INTERLEUKIN 4 PROMOTES THE GROWTH OF TUMOR-INFILTRATING LYMPHOCYTES CYTOTOXIC FOR HUMAN AUTOLOGOUS MELANOMA

By YUTAKA KAWAKAMI, STEVEN A. ROSENBERG, AND MICHAEL T. LOTZE

From the Surgery Branch, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

Adoptive immunotherapy of patients with cancer using lymphoid cells expanded from peripheral blood or directly from the autologous tumor has been extensively investigated in our laboratory (1-6). Preliminary studies suggested the possible efficacy of expanded tumor-infiltrating lymphocytes (TIL)[1] in the treatment of patients with melanoma, in whom the existence of CTL specific for autologous tumors (2-5) and the accumulation of transferred lymphocytes at the tumor site (6) have recently been shown.

IL-4 is a species-specific pleiotropic lymphokine (7, 8) acting on various cell types, including T cells, B cells, NK cells, and monocytes. High-affinity receptors for IL-4 have been detected on both hematopoietic and nonhematopoietic cells (7, 8). Human IL-4 stimulates proliferation of T cells from peripheral blood and thymus with or without other stimuli (9, 10). Preactivated T cell growth (9) and allo-specific CTL generation (11, 12) are augmented by IL-4. It appears, however, that human IL-4 inhibits IL-2-induced lymphokine-activated killer (LAK) activity from unstimulated cells (10-12). We demonstrate here that human IL-4 in conjunction with IL-2 augments the growth of TIL apparently specific for autologous melanoma, and reciprocally inhibits nonspecific killer activity.

Materials and Methods

Lymphokines, Antibodies, and Phenotypic Analysis. Human rIL-2 was kindly supplied by Cetus Corp., Emeryville, CA. Human rIL-4 (10^8 U/mg in a tonsilar B cell growth assay with anti-μ antibody) was generously provided by Dr. Steven Gillis (Immunex Corp., Seattle, WA). FITC-conjugated anti-CD3 (Leu-4), CD4 (Leu-3), and CD8 (Leu-2) antibodies and phycoerythrin (PE)-conjugated anti-NKH1 (Leu-19) antibody were purchased from Becton Dickinson & Co., Mountain View, CA. TIL were first incubated in heat-inactivated human AB serum to block nonspecific binding. After a wash, cells were incubated with FITC- or PE-conjugated mAbs for 1 h at 4°C. Cells were then washed and fixed with 1% paraformaldehyde for 10 min. Stained cells were analyzed using a FACS (model 440; Becton Dickinson & Co.) and percent positive cells were determined.

TIL Culture. Tumor-mononuclear cell suspensions were prepared from 11 metastatic melanomas resected from 10 patients as previously reported (13). Although vigorous attempts are

---

[1] *Abbreviations used in this paper:* LAK, lymphokine-activated killer; PE, phycoerythrin; TIL, tumor-infiltrating lymphocytes.

made to remove tissues that do not clearly contain tumors, some of the cells derived in these studies could be tumor- "associated" rather than tumor-infiltrating lymphocytes. Only patient 638 had previously received IL-2. Briefly, tumor specimens were minced into small pieces and incubated for 2–16 h in RPMI 1640 (Biofluids, Rockville, MD) containing 0.01% hyaluronidase type V, 0.002% DNAse type I, 0.1% collagenase type IV (Sigma Chemical Co., St. Louis, MO), 50 IU/ml penicillin, and 50 µg/ml streptomycin (Biofluids). Harvested cells were centrifuged on lymphocyte separation media (Organon Tecknika Corp., Durham, NC). The cells at the interface were collected, washed, resuspended in heat-inactivated human AB serum (Advanced Biotechnologies, Inc., Silver Spring, MD), and frozen in the vapor phase of a liquid N_2 freezer. For TIL expansion, a total of 6 ml of either fresh or thawed cell suspensions at a concentration of 10^6 cells/ml was cultured in 6-well plates (3506; Costar, Cambridge, MA) in complete medium. This consisted of RPMI 1640 supplemented with 10% heat-inactivated human AB serum, 2 mM L-glutamine (M. A. Bioproducts, Walkersville, MD), 50 IU penicillin, and 50 µg/ml streptomycin, with or without IL-2 (10 or 1,000 U/ml) or IL-4 (1,000 U/ml). During the initial culture, medium was changed one to three times by replacement of three-quaters of supernatant with new medium containing the appropriate concentration of lymphokines. During the subsequent maintenance culture, cells were harvested and adjusted to 2.5 × 10^5/ml in new media with lymphokines every 4–7 d. Irradiated (5,000 rad) autologous tumor cells were added at 5 × 10^4/ml (TIL/Tumor ratio, 5:1) in some experiments, as noted.

