Immunology 1992 **75** 343–348

Evidence that interleukin-4 suppression of lymphokine-activated killer cell induction is mediated through monocytes

B. BROOKS*, H. PARRY,† J. LAWRY & R. REES *Section of Tumour Biology and Immunology, Department of Experimental and Clinical Microbiology, University of Sheffield Medical School, Sheffield*

Accepted for publication 30 September 1991

SUMMARY

Recombinant human interleukin-4 (IL-4) and transforming growth factor-beta (TGF-β) reduce recombinant interleukin-2 (IL-2) induction of lymphokine-activated killer (LAK) cell activity from human peripheral blood mononuclear cells (PBMC). Monocytes can be removed from PBMC by adherence, leaving a peripheral blood lymphocyte population (PBL) which also responds to IL-2 to generate LAK activity. PBL generation of LAK cytotoxicity is susceptible to inhibition by TGF-β, but not by IL-4. Readdition of purified monocytes to PBL is accompanied by return of the suppressive action of IL-4 on the generation of LAK activity. Induction of LAK cytolysis from Percoll-isolated T cells (> 90% $CD3^+$) is also refractory to the inhibitory effect of IL-4. When PBMC were cultured in IL-2, with and without IL-4, subsequent sorting of $CD3^+$ and $CD3^-$ lymphocytes by flow cytometry demonstrated that IL-4 had suppressed LAK induction in both effector populations. This suggests that, although isolated $CD3^+$ cells are not susceptible to IL-4 suppression of IL-2 activation, they are sensitive to inhibition when part of a mixed PBMC population. Evidence is presented for the first time that this suppression is mediated via the action of IL-4 on monocytes.

INTRODUCTION

The culture of human PBMC in recombinant human IL-2 leads to the generation of LAK activity. These non major histocompatibility complex (MHC)-restricted killer cells are capable of lysing fresh tumour targets and tumour cell lines resistant to natural cell-mediated cytotoxicity.[1] LAK activity represents a function rather than a cell type, and there is evidence that both large granular lymphocytes (LGL) and T cells respond to IL-2 and generate LAK activity.[2-4]

Both IL-4 and TGF-β have been reported to inhibit the induction of LAK activity from PBMC.[5-8] This reduction in cytotoxic capacity is accompanied by a suppression of other IL-2-mediated responses: proliferation, Tac antigen expression and production of the cytokines tumour necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ).[9-12] IL-4 has also been shown to suppress the production of IL-1 by IL-2-activated PBMC.[13]

The suppression of IL-2-induced cytotoxic and proliferative capacities by IL-4 or TGF-β is sometimes incomplete, and partial activation can occur in the presence of these inhibitory cytokines. This suggests that suppression is due either to a general reduction in IL-2 responses in all responding PBMC populations or to a selective effect on a certain subset(s). The role of monocytes in the induction of LAK activity has been studied previous. Toledeno *et al*,[14] and Roberts *et al*.[15] showed that non-adherent PBL are capable of generating greater cytotoxicity than whole PBMC, and readdition of adherent cells has been shown to suppress LAK induction by PBL in a dose-dependent manner.[16,17] However, others have demonstrated that monocytes can augment the induction of LAK, depending on their density and activation state.[18,19] The contribution of monocytes to LAK induction may be dependent on the balance between prostaglandin E_2 (PGE_2) synthesis and membrane-associated IL-1, as suggested by Bloom and Babbit.[20]

In the present study, the role of monocytes in IL-4- and TGF-β-mediated inhibition of LAK induction was investigated. Following removal of plastic adherent cells, the resulting PBL population was not susceptible to IL-4-mediated inhibition of IL-2-induced LAK activity, but was susceptible to TGF-β-mediated suppression. Readdition of purified monocytes rendered PBL sensitive to the suppressive action of IL-4. PBMC cultured in the presence of IL-2 with and without IL-4 were sorted into $CD3^+$ and $CD3^-$ populations using a flow cytometer. Both sorted populations exhibited lower LAK activity from cultures which had contained IL-2 and IL-4 than those which had contained IL-2 alone. Purified T cells were also assessed for their response to IL-2 in the presence and absence of IL-4; IL-4 did not inhibit LAK induction from this population.

Abbreviations: IFN, interferon; IL, interleukin; LGL, large granular lymphocytes; PBL, peripheral blood lymphocytes; PBMC, peripheral blood mononuclear cells; TGF, transforming growth factor; TNF, tumour necrosis factor.

