Jpn. J. Cancer Res. 82, 585–592, May 1991

Suppressive Activity of Interleukin 4 on the Induction of Antigen-specific Cytotoxic T Cells in Humans

Uki Yamashita[1] and Yoshiya Tanaka[2]

[1]*Department of Immunology and Parasitology, School of Medicine, Hiroshima University, 1-2-3 Kasumi, Minamiku, Hiroshima 734 and* [2]*First Department of Internal Medicine, University of Occupational and Environmental Health, School of Medicine, 1-1 Iseigaoka, Yahatanishiku, Kitakyushu 807*

The effect of interleukin 4 (IL 4) on the induction of cytotoxic T cells (CTL) was studied by using human peripheral blood lymphocytes *in vitro*. IL 4 suppressed the induction of CTL specific for allogeneic antigens in a concentration-dependent manner. However, IL 4 did not suppress proliferative responses induced with allogeneic antigens or mitogens. The suppressive effect of IL 4 on CTL induction was observed when IL 4 was added at the early period of the CTL induction culture, but not at the later period. Furthermore, IL 4 did not suppress the effector function of CTL to target cells. IL 4 suppressed the production of IL 1 by monocytes/macrophages and the production of IL 2 and the expression of IL 2 receptors on T cells. Moreover, IL 4 suppressed the induction of lymphokine-activated killer cells. These results suggest that IL 4 has a suppressive activity on the induction of killer cells in humans.

Key words: Interleukin 4 — Cytotoxic T cells — Lymphokine-activated killer cells

Interleukin 4 (IL 4) was first designated as B cell stimulating factor-1 (BSF-1) from its stimulatory activity on B cells.[1] Recently, a body of evidence has been accumulated to show that IL 4 has regulatory activity not only on B cell functions, but also on T cells and macrophages.[2–8] However, some conflicting data have been obtained about the regulatory activity on T cells in mouse and human systems. For example, IL 4 could induce lymphokine-activated killer cells (LAK) in murine systems.[9, 10] However, IL 4 could not induce human LAK activity and it suppressed LAK activity induced with interleukin 2 (IL 2).[11, 12] Murine IL 4 also enhanced cytotoxic T cells (CTL) induced in the mixed lymphocyte reaction.[13] However, regarding human CTL, no definitive evidence has been reported. In order to clarify further the regulatory role of IL 4 on T cell and macrophage functions, we studied the effect of IL 4 on the induction of CTL using human peripheral blood lymphocytes (PBL). In this communication we will present definitive evidence that IL 4 has suppressive activity on the induction of CTL in humans.

MATERIALS AND METHODS

Target cells for killer cells As the stimulator and the target cells for CTL, adult T cell leukemia cell line, MT 2[14] and B cell line, IKD, established in our laboratory, and allogeneic PBL were used. All these cells are major histocompatibility complex class I and class II antigen-positive. As the target cells for LAK cells, Raji B cell blastoid cell line was used. These cells were maintained *in vitro* by culturing in RPMI 1640 medium (Nissui Seiyaku Co., Tokyo) containing 10% fetal calf serum (FCS, Grand Island Biological Co., Grand Island, NY).

Induction of CTL and LAK PBL were prepared from heparinized peripheral blood of healthy volunteers by centrifugation over lymphocyte separation medium (Litton Bionetics, Kensington, MD). PBL (6×10^6) were cultured *in vitro* with mitomycin C (MMC, 100 μg/ml, Kyowa Hakko Kogyo Co., Tokyo)-treated MT 2, IKD cells (2×10^5) or allogeneic PBL (2×10^6) in wells of 24-well Linbro culture plates (Flow Laboratories Co., Hamden, CO) in 2 ml of RPMI 1640 medium containing 10% FCS for 6 days at 37°C in 5% CO_2 and 95% air. For the induction of LAK, PBL were cultured with 100 ng/ml of human recombinant IL 2 (donated by Ajinomoto Co., Yokohama) for 3 days at 37°C in 5% CO_2 and 95% air.

