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Human recombinant IL-4 suppresses the induction of human IL-2 induced lymphokine activated killer (LAK) activity

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SUMMARY

The effect of recombinant human interleukin 4 (rhIL-4) on the induction *in vitro* of human lymphokine activated killer cell (LAK) activity was investigated. Peripheral blood mononuclear cells (PBMC) from normal healthy donors were incubated for 4 days with or without recombinant human interleukin-2 (rhIL-2) in the presence or absence of rhIL-4. LAK activity was measured against the NK-resistant colon adenocarcinoma cell line SW742, and NK mediated cytotoxicity was determined using NK sensitive K562 cells. Unlike previous reports using mouse effector cells, rhIL-4 neither induced LAK activity nor augmented the cytotoxic response induced by rhIL-2. In four out of six experiments there was a significant reduction of rhIL-2 induced LAK in the presence of rhIL-4, accompanied by a reduction of Tac antigen expression by rhIL-2 activated cells. Recombinant hIL-4 failed to influence the effector phase of the activated PBMC against SW742 or K562 targets.

Keywords lymphokine activated killer cells interleukin-2 interleukin-4 suppression

INTRODUCTION

The culture of normal peripheral blood mononuclear cells (PBMC) in the presence of recombinant human interleukin 2 (rhIL-2) leads to the generation of a population(s) of cells able to kill previously resistant fresh tumour cells and tumour cell lines resistant to NK cell killing (Grimm *et al.*, 1982). Lymphokine activated killer (LAK) cell activity represents a function rather than a distinct cell type (Ortaldo, Mason & Overton, 1986; Tilden, Itoh & Balch, 1987; Hersey & Bolhuis, 1987), recent studies inferring that both large granular lymphocytes (LGL) and T cells respond to IL-2 and generate LAK activity (Damle, Doyle & Bradley, 1986; Mingari *et al.*, 1986; Herberman, 1987). In combination with rhIL-2, LAK cells have been used in the treatment of cancer with varying degrees of success (Rosenberg, *et al.*, 1985; Rosenberg, 1988).

As with other approaches to the treatment of cancer, LAK and IL-2 infusion has toxic side effects. Thus the potential role of other cytokines in LAK therapy has become an important issue. Recently the induction and augmentation of murine LAK activity by murine interleukin 4 (IL-4) was reported (Mule, Smith & Rosenberg *et al.*, 1987). IL-4 (previously B cell stimulating factor I) is released by activated T lymphocytes (Yokota *et al.*, 1986) and exerts various biological effects on B cells, including the induction of Fc receptors and HLA-DR receptors on B cell lines (Rousset *et al.*, 1988), and the promotion of T cell proliferation (Spits *et al.*, 1987). An important issue therefore is whether human rIL-4 (rhIL-4) influences the induction of LAK activity in human PBMC, since administration of combined lymphokine therapy may reduce the multiple side effects reported with high dose IL-2 and LAK cell treatment.

In the present study rhIL-4 proved ineffective in promoting LAK activity alone or augmenting rhIL-2-induced LAK cell activity. Cytotoxicity and Tac antigen expression induced by rhIL-2 were inhibited by rhIL-4; the effector phase of rhIL-2 induced cytotoxicity was not affected by rhIL-4.

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MATERIALS AND METHODS

Effector cells

Peripheral blood from healthy donors was collected in heparin (10 units per ml) and the mononuclear cells (PBMC) separated on lymphocyte separation medium (Lymphoprep, Nyegaard & Company) as described previously (Boyum, 1968). The recovered PBMC were washed three times in phosphate-buffered saline (PBS) and resuspended in RPMI supplemented with antibiotics and 10% heat-inactivated human AB serum.

Target cells

The SW742 colon adenocarcinoma cell line (Leibovitz *et al.*, 1976) was grown as an adherent cell line in RPMI medium plus 10% fetal calf serum. Previous study of this line has shown it to be relatively insensitive to NK cytolysis but sensitive to rhIL-2 generated LAK activity. The NK sensitive K562 cell line was also used (Lozzio & Lozzio, 1975).

Cytokines

Recombinant human interleukin-2 (rhIL-2) was kindly provided by Sandoz, Vienna, Austria. In all assays rhIL-2 was used at a final concentration of 1,000 units/ml. Recombinant human interleukin 4 (rhIL-4) was kindly provided by Immunex Corporation, Seattle, Washington and used at final concentrations of 100 and 1000 units/ml. These cytokines were stored at −20°C and −80°C respectively and diluted in RPMI+0·1% bovine serum albumin before addition to PBMC.

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, New Jersey). The cells were incubated at 37°C in a 5% CO_2/95% air atmosphere for 4 days, after which each effector cell population was harvested from the 24-well plate, washed in PBS, counted, resuspended in the appropriate volume of RPMI plus 10% newborn calf serum (RPMI-NBCS), and assayed for cytotoxicity.