* Present address and correspondence: Dr B. Brooks, University Dept. Surgery, Glasgow Royal Infirmary, Alexandra Parade, Glasgow G31 2ER, U.K.

† Present address: Dept. of Immunology, Harefield Hospital, Harefield, Middlesex, U.K.

MATERIALS AND METHODS

Isolation of lymphoid subsets

PBMC. Peripheral blood from healthy donors was collected in heparin (10 U/ml) and the mononuclear cells (PBMC) separated on lymphocyte separation medium (Lympohoprep; Nyegaard, Oslo, Norway) according to Boyum,[21] washed three times in phosphate-buffered saline (PBS) and resuspended in the serum-free medium AIM V (Gibco, Paisley, U.K.).

PBL. A total of 40×10^6 PBMC were suspended in 30 ml of AIM V and incubated at 37 C for 90 min in a T75 falcon tissue culture flask (Becton-Dickinson, Lincoln Park, NJ). Non-adherent PBL were decanted and phenotyped.

T cells. A total of 50×10^6 PBL were layered onto a seven-step discontinuous Percoll gradient as described by Ortaldo.[22] Briefly RPMI + 10% foetal calf serum (FCS; 285 mOsm) were added to Percoll (285 mOsm) adjusted by 10 × PBS, and a seven-step gradient made using the following Percoll concentrations: 42·5%, 45%, 47·5%, 50%, 52·5%, 55% and 66·6%. The gradients were centrifuged at 550 ***g*** for 30 min at room temperature and each layer was removed, washed, counted and assessed for lymphocyte phenotypes. Bands between 45% and 47·5% and those between 47·5% and 50% were enriched for T cells. T-cell populations used were >90% $CD3^+$.

Monocytes

Monocytes were purified using two methods:

(1) A total of 30×10^6 PBMC were layered onto a three-step discontinous Percoll gradient consisting of layers of 40%, 45·8% and 66·6%. The gradient was centrifuged at 550 ***g*** for 30 min at 4°. The band between the top two layers was enriched for monocytes.

(2) A total of 50×10^6 PBMC, suspended in 20 ml of RPMI + 10% AB serum, were incubated for 90 min on a T175 Costar tissue culture bottle (Northumbria Biologicals, Northumberland, U.K.). Non-adherent cells were gently removed, and plates were washed five times with warmed PBS. After microscopic examination for removal of non-adherent cells, adherent cells were recovered by incubation in 5 mM ethylenediaminetetra-acetic acid (EDTA) for 30 min. Recovered adherent cells were routinely >95% $CD14^+$.

Monoclonal antibodies

Lymphocyte subsets were assessed using: CD3 (Leu-4: pan-T cell); CD56 [Leu-19: natural killer (NK) cell and activated T-cell marker]; CD57 [Leu-7; NK and T suppressor (Ts) marker]; CD16 (Leu-11c; NK, Ts and granulocyte marker); all purchased from Becton Dickinson (Oxford, U.K.); CD14 (UCHMI: monocyte marker), purchased from Seralab (Sussex, U.K.); and a mixture of CD19, CD21 and CD22 pan-B-cell markers (Royal Free Hospital, London, U.K.).

Flow cytometry

Assessment of phenotypes was performed as previously described.[6]

Cell sorting was performed using OKT3a phycoerythrin (PE)-conjugate mAb against CD3 (Ortho Diagnostics, Bucks., U.K.) on a Becton-Dickinson FACS420 with accessory CONSORT 30 computer (Becton-Dickinson, Oxford, U.K.). Scatter gates were limited to the removal of debris only. Both mAb^+ and mAb^- cells were sorted by vitue of PE presence or absence. For the purpose of sorting stained PBMC, all cells with a fluorescence greater than Channel 30 on a linear scale were considered to be mAb^+; and cells from Channel 0 to 20 were considered to be mAb^-, as determined by isotype controls. Sorted cells were collected in 10-ml conical-based centrifuge tubes containing RPMI-10% newborn calf serum (NBCS) in an ice bath, to minimize cell loss. Collected cells were then washed, counted, reanalysed, and found to be >90% pure.

Target cells

The SW742 colon adenocarcinoma cell line[23] was grown as an adherent cell line in RPMI-1640 medium plus 10% FCS. Previous study with this cell line has shown it to be relatively insensitive to NK cytolysis but sensitive to IL-2-induced LAK activity. This cell line was routinely screened for mycoplasmal contamination by DNA hybridization assay (Lab Impex, Middlesex, U.K.) and shown to be free of infection.

