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Expression of high-affinity IL-4 receptors on murine tumour infiltrating lymphocytes and their up-regulation by IL-2

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SUMMARY

Since interleukin-2 (IL-2) and IL-4 act in concert to support the development of cytotoxic T lymphocytes (CTL) and the generation of antigen-specific tumour infiltrating lymphocytes (TIL), we investigated the interaction of these cytokines with an established TIL line. TIL proliferated in an additive fashion in response to suboptimal concentrations of IL-2 and various concentrations of IL-4. TIL possessed high-affinity IL-4 receptors whether cultured in recombinant IL-2 (rIL-2) or rIL-4, but cells cultured in rIL-2 had higher numbers of IL-4 receptors than cells cultured in rIL-4. When TIL were cultured in increasing concentrations of rIL-2, a dose-dependent enhancement in IL-4 receptor number was observed. The maximum induction of IL-4 receptor expression was achieved by 4 hr of incubation with rIL-2 and was completely blocked by cycloheximide. Other cytokines, such as rIL-1, recombinant tumour necrosis factor (rTNF), recombinant interferon-alpha (rIFN-α) and rIFN-γ, had no effect on IL-4 receptor number. rIL-2 also up-regulated IL-4 receptors on CTLL-2, a murine CTL line. These data indicate that high-affinity IL-4 receptors exist on murine TIL and they can be up-regulated by IL-2. Our observation that IL-2 up-regulates IL-4 receptor may help explain the additive effects of these lymphokines on the proliferation of TIL and other cell lines. It may also help explain their co-operative effects on the generation of antigen-specific TIL and the differentiation of CTL.

INTRODUCTION

Lymphoid cells isolated from peripheral blood [e.g. interleukin-2 (IL-2)-activated lymphokine activated killer (LAK) cells] or from autologous tumour cells [such as tumour infiltrating lymphocytes (TIL)] can be expanded *in vitro* using IL-2. The adoptive transfer of such cells in immunotherapeutic regimens in combination with multiple injections of IL-2 has induced a significant regression of metastatic tumours in a variety of murine models (Rosenberg, 1986; Rosenberg, Spiess & Lafreniere, 1986, Rosenberg *et al.*, 1987) as well as in some human tumours (Rosenberg, 1986; Rosenberg *et al.*, 1988). Although many tumours contain activated lymphocytes (Galili *et al.*, 1979; Hutchinson *et al.*, 1981; Vose *et al.*, 1981; Ferrini *et al.*, 1985), there has been mixed success (Itoh, Platsoucas & Bach, 1988; Muul *et al.*, 1987) in obtaining and expanding antigen-specific TIL that can be utilized in the adoptive immunotherapy of human cancer.

Abbreviations: CTL, cytotoxic T lymphocytes; HBSS, Hanks' balanced salt solution; IL-2, interleukin-2; IL-4, interleukin-4; IL-4R, interleukin-4 receptor; IU, international units; TIL, tumour infiltrating lymphocytes.

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It is well-known that IL-2 has a central role in the development of cytotoxic T cells. We have previously shown that IL-4 can provide proliferative, differentiative and suppressive signals in cell-mediated immune responses (Horohov *et al.*, 1988). IL-4 also can selectively enhance the growth of IL-2-induced TIL, which are specifically cytotoxic to autologous melanoma cells (Kawakami, Lotze & Rosenberg, 1989). The mechanisms of the co-operative effects of IL-4 with IL-2 are not clear. Data are presently available suggesting that IL-4 can down-regulate high-affinity IL-2 receptors (IL-2R) in T- and B-cell lines (Fernandez-Botran, Sanders & Vitetta, 1989); however, the effect of IL-2 on IL-4 receptor expression is unknown. In this study we demonstrate that murine TIL possess high-affinity IL-4 receptors and that their expression can be up-regulated by IL-2.

MATERIALS AND METHODS

Recombinant cytokines

Human recombinant IL-2 (rIL-2) and human recombinant tumour necrosis factor-alpha (TNF-α) were kindly provided by Cetus Corporation (Emeryville, CA). The specific activity of rIL-2 was stated to be 3×10^6 Cetus units or $1{\cdot}8 \times 10^7$ International units (IU)/mg and of rTNF-α to be $2{\cdot}2 \times 10^7$ U/mg protein.

