Clin. exp. Immunol. (1990) **82**, 583–589

Suppression of lymphokine-activated killer (LAK) cell induction mediated by interleukin-4 and transforming growth factor-β1: effect of addition of exogenous tumour necrosis factor-alpha and interferon-gamma, and measurement of their endogenous production

B. BROOKS, K. CHAPMAN, J. LAWRY, A. MEAGER* & R. C. REES *Section of Tumour Biology and Immunology, Department of Experimental and Clinical Microbiology, University of Sheffield Medical School, Sheffield, and *National Institute for Biological Standards and Control, South Mimms, England*

(Accepted for publication 7 June 1990)

SUMMARY

Recombinant human interleukin-4 (rhIL-4) and transforming growth factor-β1 (TGF-β1) suppressed the induction of lymphokine-activated killer (LAK) activity induced by recombinant human interleukin-2 (rhIL-2) in peripheral blood lymphocytes. DNA synthesis and the expression of the p55 alpha chain of the IL-2 receptor (Tac antigen) were also inhibited. The inhibitory effect was greatest when these factors were added during the first 48 h of a 4-day culture, with reduced cytolytic activity against both natural killer (NK) resistant and NK-sensitive tumour cell line targets. The suppressive action of both cytokines was accompanied by a reduction in tumour necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) levels in lymphocyte culture supernatants. Recombinant human IFN-γ (rhIFN-γ), but not recombinant human TNF-α (rhTNF-α) was able to overcome the inhibitory effect of recombinant human interleukin-4 (rhIL-4) on LAK induction and DNA synthesis but not Tac antigen expression. However, cytotoxicity induced by rhIFN-γ alone was also suppressed by rhIL-4 and TGF-β1, inferring that rhIFN-γ-mediated abrogation of rhIL4 suppression was not simply a direct IL-2-independent effect on cytotoxicity. In addition, rhIL-4 did not increase TGF-β production from rhIL-2-activated peripheral blood mononuclear cells, suggesting that rhIL-4 did not mediate reduction of rhIL-2 responses through the induction of TGF-β release.

Keywords lymphokine-activated killer cells interleukin-2 interleukin-4 transforming growth factor-β1 interferon-gamma tumour necrosis factor-alpha

INTRODUCTION

The culture of human peripheral blood mononuclear cells (PBMC) in recombinant human interleukin-2 (rhIL-2) leads to the generation of lymphokine-activated killer (LAK) activity. These non-MHC-restricted killers are capable of killing fresh tumour targets and tumour cell lines resistant to natural cell-mediated cytotoxicity (NCMC) (Grimm *et al.*, 1982). LAK activity represents a function rather than a cell type, with evidence that both large granular lymphocytes (LGL) and T cells respond to IL-2 and generate LAK activity (Damle, Doyle & Bradley, 1986; Ortaldo, Mason & Overton, 1986). IL-2 and LAK therapy has been shown to be effective in experimental models (Papa, Mule & Rosenberg, 1986) and in clinical trials (Rosenberg, 1988), but only 20–30% of patients show a positive clinical response (reduction in tumour burden). Thus, interest has focused on the role of other cytokines in the induction of cytotoxic effector cells, with a view to optimizing immunotherapeutic protocols.

The influence of interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) on the modulation of cytotoxic function and LAK induction has been documented. IFN-γ is produced by activated T lymphocytes and LGL, and either directly or indirectly augments natural killer (NK) activity (Kasahara *et al.*, 1983; Christmas, Meager & Moore, 1987; Young & Ortaldo, 1987; Rawlinson *et al.*, 1989). Increased NK cytotoxicity and induction of LAK activity by rhIL-2 has been reported to be wholly or partially due to, or completely independent of the induction of IFN-γ release (Weigent, Stanton & Johnson, 1983; Itoh *et al.*, 1985; Sayers, Mason & Ortaldo, 1986; Braakman *et al.*, 1986; Damle & Doyle, 1987). TNF-α is produced by monocytes, activated lymphocytes and LGL (Economou *et al.*, 1989; Turner, Londei & Feldmann, 1987; Peters *et al.*, 1986); it is cytotoxic/cytostatic to some