Assay of killer cell activity The ^{51}Cr-release assay was performed according to the method of Cerottini *et al.*[15] Target cells were radiolabeled by incubating 3×10^6 cells in one ml of RPMI 1640 medium containing 10% FCS and 100 μCi of sodium [^{51}Cr]chromate (New England Nuclear, Boston, MA) in a culture tube (#2057, Falcon Plastics, Oxnard, CA) at 37°C for 2 h. After extensive washings, 1×10^4 target cells were mixed with 4 or 2×10^5 *in vitro*-activated PBL (effector/target ratio, 40:1 or 20:1) in 0.2 ml of RPMI 1640 medium containing 10% FCS in wells of round-bottomed microtiter culture plates (Nunc, Roskilde, Denmark). After effector-to-target cell

Abbreviations used in this paper: Con A, concanavalin A; CTL, cytotoxic T cells; FCS, fetal calf serum; IL, interleukin; LAK, lymphokine-activated killer; LPS, lipopolysaccharide; MMC, mitomycin C; NK, natural killer; PBL, peripheral blood lymphocytes; ^{3}H-TdR, tritiated thymidine.

interaction at 37°C for 5 h in 5% CO_2 and 95% air, the microtiter culture plates were centrifuged at 1500 rpm for 10 min, the culture supernatant in each well was harvested, and the radioactivity in the culture supernatant was counted with a gamma counter. The percentage of specific lysis was calculated from the formula: % specific lysis = [(experimental release − control release)/(maximum release − control release)] × 100, where maximum release was obtained by incubating ^{51}Cr-labeled target cells in the presence of 1% Nonidet P40 (Sigma Chemical Co., St. Louis, MO), and the control release was obtained by incubating target cells alone or target cells with unsensitized PBL. Since the standard errors were less than 10% of the means, only mean % of specific killing of triplicate cultures is given in the results for simplicity.

Assay of proliferative responses PBL (2×10^5) were cultured with MMC-treated MT 2, IKD cells (1×10^4), allogeneic PBL (1×10^5) or concanavalin A (Con A, 5 μg/ml, EY Laboratories, San Mateo, CA) in 0.2 ml of RPMI 1640 medium containing 10% FCS in wells of flat-bottomed microtiter culture plates (#3072, Falcon Plastics) at 37°C for 3 days in 5% CO_2 and 95% air. The cells were labeled during the last 18 h with 0.5 μCi of tritiated thymidine (^{3}H-TdR, specific activity, 6.0 Ci/mmol, Amersham plc, Buckinghamshire, UK) and were harvested with the aid of an automated cell harvester (Abe Kagaku Co., Chiba). The amount of radioactivity incorporated into DNA in the cells was measured with a beta counter.[16] The results were expressed as the mean counts per minute (cpm) of triplicate cultures with the standard error.

Production and assay of IL 1 PBL (5×10^6/ml) were cultured in 5 ml of RPMI 1640 medium containing 10% FCS in culture dishes (#3002, Falcon Plastics) at 37°C for 2 h. Nonadherent cells were removed by gentle washing and adherent cells were treated with anti-CD3 and anit-Ig monoclonal antibody (Becton Dickinson Co., Mountain View, CA) and rabbit complement (Cedarlane Laboratories, Toronto, Canada) at 37°C for one hour. The recovered cells (1×10^6/ml) were cultured with lipopolysaccharide from *E. coli* (LPS, 10 μg/ml, Sigma Chemical Co.) in RPMI 1640 medium containing 10% FCS in culture tubes (#2057, Falcon Plastics) at 37°C for 48 h in 5% CO_2 and 95% air. The culture supernatant was harvested by centrifugation, dialyzed against 100 volumes of RPMI 1640 medium, and used as a source of IL 1. The IL 1 activity of the culture supernatant was assayed using murine thymocytes according to the method of Mizel *et al.*[17] Thymocytes (1.5×10^6) from A.TH mice (bred in our laboratory) in 0.2 ml of Eagle Hanks Amino Acid medium as described by Corradin *et al.*[18] containing 10% FCS were cultured with serially diluted IL 1 samples in wells of flat-bottomed microtiter culture plates (#3072, Falcon Plastics) at 37°C for 3 days in 5% CO_2 and 95% air. The cells were labeled during the last 18 h with 0.5 μCi of ^{3}H-TdR, then harvested, and the radioactivity incorporated into thymocytes was counted. The results were expressed as units of IL 1 produced by 1×10^6 monocytes compared with recombinant human IL 1 (donated by Dr. M. Yamada, Dainippon Pharmaceutical Co., Osaka) as a standard.