Cytotoxicity Assay. Sequentially harvested cells from TIL cultures were used as effectors. The B cell line, Daudi, as well as autologous fresh melanoma and two other fresh allogeneic melanomas were used as targets. A 4-h ^{51}Cr release assay was performed as previously noted (13). Briefly, targets were incubated with 200 µCi of $Na^{51}CrO_4$ for 1–1.5 h followed by washing and filtering through nylon mesh. Various concentrations of effector cells at E/T ratios of 0.6–40 were mixed with 5 × 10^3 target cells in 96-well round-bottomed microplates (3799; Costar) in triplicate, centrifuged for 5 min at 500 rpm, and then incubated for 4 h at 37°C in a 5% CO_2 incubator. Supernatants were collected using a Skatron-Titertek system (Skatron, Inc., Norway) and counted on a gamma counter. Maximal or minimal lysis were measured using supernatants from wells receiving 2% SDS or media alone, respectively. Minimal lysis was usually <30% of maximal release. Percent specific lysis was calculated as: percent specific lysis = 100× (experimental release − minimal release)/(maximum release − minimal release). Cytotoxicity is expressed as LU per 10^7 cells. 1 LU is defined as the number of effector cells mediating 20% specific lysis of 5 × 10^3 target cells, determined from the dose-response curve previously described (2, 14). Total lytic activity was calculated by multiplying LU/10^7 and fold expansion.

Statistical Analysis. Statistical analysis was performed using a two-tailed student's *t* test.

Results

Tumor-infiltrating Lymphocyte Expansion. Two phases of culture are noted. The first, or initiation phase, was the period during which lymphoid cells were first grown from the tumor suspension. The second, or maintenance phase, denotes the period during which these cells were further expanded in culture after all tumor cells had been eliminated. The total time in culture (both initiation and maintenance phases) are described as "overall culture." No lymphocytes expanded in cultures grown in media or IL-4 (1,000 U/ml) alone for up to 30 d. In many cases, tumor cells grew from these cultures. An experiment performed with tumors from patient 1 is shown in detail in Fig. 1. A major advantage in terms of expansion as well as lytic activity was observed. In the initial culture (6–29 d; median 18 d), IL-4 (1,000 U/ml) addition together with 10 or 1,000 U/ml IL-2 grew cells better than IL-2 alone in all (11:11) cases (Table I). Further, TIL recovery after culture with IL-2 (10 U/ml) and IL-4 was better than that with IL-2 (1,000 U/ml) alone in most cases (7:10). During the maintenance culture (7–29 d; median 17 d) IL-4 addition with IL-2 (10 U/ml)
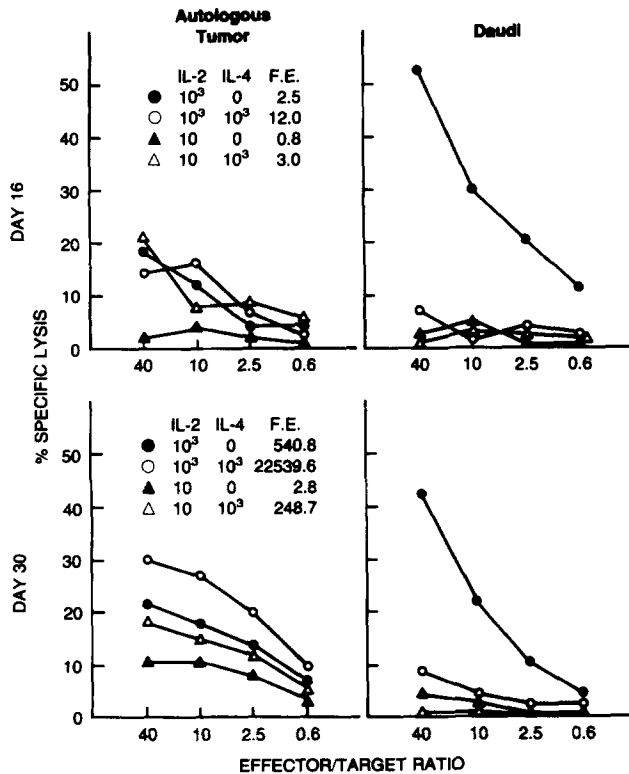
FIGURE 1. IL-4 decreases nonspecific killer activity, but increases growth of TIL capable of killing autologous melanomas. Cell expansion and percent specific lysis tested against autologous melanoma and Daudi at various E/T ratios of TIL grown from a patient with IL-2, IL-4 (U/ml) on day 16 and 30 are shown. *F. E.*, fold cell expansion.