Correspondence: Bernadette Brooks, Department of Experimental and Clinical Microbiology, University of Sheffield, Beech Hill Road, Sheffield S10 2RX, UK.

tumour cells, synergizing with IFN-γ, and augments NK activity (Sugarman *et al.*, 1985; Schiller *et al.*, 1987; Ostensen, Thiele & Lipsky, 1987). These cytokines may represent important additional factors for the induction and maintenance of IL-2-activated lymphocytes, although their precise role remains to be defined. Augmentation of LAK induction by TNF-α and TNF-β, IL-6 and IL-1 has been reported (Owen-Shaub, Gutterman & Grimm, 1988; Crump, Owen-Shaub & Grimm, 1989; Blay *et al.*, 1989; Gallagher *et al.*, 1990), whereas IFN-α, -β and -γ, transforming growth factor-β1 (TGF-β1) and TGF-β2, IL-3 and IL-4 have all been reported to reduce cytotoxic induction (Sone *et al.*, 1988; Mule *et al.*, 1988; Gallagher, Wilcox & Al-Azzawi, 1988; Toledano *et al.*, 1989).

In the present study, the suppressive effects of recombinant human IL-4 (rhIL-4) and TGF-β1 were further investigated. Both were shown to reduce LAK induction, DNA synthesis, Tac antigen expression and both TNF-α and IFN-γ production from lymphocytes stimulated with rhIL-2. Of particular interest was the finding that recombinant human IFN-γ (rhIFN-γ) abrogated rhIL-4-mediated suppression of IL-2-induced cytotoxicity and DNA synthesis without a concomitant increase in Tac antigen expression. TGF-β1 and rhIL-4 were also found to inhibit IFN-γ-induced cytotoxicity. TGF-β1 production by cultured lymphocytes was not influenced by addition of rhIL-4.

MATERIALS AND METHODS

Effector cells

Peripheral blood from healthy donors was collected in heparin (10 U/ml) and the mononuclear cells (PBMC) were separated on lymphocyte separation medium (Lymphoprep; Nyegaard, Oslo, Norway), according to Böyum (1968). The recovered PBMC were washed three times in phosphate-buffered saline (PBS) and resuspended in the serum-free media, AIM-V (GIBCO, Paisley, UK).

Target cells

The SW742 colon adenocarcinoma cell line (Leibovitz *et al.*, 1976) was grown as an adherent cell line in RPMI 1640 medium plus 10% fetal calf serum (FCS). Previous study with this cell line has shown it to be relatively insensitive to NK cytolysis, but sensitive to rhIL-2-induced LAK activity. The NK-sensitive K562 cell line was also used (Lozzio & Lozzio, 1975). Both cell lines were routinely screened for mycoplasma contamination by DNA hybridization assay (Lab Impex, Middlesex, UK) and shown to be free of infection.

Cytokines and growth factors

Recombinant human IL-2 (specific activity $8{\cdot}3 \times 10^6$ U/mg) was kindly provided by Glaxo (Geneva, Switzerland), and used in culture at a final concentration of 500 U/ml; rhIL-4 (specific activity 10^8 U/mg) was kindly provided by the Immunex Corporation (Seattle, WA), and rhIFN-γ (specific activity 2×10^7 U/mg) and rhTNF-α (specific activity 6×10^7 U/mg) were kindly provided by Boehringer Ingelheim (Bender & Co., Vienna, Austria). Porcine TGF-β1 was purchased from British Biotechnology (Oxford, UK).

LAK cell induction

PBMC were seeded at 4×10^6 cells/ml in 1-ml volumes into flat-bottomed 24-well plates (Becton Dickinson, Lincoln Park, NJ). The cells were incubated at 37°C in a 5% CO_2/95% air atmosphere for a maximum of 4 days, after which each effector population was harvested, washed in PBS, counted and resuspended in the appropriate amount of RPMI plus 10% newborn calf serum (NBCS), and assayed for cytotoxic capacity.