Murine recombinant IL-4 (rIL-4) was kindly supplied by Immunex Corporation (Seattle, WA) and had a specific activity

10^8 U/mg protein. Recombinant murine interferon-gamma (rIFN-γ) was kindly provided by Genentech Inc. (San Francisco, CA) and had a specific activity of $0{\cdot}9 \times 10^7$ U/mg protein.

Recombinant human/murine hybrid interferon-alpha A/D (rIFN-αA/D) and human interleukin-1 alpha (IL-1α) were kindly provided by Hoffmann-La Roche Inc. (Nutley, NJ). The specific activity of rIFN-A/D was in the range of $6\text{–}10 \times 10^4$ U/mg protein. The specific activity of rIL-1α was $2{\cdot}5 \times 10^9$ U/mg protein.

Generation of murine TIL

TIL were generated from subcutaneous MC-38 tumour from C57BL/6 mice as described previously (Spiess, Yang & Rosenberg, 1987), with slight modifications. Briefly, tumours were digested with triple enzymes (DNase, collagenase and hyaluronidase; Sigma Chemical Co., St Louis, MO) and the resulting tumour cell suspension was exposed to magnetic dyna beads (Dynal Inc., Great Neck, NY) pretagged with anti-Thy-1·2 monoclonal antibody (mAb). Beads with bound Thy-1·2$^+$ T lymphocytes were removed from the tumour mixture using a magnet, washed, and cultured with 5000 IU/ml IL-2 in a 24-well plate for 24–48 hr in a 37° incubator. This resulted in detachment of cells from the magnetic beads. Cells were then harvested, and the beads were removed by magnetic fields. The cells, composed mainly of lymphocytes, were subsequently cultured in medium supplemented with IL-2 (5000 IU/ml). Cultures were supplemented with gamma-irradiated (3000 rads) splenocytes as feeder cells and were split every 3–5 days. TIL cultures were stimulated with pre-irradiated (3000 rads) autologous tumour cells every 3–4 weeks. The surface phenotype of TIL and their *in vivo* therapeutic activity against MC-38 tumour implanted in the lungs of syngeneic mice were tested at various time-points of the TIL culture (R. K. Puri, manuscript in preparation). TIL were found to be Lyt-2$^+$, Thy-1·2$^+$ and L3T4$^-$ and were able to cause significant regression of the MC-38 tumours in mice when co-administered with multiple doses of IL-2.

Radiolabelling of rIL-4

Recombinant murine IL-4 was labelled with ^{125}I by the technique described for murine recombinant granulocyte–macrophage colony-stimulating factor (GM-CSF) (Park *et al.*, 1987). The specific activity of radiolabelled IL-4 was estimated to be $3\text{–}8 \times 10^{10}$ c.p.m./mg based on a molecular weight (MW) of 17,000 (based on SDS–PAGE of the recombinant material). The bioactivity of IL-4 was not altered by the iodinization procedure, as determined by the proliferative response of CT.4S (a kind gift of Dr William Paul, NIH) cell line in response to ^{125}I-rIL-4 (data not shown).

Binding of ^{125}I-rIL-4 to TIL

The binding of ^{125}I-rIL-4 was measured according to the method previously described for IFN-γ (Finbloom *et al.*, 1985) and IL-4 (Park *et al.*, 1987). Briefly, aliquots of cells ($2{\cdot}5 \times 10^6$) were incubated in the presence of ^{125}I-rIL-4 for 2 hr at 4°. Duplicate samples were then centrifuged through a cushion of phthalate oils to separate cell-bound ^{125}I-IL-4 from non-bound (free) ^{125}I-IL-4. Non-specific binding was determined in the presence of 100–200-fold molar excess of unlabelled rIL-4. The affinity and number of molecules bound per cell were calculated by Scatchard analysis (Scatchard, 1949) of the equilibrium binding data.