Cytokines

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, previously determined to be optimal for LAK induction; rhIL-4 (specific activity 10^7 U/mg) was kindly provided by the Immunex Corporation (Seattle, WA). Porcine TGF-β1 was purchased from British Biotechnology (Oxford, U.K.). IL-4 and TGF-β were used at 500 U/ml and 5 ng/ml, respectively, as prior titration has shown these concentrations to give maximal suppression of LAK induction.

LAK-cell induction

Lymphocyte subsets were suspended in AIM V medium and seeded at 3 or 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° in a 5% CO_2/95% air atmosphere for 4 days, after which each effector population was harvested, washed in PBS, counted, resuspended in the appropriate amount of RPMI + 10% NBCS, and assayed for cytotoxic capacity.

^{51}Cr-release test

A 4-hr ^{51}Cr-release assay was performed as described previously.[6] Briefly, labelled target cells (0·1 ml/well) were incubated with effector (0·1 ml/well) at the ratios indicated, in 96-well plates, in triplicate. Test plates were incubated at 37° in a 5% CO_2/95% air, humidified atmosphere for 4 hr and ^{51}Cr release and cytotoxicity were calculated as previously described.[6]

Proliferation assay

Cells harvested from lymphocyte cultures were seeded into 96-well tissue culture plates (Becton-Dickinson) at 1×10^5 cells/0·1 ml/well in triplicate and 0·5 μCi of [^{3}H]thymidine added. Cells were incubated for 4 hr and harvested onto filter paper using an automated cell harvester (Skatron, Norway). Incorporation of [^{3}H]thymidine was measured by counting the filter paper in scintillation fluid using a β-spectrophotometer.

Statistical analysis

Inhibitory or augmentory effects were determined as significant using Student's *t*-test.

Table 1. Effect of removal of monocytes on rhIL-2-mediated induction of LAK activity and proliferation

	No. of experiments in which the observation was recorded	
	Response measured*	
Observation	LAK activity	Proliferation
Increased†	1/17	2/11
Decreased†	11/17	7/11
No change	5/17	2/11

* Lymphoid cells were incubated in AIM V medium with 500 U/ml rhIL-2 for 4 days. LAK activity was assessed against SW742 targets, and proliferation by uptake of [^{3}H]thymidine.

† Significant as determined by Student's *t*-test: $P < 0{\cdot}01$.

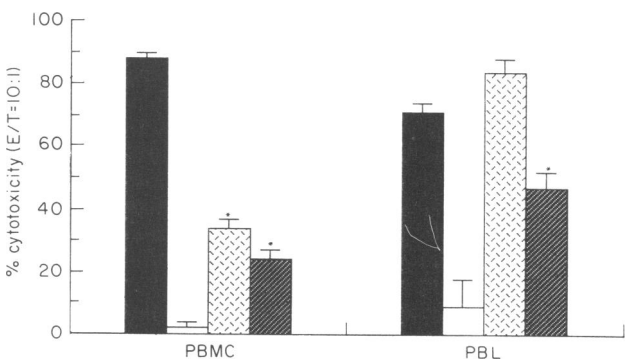

Figure 1. Effect of IL-4 and TGF-β on LAK induction by PBMC and PBL precursors. Lymphocyte populations were cultured for 4 days in AIM V medium containing: (■) IL-2 (500 U/ml); (□) 0; (▨) IL-2+IL-4 (500 U/ml); (■) IL-2+TGF-β (5 ng/ml). Cytotoxicity was measured against the SW742 cell line. * Suppression was significant as determined by Student's *t*-test: $P < 0{\cdot}01$.

RESULTS

Removal of adherent cells

Monocyte-depleted PBL cultured in the presence of IL-2 were capable of exhibiting LAK activity against NK-resistant SW742 cell line, and proliferating, as determined by [^{3}H]thymidine incorporation, in response to IL-2. As shown in Table 1, LAK activity and proliferation were usually lower in the absence of monocytes, although there was variability between the donors used.

The effect of TGF-β and IL-4 on these responses was studied, both factors being added (at concentrations previously determined to give optimal suppression of LAK induction) at the onset of culture of PBL with IL-2. Figure 1 is representative of a series of experiments performed to compare LAK induction by PBL and PBMC, which are summarized in Table 2. When cultured in the absence of IL-2, PBMC and PBL were routinely shown to cause little or no lysis of SW742 targets. Although IL-4

Table 2. Summary of the responses of PBL to rhIL-2 with added rhIL-4 or TGF-β

	No. of experiments in which the observation was recorded	
	Response measured*	
Observation	LAK activity	Proliferation
(a) *Effect of rhIL-4*		
Increase†	4/16	4/10
Decrease†	0/16	4/10
No effect	12/16	2/10
(b) *Effect of TGF-β*		
Increase†	0/4	2/4
Decrease†	3/4	0/4
No effect	1/4	2/4

* PBL were incubated in AIM V medium containing 500 U/ml rhIL-2 and either 500 U/ml rhIL-4 or 5 ng/ml TGF-β. In all experiments, corresponding PBMC cultured under identical conditions exhibited reduced responses to rhIL-2 in the presence of either rhIL-4 or TGF-β.

