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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

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Human rIL-4 was studied for its capacity to induce lymphokine-activated killer (LAK) cell activity. In contrast to IL-2, IL-4 was not able to induce LAK cell activity in cell cultures derived from peripheral blood. IL-4 added simultaneously with IL-2 to such cultures suppressed IL-2-induced LAK cell activity measured against Daudi and the melanoma cell line MEWO in a dose-dependent way. IL-4 also inhibited the induction of LAK cell activity in $CD2^+$, $CD3^-$, $CD4^-$, $CD8^-$ cells, suggesting that IL-4 acts directly on LAK precursor cells. IL-4 added 24 h after the addition of IL-2 failed to inhibit the generation of LAK cell activity. Cytotoxic activity of various types of NK cell clones was not affected after incubation in IL-4 for 3 days, indicating that IL-4 does not affect the activity of already committed killer cells. No significant differences were observed in the percentages of Tac^+, $NKH\text{-}1^+$ and $CD16^+$ cells after culturing PBL in IL-2, IL-4 or combinations of IL-2 and IL-4 for 3 days. IL-4 also inhibited the activation of non-specific cytotoxic activity in MLC, as measured against K-562 and MEWO cells. In contrast, the Ag-specific CTL activity against the stimulator cells was augmented by IL-4. Collectively, these data indicate that IL-4 prevents the activation of LAK cell precursors by IL-2, but does not inhibit the generation of Ag-specific CTL.

IL-2 has the ability to activate a killer cell population of PBL (1–6) and thymocytes (7–9). These LAK[2] cells are able to lyse fresh tumor cells as well as cells from in vitro tumor cell lines that are not lysed by freshly isolated NK cells. It has been shown recently that both the precursors and the effector cells have the characteristics of NK cells (4–6). It was demonstrated that the majority of the LAK cell activity is mediated by $NKH\text{-}1^+$ cells that do not express CD3 and that the precursors of these cells have the same phenotype (5, 6). In addition, there appears to be some contribution of $CD3^+$, $NKH\text{-}1^+$ T cells to the LAK cell activity induced from PBL, but the kinetics of appearance of LAK cell activity mediated by these cells is delayed compared with the LAK cell activity mediated by $CD3^-$, $NKH\text{-}1^+$ cells (5, 6). Little is known about the mechanism of induction of LAK cell activity. It has been reported that activation of NK cells by IL-2 is not inhibited by mAb against the 55-kDa Tac Ag, whereas these antibodies block T cell proliferation (10, 11). This suggests that induction of LAK cell activity involves a different IL-2 R than T cell proliferation. Indeed, evidence has been obtained that IL-2 activates NK cells via a 73-kDa peptide that binds IL-2 with an intermediate affinity (12–14). The high affinity IL-2R appears to consist of a complex of the Tac Ag with the 73-kDa protein (15).

Recently, a cDNA clone that encodes human IL-4 has been obtained from the human T cell clone 2F1 (16). We reported that human rIL-4 can support the growth of human T cells and that the growth-promoting activity of IL-4 was not restricted to a certain subset of T cells (17). $CD3^+$ as well as $CD3^-$ T cell clones could respond to IL-4 (17). These findings raised the question of whether IL-4 could also induce LAK cell activity. In this paper, we investigate the effect of IL-4 on the induction of cytolytic activity of human cells against the lymphoblastoid Burkitt lymphoma line Daudi and against the short term cultured melanoma cell line MEWO. It was found that IL-4 was not only unable to induce cytotoxic activity, but that it inhibited the IL-2-mediated induction of cytolytic activity against these cell lines. IL-4 was also found to block non-specific cytotoxicity generated in a MLC. In contrast, MLC-generated Ag-specific cytotoxicity was augmented by IL-4.

MATERIALS AND METHODS

IL. An IL-4-encoding cDNA clone was isolated from a cDNA library of the human helper T cell clone 2F1 (16). Cos-7 monkey kidney cells were transfected with this cDNA clone in a mammalian expression vector, and supernatants containing IL-4 were harvested after 48 h (16). One unit of IL-4 is defined as the amount of IL-4 required to cause half-maximal proliferation of 2×10^4 PHA-preactivated T cells. In some experiments, IL-4 from Cos-7 supernatants, partially purified by passage over S-Sepharose and Sephadex G-100 columns was used.

The IL-2 preparation was an *Escherichia coli*-produced IL-2. The biologic activity of IL-2 was determined by using the IL-2-dependent murine T cell line CTLL-2, as described by Gillis et al. (18).

Preparation of lymphocyte populations. PBL were isolated from healthy donors by Ficoll-Hypaque density gradient centrifugation. To enrich for $CD3^-$ cells, PBL were first rosetted with SRBC at a lymphocyte to SRBC ratio of 1:50 for 15 h at 4°C in a modification of Iscoves medium (Gibco Laboratories, Glasgow, Scotland), described by Yssel et al. (19), containing 1% human AB^+ serum. The rosetted cells were centrifuged through a Ficoll-Hypaque gradient, followed by lysis of the SRBC in an isotonic NH_4Cl buffer for 10 min

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[2] Abbreviations used in this paper: LAK, lymphokine-activated killer; HRBC, human RBC; GAM, goat anti-mouse Ig; LU, lytic units.

at 4°C. The cells (5×10^7/ml) were washed twice and incubated with a 1/100 dilution of the anti-CD3 mAb SPV-T3b for 1 h at 4°C. After three washes, the cells were incubated for 20 min with HRBC coated with GAM using $CrCl_3$ as described previously (20). After vortexing, the cells were depleted from the rosette-forming ($CD3^+$) cells by centrifugation over Ficoll-Hypaque. The $CD3^-$ population was also negative for CD4 and CD8 and reacted weakly with the mAb NKH-1. Alternatively, PBL were incubated for 45 min at 37°C in tissue culture flasks (Falcon; Becton Dickinson, Mountain View, CA) to remove adherent cells. Subsequently, the non-adherent cells were incubated with a mixture of the anti-CD8 mAb SPV-T8 and the anti-CD4 mAb RIV-6. The $CD4^+$ and $CD8^+$ cells were removed with the reversed rosetting method described above. The remaining cells contained 5 to 10% $CD3^+$ cells, but were virtually negative for CD4 and CD8, as determined with a FACS 440 (Becton Dickinson).

Cultures for induction of LAK cell activity. PBL or subpopulations of PBL were usually incubated with 20 U/ml IL-2 at a concentration of 10^6 cells/ml for 3 days in Yssel's medium containing 1% human AB^+ serum. The cultures were performed in 1-ml wells of a Linbro 24-well plate (Flow Laboratories, McLean, VA). After the culture period, the cells were harvested and washed twice before use.