For cells that had been cultured in the presence of unlabelled IL-4, one of two approaches to the removal of IL-4 was employed prior to the radioiodine binding assay. In some experiments cells which were cultured in rIL-4 were washed two times with Hanks' balanced salt solution (HBSS), incubated in complete media for 1 hr at 37° without any lymphokine addition, and then washed twice again. Preliminary experiments indicated that this procedure removed greater than 95% of bound ^{125}I-IL-4 without affecting the IL-4 receptor binding ability. In other experiments, cells were treated with 1·0 ml of cold 0·025 M glycine-HCl buffer (pH 2·7) with 0·15 M NaCl for 1–2 min to strip bound IL-4 from its receptor. This technique also removed greater than 95% of ^{125}I-rIL-4 bound to the cell at 4° without affecting the IL-4 receptor binding ability.

RESULTS

Effects of IL-2 and IL-4 on TIL proliferation

The proliferative responses of TIL to IL-2 and IL-4 alone and in combination were assessed by measuring [^{3}H]thymidine uptake (Table 1). TIL proliferated in response to IL-2 in a dose-dependent manner and maximum proliferation occurred at 500 IU/ml rIL-2. Proliferation of TIL in response to rIL-4 plateaued at 1000 U/ml rIL-4 (higher concentrations were tested but not shown). However, when TIL were cultured in mixtures of a suboptimal concentration of IL-2 (5–50 IU/ml) plus IL-4 (10–1000 U/ml), thymidine uptake exceeded the sum of that which resulted from either lymphokine alone. At higher concentrations of rIL-2, the combined effect of rIL-4 was not observed.

Expression of IL-4 receptors on TIL

Since TIL responded to rIL-4, we next examined the expression of IL-4R on these cells by radioligand binding assays. TIL were incubated in rIL-2, rIL-4 or both and the number of IL-4 molecules bound/cell and the affinity of the receptor for IL-4 were determined. The equilibrium binding data for a typical TIL preparation are shown in Fig. 1a. The data represent specific binding, non-specific binding being $< 10\%$ of the total radioactivity bound to the cell. Specific binding of IL-4 increased with concentrations of ^{125}I-rIL-4 up to 500 pM, at which point saturation of binding occurred. Scatchard plot-analysis of these data (Fig. 1b) was consistent with a single class of high-affinity IL-4 receptors in cells treated with rIL-2, rIL-4 or both. TIL expressed high-affinity IL-4 receptors whether cultured in rIL-2 alone ($K_d = 70$ pM), rIL-4 alone ($K_d = 38$ pM) or both ($K_d = 68$ pM). The affinity of rIL-4 to its receptors from all three groups of cells did not seem to differ from each other.

Table 2 shows a summary of four experiments demonstrating the expression of higher numbers of IL-4 receptors in the presence of rIL-2. In Exps 1 and 2, TIL were washed thoroughly and cultured with either rIL-2, rIL-4 or both lymphokines for 1–2 days. They were then stripped of surface IL-4 and the IL-4 binding assay was performed. The number of IL-4 molecules bound/cell was more than two times higher in rIL-2-treated cells than in rIL-4-treated cells. Receptor numbers in rIL-2 plus rIL-4-treated cells were similar to those in the rIL-2-treated cells. Thus it appears that IL-2 is able to maintain a high number of IL-4 receptors on TIL, while IL-4 in the presence of IL-2 has little additional effect.

Table 1. Proliferation of TIL in response to IL-2 and IL-4*

		[^{3}H]Thymidine uptake (c.p.m./culture ± SEM)			
	IL-2	IL-4 (U/ml)			
Exp. no.	(IU/ml)	0	10	100	1000
1	0	167±24	433±90	840±83	8109±949
	5	1392±145	2203±179	6484±660	13,075±283
	50	5200±272	7233±355	8253±585	15,341±652
	500	22,042±3449	18,625±2799	13,966±2908	19,181±2443
2	0	967±187	1521±104	5866±414	17,703±1073
	50	12,870±646	12,744±1679	23,797±882	50,534±6291
	500	148,286±5515	152,572±2148	130,492±7036	133,314±4363
	5000	181,077±4165	172,242±4338	140,166±7574	131,090±13654

* TIL were cultured at a density of 10^5/well (5×10^4/well in Exp. 1) in 0·2 ml of complete media containing varying amounts of lymphokines. On Day 3 of culture cells were pulsed with [3H]thymidine (0·5 μCi/well) and harvested after 16 hr.