Chromium-51 release test

Target cells in 0·1 ml of their culture medium were radio-labelled by the addition of 100 μCi of sodium chromate (New England Nuclear Products, Hertfordshire, New England) and incubated for 1 h at 37°C in a 5% CO_2/95% air humidified atmosphere. The cells were then washed once in RPMI-NBCS and resuspended in 10 ml of medium and incubated for a further 1 h at 37°C. The cells were subsequently washed once in RPMI-NBCS, counted and resuspended at $1{\cdot}0 \times 10^5$ cells/ml in RPMI-NBCS. Assays were performed in triplicate in round-bottomed 96-well microtest plates (Falcon Microtest III flexible assay plates, Becton Dickinson). Target cells (0·1 ml/well) were incubated together with effector cells (0·1 ml/well) at the ratios indicated, test plates were incubated at 37°C in a 5% $C0_2$/95% air humidified atmosphere for 4 h, and ^{51}Cr-release and cytotoxicity calculated as previously described (Rees *et al.*, 1986).

Monoclonal Antibodies (MoAb)

Mouse anti-HLA-DR and anti-Tac were purchased from DAKO (Buckinghamshire, UK). The antibodies were used at 1/40 and 1/20 dilutions respectively. Fluorescein isothiocyanate (FITC)—conjugated goat anti-mouse IgG was purchased from Tissue Culture Services (Slough, Berkshire, UK) and used at 1/40 dilution. All dilutions were in PBS.

Flow cytometry

Samples of each effector population were incubated with 100 μl of each MoAb for 20 min at 4°C. After washing, cells were resuspended in 100 μl FITC-conjugated goat anti-mouse IgG. Control samples were incubated in the FITC conjugate alone. After incubation for 20 min at 4°C, all samples were washed and analysed on a Flow cytometer-FACS 420 B-D (FACS Systems, Becton Dickinson, Sunnyvale, CA). The results are expressed as a percentage of positive cells with respect to control samples which were used to indicate background fluorescence. Mean channel values were measured in arbitrary linear units considered to be proportional to antigen density. Excitation laser wavelength was 488 nm, suitable for the FITC label used.

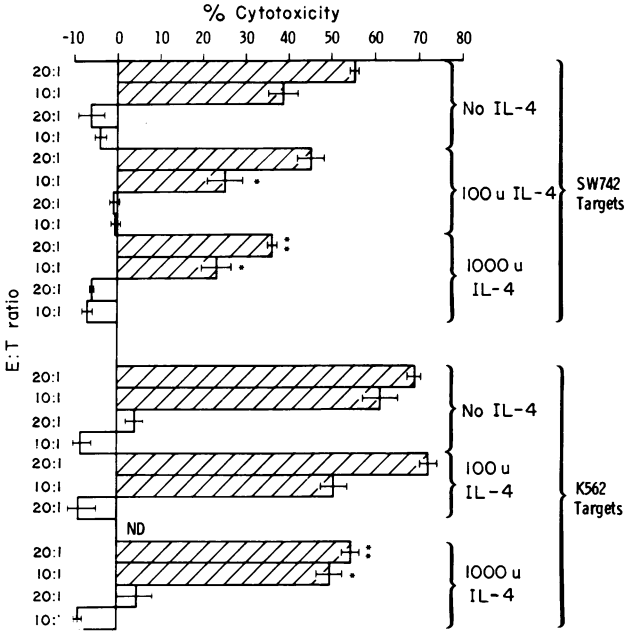

Fig. 1. Cytotoxicity of lymphocytes incubated with rhIL-2 in the presence (▨) or absence (□) of rhIL-4. Cytotoxicity was assessed against SW742 and K562 targets at 20:1 and 10:1, E:T ratios. * $P<0{\cdot}05$; ** $P<0{\cdot}001$.

RESULTS

PBMC were cultured in RPMI +10% human AB serum in the presence or absence of rhIL-2 (1,000 units/ml) and with or without the addition of rhIL-4 at 100 or 1,000 units/ml. Following incubation for 4 days at 37°C the cells were harvested and assayed for cytolytic activity against the NK resistant SW742 cell line or the NK-sensitive K562 targets. In a series of six experiments rhIL-4 failed to potentiate the cytotoxic activity of PBMC and no additive or synergistic effect was noted when PBMC were cultured in the presence of rhIL-2 and rhIL-4. The results of one of the six experiments performed is shown in Fig. 1. Four out of six individual PBMC preparations showed a suppressed response to rhIL-2 when rhIL-4 was present during the induction phase of the assay, which was particularly apparent when IL-4 was used as 1,000 units/ml. The decreased level of killing as manifest against both SW742 and K562 targets. These results, in contrast to those obtained using murine spleen cells, showed that rhIL-2-induced cytotoxicity can be suppressed in the presence of rhIL-4. The rhIL-4-mediated suppression of cytotoxicity was accompanied by a decreased expression of Tac antigen by rhIL-2-activated cells (Table 1); changes in MHC class II antigen expression were not consistently observed.