MLC. PBL (10^6/ml) were cultured with allogeneic irradiated (3000 rad) PBL at a responder to stimulator ratio of 1:1 in Yssel's medium plus 1% AB^+ serum for 6 days in 1-ml wells of a 24-well Linbro plate. The cells were harvested, washed twice, and used as effector cells in a ^{51}Cr release assay. For a secondary MLC, the responder cells were washed 10 days after the onset of the primary MLC and restimulated with the irradiated stimulator cells at a 1:1 ratio under the same conditions as used for the primary MLC, except that the mixtures were incubated for 4 days before testing the responder cells. PBL of the stimulator cell type that were stimulated 4 days before the test with 0.1 μg/ml purified PHA (Wellcome, Beckenham, England), were used as target cells.

^{51}Cr release assay. The ^{51}Cr release assay was carried out as described previously (21). Briefly, 1000 ^{51}Cr-labeled target cells were mixed with varied numbers of effector cells in Iscove's medium containing 0.25% BSA (Sigma Chemical Co., St. Louis, MO). The tests were carried out in U-shaped 96-well Linbro plates. The plates were centrifuged for 5 min at 50 × *g* before an incubation for 4 h at 37°C in a humidified 5% CO_2 atmosphere. The samples were harvested by using a Skatron harvester (Lier, Norway) and counted in a gamma counter (LKB, Bromma, Sweden).

Target cells. The cell lines used in this study were the erythroid/myeloid cell line K-562, the Burkitt lymphoma line Daudi, and the melanoma cell line MEWO. These cell lines were cultured in Yssel's medium containing 1% AB^+ human serum.

Establishment of LAK cell clones. Clone WM-14 has been isolated from $WT\text{-}31^-$ cord blood cells as has been described previously (22). The clone expresses TCRγ, which is associated with the CD3 complex and reacts with anti-TCRδ1 (23). WM-14 does not express CD4 or CD8, but is positive for NKH-1. Clone HY-133 ($CD3^+$, $WT\text{-}31^+$, $CD4^-$, $CD8^+$, $NHK\text{-}1^+$) has been described previously (22). Clone HY-212 is a $CD3^-$ clone derived from the same cloning as clone HY-133. The clones NL-5 ($CD3^+$, $WT\text{-}31^-$, $TCR\delta1^+$), NL-1 ($CD3^-$), and NL-6 ($CD3^-$) are derived from peripheral blood T cells that were depleted of $CD4^+$ and $CD8^+$ cells by reversed rosetting. The clones were maintained by weekly stimulations in a feeder cell mixture containing irradiated PBL (4000 rad), irradiated EBV-transformed B cells (5000 rad), and PHA (0.1 μg/ml) as described previously for cloned allospecific CTL (21). The clones were cultured in Yssel's medium.

mAb. The mAb used in this study for separation by means of reversed rosetting and for phenotyping were SPV-T3b (anti-CD3) (24), RIV-6 (anti-CD4; a kind gift from Dr. Kreeftenberg, National Institute of Health (RIV), Bilthoven, The Netherlands), the anti-CD8 mAb SPV-T8 (4), WT-31 (anti-TCRα, β) (25), NHK-1 (reacts with NK cells; purchased from Coultronics, Margency, France) (26), and Leu 11 (anti-CD16, Becton Dickinson). The anti-Tac mAb, IOT-14 (27), was purchased from Immunotech (Luminy, Marseille, France). The anti-TCRδ1 antibody that reacts with a framework determinant on the TCRδ chain (23) was a kind gift from Dr. M. Brenner (Dana Farber Cancer Institute, Boston, MA).

Phenotyping of cell samples. The separated cell samples and T cell clones were typed for the expression of cell surface Ag by flow cytometry. The samples were labeled with mAb and FITC-labeled GAM $F(ab')_2$ fragments (Grub, Vienna, Austria) as described previously (21). The samples were analysed with a FACS-440.

RESULTS

The effect of IL-4 on the induction of LAK cells. Freshly isolated PBL were incubated with IL-2, with IL-4 semi-purified from Cos-7 monkey kidney cell supernatants (see *Materials and Methods*), or with combinations of IL-2 and IL-4. Strong LAK cell-mediated cytotoxicity induced by IL-2 was observed at an E:T cell ratio of 30:1. Freshly isolated PBL or PBL cultured in medium only were ineffective in lysing Daudi or MEWO cells. In Figure 1 it is shown that IL-4, in contrast to IL-2, was unable to induce cytotoxic activity against Daudi cells (Fig. 1*A*) or against the melanoma cell line MEWO (Fig. 1*B*). Strikingly, it was observed that IL-4 reduced the level of LAK cell activity induced by IL-2 when these lymphokines were added simultaneously to the PBL. In Figure 1*A* it is shown that the inhibiting effection of IL-4 was dose dependent. Approximately 50% inhibition was observed at an IL-4 concentration of 20 U/ml. The LAK cell activity generated by IL-2 against MEWO was already completely blocked by 20 U/ml IL-4 (Fig. 1*B*). The results in Figure 1, *A* and *B* reflect the general finding that the IL-2-induced LAK cell activity against MEWO cells is more sensitive to blocking with IL-4 than the activity against Daudi. The reason for this difference in sensitivity is presently unclear.

The effect of IL-4 on induction of LAK cell activity in $CD2^+$, $CD3^-$ precursor cells. The results presented in Figure 1 indicate that IL-4 inhibits the generation of LAK cell activity mediated by IL-2. To determine whether IL-4 acts directly on the precursor cell, induces the generation