^{51}Cr release test

A 4-h ^{51}Cr release assay was performed as described previously (Brooks & Rees, 1988). Briefly, labelled target cells (0·1 ml/well) were incubated with effector cells (0·1 ml/well) at the ratios indicated, in 96-well plates, in triplicate. Test plates were incubated at 37°C in a 5% CO_2/95% air humidified atmosphere for 4 h and ^{51}Cr release and cytotoxicity were calculated as previously described.

Monoclonal antibodies (MoAbs) and flow cytometry

Mouse anti-Tac was purchased from Dako (High Wycombe, UK), and flow cytometry analysis performed as previously described (Brooks & Rees, 1988).

ELISAs

For TNF-α. Supernatants from PBMC were harvested, stored at −70°C and assayed for TNF-α content in a sandwich ELISA, performed essentially according to a method developed by Meager *et al.* (1987). However, streptavidin-conjugated alkaline phosphatase (1/100 000, Amersham International, Amersham, UK) was used in preference to peroxidase and the assay developed using an ELISA amplification kit (GIBCO). RhTNF-α (Boehringer) was used as a reference standard.

For IFN-γ. Titres of IFN-γ were determined as described previously (Christmas *et al.*, 1987), using a sandwich ELISA.

Bioassay for TGF-β

Mink lung cells, MV-3D9 (a generous gift of Dr P. Lindquist, Genentech, South San Francisco, CA) were plated at 2×10^4 cells/well in microtitre plates in MEM + 10% FCS. Supernatants were acidified to pH 2 with 1 M HCl for 30 min and re-neutralized with 1 M NaOH prior to dilution in order to activate latent TGF-β. Following 3-h incubation of cells at 37°C, serial dilutions of TGF-β standard (NIBSC 89/514, 3000 U/ampoule, interim standard; 6 U = 1 ng) or cell supernatants were added in duplicate.

After a further 24-h incubation at 37°C, cells were pulsed with ^{3}H-thymidine (Amersham International), 1 μCi/well, for 4 h at 37°C. Cells were then removed using trypsin–versene, processed with a cell harvester, and counted in a beta-scintillation counter. Inhibition of thymidine uptake for each dilution of standard or supernatant was calculated using the formula:

$$\% \text{ inhibition} = 1 - \frac{\text{ct/min of sample}}{\text{ct/min of cell control}} \times 100$$

Titres of TGF-β activity were interpolated from the dose–response curve, and corrected to the assigned potency of the TGF-β standard.

Assessment of cell proliferation

Cells harvested from lymphocyte cultures were seeded into 96-well plates (Becton Dickinson) at 10^5 cells/0·1 ml per well in triplicate and 0·5 μCi ^{3}H-thymidine added. Cells were incubated for 4 h, and harvested onto filter paper using an automated cell