† Significant as determined by Student's *t*-test: $P < 0{\cdot}01$.

and TGF-β both inhibited IL-2-induced LAK activity and proliferation by PBMC, using autologous PBL, IL-4 either augmented or had no effect on IL-2 responses, whereas TGF-β suppressed the induction of LAK activity (although, in the absence of monocytes, this suppressive action was reduced), but not of proliferation. Increasing IL-4 concentrations to 2000 U/ml did not alter the level of these responses (data not shown).

The phenotypes of PBL populations were assessed by using monoclonal antibodies which recognize monocytes (CD14), T lymphocytes (CD3), NK cells (CD56, CD16 and CD57) and B cells (CD19, CD21 and CD22); three examples of a series of experiments are shown in Table 3. Adherence did not affect the percentage of cells expressing CD16 (five experiments); CD57 was increased in PBL, compared with PBMC, in two out of five experiments; after adherence, CD3 increased, whilst CD14$^+$ cells were generally decreased to less than 5%. Adherence sometimes removed CD56$^+$ and B cells, but this had no influence on the responses observed.

Addition of monocytes to PBL

To determine whether monocytes were responsible for IL-4-mediated suppression of LAK induction, isolated monocytes (>95% purity, using adherence) were added to PBL. Readdition of monocytes to PBL re-established IL-4-mediated suppression of LAK induction: Figure 2 is an example of four experiments performed. Monocytes were also isolated on Percoll gradients and, although these populations were only 46–64% CD14$^+$, they could also mediate the suppressive effects of IL-4. In two out of three experiments, monocytes rendered PBL susceptible to IL-4-mediated inhibition of IL-2-induced proliferation (data not shown).

Table 3. Phenotypic analysis of PBMC and PBL

Phenotype	Percentage positive cells*						
	Control†	CD3	CD14	CD16	CD57	CD56	B cells‡
Experiment 1							
PBMC	3	43	30	19	25	17	9
PBL	3	65	6	19	38	27	6
Experiment 2							
PBMC	4	63	12	26	19	28	ND
PBL	1	64	2	25	16	28	ND
Experiment 3							
PBMC	3	61	26	ND	ND	12	7
PBL	2	76	3	ND	ND	14	3

* The percentage of positive cells as assessed by flow cytometry.
† FITC conjugate only.
‡ A cocktail of CD19, CD21 and CD22 was used for B-cell analysis.
ND = not determined.

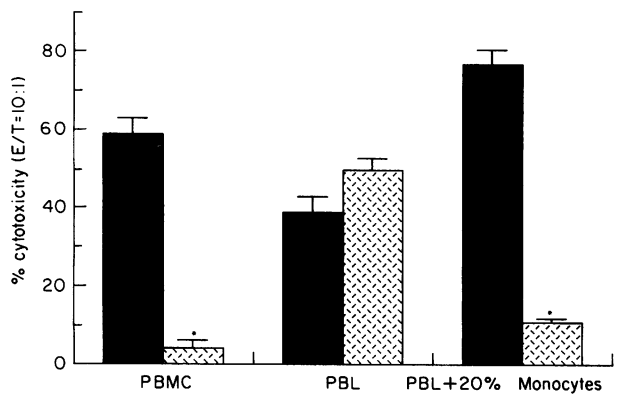

Figure 2. Addition of monocytes to PBL. Lymphocyte populations were cultured for 4 days in AIM V medium containing: (■) IL-2 (500 U/ml) or (▨) IL-2+IL-4 (500 U/ml). Cytotoxicity was measured against the SW742 cell line. * Suppression was significant as determined by Student's *t*-test: $P < 0.01$.