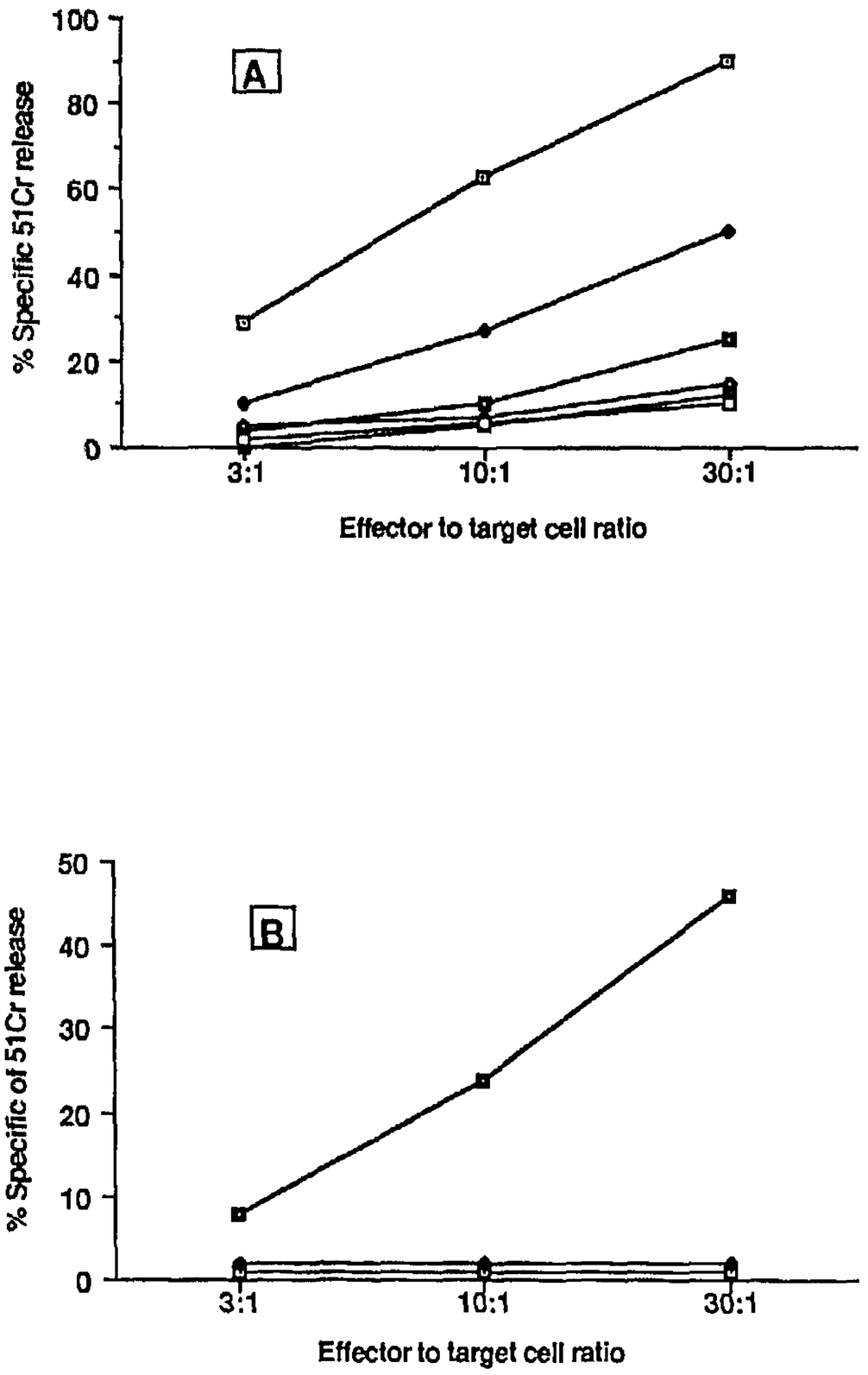


Figure 1. The effect of purified IL-4 on the induction of cytotoxic activity and IL-2-induced LAK cell activity against Daudi cells (*A*) and MEWO cells (*B*). □, Medium control; ◆, IL-4 (200 U/ml); □, IL-4 (20 U/ml); ◇, IL-2; ■, IL-2 plus IL-4 (200 U/ml); and □, IL-2 plus IL-4 (20 U/ml). IL-2 was added at a concentration of 20 U/ml. The IL-4 used in this experiment was semi-purified from Cos-7 supernatants as indicated in *Materials and Methods*.

of suppressor T cell activity, or, alternatively, induces the synthesis of an inhibitory factor from mature T cells, $CD3^-$ cells were isolated from $CD2^+$ cells by using a two-step separation procedure. In the first experiment shown in Table I, non-adherent, SRBC rosette-positive T cells were incubated for 1 h with the anti-CD3 mAb SPV-T3b. The $CD3^+$ cells were subsequently removed by rosetting with GAM-labeled HRBC, followed by centrifugation over a Ficoll-Hypaque gradient. Less than 2% of the interface cells expressed CD3 as determined by flow cytometry. In Table I it is shown that IL-2 could induce a high cytotoxic activity in the $CD3^-$ cell population. IL-4-containing Cos-7 supernatant strongly blocked the induction of this LAK cell activity. The same results were obtained with $CD4^-$, $CD8^-$ cells that were obtained after removal of the $CD4^+$ and $CD8^+$ cells from non-adherent PBL by the same procedure. In that case, the non-adherent cells were incubated with a mixture of anti-CD4 and anti-CD8 mAb, followed by rosetting with GAM-labeled HRBC (Table I). Although not shown, supernatants of mock-transfected Cos-7 cells have no effect on IL-2-induced LAK cell activity. These results suggest that IL-4 inhibits the induction of LAK cell activity at the level of the precursor cell.

The effects of sequential addition of IL-2 and IL-4 on the induction of LAK cell activity in PBL. The results of the experiments shown in Table I suggest that the inhibition of LAK cell generation by IL-4 is not an indirect effect. It was, however, not clear whether the inhibition of LAK cell activity is based on the inhibition of the inducing event itself or on the inactivation of the effector cells mediating the cytolytic activity. In the latter case, we would expect that incubation with IL-4 of cells that are already activated with IL-2 would inhibit their lytic activity. In Table II it is shown that the addition of IL-4 24 h after the addition of IL-2 did not affect the lytic activity of the activated killer cells, whereas simultaneous addition of IL-4 and IL-2 strongly inhibited this activity.

In a second set of experiments, we investigated the possibility that pre-incubation with IL-4 somehow inactivates the precursor of the cells that mediate non-specific cytotoxic activity. PBL (10^6/ml) were incubated in medium or in 100 U/ml IL-4 for 24 h, then washed three times, and incubated for 3 days in medium alone or containing IL-2, IL-4, or IL-2 plus IL-4. It is shown in Table III that IL-2 induced LAK cells with a cytotoxic activity of 17 and 7 LU/10^5 cells, respectively, against Daudi and MEWO cells. This activity was reduced to 2 and <1 LU/10^5 cells, respectively, when IL-2 was added together with IL-4. The PBL pre-incubated in IL-4 and subsequently cultured in IL-2 had the same cytotoxic activity against Daudi cells (17 LU/10^5 cells) as the PBL pre-incubated in medium, whereas the activity against MEWO was slightly reduced (5 LU/10^5 cells) in the PBL pre-incubated with IL-4. These results demonstrate that 24 h of pre-incubation in IL-4 does not inactivate the LAK cell precursors.