grew TIL better than IL-2 (10 U/ml) alone, but less well than with IL-2 (1,000 U/ml) alone. In contrast to the initial culture, TIL recovery with IL-2 (1,000 U/ml) and IL-4 was less than that with IL-2 (1,000 U/ml) alone in some cases (4:10) (data not shown). Finally, over the entire culture period ("overall," 23–48 d; median 34 d), IL-4 plus IL-2 grew TIL better than IL-2 alone in most cases (8:10). TIL did not grow in three cases with IL-2 (10 U/ml) alone, in two cases with combinations of IL-2 (10 U/ml) and IL-4, and in one case with high levels of IL-2 (1,000 U/ml). TIL grew with IL-2 (1,000 U/ml) and IL-4 in all cases (11:11) (Table I).

Weekly addition of 5,000 rad irradiated autologous tumor cells led to better TIL expansion than cultures without tumor restimulation in most (4:5) cases (Table II). With low-dose IL-2 (10 U/ml) alone, tumor addition sometimes appeared to inhibit TIL growth. TILs maintained with IL-2 and IL-4 could subsequently grow when transferred into media containing IL-2 (10 U/ml) alone. The expansion under these conditions was less than that observed with IL-2 (10 U/ml) and IL-4 in all cases (data not shown).

Cytotoxic Activity and Specificity of TIL.   TIL grown with IL-2 (10 U/ml) and IL-4 and tested after the initial culture (Table III) did not have significant nonspecific LAK-like cytolytic activity against Daudi and two allogeneic melanoma targets in most cases (7:9). However, IL-4 with IL-2 (10 U/ml) usually augmented total lytic activity observed against autologous melanoma (5:7). IL-4 addition with IL-2 (1,000