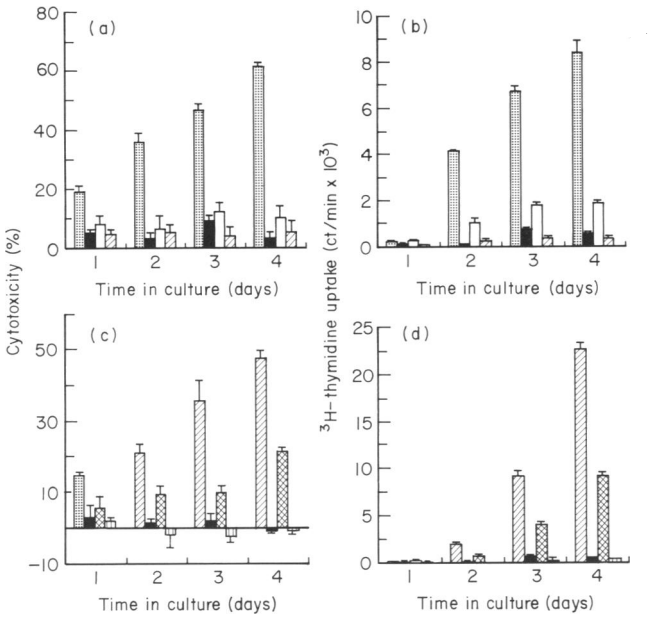

Fig. 1. Effect of 500 U/ml recombinant human interleukin-4 (rhIL-4) (a, b) and of 2 ng/ml transforming growth factor-β1 (TGF-β1) (c, d) on cytotoxicity and proliferation induced by 500 U/ml recombinant human interleukin-2 (rhIL-2). Peripheral blood mononuclear cells were cultured in AIM-V, harvested daily, washed and assessed for cytolytic capacity against the SW742 cell line (at effector-to-target cell ratio 10/1), and proliferation was measured by uptake of ^{3}H-thymidine. ▨, rhIL-2; ■, control; □, rhIL-2+rhIL-4; ■, rhIL-4; □, rhIL-2+TGF-β1; ■, TGF-β1.

harvester (Skatron, Norway). Incorporation of ^{3}H-thymidine was measured by counting the filter paper in scintillation fluid using a beta-spectrophotometer.

Statistical analysis

Non-parametric analysis of significance was assessed by Mann–Whitney *U*-test.

RESULTS

The addition of rhIL-4 or TGF-β to lymphocytes cultured in the presence of rhIL-2 was shown to reduce the induction of cytotoxicity (against the NK-resistant colon carcinoma cell line SW742 and the NK-sensitive cell line K562), DNA synthesis (Fig. 1) and Tac antigen expression (Table 1). Induction of cytotoxicity against SW742 targets was more sensitive to inhibition than the increased cytotoxicity observed against K562; only results using SW742 are shown. The suppressive effects were dose-dependent and greatest when rhIL-4 or TGF-β were added during the first 24 h of culture (data not shown). The results given in Fig. 1 and Table 1 are representative of a series of experiments performed. Supernatants collected from PBMC cultured in the presence of rhIL-2 and either rhIL-4 or TGF-β were assayed for the presence of TNF-α and IFN-γ, and the results shown in Table 2 are representative of several experiments. TNF-α release by PBMC cultured with rhIL-2 was reduced by rhIL-4 (15/15 experiments; $P < 0.001$) and by TGF-β (7/7 experiments; $P < 0.05$). Reduction of rhIL-2-induced IFN-γ release was also shown in the presence of rhIL-4 (10/10 experiments; $P < 0.01$) and TGF-β (4/4 experiments; $P = 0.014$).

Table 1. Tac antigen expression by peripheral blood mononuclear cells cultured with cytokines and growth factor

Cells cultured in medium plus	Percentage positive cells (intensity as measured by channel number)*			
	Day 1		Day 4	
	Control	% total	Control	% total
—	0·26	2·80 (30)	4·08	5·22 (42)
rhIL-2	0·24	18.24 (61)	0·48	21·98 (92)
rhIL-2+rhIL-4	0·38	10·48 (43)	1·00	12·14 (92)
rhIL-4	0·22	3·48 (38)	4·14	5·92 (64)
—	1·16	3·64 (37)	4·22	7·44 (38)
rhIL-2	0·48	8·06 (48)	3·64	22·90 (89)
rhIL-2+TGF-β1	0·90	4·22 (45)	2·42	10·26 (69)
TGF-β1	0·98	3·26 (32)	3·00	9·04 (33)

* The percentage positive cells and intensity of staining of lymphocytes incubated with rhIL-2 (500 U/ml) and either rhIL-4 (500 U/ml) or TGF-β1 (2 ng/ml) as measured by flow cytometry. Controls, FITC conjugate only.

rhIL-2, recombinant human interleukin-2; rhIL-4 recombinant human interleukin-4; TGF-β1, transforming growth factor-β1.

These reductions in cytokine release by rhIL-4 and TGF-β could be observed after 24 h of culture.