Assay of IL 2 PBL (5×10^6/ml) were cultured with MMC-treated MT 2 cells (2×10^5/ml) in one ml of RPMI 1640 medium containing 10% FCS in culture tubes (#2057, Falcon Plastics) at 37°C for 48 h in 5% CO_2 and 95% air. The culture supernatant was recovered by centrifugation and dialyzed against RPMI 1640 medium. The IL 2 activity in the culture supernatant was assayed with a murine cytotoxic T cell line (CTLL).[19] CTLL (1×10^4) were cultured with serially diluted IL 2 samples in 0.2 ml of RPMI 1640 medium containing 10% FCS in flat-bottomed microtiter culture plates (#3072, Falcon Plastics) at 37°C for 48 h in 5% CO_2 and 95% air. The cells were pulsed with 0.5 μCi of ^{3}H-TdR for the last 12 h, then harvested and the radioactivity incorporated into CTLL was counted. The results were expressed as units of IL 2 produced by 5×10^6 PBL compared with recombinant human IL 2 as a standard.

Assay of the expression of IL 2 receptors PBL (5×10^6) were cultured with MMC-treated MT 2 cells (2×10^6) in one ml of RPMI 1640 medium containing 10% FCS in culture tubes (#2057, Falcon Plastics) at 37°C for 48 h in 5% CO_2 and 95% air. The recovered cells (5×10^5) were incubated with a fluoresceinated anti-IL 2 receptor monoclonal antibody (CD 25, Becton Dickinson Co.) at 4°C for one hour. After washings, the IL 2 receptor-positive cells were detected with an Epics 752 flow cytofluorometer (Coulter Co., Hialeah, FL).[20] The results were expressed as the percentage and the mean fluorescence intensity (MFI) of IL 2 receptor-positive cells.

Source of IL 4 Recombinant human IL 4 was kindly donated by Dr. K. Hama (Ono Pharmaceutical Co., Osaka).[4] The culture supernatant of Cos cells into which IL 4 complementary DNA had been introduced was purified by ammonium sulfate precipitation, gel-filtration, ion exchange chromatography and high-pressure liquid chromatography. The purity of IL 4 was more than 98% as detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver staining. The specific activity as detected by the stimulation of proliferative responses of phytohemagglutinin-induced T cell blasts was 1.65×10^6 units/mg protein.

RESULTS

Induction of CTL by allogeneic cells PBL were cultured *in vitro* with MT 2, IKD or allogeneic PBL for 6 days

Table I. Induction of Antigen-specific CTL by Allogeneic Cells

Exp. No.	Responder cells	Stimulator cells	% specific killing of target cells[a]					
			MT 2		IKD		Allogeneic PBL	
			40:1	20:1	40:1	20:1	40:1	20:1
I	PBL	MT 2	58.8	42.2	28.2	19.7	4.9	−2.8
		IKD	14.7	4.9	50.3	32.8	5.2	1.2
		Allogeneic PBL	8.3	−1.6	4.1	1.1	20.1	7.5
II	PBL	MT 2	54.2	39.7	16.1	7.8	ND[b]	ND
		IKD	10.7	4.3	41.3	17.7	ND	ND

a) PBL (6×10^6) were cultured with MMC-treated MT 2, IKD cells (2×10^5) or allogeneic PBL (2×10^6) for 6 days. The induced CTL activity was measured by culturing with ^{51}Cr-labeled MT 2, IKD or allogeneic PBL as the target at the effector-to-target cell ratio of 40:1 or 20:1 for 5 h. The results are expressed as mean percentage of specific killing of target cells of triplicate cultures.
b) Not determined.

Table II. Effect of IL 4 on CTL Induction

Responder cells	Stimulator cells[a]	IL 4 (ng/ml)	Exp. I			Exp. II		
			% cell recovery[b]	% killing		% cell recovery[b]	% killing	
				40:1	20:1		40:1	20:1
PBL	MT 2	0	35.0	42.3	27.3	47.0	54.2	39.7
		1	32.0	18.6	6.5	52.0	16.4	10.3
		10	30.0	18.2	6.5	43.0	13.8	6.0
		100	29.0	12.5	3.4	46.0	10.9	3.0
PBL	IKD	0	40.0	50.3	32.8	13.3	43.0	29.3
		1	33.0	29.3	27.8	23.3	28.9	16.1
		10	41.7	24.0	17.6	21.7	4.9	0.7
		100	30.0	11.1	4.1	20.0	2.5	0.1
PBL	Allogeneic PBL	0	20.0	38.4	30.3	30.0	20.1	7.5
		1	28.3	25.2	21.0	31.7	13.2	4.1
		10	23.3	18.6	5.9	26.7	2.0	−4.7
		100	23.3	2.8	0.5	ND[c]	ND	ND

a) PBL were cultured with MT 2, IKD or allogeneic PBL in the presence of IL 4 for 6 days.
b) Cell recovery is expressed as the % of the original cell number cultured.
c) Not determined.