TABLE I

IL-4 Augments Tumor-infiltrating Lymphocyte Growth with IL-2

Patient	Tumor site	Percent MNC	Days	Initial culture (fold expansion) 10 U IL-2		1,000 U IL-2		Days	Overall culture (fold expansion) 10 U IL-2		1,000 U IL-2	
				−IL-4	+IL-4	−IL-4	+IL-4		−IL-4	+IL-4	−IL-4	+IL-4
1. 560	Lung	20	16	0.8	3.0	2.5	12.0	30	2.8	248.7	540.8	22,539.6
2. 544B	Skin	20	16	1.6	7.3	2.5	14.0	30	23.8	223.4	246.5	1,197.0
3. 603	Lymph node	32	19	2.2	5.0	7.5	7.8	48	0.3	1,547.5	3,463.5	1,820.5
4. 551	Skin	21	16	3.2	7.1	3.3	12.1	23	3.2	18.5	6.3	36.3
5a. 553B	Mesenteric lymph node	21	25	0.0	5.0	3.0	14.3	44	0.0	248.5	427.8	194.5
5b. 553C	Skin	28	16	0.0	0.0	3.2	6.3	30	0.0	0.0	9.9	92.0
6. 634	Small bowel	5	29	0.0	0.0	0.0	18.5	40	0.0	0.0	0.0	29.6
7. 619	Skin	7	28	1.0	25.7	5.3	21.4	47	4.1	300.7	736.2	1,921.7
8. 648	Lung	34	26	3.1	7.0	5.3	12.9		ND	ND	ND	ND
9. 587	Pleural effusion	12	18	6.4	20.0	6.6	46.4	36	21.1	76.8	71.9	371.2
10. 638	Lymph node	40	6	3.3	3.8	4.5	5.3	32	63.7	82.1	14,054.4	24,795.5
		21.9 ± 11.0* (11)		2.0 ± 2.0 (11)	7.6 ± 8.0 (11)	4.0 ± 2.1 (11)	15.5 ± 11.3 (11)		11.9 ± 20.2 (10)	274.6 ± 461.6 (10)	1955.7 ± 4376.1 (10)	5299.8 ± 9721.7 (10)

10^6/ml of tumor-mononuclear cell suspension was cultured with or without IL-2 (10 or 10^3 U/ml) and/or IL-4 (10^3 U/ml) for a median period of 18 d (initial culture) and subsequently expanded for a median 34 d (overall culture). Fold expansion was calculated at each cutback. Shown in days are the number of days in culture for both initial and overall time for each tumor studied. The time in maintenance culture can be derived by subtracting the time in initial culture from the total time ("overall") in culture.

* Mean ± SD, number in parentheses is n.

TABLE II

The Addition of Irradiated Autologous Tumor Stimulates Growth of TIL

Tumor	Culture duration		10 U IL-2						1,000 U IL-2					
		Tumor	−IL-4			+IL-4			−IL-4			+IL-4		
			+TU	−TU	Ratio	+TU	−TU	Ratio	+TU	−TU	Ratio	+TU	−TU	Ratio
	d					*Fold expansion*								
3	29		0.1	53.1	(0.003)	309.5	43.9	(7.1)	461.8	1,374.9	(0.3)	233.4	779.3	(0.3)
5a	19		*	*	(*)	49.7	153.6	(0.3)	142.6	96.0	(1.5)	13.6	3.2	(4.3)
7	19		*	*	(*)	11.7	1.9	(6.1)	138.9	59.5	(2.3)	89.8	42.0	(2.1)
9	18		3.3	2.0	(1.7)	3.8	1.6	(2.3)	10.9	5.4	(2.0)	8.0	3.1	(2.6)
10a	26		19.3	22.5	(0.9)	21.6	2.3	(9.4)	3,123.2	2,802.7	(1.1)	4,678.4	608.2	(7.7)
	22.2 ± 5.0‡				(0.5 ± 0.8)			(5.0 ± 3.7)			(1.4 ± 0.8)			(3.4 ± 2.8)

2.5 × 10⁵/ml of TIL harvested from the initial culture was maintained under various conditions with or without 5 × 10⁴/ml of 5,000-rad irradiated autologous tumor (TU) for 18–29 d. Every 4–7 d, media containing lymphokines was completely changed and cells were adjusted to 2.5 × 10⁵/ml with or without 5 × 10⁴/ml of irradiated tumors. Data were shown as fold expansion with or without irradiated tumors.
* Cells did not grow.
‡ Mean ± SD.