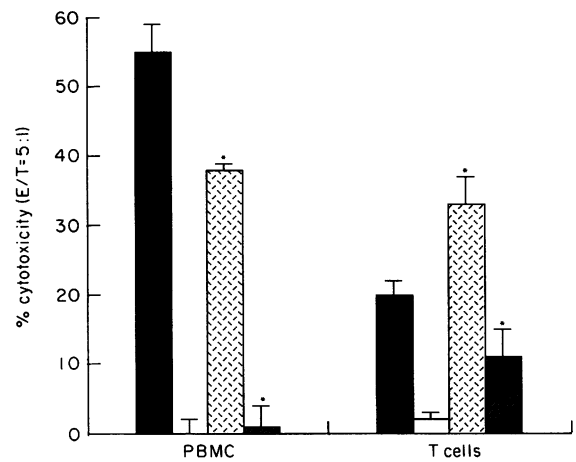

Figure 3. Effect of IL-4 and TGF-β on LAK induction by PBMC and T-cell precurors. Lymphocyte populations were cultured for 4 days in AIM V medium containing: (■) IL-2 (500 U/ml); (□) 0; (▨) IL-2+IL-4 (500 U/ml); (■) IL-2+TGF-β (5 ng/ml). Cytotoxicity was measured against the SW742 cell line. *Suppression or augmentation of LAK activity by IL-4 or TGF-β was significant as determined by Student's *t*-test: $P < 0.01$.

Purification of T cells

T cells were further purified from PBL using seven-step Percoll gradients, and cells of >90% $CD3^+$ were used. As shown in Fig. 3 (one of four experiments performed), TGF-β suppressed LAK induction by these T-cell progenitors, whereas IL-4 did not. In three out of four experiments IL-4 augmented LAK induction by PBL precursors, and had no effect in one of them.

Sorting of activated cells

PBMC were cultured for 4 days in medium containing either IL-2 or IL-2+IL-4. These effectors were then sorted into $CD3^+$ and $CD3^-$ subsets using a flow cytometer, which yielded populations of >90% purity. As shown in Fig. 4 (one of two experiments), both populations exhibited decreased cytotoxicity when IL-4 was present during induction. In both experiments, LAK activity was lower in $CD3^+$ effectors than in $CD3^-$ populations. Controls included effectors stained with mAb and stained cells which had been run through the flow cytometer under standard conditions. It was found that these cells had unaltered cytotoxic capacity compared with effectors that had not been stained or sorted, eliminating any possibility of induced effects on sorted effectors. Thus, when cultured alone, $CD3^+$ T cells are not susceptible to IL-4-mediated inhibition of LAK induction. In contrast, when cultured in a PBMC population, $CD3^+$ cells are sensitive to IL-4 regulation of IL-2 induced cytotoxicity.

DISCUSSION

In the present study, PBL were shown to be capable of responding to IL-2 to proliferate and generate LAK activity, but

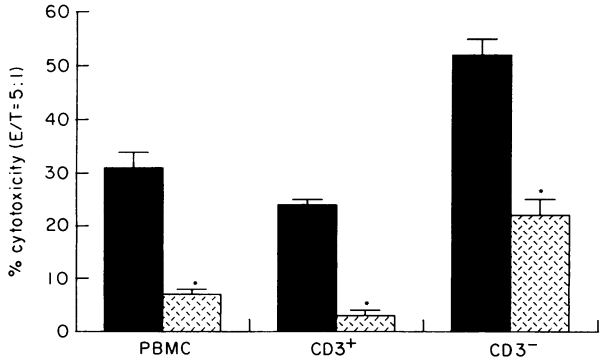

Figure 4. FACS sorting of activated killer cells. PBMC were cultured for 4 days in AIM V medium containing: (■) IL-2 (500 U/ml) or (▨) IL-2 + IL-4 (500 U/ml). Both groups were then stained with anti-CD3 mAb, and sorted into positive and negative populations. Cytotoxicity was measured against the SW742 cell line, and was unaffected in control groups. * Suppression was significant as determined by Student's *t*-test: $P < 0.01$.

this response was generally lower than that observed in corresponding PBMC. The variation in the results obtained upon removal of adherent cells may reflect differences in the activation state of monocytes present, their removal therefore having contrasting effects on the response of cells from different donors. Although IL-2 induction of LAK activity from PBL was reduced by TGF-β, IL-2-induced proliferation was found to be refractory to its action; furthermore, the inhibitory effect of TGF-β on LAK induction was less pronounced in the absence of monocytes. PBL responses to IL-2 were either not affected or augmented by IL-4, as were those of purified T cells. This is in contrast to the observations of Damle and Doyle,[24] who demonstrated that the generation of LAK cytotoxicity by purified T cells (> 95% $CD3^+$) is suppressed by IL-4. Han *et al.*[9] showed that purified PBL, but not T cells, generated cytotoxity in response to IL-2 which was also susceptible to the inhibitory effects of IL-4. These disparities with the present study could be due to experimental differences, including the media used during IL-2 activation and the target cells employed to measure LAK activity. In keeping with the present study, Ortaldo[25] has shown that IL-4 enhances IL-2-mediated LAK induction by human T cells.