The effect of IL-4 and IL-2 on the cytolytic activity of NK clones. To further determine whether IL-4 can affect the lytic activity of already committed killer cells, we tested the effect of incubation with IL-4 on the cytolytic activity of activated killer cell clones. The clones NL-1 and NL-6 ($CD3^-$, NHK-1^+, Leu 11^+), clones NL-5 and WM 14 ($CD3^+$, WT-31^-, TCR$\delta 1^+$, NKH-1^+, Leu 11^-), and clone HY-133 ($CD3^+$, WT-31^+, TCR$\delta 1^-$, NKH-1^+, Leu 11^-) represent the various subpopulations of LAK cells as described by Phillips and Lanier (5) (Table IV). All these clones were cytotoxic for Daudi cells. Previously, we have found that IL-4 could promote the growth of such clones (17). Six days after stimulation with feeder cells, the activated killer clones were washed extensively and in-

TABLE I

Effect of IL-4 on IL-2-mediated induction of LAK cell activity in $CD4^-$ $CD8^-$ and $CD3^-$ cells isolated from PBL against MEWO cells

Cell Population[a]	Lymphokine Added[b]	% ^{51}Cr Release at an E:T Ratio of			
		1:1	3:1	10:1	30:1
Expt. 1					
PBL	None	ND	0	1	2
	IL-2	ND	6	15	28
	IL-4	ND	0	1	0
	IL-2 + IL-4	ND	4	6	13
$CD4^-$ $CD8^-$	None	ND	0	0	6
	IL-2	ND	18	33	54
	IL-4	ND	0	0	0
	IL-2 + IL-4	ND	5	9	22
Expt. 2					
$CD3^-$	None	2	3	10	ND
	IL-2	47	58	70	ND
	IL-4	1	2	5	ND
	IL-2 + IL-4	16	24	34	ND

[a] The $CD4^-CD8^-$ and the $CD3^-$ cells were obtained as described in *Materials and Methods*. The $CD4^-CD8^-$ cell sample in experiment 1 contained 10% $CD3^+$ cells and no $CD4^+$ or $CD8^+$ cells, whereas the $CD3^-$ cell sample in experiment 2 contained 3% $CD3^+$ cells.

[b] IL-2 was added at a concentration of 20 U/ml. The concentration of IL-4 in experiment 1 was 100 U/ml (2% of supernatant of Cos-7 cells transfected with IL-4 cDNA, containing 5000 U/ml). In experiment 2, the IL-4 was purified from transfected Cos-7 supernatants and used at a concentration of 100 U/ml.

TABLE II

Effect of sequential addition of IL-4 on the IL-2-induced LAK cell activity in PBL against MEWO cells

Lymphokine Added[a]	% ^{51}Cr Release at an E:T Ratio of	
	30:1	10:1
Medium	11	7
IL-2	47	29
Il-2 + IL-4	5	3
IL-2 + IL-4 at 24 h[b]	46	29

[a] The concentration of IL-2 used in this experiment was 20 U/ml. IL-4 (100 U/ml final concentration) was added as a supernatant of transfected Cos-7 cells, as in Table I.

[b] IL-4 was added 24 h after the addition of IL-2.

TABLE III

Effect of pre-incubation of PBL in IL-4 for 24 h on the subsequent induction of LAK cell activity by IL-2 or IL-2 plus IL-4

Pre-incubation[a]	Incubation[b]	LU/10^5 Cells[c]	
		Daudi	*MEWO*
Medium	Medium[d]	<1	<1
	IL-4	<1	<1
	IL-2	17	7
	IL-2 + IL-4	3	<1
IL-4	Medium[d]	<1	<1
	IL-4	<1	<1
	IL-2	17	5
	IL-2 + IL-4	2	<1

[a] PBL (10^6) were pre-incubated in 100 U/ml IL-4 or medium for 24 h.

[b] IL-2 and IL-4 were added at concentrations of 20 and 100 U/ml, respectively.

[c] One LU was defined as the number of effector cells necessary to cause 30% specific ^{51}Cr release of 10^3 target cells. LU were calculated from a dose-response curve in which the percent ^{51}Cr release was plotted against the E:T ratios 90:1, 30:1, 10:1, and 3:1.

[d] Cell recoveries were similar for all cultures.

TABLE IV

Effect of incubation in IL-2, IL-4, and IL-2 plus IL-4 on the cytolytic activity of NK clones against Daudi cells

Clone	Phenotype	Incubation[a]	% ^{51}Cr Release at an E:T Ratio of	
			30:1	3:1
NL-1	CD3$^-$, NHK-1$^+$, Leu 11$^+$	IL-2	35	17
		IL-4	31	17
		IL-2 + IL-4	42	21
NL-5	CD3$^+$ WT31$^-$, NKH-1$^+$, TCRδ1$^+$, Leu 11$^-$	IL-2	64	50
		IL-4	62	55
		IL-2 + IL-4	66	47
NL-6	CD3$^-$, NKH-1$^+$, Leu 11$^+$	IL-2	41	21
		IL-4	51	30
		IL-2 + IL-4	48	19
WM-14	CD3$^+$, WT31$^-$, NKH-1$^+$, TCRδ1$^+$, Leu 11$^-$	IL-2	59	37
		IL-4	73	48
		IL-2 + IL-4	70	20
HY-133	CD3$^+$, WT31$^+$, NKH-1$^+$, Leu 11$^-$	IL-2	41	21
		IL-4	39	18
		IL-2 + IL-4	45	19

[a] The clones were washed two times 6 days after stimulation with feeder cells and were incubated with the lymphokines for another 3 days. IL-2 and IL-4 were added at concentrations of 40 and 100 U/ml, respectively.

TABLE V

Effect of culturing PBL in IL-2, IL-4, and IL-2 plus IL-4 for 3 days on the phenotype of PBL

mAb	% of Positive Cells After Culture in[a]			
	Medium	IL-2	IL-4	IL-2 + IL-4
SPV-T3b	62	62	65	61
WT-31	61	60	60	60
NKH-1	5	6	6	7
Leu 11	10	10	13	17
IOT-14 (anti-Tac)	2	6	2	4

[a] IL-2 and IL-4 were added at concentrations of 40 and 100 U/ml, respectively. The cell concentration in the cultures was 10^6 cells/ml.

cubated in IL-2, IL-4, or IL-2 plus IL-4 for three days. In Table IV it is shown that none of these clones lost their cytolytic activity against Daudi cells upon incubation in IL-2, IL-4, or a mixture of IL-2 and IL-4. One of the clones, WM-14, was also cytotoxic for MEWO cells. The activity of WM-14 against MEWO was not affected by incubation in IL-4 (results not shown).

Taken together, these results indicate that pre-incubation of PBL in IL-4 for 24 h does not inactivate the precursor cell nor the effector cell that mediates activated killer cell activity. Moreover, IL-4 appears to inhibit the generation of LAK cell activity only when it is added simultaneously with IL-2 to the PBL, indicating that IL-4 somehow prevents the activation event mediated by IL-2.