Further studies were undertaken to determine the effect of adding at the onset of culture exogenous rhTNF-α or rhIFN-γ to PBMC cultured with rhIL-2 and either rhIL-4 or TGF-β (where cytotoxicity and DNA synthesis was suppressed). In three out of six experiments, rhTNF-α (at concentrations ranging from 50 to 5000 U/ml) caused a small but significant reduction of rhIL-4-mediated suppression of cytotoxicity; in the other three experiments it had no effect (data not shown). RhTNF-α was able to reduce the suppression of DNA synthesis mediated by rhIL-4 in six out of six experiments, but completely only in one experiment (data not shown). The addition of exogenous rhIFN-γ abrogated the rhIL-4-mediated suppression of rhIL-2-induced cytotoxicity and DNA synthesis (nine out of 14 and four out of seven, respectively) (Fig. 2). Where abrogation of rhIL-4-mediated suppression was observed, Tac antigen expression was not returned to the level expressed by PBMC cultured in rhIL-2 alone. Thus, PBMC incubated with rhIL-2 plus rhIL-4 showed 8% of the cells positive for Tac, compared with 18% for PBMC cultured with rhIL-2 alone; the addition of rhIFN-γ to PBMC in the presence of rhIL-4 and rhIL-2 showed only 11% of the cells expressing the p55 (Tac) antigen. This effect was shown to be reproducible.

rhIFN-γ did not affect the level of Tac expression on cells cultured with or without rhIL-2 in the absence of rhIL-4 (results not shown).

The effect of rhTNF-α and rhIFN-γ on TGF-β-mediated reduction of rhIL-2 responses was also studied. When added at the onset of culture, rhIFN-γ (at a range of 10–1000 U/ml) reduced TGF-β-mediated suppression of rhIL-2-induced cytotoxicity in five out of eight experiments (complete in three) and of DNA synthesis in seven out of seven experiments (complete in three). In complementary experiments, rhTNF-α (at a range of

Table 2. Interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) release from peripheral blood mononuclear cells (PBMC) cultured in medium with added cytokines or growth factor

Culture supernatant collected on day	Culture supernatant assayed for	PBMC cultured in medium containing					
		0	rhIL-2	rhIL-2 +rhIL-4	rhIL-2 +TGF-β1	rhIL-4	TGF-β1
1	TNF-α	78	680	220	340	36	44
	IFN-γ	2	80	2	2	2	4
4	TNF-α	70	1760	128	360	36	30
	IFN-γ	2	220	3·4	9·2	4·1	2·55

rhIL-2, recombinant human interleukin-2 (500 u/ml); rhIL-4, recombinant human interleukin-4 (500 u/ml); TGF-β1, transforming growth factor −β1 (2 ng/ml).

PBMC were cultured in serum free media and supernatant collected on days 1 and 4 and stored at −70°C until assayed. TNF-α given as reference units/ml and IFN-γ as international units/ml.