When PBMC were activated with IL-2 in the presence of IL-4 and subsequently sorted into $CD3^+$ and $CD3^-$ cells, both populations exhibited reduced LAK activity in comparison with equivalent populations sorted from autologous PBMC incubated in IL-2 alone, demonstrating that culturing a mixed cell population renders T cells susceptible to IL-4-mediated suppression, in contrast with the response of T cells activated following purification. Furthermore, addition of adherence-purified monocytes to PBL restored their susceptibility to IL-4 suppression. Since these adherent monocytes could be considered to be activated,[26] monocytes purified by discontinuous Percoll gradients were used and shown to be equally effective in causing IL-4-dependent suppression.

IL-4 is produced by a limited number of T cells of both CD4 and CD8 phenotypes,[27,28] promotes IL-2-independent T-cell growth[29] and enhances the induction of antigen-specific cytotoxicity.[5,30,31] The results presented here implicate monocytes in the regulation of the induction of non-MHC-restricted LAK activity by PBMC precursors, possibly by an indirect mechanism. It is relevant to note that IL-4 has been shown to affect monocyte function, including down-regulating lipopolysaccharide (LPS)-induced IL-6 and IL-1 production;[32,33] inhibiting IL-8 mRNA expression and protein production upon LPS, TNF-α and IL-1 stimulation;[34] reducing LPS-induced TNF-α mRNA expression and protein secretion;[35] and decreasing CD14 expression.[36]

Although the mechanism by which IL-4 reduces LAK induction is unknown, it is possible that PBMC LAK effectors are generated from two distinct populations one being monocyte independent and one requiring the release of a monocyte-derived factor(s), such as IL-1, or IL-6, both of which are known to enhance LAK induction.[17,37,38] It has been shown that IL-1, which is predominantly produced by adherent cells, can reverse the inhibitory effects of IL-4 on LAK induction.[13] Since IL-4 suppresses IL-1 and IL-6 production by monocytes, this provides a possible mechanism for its regulatory action on LAK induction. It is unlikely that IL-4 mediates its suppression via the induction of PGE_2, since IL-4 has been shown to reduce PGE_2 production by monocytes;[39] however, it could be postulated that IL-4 induces the release of TGF-β from monocytes, which, in turn, mediates suppression of IL-2 responses. To date, TGF-β released by PBMC into culture media, as measured by bioassay, has been found to be unaffected by IL-4,[10] although the more precise measurement of TGF-β in culture supernatants using recently reported enzyme-linked immunosorbent assay (ELISA) reagents[40] would provide more reliable data regarding this issue. The proposition that IL-4 acts by down-regulating rather than inducing monocyte activity is strengthened by the recent report of Lauener *et al.*[36] demonstrating a reduced expression of CD14 on monocytes in the presence of IL-4; since CD14 may be involved in monocyte activation, it was suggested that IL-4 causes a 'dampening' of monocyte function. The mechanism involved in monocyte-dependent IL-4 suppression is currently under investigation: preliminary data indicate that PBMC produce IL-6 in response to IL-2, and that this response is suppressed by IL-4. PBL-conditioned media contain much lower levels of IL-6.

ACKNOWLEDGMENTS

This work was funded by The Yorkshire Cancer Research Campaign.

REFERENCES

1. Grimm E.A., Mazumber A., Zhang H. & Rosenberg S.A. (1982) Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumour cells by interleukin 2-activated autologous human peripheral blood lymphocytes. *J. exp. Med.* **155,** 1823.
2. Damle N.K., Doyle L.V. & Bradley E.C. (1986) Interleukin 2-activated human killer cells are derived from phenotypically heterogeneous precursors. *J. Immunol.* **137,** 2814.
3. Ortaldo J.R., Mason A. & Overton R. (1986) Lymphokine-activated killer cells. Analysis of progenitors and effectors. *J. exp. Med* **164,** 1193.
4. Sawada H., Abo T., Sugawara S. & Kumagai K. (1988) Prerequisite for the induction of lymphocyte-activated killer cells from T lymphocytes. *J. Immunol.* **140,** 3668.
5. Widmer M.B., Acres R.B., Sassenfeld H.M. & Grabstein K.H. (1987) Regulation of cytolytic cell populations from human peripheral blood by B cell stimulatory factor 1 (interleukin 4). *J. exp. Med.* **166,** 1447.