TABLE III

Lytic Activity of TIL after the Initial Culture

Exp.	Autologous fresh melanoma				Daudi				Allogeneic fresh melanoma*			
	10 U/ml‡		10^3 U/ml		10 U/ml		10^3 U/ml		10 U/ml		10^3 U/ml	
	0 U/ml§	10^3 U/ml	0 U/ml	10^3 U/ml	0 U/ml	10^3 U/ml	0 U/ml	10^3 U/ml	0 U/ml	10^3 U/ml	0 U/ml	10^3 U/ml
1.	<1.0	54.0	37.0	34.5	<1.0	<1.0	869.6	<1.0	NT	NT	NT	NT
2.	10.0	5.7	40.0	454.5	5.0	<1.0	2,000.0	312.5	<1.0	<1.0	<1.0	<1.0
3.	11.8	14.3	87.0	83.3	10.0	11.8	2,105.3	2,000.0	<1.0	<1.0	<1.0	<1.0
4.	<1.0	<1.0	1.4	16.7	<1.0	<1.0	1,250.0	20.8	<1.0	<1.0	2.0	<1.0
5a.	NT	NT	<1.0	28.6	NT	NT	<1.0	<1.0	NT	<1.0	<1.0	<1.0
5b.	NT	NT	29.4	35.8	NT	NT	83.3	<1.0	NT	NT	<1.0	<1.0
6.	NT	NT	NT	<1.0	NT	NT	NT	<1.0	NT	NT	NT	<1.0
7.	NT	<1.0	11.8	1.5	<1.0	<1.0	71.4	<1.0	<1.0	<1.0	1.0	<1.0
8.	<1.0	6.9	31.2	26.3	<1.0	<1.0	153.8	1.1	<1.0	<1.0	<1.0	<1.0
9.	<1.0	38.5	51.3	32.3	<1.0	<1.0	1.0	<1.0	<1.0	<1.0	1.0	<1.0
10.	<1.0	<1.0	15.4	15.4	1.2	1.0	111.1	76.9	<1.0	<1.0	<1.0	<1.0

Cytotoxic activity against autologous melanoma. Daudi, or allogeneic melanoma were tested after the initial culture (median 18 d). Data were shown as LU/10^7 cells. NT: not tested because of insufficient cell recovery for cytotoxicity assay. LUs were defined from experiments conducted at four E/T ratios ranging from 40:1 to 0.6:1.
* Allogeneic melanoma were lysable by appropriate TIL.
‡ IL-2.
§ IL-4.

U/ml) decreased nonspecific cytolytic activity against Daudi or a number of LAK-sensitive allogeneic fresh melanomas (data not shown) compared with that in IL-2 alone in many cases (7:10 per cell and 6:10 on a per culture basis after the initial culture; 3:5 per cell and 2:4 on a per culture basis after overall culture [data not shown]). Reciprocally, IL-4 addition increased the total lytic activity against the autologous melanoma in most cases (6:10 after the initial culture and 5:7 after overall culture on a per culture basis [data not shown]).

Phenotype of Growing TIL. TIL grown IL-2 and/or IL-4 were mostly CD3$^+$ (>80%) T cells and either CD8$^+$ (9:11) or CD4$^+$ (2:11) predominant in most cases. In one instance, IL-4 addition grew mainly CD4$^+$ T cells, while CD3$^-$ Leu-19$^+$ NK-like cells grew with IL-2 (1,000 u/ml) alone (data not shown). TIL 648 (day 40) grown with IL-2 and IL-4 was primarily CD4$^+$ T cells. These cells proliferated best with an autologous tumor in the presence of 50 U/ml of IL-2, when measured by [^{3}H]thymidine incorporation (data not shown) after 4 d of incubation.