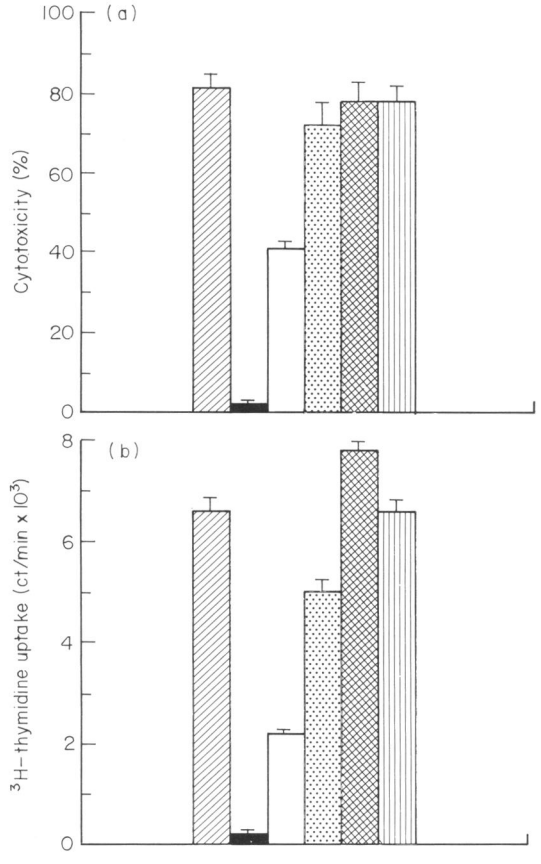

Fig. 2. Effect of recombinant human interferon-gamma (IFN-γ) on reduction (a) of cytotoxicity and (b) proliferation induced by interleukin-2 (IL-2), mediated by 500 U/ml recombinant human interleukin-4 (rhIL-4). Peripheral blood mononuclear cells were cultured for 4 days in AIM-V with combinations of cytokines: ▩, 500 U/ml rhIL-2; ■, control; □, 500 U/ml rhIL-4; ▨, 10 U/m rhIFN-γ+IL-2+IL-4; ▩, 100 U/ml rhIFN-γ+IL-2+IL-4; ■, 1000 U/ml rhIFN-γ+IL-2+IL-4. All cytokines were added at the onset of culture. Cytotoxicity was measured against the SW742 cell line (at effector-to-target cell ratio 10:1), and proliferation was measured by uptake of ^{3}H-thymidine.

50–5000 U/ml) reduced TGF-β suppression of rhIL-2-induced cytotoxicity in four out of eight experiments (complete in none) and DNA synthesis in seven out of seven experiments (complete in two) (data not shown). Thus, rhIFN-γ is a more consistent abrogator of TGF-β suppression of rhIL-2 responses than rhTNF-α, at the range of concentrations used. These results also highlight the variation in response to cytokines between individual donors.

It was further shown that PBMC cultured in the presence of rhIFN-γ (10–10000 U/ml) for 4 days generated cytotoxicity against the NK-resistant target cell line SW742 although this cytotoxicity was not as great as that induced by rhIL-2. Both TGF-β and rhIL-4 suppressed the induction of this cytotoxic activity (Fig. 3).

In order to determine whether rhIL-4 influenced TGF-β production by PBMC, supernatants were assayed for total and active TGF-β, using a mink lung bioassay. The addition of rhIL-4 to PBMC cultured with or without rhIL-2 did not increase the total amount of TGF-β released in five (out of five) experiments (Table 3). Active TGF-β (assayed before acid treatment of supernatants) was measured in two experiments. No difference was seen between samples, with levels less than 1 ng/ml. In one experiment where PBMC were cultured with or without rhIL-2, in the presence or absence of TGF-β, supernatants contained no detectable IL-4 (as assessed by immunoradiometric assay; Dr Chris Bird, NIBSC, personal communication). Effector function of rhIL-2-induced LAK was not affected by the addition of rhIL-2 (500 U/ml), rhIL-4 (500 and 1000 U/ml), rhTNF-α (5–1000 U/ml), rhIFN-γ (10–1000 U/ml), or TGF-β (2 ng and 5 ng/ml) to 4-h ^{51}Cr release assays; rhIL-2 alone was the only one of these cytokines which consistently increased natural killing against K562 and SW742 targets when added to fresh PBMC in a 4-h NCMC assay. This increased killing was not affected by addition of rhIL-4 or TGF-β (data not shown).

DISCUSSION

PBMC cultured in IL-2 exhibit LAK activity, which is mediated by a heterogeneous population of effector cells. The regulation of the induction of LAK activity by IL-2 and the contribution of other cytokines in this process is not fully understood. IL-4