The effects of IL-4 and IL-2 on the phenotype of short term cultured PBL. The results of the previous experiments suggest that IL-4 prevents the IL-2-induced activation of the precursors of LAK cells. To further investigate this point, we compared the phenotype of PBL cultured in IL-2, IL-4, IL-2 plus IL-4, or medium only. In Table V it is shown that after 3 days of culture no differences in the phenotype of the PBL in the four cultures could be detected. The finding that no significant differences in the distribution of NKH-1 and Leu 11 were observed after 3 days indicates that the inhibition of the generation of LAK cell activity by IL-4 is not due to a selective disappearance of NKH-1$^+$ or CD16$^+$ cells in the first 3 days.

The effect of IL-4 on the activity of NK cells against K-562. In view of the discussion about the relationship between LAK cells and NK cells, it was of interest to know which effect IL-4 has on the constitutive NK cell activity and whether IL-4 can inhibit the enhancement of NK activity by IL-2 (10, 28). To this end, PBL were cultured for 48 h with 100 U/ml IL-4, 20 U/ml IL-2, or a combination of these lymphokines, It is shown in Table VI that IL-4 does not affect the constitutive cytotoxic activity against K-562, but blocked the IL-2-mediated enhancement of this activity. These findings support the notion that once there is a non-specific (NK-like) cytotoxic activity, incubation for a limited period of time in IL-4 does not inhibit this activity. Moreover, IL-4 inhibited the IL-2-mediated enhancement of NK activity against K-562.

The effect of IL-4 on cytotoxic activities generated in MLC. The data thus far indicate that IL-4 inhibits IL-2-induced cytotoxic activity. Recently it has been reported that murine IL-4 augmented the cytotoxic activity of murine CTL generated in an in vitro MLC (29). It has been demonstrated that, in addition to specific CTL activity, non-specific cytotoxic activity is also generated in a MLC (30). It was of interest therefore to compare the effects of IL-4 on the generation of specific and non-specific cytotoxic activities in the human system. In Figure 2 it is shown that administration of IL-4 to a MLC augmented the cytotoxic activity of the MLC responder cells against the PHA-pre-activated cells of the stimulator cell type (Fig. 2*A*). IL-2 at 20 U/ml hardly affected the cytotoxic activity against the specific stimulator cells on a per cell basis, but enhanced the cytotoxicity against MEWO (Fig. 2*B*) and K-562 (Fig. 2*C*). In contrast, IL-4 blocked the generation of cytolytic activity against K-562 and MEWO cells. It should be noted here that the cell recoveries of the cultures in IL-2 and IL-4 were comparable and only slightly higher (10 to 20%) than those of the MLC cultured in medium only (data not shown). IL-4 also stimulated the specific cytotoxicity, but blocked the non-specific cytotoxicity, in secondary MLC. PBL were stimulated with irradiated allogeneic PBL. Ten days later, 10^6 responder PBL/ml were restimulated with 10^6 irradiated stimulator PBL/ml in medium or in medium supplemented with 20 U/ml IL-2 or 100 U/ml IL-4. The recoveries of these cultures after 4 days were 1.2×10^6 T cell blasts/ml in medium, 3.2×10^6 T cell blasts/ml in IL-2, and 5×10^6 T cell blasts/ml in IL-4.

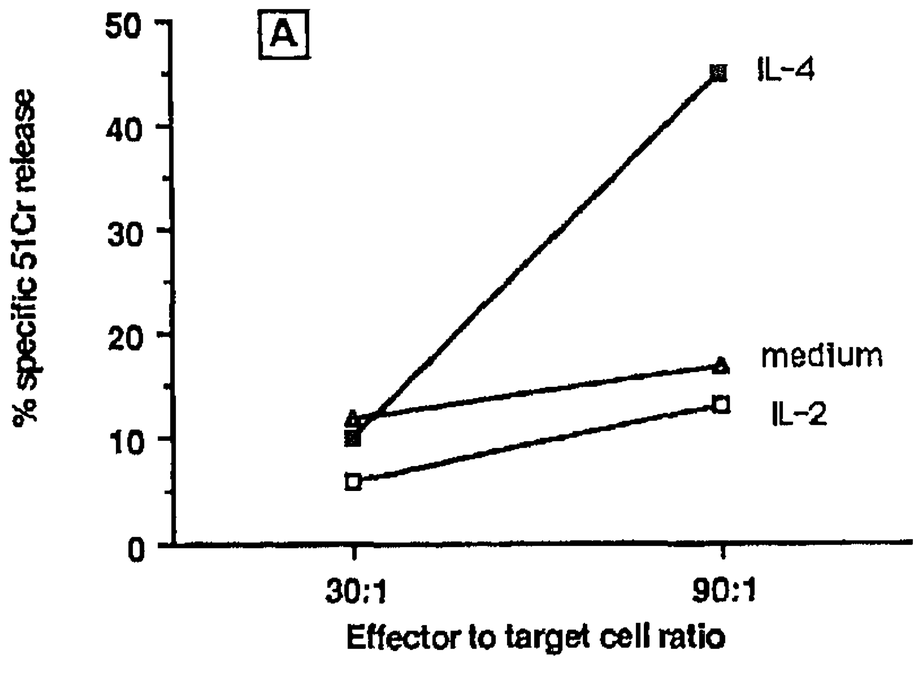
In Figure 3 it is shown that on a per cell basis the

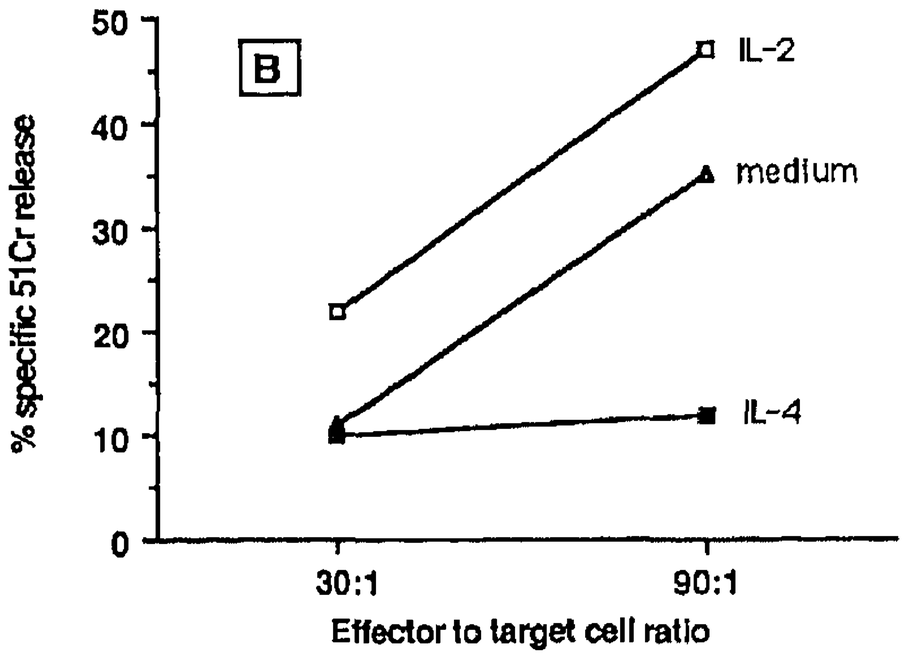
TABLE VI

Effect of IL-4 on NK cell activity against K-562 and the enhancement of NK cell activity induced by IL-2[a]

	% ^{51}Cr Release at an E:T Ratio of			
	60:1	20:1	6:1	2:1
Control	24	12	4	0
IL-4	23	10	4	1
IL-2	76	50	29	10
IL-2 + IL-4	53	37	7	3

[a] PBL (10^6/ml) were incubated for 48 h in medium with or without 100 U/ml IL-4, 20 U/ml IL-2, or a combination of these lymphokines.