Discussion

In murine models the transfer of 10–100-fold fewer tumor-specific T cells than LAK cells is required for comparable antitumor effects in adoptive cellular immunotherapy experiments (15). Based on our murine studies, it is estimated that >2 × 10^{11} TIL would be required to effectively treat patients bearing metastatic cancer. Better methods to expand cells with specific antitumor activity are required. IL-4 does stimulate T cell proliferation after allostimulation or with the mitogens, PHA, Con A, or PMA (9, 10), and also augments the induction of allospecific CTL (11, 12). In contrast to murine studies (14), human IL-4 inhibits IL-2 induction of LAK activity from unstimulated lymphocytes of various lymphoid organs by direct inhibition of precursors to proliferate and differentiate into nonspecific killer cells and to express IL-2-R (Tac). This could, in turn, suppress further proliferation (10–12, Kawakami, Y., et al., submitted for publication). Since asialo-GM1$^+$ LAK-like cells seem to be responsible for the capillary leak syndrome in the mouse (16), it may be reasonable to use human IL-4 for the selective growth of tumor-specific T cells with IL-2 in vitro or in vivo. IL-4, together with IL-2, augmented T cell growth and cytolysis against autologous melanomas but decreased nonspecific cytolysis against Daudi or allogeneic melanomas. Some difference was observed from the previously reported allospecific CTL, in that IL-4 alone can augment CTL induction but simultaneous addition with IL-2 rather inhibits CTL generation as well as nonspecific killer cells, obscuring in part the specific response (11). IL-4 may augment TIL growth by directly acting on T cells as an additive growth factor, by inducing T cell subsets that cannot be grown with IL-2 alone, by increasing the antigen-presenting function of macrophages contained in tumors (8), or alternatively, by inhibiting the suppressive activity of tumors. Addition of irradiated tumor during culture led us to enhanced TIL expansion possibly because of restimulation of tumor-specific T cells with putative tumor antigens.

IL-4 used with high-dose IL-2 sometimes decreased TIL growth during maintenance culture. IL-4 could be inhibitory to T cell growth under certain conditions by mechanisms that involve IL-4 inhibition of IL-2 stimulated IL-2 R(Tac) expression. Accessory cells or factors such as IL-1 may be necessary to maintain T cell responsiveness to IL-4 as has been demonstrated with murine Th2 cells (17) and

may be insufficient under our maintenance conditions. In our study, TIL maintained with IL-2 (10 U) and IL-4 expanded better in subsequent culture with low-dose IL-2 (10 U) than TIL grown with IL-2 (1,000 U) and IL-4 (data not shown). T cells maintained with such low doses of IL-2 in vitro may preferentially grow when adoptively transferred to patients given IL-2 in whom attainable IL-2 levels are expected to be lower and possibly with less negative effects on the subsequent T cell responsiveness to IL-2 (18).

Our data suggests that IL-4 selectively enhances the growth of T cells cytotoxic to autologous melanomas with IL-2. This strategy may be useful for expanding cells with specific reactivity from inflammatory sites as well as other tumors.

Summary

Addition of IL-4 (1,000 U/ml) to either high or low concentrations of IL-2 augmented tumor-infiltrating lymphocytes (TIL) growth from human melanoma. Weekly restimulation with irradiated tumor cells in conjunction with IL-4 allowed enhanced growth of TIL. With low-dose IL-2 (10 U/ml) and IL-4, expanded TIL had little cytolytic activity against Daudi or allogeneic tumors. Further, IL-4 augmented the total lytic activity against autologous tumors in most cases. With high-dose IL-2 (1,000 U/ml), IL-4 addition decreased nonspecific killing activity against Daudi or allogeneic melanomas in many cases, and reciprocally augmented cytolytic activity against the autologous melanoma in many cases. This suggests the possible use of IL-4 in cancer therapy, especially in adoptive cellular immunotherapy using TIL or in conjunction with IL-2 administration.

References

1. Rosenberg, S. A., M. T. Lotze, L. M. Muul, A. E. Chang, F. P. Avis, S. Leitman, W. M. Linehan, C. N. Robertson, R. E. Lee, J. T. Rubin, C. A. Seipp, C. G. Simpson, and D. E. White. 1987. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high dose interleukin-2 alone. *N. Engl. J. Med.* 316:889.

2. Muul, L. M., P. J. Spiess, E. P. Director, and S. A. Rosenberg. 1987. Identification of specific cytolytic immune responses against autologous tumor in humans bearing malignant melanoma. *J. Immunol.* 138:989.

3. Topalian, S. L., L. M. Muul, D. Solomon, and S. A. Rosenberg. 1987. Expansion of human tumor infiltrating lymphocytes for use in immunotherapy trials. *J. Immunol. Methods.* 102:127.