The influence of rhIL-4 on the effector phase of rhIL-2 generated LAK activity was investigated. The results show that rhIL-4, when present during the cytotoxicity assay, does not inhibit LAK effector cell function (Table 2). These results demonstrate rhIL-4 to be inhibitory to the induction phase but not the effector phase of the LAK response.

Table 1. Effect of rhIL-4 on HLA DR and Tac antigen expression

	Cultured lymphocytes stained with	Percentage positive cells (Intensity as measured by channel number)* Lymphocytes cultured with			
		Nil	IL-2	+IL-4	IL-2+IL-4
Experiment 1	Control	4·08	0·48	4·14	1·00
	class II MoAb	11·14 (157)	17·34 (94)	13·44 (512)	11·06 (97)
	Tac MoAb	5·22 (42)	21·98 (141)	5·92 (64)	12·14 (92)
Experiment 2	Control	6·56	5·98	4·36	3·52
	class II MoAb	33·36 (163)	40·66 (360)	26·68 (400)	41·96 (358)
	Tac MoAb	10·74 (49)	36·46 (92)	12·68 (49)	19·64 (93)

* The percentage positive cells and intensity of staining of lymphocytes incubated for 4 days with rhIL-2 (1,000 u/ml) and/or rhIL-4 (1,000 u/ml) was measured by flow cytometery. Controls FITC—conjuate only.

Table 2. The effect of rhIL-4 on the LAK effector phase

	% Cytotoxicity			
	SW742 targets		K562 targets	
	−IL-4	+IL-4	−IL-4	+IL-4
Experiment 1	49·8 (2·7)	47·0 (3·7)	70·8 (7)	74·9 (1·9)
Experiment 2	25·6 (0·2)	26·4 (1·0)	57·3 (5·8)	58·8 (5·5)

Effector cells (incubated for 4 days in rhIL-2) were incubated with 1000 u/ml of rhIL-4 during the 4 h Chromium-release assay. E:T = 10:1.

DISCUSSION

The relative success of using lymphokine activated killer (LAK) cells and rhIL-2 in the treatment of human malignant disease has generated considerable interest in the potential use of cytokines to promote anti-tumour immune responses. Cytokines interact to regulate the immune system (Guillou, 1987) and some may act synergistically (Nedwin *et al.*, 1985; Schiller *et al.*, 1987) or antagonistically (Hoffman, 1986; Rosztoczy, Siroki & Béládi, 1986) with each other. Thus, the interaction between different cytokines in inducing non-MHC-restricted cytotoxicity is an important consideration. Recently Mule *et al.* (1987) reported the induction and augmentation of LAK from mouse splenocytes using murine IL-4 and thus inferred the combined use of these agents would prove to be of value in promoting LAK activity. We report here the effect of human recombinant IL-4 (rhIL-4) on human PBMC cultured in the presence or absence of rhIL-2. Unlike the results reported in mice, rhIL-4 failed to augment cytotoxicity above background killing of PBMC cultured for 4 days without rhIL-2, and rhIL-4 failed to augment rhIL-2-induced LAK cytotoxicity. In contrast, in four out of six experiments performed using PBMC prepared from different donors IL-4 was shown to suppress rhIL-2-induced cytotoxicity against both NK sensitive and insensitive target cells. Although the mechanism of the suppressive effect of IL-4 is not known the results of the present study suggest that, in part, this may be due to a decrease in the expression of the low affinity IL-2 receptor, as measured by flow cytometry using anti-tac monoclonal antibody. Since only a partial reduction of LAK activity was observed in this study it is pertinent to speculate that a proportion, possibly a subpopulation, of lymphocytes fail to respond to rhIL-2 when co-cultured with rhIL-4. This could be accounted for by a decrease in the number of cells expressing the low affinity 55 kD IL-2 receptor. Alternatively IL-4 could interfere in some way with transmembrane signalling mediated through the high affinity IL-2 receptor, which would be required for cell proliferation, and induction of cytotoxic activity, although it is not clear whether these two events are directly related (Malkovsky *et al.*, 1987; Ramsdell, Shau & Golub, 1988). The significance of these results suggests firstly a difference between murine and human effector cells and their response to IL-4, and secondly emphasizes the possible interactions which may determine the cytotoxic potential of individual effector cell populations. This is an important consideration, which should be addressed when assessing the therapeutic potential of cytokines.

ADDENDUM

Additional studies have indicated that rhIL-4 reduces rhIL-2 induced DNA synthesis (as assessed by propidium iodide staining and FACS analysis). Furthermore, rhIL-4 suppression of rhIL-2 induced cytotoxicity is apparent 24 h after the beginning of LAK induction.

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