and their cytotoxic activity was determined by further culturing with ^{51}Cr-labeled MT 2, IKD or allogeneic PBL for 5 h. As shown in Table I, PBL cultured with MT 2 cells markedly killed MT 2 cells, but had only a weak killing action on IKD cells and allogeneic PBL. PBL cultured with IKD cells or allogeneic PBL also preferentially killed IKD cells and allogeneic PBL, respectively. This activity was mediated by $CD8^+$ T cells, because it was abrogated by the treatment of PBL with anti-CD3 antibody and anti-CD8 antibody and complement (data not shown). Thus, the *in vitro* culture of PBL with MT 2, IKD or allogeneic PBL induced antigen (alloantigen)-specific CTL.

Suppressive effect of IL 4 on the induction of CTL When human recombinant IL 4 was added to the culture of PBL and the stimulator cells, the development of CTL was suppressed in a concentration-dependent manner, as shown in Table II. The addition of 10–100 ng/ml of IL 4 almost completely suppressed CTL induction. Although we have presented only the data at the responder/target cell ratios of 40:1 and 20:1 in the tables, the suppression of CTL activity in the IL 4-added group was observed at all ratios from 80:1 to 5:1 studied. The suppression of CTL induction by IL 4 was not due to the cytotoxic activity of IL 4 on PBL, because the cell recovery after 6 days of culture was not different between the control

Table III. Effect of IL 4 on Proliferative Responses of PBL

IL 4 (ng/ml)	[^{3}H]Thymidine uptake of PBL[a] stimulated with				
	(−)	Con A	MT 2	IKD	Allogeneic PBL
0	263±30	4,567±655	14,536±142	10,357±467	5,264±602
1	257±12	5,947±379	15,572±572	10,715±640	6,846±582
10	316±14	6,747±379	17,185±911	11,908±640	7,576±424
100	407±13	6,688±113	17,652±815	14,716±440	4,588±892

a) PBL (2×10^5) were cultured with concanavalin A (Con A, 5 μg/ml), MMC-treated MT 2, IKD cells (1×10^4) or allogeneic PBL (1×10^5) for 3 days and [^{3}H]thymidine uptake of BPL was measured.

Table IV. Effect of IL 4 on CTL Induction: Time Course Study

Responder cells	Stimulator cells	IL 4 10 ng/ml	% specific killing of target cells[a]			
			Exp. I		Exp. II	
			40:1	20:1	40:1	20:1
PBL	MT 2	—	73.4	47.7	36.8	24.1
		Added on day 0[b]	20.7	7.1	20.3	10.0
		1	36.2	18.3	22.9	11.7
		2	42.3	19.9	28.3	25.6
		3	59.5	36.0	31.7	27.4
		4	61.1	39.3	36.5	24.6
		5	62.5	41.8	37.6	27.1
		—	41.4	25.4	33.6	19.8
		Washed on day 1[c]	31.4	19.0	31.5	13.8
		2	22.1	11.3	27.1	12.4
		3	11.3	6.4	20.2	9.7
		4	9.4	3.7	14.6	5.7
		5	3.9	1.1	11.5	4.4
		6	3.3	0.1	10.0	2.2

a) PBL were cultured with MT 2 for 6 days.
b) IL 4 (10 ng/ml) was added on the days indicated.
c) The mixture of PBL, MT 2 and IL 4 was washed on the days indicated and cultured to make a total of 6 days.

group and the suppressed group. Furthermore, the suppression of CTL development by IL 4 was not due to a kinetic difference of CTL development, because the CTL activity was always suppressed by IL 4 when assayed on days 4, 6 and 8 of the culture of PBL. These results suggest that IL 4 has specific suppressive activity on the induction of CTL in humans. In the following experiments we analyzed the mechanisms of IL 4-induced suppression of CTL development using MT 2 cells.