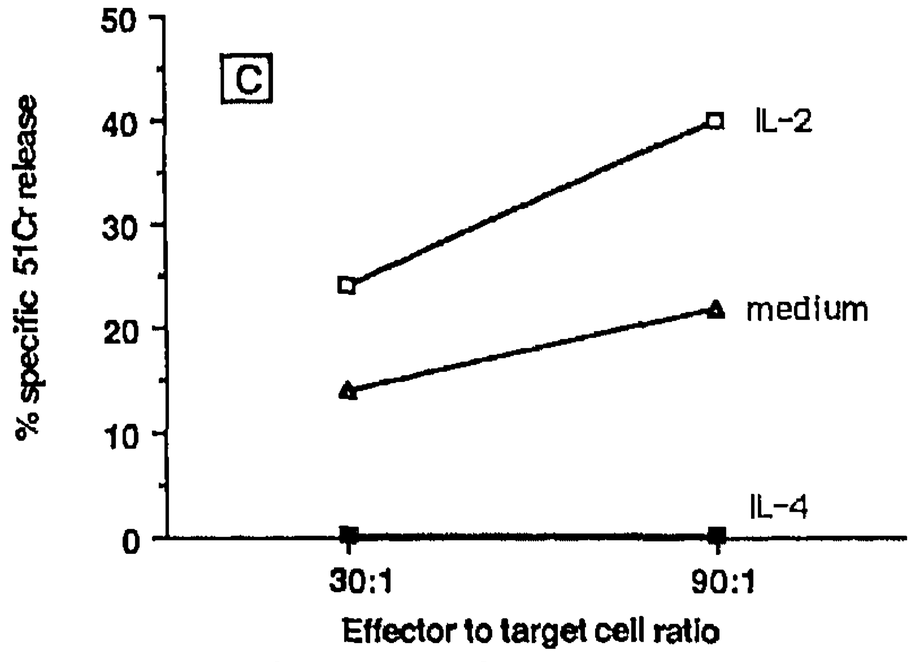
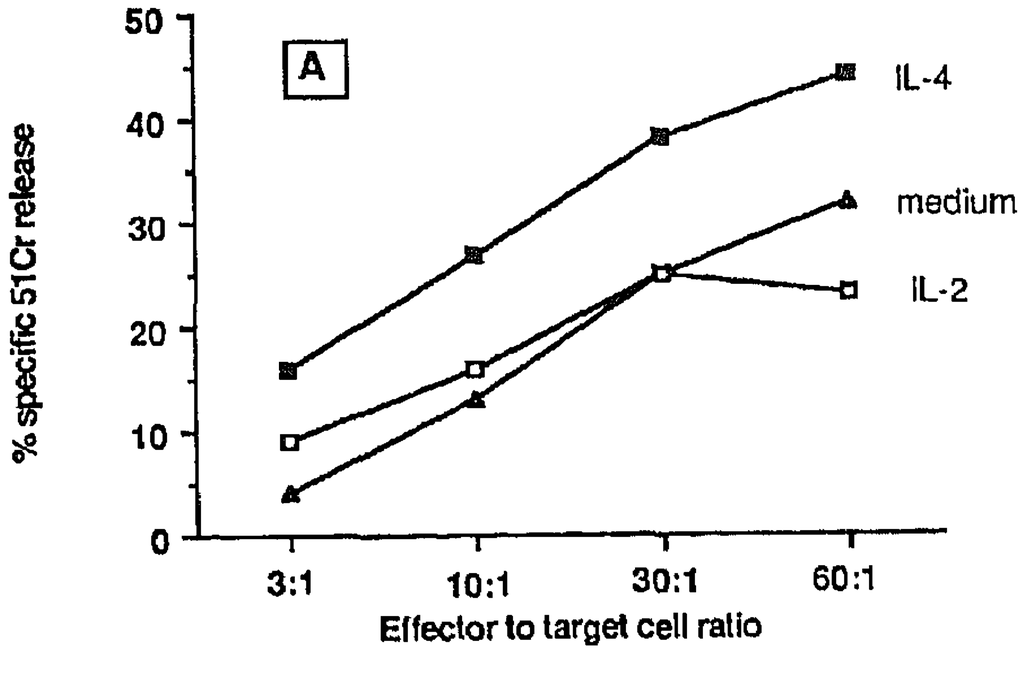
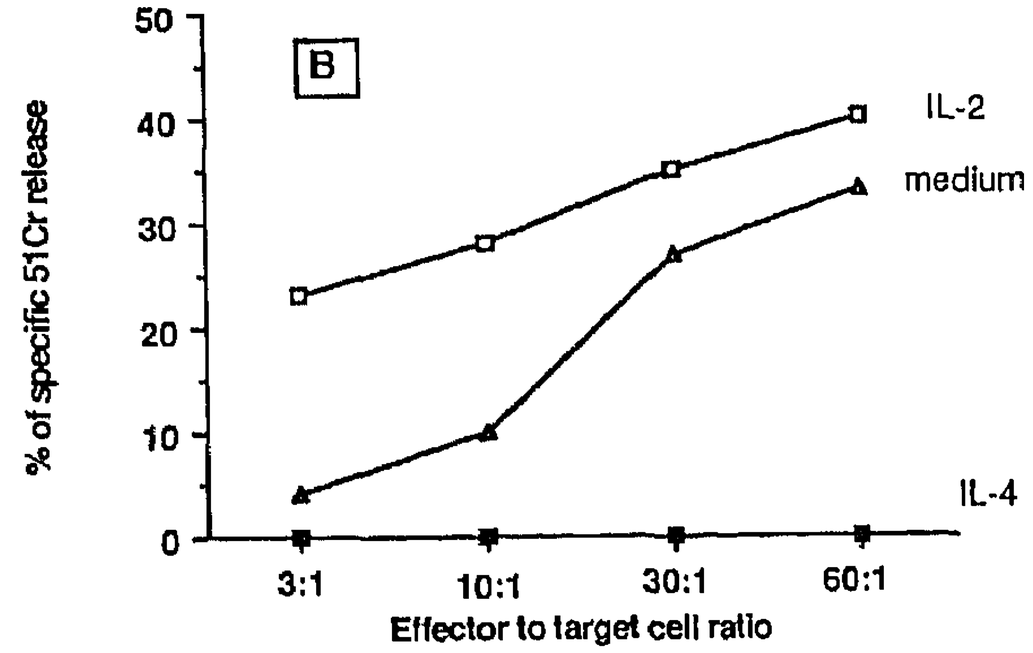