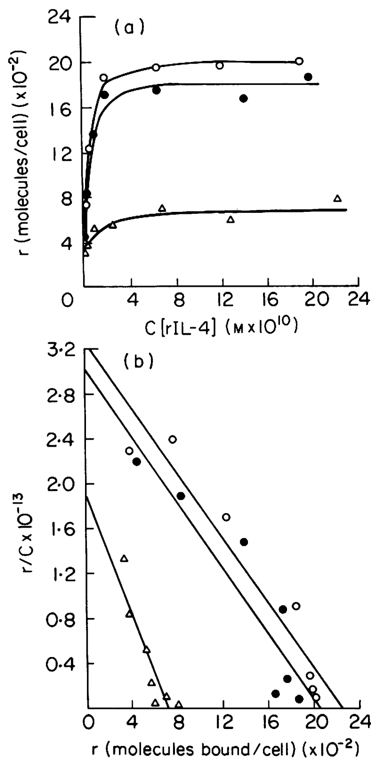

Figure 1. (a) Equilibrium binding of ^{125}I-rIL-4 to TIL. Cells were extensively washed and cultured overnight with rIL-2(●), rIL-4(△) or both(○). TIL were then washed, incubated for 1 hr and washed again to remove free and surface-bound IL-4. Cells ($2{\cdot}5 \times 10^6$) were then incubated with ^{125}I-rIL-4 in increasing concentrations with or without 100–200-fold excess of unlabelled rIL-4. Receptor-bound ligand was separated from free ligand by centrifugation of 50-μl aliquot of cells through a phthalate oils mixture. The tips of the tubes containing the cell pellets were then cut off (bound) and remaining supernatants (free) counted. The specific binding of ^{125}I-rIL-4 is depicted. (b) Scatchard analysis of the binding data shown in (a). The K_d = 70 pM, 38 pM and 68 pM in cells cultured in rIL-2 (●), rIL-4 (△) or both (○), respectively, was calculated from the slopes.

Table 2. Removal of TIL from IL-2 results in reduced IL-4 receptor expression

	IL-4 molecules bound/cell			
	Cells precultured in IL-2*		Cells precultured in IL-4†	
Lymphokine treatment	Exp.-1	Exp.-2	Exp.-3	Exp.-4
None	—‡	—	446	—
rIL-4	771	720	713	577
rIL-2	1924	2040	1617	1216
rIL-2+rIL-4	1512	2240	—	1761

* TIL grown in IL-2 were washed three times and incubated with either 5000 IU/ml rIL-2 or 1000 U/ml rIL-4 or both for 2 days (Exp. 1) or 1 day (Exp. 2) before the IL-4 binding assay was performed using various concentrations of ^{125}I-rIL-4. In Exp. 1 (Fig. 1) and Exp. 2, IL-4 molecules bound/cell were determined from Scatchard plots. In Exps 3 and 4, saturating concentrations of (300–600 pM) ^{125}I-IL-4 were used in binding assays.

† TIL grown in rIL-2 were washed three times with HBSS and cultured with 500–1000 U/ml rIL-4 for 24 hr. Cells were then washed two times, incubated in complete medium for 1 hr at 37° and washed two additional times. Cells were then incubated with either rIL-2 (500 IU/ml in Exp. 3 and 5000 IU/ml in Exp. 4), rIL-4 (500 U/ml in Exp. 3 and 1000 U/ml in Exp. 4), neither or both lymphokines for an additional 24 hr before IL-4 binding assay was performed. Cells were then washed, incubated in medium for 1 hr, and washed to remove free and bound IL-4 prior to measuring ^{125}I-rIL-4 binding using a saturating concentration.

‡ Denotes not done.

In Exps 3 and 4 of Table 2, TIL were precultured in rIL-4 but not IL-2 for 24 hr at 37°. Cells were then washed to remove free and bound IL-4 and cultured for an additional 24 hr in the presence of rIL-4, rIL-2 or neither. They were then stripped of surface IL-4 before IL-4 binding was assayed. Again, the presence of rIL-2 during the final 24 hr incubation increased the

Table 3. Up-regulation of IL-4 receptor by rIL-2 on murine TIL. Effect of variation of rIL-2 concentration

rIL-2 (IU/ml)	IL-4 molecules bound/cell*		
	Exp. 1	Exp. 2	Exp. 3
25	542	—†	—
50	539	—	348
250	—	1283	—
500	1232	1202	1028
5000	—	1260	830

*TIL grown in rIL-2 were washed extensively and then were cultured with various concentrations of rIL-2 for an additional 3–5 days before the cells were harvested and an IL-4 receptor binding assay was performed as described in Table 2.