6. Brooks B. & Rees R.C. (1988) Human recombinant IL-4 suppresses the induction of human IL-2 induced lymphokine activated killer (LAK) activity. *Clin. exp. Immunol.* **74,** 162.
7. Gallagher G., Wilcox F. & Al-Azzawi F. (1988) Interleukin-3 and interleukin-4 each strongly inhibit the induction of human LAK cells. *Clin. exp. Med.* **74,** 166.
8. Mule J.J., Schwartz S.L., Roberts A.B., Sporn M.B. & Rosenberg S.A. (1988) Transforming growth factor-beta inhibits the in-vitro generation of lymphokine-activated cells and cytotoxic T cells. *Cancer Immunol. Immunother.* **26,** 95.
9. Han X., Itoh K., Balch C.M. & Pellis N.R. (1988) Recombinant interleukin-4 (RIL4) inhibits interleukin 2-induced activation of peripheral blood lymphocytes. *Lymphokine Res.* **7,** 227.
10. Brooks B., Chapman K., Meager A., Lawry J. & Rees R.C. (1990) Suppression of lymphokine-activated killer cell induction mediated by Il-4 and transforming growth factor-β1: effect of addition of exogenous tumour necrosis factor-alpha and interferon-gamma, and measurement of their endogenous production. *Clin. exp. Immunol.* **82,** 583.
11. Swisher S.G., Economou J.S., Holmes E.C. & Golub S.H. (1990) TNF-α and IFN-γ reverse IL-4 inhibition of lymphokine-activated killer cell function. *Cell. Immunol.* **128,** 450.
12. Espevik T., Figari I.S., Ranges G.E. & Palladino M.A. (1988) Transforming growth factor-α1 (TGF-β1) and recombinant human tumour necrosis factor-α reciprocally regulate the generation of lymphokine-activated killer cell activity. Comparison between natural porcine platelet-derived TGF-β and TGF-β2, and recombinant human TGF-β1. *J. Immunol.* **140,** 2312.
13. Ebina N., Galladaro D., Shau H. & Golub S.H. (1990) IL-1 and IL-4 as reciprocal regultors of IL-2 induced lymphocyte cytotoxicity. *Br. J. Cancer* **62,** 619.
14. Toledeno M., Mathiot C., Michon J., Andreu G., Lando D., Brandley M. & Fridman W.H. (1990) Interferon-γ (IFN-γ) and interleukin-2 in the generation of lymphokine-activated killer cell cytotoxicity–IFN-γ-induced suppressive cytotoxicity. *Cancer Immunol. Immunother.* **30,** 57.
15. Roberts K., Lotz M.T. & Rosenberg S.A. (1987) Separation and functional studies of the human lymphokine-activated killer cells. *Cancer Res.* **47,** 4366.
16. Ibayashi Y., Hoon S.D.B. & Golub S.H. (1987) The regulatory effect of adherent cells on lymphokine activated killer cells. *Cell. Immunol.* **110,** 365.
17. Hoyer M., Meineke T., Lewis W., Zwilling B. & Rinehart I. (1986) Characterisation and modulation of human lymphokine (interleukin-2) activated killer cell induction. *Cancer Res.* **46,** 2834.
18. Nii A., Sone S., Utsugi T., Yanagawa H. & Ogura T. (1988) Up- and down-regulation of human lymphokine (IL-2) activated killer cell induction by monocytes, depending on their functional state. (1988) *Int. J. Cancer* **41,** 33.
19. Silvennoinen O., Vakkila J. & Hurme M. (1988) Accessory cells, dendritic cells, or monocytes are required for the lymphokine-activated killer cell induction from resting T cell but not from natural killer cell precursors. *J. Immunol.* **141,** 1404.
20. Bloom E.T. & Babbit J.T. (1990) Prostaglandin E2, monocyte adherence and interleukin-1 in the regulation of human natural killer cell activity by monocytes. *Nat. Immunol. Cell Growth Regul.* **9,** 36.
21. Boyum A. (1968) Isolation of mononuclear cells and granulocytes from human blood. *Scand J. Immunol.* **21,** Suppl. 77.
22. Ortaldo J.R. (1986) Cytotoxicity by natural killer cells: analysis of large granular lymphocytes. *Methods Enzymol.* **132,** 445.