Figure 2. The effects of additions of IL-2 (20 U/ml) and IL-4 (100 U/ml) on cytotoxic activities generated against PHA blasts of the stimulator cell type (*A*), K-562 (*B*), and MEWO (*C*). The PHA blasts were obtained by stimulation of the PBL with 0.1 μg of purified PHA for 4 days. None of the cultures were cytotoxic for the PHA blasts of the responder cell type.





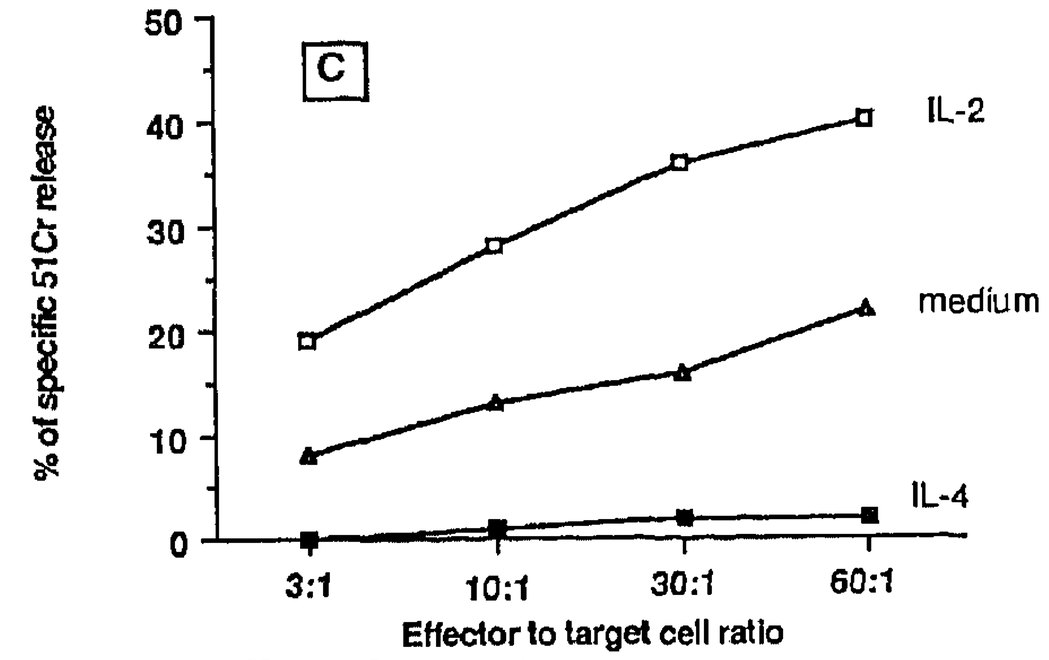


Figure 3. The effects of IL-2 and IL-4 on cytotoxic activities generated in secondary MLC. The cytotoxic activities were determined 6 days after initiation of the primary MLC and 4 days after the onset of the secondary MLC (see *Materials and Methods*). The cytotoxic activities of the primary MLC blasts at an E:T ratio of 30:1 were 7% against PHA blasts of the stimulator cell type, 15% against MEWO, and 34% against K-562. The cell recoveries of the secondary MLC were 1.3×10^6 cells/ml in medium, 3.2×10^6 cells/ml in IL-2 (20 U/ml) and 5×10^6 cells/ml in IL-4 (100 U/ml). The secondary MLC T blasts were tested against PHA blasts of the stimulator cell type (*A*), K-562 (*B*), and MEWO (*C*).

cytotoxic activity of the T cell blasts against the PHA blasts of the stimulator cell type is similar for the cells cultured in medium compared with those cultured in IL-2, but inasmuch as the IL-2-supplemented culture contained approximately 3 times more cells than the cultures incubated in medium only, IL-2 enhanced the specific CTL activity per culture. IL-4 enhanced the specific CTL activity both per cell as well as per culture. It is shown in Figure 3, *B* and *C*, that IL-4 completely abrogated the non-specific cytotoxic activities against K-562 and MEWO, respectively, whereas IL-2 enhanced these activities. These results demonstrate that IL-4 also blocks the generation of Ag-non-specific cytotoxic activity in a MLC, whereas it augments the Ag-specific cytotoxic activity.

DISCUSSION

Recently, we have found that IL-4 can promote the growth of mature $CD4^+$ and $CD8^+$ T cells, as well as of $CD3^-$ NK clones (17). It was therefore of interest to know whether IL-4, like IL-2, could also induce activated killer cell activity. It is reported here that IL-4 does not induce activated killer cells. On the contrary, we observed that IL-4 inhibits the induction of activated killer cells by IL-2. We did not test the activity of the killer cell populations activated in our system against fresh tumor cells. One of the target cells used in these studies, however, is a melanoma cell line (MEWO) in an early passage after establishment. We consider it therefore very likely that our observations reflect the inhibition by IL-4 of the induc-

tion of LAK cell activity (defined a priori as an activity against fresh tumor cells). Interestingly, IL-4 also inhibited the augmentation of NK cell activity by IL-2 as measured against K-562, but did not affect the constitutive NK cell activity after an incubation of PBL for 48 h.

The finding that depletion of mature T cells from PBL does not affect the inhibition by IL-4 of IL-2-mediated induction of LAK cells excludes the possibility that IL-4 induces the production of inhibitory factors by mature T cells. Our data, rather, suggest that IL-4 inhibits the induction of LAK cell activity directly at the level of the precursor cell. It can presently not be excluded that the effect of IL-4 is an indirect one, namely, inducing the production of inhibitory factors by the LAK precursor itself or by a cell type that is neither a T cell nor a LAK precursor, but which co-purified with the LAK precursor cells. It is clear from the data presented in Table III that pre-incubation of PBL in IL-4 for 24 h does not inactivate the cytotoxic precursor cells, inasmuch as PBL pre-incubated in IL-4, washed, and subsequently incubated in IL-2 develop activated killer cell activity to an extent similar to the PBL pre-incubated in medium for 24 h.