† Denotes note done.

IL-4 binding to its receptor by more than threefold. In another experiment, after culture with IL-2, IL-4 or both, TIL were either washed, incubated and washed again, or stripped with glycine buffer before the ^{125}I-IL-4 binding assay was performed. The data show that by either technique comparable numbers of IL-4 receptors were observed (data not shown).

Effect of IL-2 concentration on IL-4R expression

The presence of higher numbers of IL-4R on TIL cultured in rIL-2 than on cells cultured in rIL-4 prompted us to investigate the effect of various concentrations of rIL-2 on IL-4 receptor expression. TIL were maintained in 500 IU rIL-2/ml and were washed prior to culture in various concentrations of rIL-2 for 3–5 days. As observed in the experiments summarized in Table 3, receptor numbers were greater on TIL cultured in 500 IU/ml or higher concentrations of rIL-2 than on TIL cultured in 50 IU/ml rIL-2 or lower.

Kinetics of up-regulation of IL-4R

To determine the time-course of IL-2 up-regulation of IL-4 receptor, we cultured TIL for 24 hr with 1000 U/ml rIL-4 and no IL-2 to allow decreased expression of IL-4R. Cells were then washed free of bound rIL-4 and cultured at 37° with 5000 IU/ml rIL-2 for various periods of time. By 4 hr of culture in rIL-2, IL-4R expression had increased to a level comparable to that on cells continuously cultured in rIL-2, and the receptor expression did not change over the following 20 hr (data not shown). Having demonstrated that IL-2 up-regulated IL-4R on TIL, we next examined whether IL-2 up-regulation required receptor protein synthesis. Extensively washed TIL were preincubated at 37° with rIL-4 (1000 U/ml) for 20–24 hr and then washed free of cell-surface bound IL-4. TIL were then incubated at 37° with 5000 IU/ml of IL-2 with or without 10 μg/ml cycloheximide (Sigma Chemical Co.) to block new protein synthesis (Fig. 2). TIL incubated with IL-2 alone began to express higher numbers of IL-4R after 1 hr incubation, reaching a maximum level at 4 hr which did not alter over 24 hr. Cycloheximide blocked the up-regulation of IL-4 receptors. Incubation of cells in cycloheximide did not affect the viability of TIL, as measured by trypan

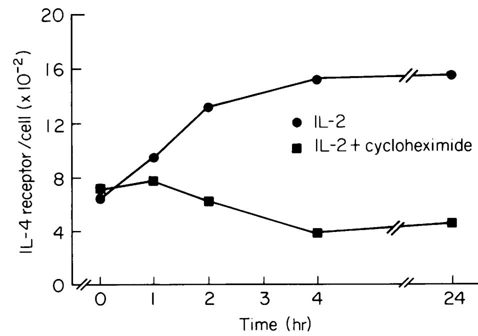

Figure 2. Up-regulation of IL-4 receptor by IL-2. TIL were preincubated at 37° for 24 hr with 1000 U/ml rIL-4 and then extensively washed (as described in the Materials and Methods) to remove free and surface-bound IL-4. Cells were cultured with 5000 IU/ml rIL-2 in the presence (■) or absence (●) of cycloheximide (10 μg/ml). At indicated time-points, cells were harvested and ^{125}I-rIL-4 binding to receptor was determined as described in the Materials and Methods. Three-hundred to 600 pM ^{125}I-rIL-4 were used for the binding assay and IL-4 molecules bound/cell from one typical experiment is presented.

blue dye exclusion. These results indicate that the observed increase in the number of IL-4R per cell induced by IL-2 was probably due to receptor biosynthesis.