23. Leibovitz A., Stinson J., McCombs W.B., McCoy L.E., Mazur K.C. & Mabry N.D. (1976) Classification of human colorectal adenocarcinoma cells lines. *Cancer Res.* **36,** 4562.
24. Damle N.K. & Doyle L.V. (1989) Distinct regulatory effects of IL-4 and TNF-α during CD3-dependent and CD3-independent initiation of human T cell activation. *Lymphokine Res.* **8,** 85.
25. Ortaldo J.R. (1990) Lymphokine activated killer cells: regulation of activity. In: *The Biology and Clinical Applications of Interleukin 2.* IRL Press, Oxford.
26. Haskill S., Johnson C., Eierman D., Becker S. & Warren K. (1988) Adherence induces selective mRNA expression of monocyte mediators and proto-oncogenes. *J. Immunol.* **140,** 1690.
27. Lewis B.D., Prickett K.S., Larsen A., Grabstein K., Weaver M. & Wilson C.B. (1988) Restricted production of interleukin 4 by activated human T cells. *Proc. natl. Acad. Sci. U.S.A.* **85,** 9743.
28. Paliard X., de Waal Malefijt R., Yssel H., Blanchard D., Chretein I., Abrams J., de Vries J.E. & Spits H. (1988) Simultaneous production of IL-2, IL-4 and IFN-γ by activated human CD4$^+$ and CD8$^+$ cell clones. *J. Immunol.* **141,** 849.
29. Spits H., Yssel H., Takebe Y., Arai N., Yokota T., Lee F., Arai K., Banchereau J. & de Vries J.E. (1987) Recombinant interleukin 4 promotes the growth of human T cells. *J. Immunol.* **139,** 1142.
30. Spits H., Yssel H., Palliard X., Kastelein R., Figdor C. & de Vries J.E. (1988) IL-4 inhibits IL-2 mediated induction of human lymphokine activated killer cells, but not the generation of antigen-specific cytotoxic T lymphocytes in mixed leukocyte cultures. *J. Immunol.* **141,** 29.
31. Kawakami Y., Rosenberg S.A. & Lotze M.T. (1988) Interleukin 4 promotes the growth of tumour-infiltrating lymphocytes cytotoxic for human autologous melanoma. *J. exp. Med.* **168,** 2183.
32. Gibbons R., Martinez O., Matti M., Heinzel F., Bernstein M. & Warren F. (1990) Recombinant IL-4 inhibits IL-6 synthesis by adherent peripheral blood cells in vitro. *Lymphokine Res.* **9,** 283.
33. Hurme M., Palkama T. & Sihova P. (1988) Interleukin-4 inhibits interleukin-1 synthesis by a post transcriptional method. *Biochem. biophys. Res. Comm.* **157,** 86.
34. Standiford T.J., Streiter R.M., Chensue S.W., Westwick J., Kasahara K. & Kunkel S.L. (1990) IL-4 inhibits the expression of IL-8 from stimulated human monocytes. *J. Immunol.* **145,** 1435.
35. Essner R., Rhoades K., McBride W.H., Morton D.L. & Economou J.S. (1989) IL-4 down-regulates IL-1 and TNF gene expression in human monocytes. *J. Immunol.* **145,** 1435.
36. Lauener R.P., Goyert S.M., Geha R.S. & Vercelli D. (1990) Interleukin 4 down-regulates the expression of CD14 in normal human monocytes. *Eur. J. Immunol.* **20,** 237.
37. Gallagher G., Stimson W.H., Findlay J. & Al-Azzawi F. (1990) Interleukin-6 enhances the induction of human lymphokine-activated killer cells. *Cancer Immunol. Immunother.* **31,** 49.
38. Crump W.L., Owen-Schaub L.B. & Grimm E.A. (1989) Synergy of human recombinant interleukin 1 with interleukin 2 in the generation of lymphokine-activated killer cells. *Cancer Res.* **49,** 149.
39. Hart P., Vitti G.F., Burgess D.R., Whitty G.A., Piccoli D.S. & Hamilton J.A. (1989) Potential anti-inflammatory effects of interleukin-4: Suppression of human monocyte tumour necrosis factor *a*, interleukin 1 and prostaglandin E2. *Proc. natl. Acad. Sci. U.S.A.* **86,** 3803.
40. Danielpour D., Kim K.Y., Dart L.L., Watanabe S., Roberts A.B. & Sporn M.B. (1989) Sandwich enzyme-linked immunosorbent assays (SELISAs) quantitate and distinguish two forms of transforming growth factor-beta (TGF-β1 and TGF-β2) in complex biological fluids. *Growth Factors* **2,** 61.