this spontaneous release of TNF-α. Other cytokines investigated included IFN-γ. This cytokine was not secreted spontaneously by CML cells ($n = 17$), regardless of the presence or absence of IFN-α, IL 2 or both (data not shown).

Fig. 2 Influence of IL 2 and IFN-α on IL 1β release in vitro. Procedure as in Fig. 1 legend, using IL 1β-specific ELISA

Discussion

Tumours exploit many mechanisms to escape immune recognition and destruction by the host [22]. One of the commonest is the secretion of immunosuppressive and/or immunomodulatory factors such as TGF-β and IL 10, which may block immune responses to the tumours or divert Th1 responses towards Th2 responses, which are thought to be less effective against tumours [22]. Peripheral cells from chronic phase CML patients (which consist mostly of tumour cells) spontaneously secrete IL 10 when cultured in vitro [19]. IL 10 is also detectable in the serum of CML patients (C.A. Müller, personal communication). This has functional consequences in-vitro at least, in that T cells of patients producing high levels of IL 10 are unable to respond by proliferation to autologous tumour cells, whereas cells from patients producing little IL 10 can do so [19]. Moreover, neutralisation of endogenously produced IL 10 with antisera enabled the "non-responders" to mount T cell proliferative responses against their autologous CML cells [19]. These observations may help to explain our previous findings that there was no correlation between proliferation in such autologous mixed lymphocyte/tumour cell cultures and the clinical course of IFN-α treatment, because we did not take variations in IL 10 production into account in those experiments [16]. If this is the case, it implies that lymphokine-activated killer (LAK) cell development is not susceptible to the effects of variable quantities of IL 10, because there was a steady increase of LAK activity over a one year IFN-α treatment period [16]. This is indeed consistent with reports that IL 10 in fact does not inhibit LAK [7]. However, it is clear that IL 10 adversely affects both antigen-presenting cells and responding T cells themselves [13], hence preferentially regulating adaptive rather than innate immunity. We are in the process of patient accrual to test whether clinical



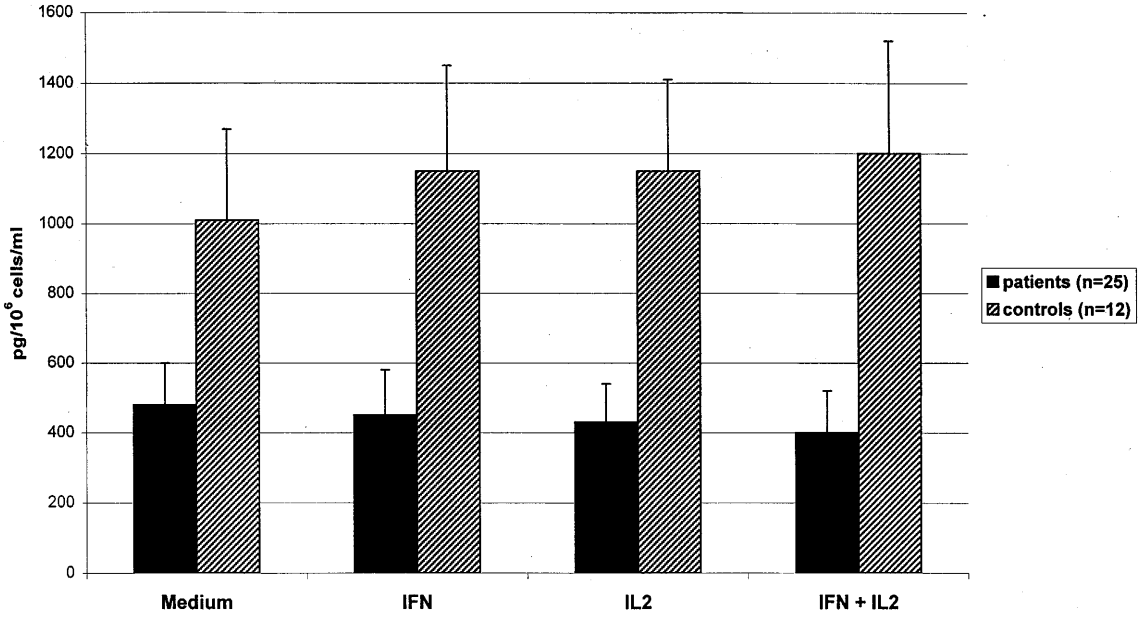


Fig. 3 Influence of IL 2 and IFN-α on TNF-α release in vitro. Procedure as in Fig. 1 legend, using TNF-α-specific ELISA

responses to IFN-α do correlate with T cell reactivity in MLTC when IL 10 production is taken into account. This would be predicted on the basis of the findings discussed here, and those of others [15].

We demonstrate here that spontaneous IL 10 secretion by CML cells is depressed by adding 100 U/ml IFN-α to the culture medium. In contrast, low level IL 10 secretion by control PMBC is if anything slightly enhanced by IFN-α. The latter finding is consistent with previous reports that IFN-α enhances IL 10 production by activated CD4+ cells and monocytes [1, 23]. In our experiments, the minimal level and enhancement reflects a lack of deliberate stimulation of the cells. The clearly suppressive effects of IFN-α on IL 10 production by CML cells suggests that cells of a different histological type can be differently regulated by IFN-α. If one mechanism of the beneficial action of IFN-α in CML is this inhibition of IL 10 production, then the data obtained with IL 2 treatment in vitro augur badly for its use in vivo. As can be seen in Fig. 1, addition of IL 2 enhances rather than suppresses the production of IL 10 by CML cells, while having essentially no effect on normal cells. However, a combination of IFN-α and IL 2, as employed in oncological practice for certain solid tumours and in a limited number of cases experimentally in CML [10, 14] might avoid this problem. Thus the in vitro data in Fig. 1 suggest that the IL 10 inhibiting effect of IFN-α is dominant over the stimulatory effect if IL 2. Since IFN-α itself may enhance IL 2 production [6], this finding may be viewed positively even in the context of IFN-α monotherapy.

In contrast to its inhibitory effects on IL 10 release, IFN-α strongly enhanced the secretion of IL 1β by CML cells and slightly enhanced its production by normal donors' cells. This result is also consistent with a previous report that IFN-α enhanced IL 1 production by normal PBMC [6]. In addition to the decrease of IL 10 production, this IFN-α-stimulated increase of IL 1β secretion might be immunostimulatory, as previously observed for IL 1α in these same patients [19]. Unlike the results with IL 1, the depressed TNF-α production by CML cells was not corrected by IFN-α in vitro, and normal controls were also unaffected.

Similarly, it has been previously reported that TNF-α production by some other human leukocyte subsets is not affected by IFN-α [8, 9]. The meaning of this for T cell responses is not clear since TNF-α may not have exclusively positive effects on T cells [18].

Further studies monitoring CML patients receiving IFN-α-treatment and comparing these with patients treated by chemotherapy will be required to demonstrate that the reduction in IL 10 release observed in vitro is also seen in vivo, and to investigate whether this parallels T cell immunity against autologous tumour, and finally whether this correlates with clinical response.

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