Effect of other cytokines on the expression of IL-4R on TIL

We also evaluated the effect of other cytokines on IL-4R expression on TIL. Cells were cultured for 24 hr in rIL-4 (1000 U/ml), washed extensively and incubated at 37° in complete medium to remove free and surface-bound IL-4. Cells were then cultured with rIL-2 (5000 U/ml), rIL-4 (1000 U/ml), rIFN-α (5000 U/ml), rIFN-γ (10,000 U/ml) or these cytokines plus rIL-2 (5000 IU/ml) for an additional 24 hr period. In another experiment, after incubation with rIL-4, cells were washed free of bound IL-4 and then cultured in rIL-2 (5000 IU/ml), rIL-1α (100,000 U/ml), rTNF-α (400 ng/ml) or no cytokine for 4 hr at 37°. Cells were again extensively washed and IL-4 binding was measured. IL-2 was able to induce a high number of IL-4R. With rIFN-α alone or rIFN-γ alone, no viable TIL were obtained, but these cytokines had no additive effects on IL-4R when used together with rIL-2. rIL-1α or rTNF-α had no augmenting or suppressive effects on IL-4R number (data not shown).

IL-4 receptors on CTLL-2 and their modulation by IL-2

We next examined whether IL-2 regulation of IL-4R was observed on cells other than TIL. Cells of the murine cytotoxic T-lymphocyte line, CTLL-2 (American Tissue Culture Collection, Rockville, MD), were washed free of the IL-2 usually present in their cultures and cultured with rIL-4 (1000 U/ml) for 24 hr at 37°. Cells were then extensively washed and incubated at 37° with rIL-2 (5000 IU/ml) for an additional 4 hr period. Table 4 shows that within 4 hr IL-2 was able to increase IL-4 binding from 379 molecules/cell to 1265 molecules per cell. TIL treated in a similar manner also showed an increase of IL-4 receptor. In another experiment CTLL-2 cells were grown in various concentrations of IL-2 for 3 days and IL-4 receptor assay was performed. On these cells, we observed an IL-2 dose-

Table 4. Up-regulation of IL-4 receptor on CTLL-2 by IL-2*

Time incubation with rIL-2 (min)	IL-4 molecules bound/cell	
	CTLL-2	TIL
0	379	270
240	1265	1041

*CTLL-2 and TIL grown in IL-2 were washed extensively and cultured overnight with rIL-4 (1000 U/ml). Cells were washed, incubated and washed and cultured again with 5000 U/ml rIL-2 for the time indicated. IL-4 binding was assayed as described in the Materials and Methods.

dependent increase in IL-4R similar to that previously observed on the TIL line (data not shown).

DISCUSSION

Since its discovery (Howard *et al.*, 1982), IL-4 has been shown to participate with other cytokines in the regulation of a diverse array of biological activities in many cell types; B cells, T cells, mast cells, monocytes and other cells (reviewed by Ohara, 1989). In accordance with the diverse functions of IL-4, receptors for this cytokine have been identified on a wide variety of cell lineages (Park *et al.*, 1987; Lowenthal *et al.*, 1988). Some of the reported interactions between IL-4 and other cytokines may be explained in part by the modulation of IL-4R expression by other cytokines.

In this report we have investigated the presence of IL-4 receptors on TIL and the regulation of IL-4 receptor expression by cytokines. We demonstrated that TIL could proliferate in response to either IL-2 or IL-4 and exhibited an additive proliferative response in the presence of suboptimal concentrations of both lymphokines. TIL were found to possess high-affinity receptors for IL-4. Culture of TIL and of a CTL line in high concentrations of IL-2 was shown to up-regulate IL-4 receptor expression. The expression of higher numbers of IL-4R on cells grown in rIL-2 than cells grown in rIL-4 could result from either up-regulation by IL-2 or down-regulation by IL-4. Since the TIL required rIL-2 or rIL-4 to maintain viability, control cells receiving neither lymphokine are not of great value in distinguishing between these possibilities. In this study we have demonstrated two different ways by which up-regulation of IL-4R is directly attributable to rIL-2. First, when cells were maintained in rIL-4 throughout, the addition of rIL-2 up-regulated IL-4R levels. Second, in the absence of IL-4, cells cultured in high concentrations of rIL-2 expressed higher IL-4R levels than those cultured in lower concentrations of rIL-2. To our knowledge, this is the first report of regulation of IL-4 receptor expression by IL-2. Cycloheximide blocked the up-regulation of IL-4 receptors, suggesting that new protein synthesis was required for receptor up-regulation. Incubation of TIL with other cytokines such as IFN-α, IFN-γ, IL-1α, and TNF-α did not show any effect on the number of IL-4R.