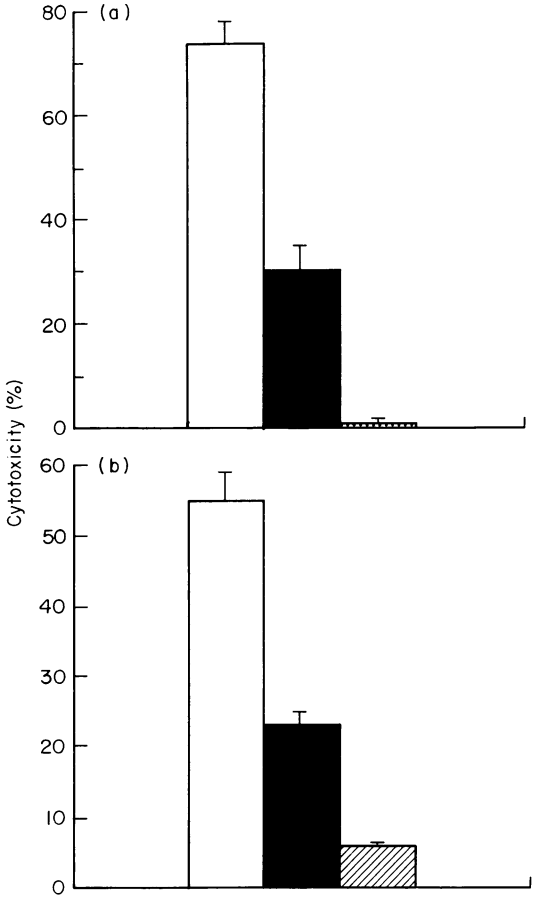

Fig. 3. Effect of (a) 500 U/ml recombinant human interleukin-4 (rhIL-4) (a) and of (b) 2 ng/ml transforming growth factor-β1 (TGF-β1) on cytotoxicity induced by 100 U/ml recombinant human interferon-gamma (IFN-γ). Peripheral blood mononuclear cells were cultured in AIM-V. All cytokines were added at the onset of culture. rhIL-2 was added at 500 U/ml. Cytotoxicity was measured against the SW742 cell line. Titration of rhIFN-γ up to 10 000 U/ml showed that this cytokine alone was not capable of inducing the same level of cytotoxicity as rhIL-2 alone. □, rhIL-2; ■, rhIFN-γ; ▨, rhIFN-γ+rhIL-4; ■, rhIFN-γ+TGF-β1.

Table 3. Transforming growth factor-β (TGF-β) release by cultured peripheral blood mononuclear cells (PBMC)

	PBMC cultured in medium containing			
Experiment	0	rhIL-2	rhIL-2 +rhIL-4	rhIL-4
1	3·0	3·2	2·7	2·9
2	4·0	3·7	4·0	2·1
3	8·0	6·5	6·4	3·7

PBMC were cultured for 4 days, and the supernatants collected and stored at −70°C before bioassay for TGF-β using the mink cell line. Results shown represent ng/ml of total TGF-β in culture supernatant.

rhIL-2, recombinant human interleukin-2; rhIL-4, recombinant human interleukin-4.

(formerly known as B cell simulating factor-1) has been implicated in IL-2-independent T cell growth, and the generation of antigen-specific cytotoxic T lymphocytes (CTL) (Widmer *et al.*, 1987; Laing & Weiss, 1988); however, the induction of human non-specific LAK effectors has been shown to be inhibited in the presence of rhIL-4. TGF-β, which belongs to a family of multi-functional polypeptides (reviewed by Roberts & Sporn, 1988), has also been shown to be a potent immunosuppressive agent, and prevents the induction of IL-2 responses (Rook *et al.*, 1986; Wahl *et al.*, 1988; Kasid, Bell & Director, 1988). Essentially all cells express TGF-β receptors and T cells differentially express TGF-β1-binding proteins upon activation (Roberts & Sporn, 1988; Ellingsworth *et al.*, 1989). TGF-β is produced by many cell types of neoplastic and non-neoplastic origin, including mitogen-stimulated T cells, and is released in an inactive high molecular weight form (Kehrl *et al.*, 1986; Roberts & Sporn, 1988). The regulation of TGF-β action may depend on the level of activation of the latent molecule, achieved by extremes of pH *in vitro*, but *in vivo* the mechanism of cleavage is not fully understood (Wakefield *et al.*, 1987; Roberts & Sporn, 1988).