Once the effector cells mediating LAK cell activity are generated, IL-4 does not affect the cytolytic activity. In the first place, it was found that IL-4, added 24 h after addition of IL-2, does not affect the subsequent development of LAK cell activity. Secondly, incubation in IL-4 of cloned lines of killer cells that had the phenotypic characteristics of LAK cells (5, 6) does not alter the cytotoxic activity measured against the LAK cell-sensitive target cell Daudi. Our findings suggest that IL-4 interferes with the IL-2-mediated inductive signal. It should be noted here that the inhibition of induction of LAK cell activity contrasts with the effect of IL-4 on the IL-2-mediated growth promotion of T cells. IL-4 can either act in an additive or in a synergistic way with IL-2 in the promotion of T cell growth and does not inhibit the proliferation of T cells induced by IL-2 (17). Therefore, the inhibitory effect of IL-4 on the induction of LAK cell activity indicates that the mechanism of IL-2-induced growth promotion is different from the IL-2-induced activation of killer cell activity. This latter notion is supported by findings reported previously that antibodies against the 55-kDa Tac Ag, although inhibiting T cell growth promotion by IL-2, do not affect the induction of LAK cell activity mediated by IL-2 (10, 11).

Recently, the existence of a second IL-2-binding protein that binds IL-2 with an intermediate affinity has been reported (12–15). A complex of this 73-kDa receptor (referred to as p70) with the Tac protein has a high affinity for IL-2, whereas the Tac protein itself has a low affinity. It has been shown that fresh NK cells express the p70 protein (12–14). Interestingly, Siegel et al. (14) recently reported evidence suggesting that the p70 protein mediates the initial phase of the induction of LAK cells. Activation of LAK cell precursors via this protein induces the formation of high affinity receptors that are involved in proliferation and a further augmentation of LAK cell activity (14). If this scheme is correct, IL-4 could interfere with signal transduction via the p70 protein. We speculate that once the high affinity receptor is expressed, IL-4 is unable to influence LAK cell activity. This idea would explain why IL-4 does not block the generation of Ag-specific cytotoxicity, inasmuch as in that situation the high affinity receptor appears on the cell membrane after the interaction of Ag with the TCR/CD3 complex. We have observed that the expression of Tac on T cell cultures stimulated by mitogens or alloantigens is not affected by IL-4. Moreover, IL-4 has no inhibitory or stimulatory effects on Tac expression by T cell lines (H. Yssel and H. Spits unpublished observation), supporting the notion that IL-4 does not affect the induction of Tac by occupancy of the TCR/CD3 complex. Thus, IL-4 could prevent the transition of $p70^+$, Tac^- cells to $p70^+$, Tac^+ cells as a consequence of activation by IL-2, but not after activation via the TCR/CD3 complex. This hypothesis would predict that IL-4 prevents the IL-2-mediated appearance of Tac. After 3 days, however, Tac expression on IL-2-activated cells is not significantly higher than on IL-4-plus-IL-2-activated cells. In preliminary experiments however, we have found that, after 7 days, Tac expression is indeed higher on IL-2 than on IL-4-plus-IL-2-activated cells (results not shown). This finding suggests that the kinetics of Tac appearance as a consequence of IL-2 activation is retarded, compared with the induction of LAK cell activity. This point is currently under investigation.

We have found that addition of IL-4 to MLC resulted in strong inhibition of cytotoxic activity against K-562 and MEWO cells generated in MLC. These findings, along with the observation that IL-4 inhibits the IL-2-induced LAK cell activity, suggest that non-specific cytotoxic activity in MLC is induced by IL-2 produced by the responding T cell blasts. In contrast, specific cytotoxic activity, generated in a MLC in the presence of IL-4, was increased compared with the specific cytotoxicity generated in the presence of IL-2 or in medium only. These results demonstrate that IL-4 is not an inhibitor of the induction of cellular cytotoxicity in general, but that this ability is restricted to the induction of LAK cell activity. Presently, the mechanism of the enhancement of specific CTL activity by IL-4 remains to be determined. MLC carried out in the presence of IL-4 contain 10 to 20% more $CD8^+$ cells than the MLC in medium only, but it is unclear whether this is the reason for the augmented CTL activity. In the secondary MLC, IL-2 and IL-4 clearly augmented the number of responding cells and enhanced the CTL activity per culture. Moreover IL-4 also stimulated slightly the CTL activity per cell in the secondary MLC. Because IL-2 and other lymphokines are produced in MLC, the exact role of IL-4 in the generation of CTL activity is difficult to assess. It is, for example, possible that IL-4 mediates its augmenting effect on CTL activity in combination with other lymphokines, such as IL-2. It is therefore premature to conclude from our present data that IL-4 is a major helper factor in the generation of Ag-specific CTL.

Presently we are performing a clonal analysis of CTL clones isolated in IL-4 compared with those isolated in IL-2 to determine the effects of these lymphokines on the level of a single cell. Preliminary results do not reveal significant differences in the clonal efficiencies and in the cytotoxic activities against the specific stimulator cells of the isolated CTL clones in IL-2 or IL-4 (X. Paliard and H. Spits unpublished observation). It is nevertheless noteworthy that our findings with bulk MLC cultures are in line with those of Widmer and Grabstein in a mouse model (29). These authors observed that IL-4 can enhance specific cytotoxic activity in mouse MLC. Thus, it seems

that mouse and human IL-4 share the ability to augment Ag-specific CTL activity. However, IL-4 has a different effect on the generation of LAK cell activity in the murine system than in the human. Mulé et al. (31) found that murine IL-4 induces LAK cell activity in spleen cells. Moreover, IL-4 enhanced the induction of LAK cell activity by sub-optimal doses of IL-2. The reason for the discrepancy between our results and those of Mulé et al. is unclear. Apart from the possibility that the ability of IL-4 to block the induction of LAK cell activity is species restricted, it might be possible that mouse spleen cells contain already activated precursor cells. As shown here, IL-4 does not inhibit the activity once the precursor LAK cells are activated. It would be interesting to see whether IL-4 also induces LAK cell activity in mouse PBL. We have found that IL-4 inhibits the IL-2-induced LAK cell activity in spleen cells, indicating that human spleen cells do not contain activated LAK cell precursors.

It is possible that the IL-4-mediated suppression of the generation of LAK cell activity is relevant in the regulation of Ig production, inasmuch as it has been shown that NK cells can suppress Ig synthesis in a non-specific way (32, 33). It is tempting to speculate that IL-4 production during T cell-B cell collaboration prevents unwanted suppression by activated killer cells induced by IL-2.

Note added in proof. After this manuscript was completed, we became aware that Widmer et al. (34) have reported findings similar to ours. The data reported here confirm and extend the findings of Widmer et al. (34).

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