The up-regulation of IL-4 receptors by IL-2 is a mechanism which may help explain the co-operative effects of these lymphokines on TIL as well as other T-cell lines (Fernandez-Botran *et al.*, 1988). In addition to the combined effects on TIL proliferation reported herein, Kawakami *et al.* (1989) have shown that co-culture of melanoma-derived TIL with IL-2 and IL-4 from the onset selectively enhances the development of TIL specifically cytotoxic to autologous melanoma cells. Our laboratory has shown that the sequential addition of IL-2 and IL-4 to influenza virus-infected lymphocyte cultures results in the optimal development of CTL (Horohov *et al.*, 1988). Others have reported synergistic effects of IL-2 and IL-4 on the proliferation of T-helper cells (Th) of both Th1 and Th2 subtypes (Fernandez-Botran *et al.*, 1988). Further studies will be necessary to determine in which, if any, of these systems the up-regulation of IL-4 receptors by IL-2 is of physiological significance.

On resting T and B lymphocytes, relatively low numbers of IL-4 receptor have been reported (Park *et al.*, 1987; Ohara & Paul, 1988). Upon activation of B cells by lipopolysaccharide or anti-IgM and of resting T cells with concanavalin A, a stimulus which leads to IL-2 secretion, their expression of IL-4 receptors and their responsiveness to IL-4 increases (Park *et al.*, 1987; Ohara & Paul, 1988). Up-regulation of IL-4R by IL-4 has also been reported on resting T lymphocytes (Ohara & Paul, 1988; Foxwell, Woerly & Ryffel, 1989). In Ohara & Paul's (1988) study, IL-2 was not able to increase IL-4R on resting T lymphocytes. This result is at variance with our finding that IL-2 up-regulates IL-4 receptors. Two key differences in the experimental design may account for this discrepancy. First, the T cells used in our studies were activated and cultured continuously in the presence of IL-2, in contrast with the resting T cells studied by Ohara & Paul (1988). Secondly, in their study the amount of IL-2 used to treat resting T cells was very small (60 IU/ml or 10 Cetus units/ml) in comparison to the amount we found to be optimal for up-regulation of IL-4 receptors. We observed an IL-2 dose-dependent increase in IL-4 receptor with optimal effects only observed at concentrations in excess of 250 IU/ml.

Our observations regarding the responses of TIL to various concentrations of IL-2 have interesting implications regarding IL-2 receptor function. The TIL we studied, as activated lymphocytes, would be expected to express high-affinity IL-2 receptors (K_d approximately 10 pM). This is evidenced by their ability to be maintained and expanded in IL-2 at concentrations as low as 5 IU/ml (20 pM). Since their high-affinity IL-2 receptors would be essentially saturated at an IL-2 concentration of 50 IU/ml (200 pM), our observation of greater proliferation and IL-4R expression in the presence of 2nM IL-2 than in the presence of 200 pM suggests that these phenomena may be mediated by low or intermediate affinity IL-2 receptors. Although we and others have shown that an intermediate affinity IL-2 receptor can mediate signals for LAK, natural killer and proliferative activities on resting lymphocytes (Siegel *et al.*, 1987), it has yet to be demonstrated that cells expressing high-affinity IL-2 receptors receive signals from IL-2 other than through these receptors. The antibody against intermediate-affinity IL-2 receptor (IL-2R beta chain) may prove useful in further investigations of the role of intermediate-affinity IL-2 receptor in the up-regulation of the IL-4 receptor, but is not yet available. The nature of the IL-2 receptor which mediates IL-2-dependent IL-4 up-regulation is under study.

In conclusion, this study demonstrated that IL-2 can regulate IL-4 receptor expression. Fernandez-Botran *et al.* (1988) have shown that IL-4 can regulate IL-2R expression. The impact of IL-2 on the expression of IL-4 receptor and vice versa

may help explain synergistic signals of these cytokines to lymphocytes. Further understanding of the mechanisms, kinetics and implications of this receptor interaction may enhance our capability to modulate the expansion and activation of antigen-specific lymphocytes *in vitro* or *in vivo* and thus may lead to improved immunotherapy for cancer and other diseases.

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