4. Topalian, S. L., D. Solomon, F. P. Avis, A. E. Chang, W. M. Linehan, M. T. Lotze, C. N. Robertson, C. A. Seipp, P. Simon, C. G. Simpson, and S. A. Rosenberg. 1988. Therapy of patients with advanced cancer using tumor infiltrating lymphocytes and recombinant interleukin-2: a pilot study. *J. Clin. Oncol.* 6:839.

5. Lotze, M. T., S. Tomita, and S. A. Rosenberg. 1988. Human tumor antigens defined by cytotoxic and proliferative T cells. *In* Human Tumor Antigens and Specific Tumor Therapy. R. Metzgar and M. Mitchell, editors. Alan R. Liss, Inc., New York. In press.

6. Fisher, B., B. Packard, J. Carrasquillo, E. J. Read, C. S. Carter, S. Topalian, J. Yang,

P. Yolles, S. Larson, and S. A. Rosenberg. 1988. Traffic of tumor infiltrating lymphocytes (TIL) to metastatic tumor deposits: human studies. *Proc. Am. Soc. Clin. Oncol. Annu. Meet.* 247. (Abstr.).

7. Paul, W. E., and J. Ohara. 1987. B-cell stimulatory factor-1/interleukin 4. *Annu. Rev. Immunol.* 5:429.

8. Yokota, T., N. Arai, J. De Vries, H. Spits, J. Banchereau, A. Zlotnik, D. Rennick, M. Howard, Y. Takeba, S. Miyatake, F. Lee, and K. Arai. 1988. Molecular biology of interleukin 4 and interleukin 5 genes and biology of their products that stimulate B cells, T cells, and hemopoietic cells. *Immunol. Rev.* 102:137.

9. Spits, H., H. Yssel, Y. Takebe, N. Arai, T. Yokota, F. Lee, K. Arai, J. Banchereau, and J. E. De Vries. 1987. Recombinant interleukin 4 promotes the growth of human T cells. *J. Immunol.* 139:1142.

10. Kawakami, Y., M. Custer, S. A. Rosenberg, and M. T. Lotze. 1988. Human interleukin 4 (IL4) inhibits interleukin 2 (IL2) induction of human lymphokine activated killer (LAK) activity from peripheral blood and spleen cells. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 2:A660. (Abstr.).

11. Widmer, M. B., R. B. Acres, H. M. Sassenfeld, and K. H. Grabstein. 1987. Regulation of cytolytic cell populations from human peripheral blood by B cell stimulatory factor 1 (interleukin 4). *J. Exp. Med.* 166:1447.

12. Spits, H., H. Yssel, X. Paliard, R. Kastelein, C. Figdor, and J. de Vries. 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.

13. Lotze, M. T., E. Grimm, A. Mazumder, J. L. Strausser, and S. A. Rosenberg. 1981. Lysis of fresh and cultured autologous tumor by lymphocytes cultured in T cell growth factor (TCGF). *Cancer Res.* 41:4420.

14. Mule, J. J., C. A. Smith, and S. A. Rosenberg. 1987. Interleukin 4 (B cell stimulatory factor 1) can mediate the induction of lymphokine-activated killer cell activity directed against fresh tumor cells. *J. Exp. Med.* 166:792.

15. Rosenberg, S. A., P. Speiss, and R. Lafreniere. 1986. A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. *Science (Wash. DC).* 233:1318.

16. Ettinghausen, S. E., R. K. Puri, and S. A. Rosenberg. 1988. Increased vascular permeability in organs mediated by the systemic administration of lymphokine-activated killer cells and recombinant Interleukin-2 in mice. *J. Nat. Cancer Inst.* 80:177.

17. Kurt-Jones, E. A., S. Hamberg, J. Ohara, W. E. Paul, and A. K. Abbas. 1987. Heterogeneity of helper/inducer T lymphocytes. I. Lymphokine production and lymphokine responsiveness. *J. Exp. Med.* 166:1774.

18. Ashwell, J. D., R. J. Robb, and T. R. Malek. 1986. Proliferation of T lymphocytes in response to interleukin 2 varies with their state of activation. *J. Immunol.* 137:2572.