Here we have extended previous work demonstrating that both rhIL-4 and TGF-β inhibit the generation of human LAK activity (Widmer *et al.*, 1987; Brooks & Rees, 1988; Gallagher *et al.*, 1988; Espevik *et al.*, 1988; Mule *et al.*, 1988). These factors were shown to prevent rhIL-2-induced cell proliferation, the expression of the alpha chain of the IL-2 receptor (Tac) and inhibit the production of TNF-α and IFN-γ. Inhibitory effects were dose dependent and greatest when added during the first 24–48 h of culture.

The augmentation of LAK induction from LGL precursors by rhTNF-α in the presence of suboptimal rhIL-2 concentrations has been reported (Blay *et al.*, 1989). RhIFN-γ can augment or suppress LAK induction, depending on the presence of adherent mononuclear cells (Toledano *et al.*, 1989). Synergy between rhIL-2 and rhTNF-α or rhIFN-γ is not observed when optimal doses of rhIL-2 are used with whole PBMC in a 4-day induction (unpublished results). The addition of rhIFN-γ to PBMC cultured with rhIL-2 and rhIL-4 restored their cytolytic and proliferative capacities in agreement with other reports on the antagonistic roles of IL-4 and IFN-γ in immunoregulation (Wagner *et al.*, 1989; Vercelli *et al.*, 1990). However, reduction of Tac antigen expression induced by rhIL-4 was not abrogated by rhIFN-γ, suggesting that high levels of Tac antigen are not necessary for rhIL-2-induced cytotoxicity and proliferation, and inferring the relative importance of p70 (beta) chain of the IL-2 receptor in the early events of cytolytic induction by rhIL-2 (Siegel *et al.*, 1987).

The restoration of lymphocyte DNA synthesis by rhTNF-α and rhIFN-γ did not always correlate with the restoration of cytotoxic function, consistent with reports dissociating the induction of lymphocyte cytolytic activity from cell proliferation (Malkovsky *et al.*, 1987; Owen-Schaub *et al.*, 1988; Jin *et al.*, 1989). The reversal of TGF-β suppression was occasionally demonstrated using either rhTNF-α or rhIFN-γ, the latter being more effective in reversing the suppressive effects. Studies are now under way to determine the effect of adding exogenous rhTNF-α and rhIFN-γ, in combination, on rhIL-4- and TGF-β-mediated suppressive effects.

Although TNF-α and IFN-γ are released during the generation of rhIL-2-activated killer cells, it is not known whether they

are essential for the induction of proliferation and LAK cytotoxicity (Limb *et al.*, 1989). They are both newly synthesized on activation with rhIL-2, since mRNA transcripts, which are not present in unstimulated PBMC, can be detected after stimulation with rhIL-2 (Kovaks *et al.*, 1989). RhIL-4, TGF-β, rhIFN-γ, rhTNF-α and rhIL-2 had no effect on spontaneous Cr release by target cells, or cytolytic function of rhIL-2-activated cells when added during the cytotoxicity assay. To investigate whether rhIL-4 suppressed the induction of LAK activity in PBMC by inducing TGF-β release, culture supernatants were assayed for the presence of TGF-β. Our results show no difference in the levels of total or active TGF-β from PBMC cultured in the presence or absence of rhIL-4. Using neutralizing antisera to TGF-β, Kawakami *et al.* (1989), reported that rhIL-4-mediated suppression of IL-2-induced proliferation was TGF-β independent.

The reduction of four responses to rhIL-2 by rhIL-4 and TGF-β have been observed in the present study: LAK induction; DNA synthesis; TNF-α and IFN-γ release; and p55 α-chain (Tac antigen) expression. Furthermore, rhIFN-γ abrogated the suppressive effects of rhIL-4 on rhIL-2-induced cytotoxicity and DNA synthesis, but this was not accompanied by reversal of reduced Tac antigen expression.

ACKNOWLEDGMENTS

This work was supported by the Yorkshire Cancer Research Campaign. We wish to thank Mrs C. Mullan for typing this manuscript, and acknowledge Mr N. Sharma for his excellent technical assistance.

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