IL 4 does not suppress proliferative responses induced with alloantigens and mitogens Next, we studied the effect of IL 4 on proliferative responses of PBL. As shown in Table III, the spontaneous proliferative responses of PBL and the proliferative responses induced by stimulation with concanavalin A, MT 2, IKD and allogeneic PBL were not suppressed, but were slightly enhanced. These results are consistent with the results that IL 4 has stimulatory activity on T cell and B cell proliferative responses.[2-5]

Time course study of the suppression of CTL induction by IL 4 Next, we studied the effect of the time of addition of IL 4 on the CTL induction. As shown in the upper part of Table IV, when IL 4 was added at the initiation of the culture, the induction of CTL was markedly suppressed. However, the suppression was gradually decreased as the addition of IL 4 was delayed. Furthermore, IL 4 did not suppress the effector function of CTL to target cells as shown in Table V. These results suggest that IL 4 affects an early step, i.e., the antigen-recognition step, of the CTL induction. In the experiment of the lower part of Table IV, the mixture of PBL, MT 2 and IL 4 was cultured for 1 to 6 days, washed to remove IL 4

Table V. Effect of IL 4 on CTL Effector Function

Responder cells	Stimulator cells	IL 4 (ng/ml)	% specific killing of target cells[a]			
			Exp. I		Exp. II	
			40:1	20:1	40:1	20:1
PBL	MT 2	0	51.5	25.4	44.1	20.4
		1	52.8	19.2	42.8	23.6
		10	53.6	17.3	45.5	21.8
		100	51.1	21.7	44.8	22.5

a) PBL and MT 2 were cultured for 6 days to induce CTL. The induced CTL activity was measured by culturing with ^{51}Cr-labeled MT 2 as the target in the presence of various concentrations of IL 4 as indicated for 5 h.

Table VI. Effect of IL 4 on IL 1 Production by Monocytes

Monocytes stimulated with	IL 4 (ng/ml)	Production of IL 1[a] (units/10^6 monocytes)	
		Exp. I	Erp. II
—	0	5.7	3.2
LPS	0	89.4	70.0
	1	58.8	19.4
	10	47.6	13.9
	100	33.9	5.0

a) Monocytes (1×10^6) were cultured with lipopolysaccharide (LPS, 10 μg/ml) in the presence of various concentrations of IL 4 for 2 days and the IL 1 activity in the culture supernatant was measured in terms of murine thymocyte proliferative responses. The results are expressed as units of IL 1 produced by 1×10^6 monocytes using recombinant IL 1 as a standard.

Table VII. Effect of IL 4 on IL 2 Production by PBL

Responder cells	Stimulator cells	IL 4 (ng/ml)	Production of IL 2[a] (units/5×10^6 PBL)	
			Exp. I	Exp. II
PBL	—	0	2.0	1.0
	MT 2	0	28.6	5.9
		1	18.6	5.2
		10	10.9	3.6
		100	5.0	2.7

a) PBL (5×10^6) were cultured with MT 2 (2×10^5) in the presence of various concentrations of IL 4 for 2 days and the IL 2 activity in the culture supernatant was measured by using an IL 2-dependent murine cytotoxic T cell line. The results are expressed as units of IL 2 produced by 5×10^6 PBL using recombinant IL 2 as a standard.

Table VIII. Effect of IL 4 on IL 2 Receptor Expression

Responder cells	Stimulator cells	IL 4 (ng/ml)	Expression of IL 2 receptor[a]	
			Exp. I	Exp. II
PBL	—	0	7.8 (53.7)	7.8 (60.5)
	MT 2	0	26.0 (70.2)	16.1 (76.5)
		1	22.0 (65.6)	14.9 (71.1)
		10	17.7 (62.7)	14.2 (72.3)
		100	16.7 (60.3)	13.7 (73.1)

a) PBL (5×10^6) were cultured with MT 2 (2×10^5) in the presence of various concentrations of IL 4 for 2 days and the expression of IL 2 receptors on T cells was measured with a flow cytofluorometer using FITC-conjugated anti-IL 2 receptor monoclonal antibody (CD25). The results are expressed as percentage of IL 2 receptor-positive cells and (mean fluorescence intensity).

and then further cultured for a total time of 6 days. As can be seen, the suppression of CTL induction was marked when PBL were cultured with IL 4 for more than 2 or 3 days. However, when PBL were cultured for less than 1 or 2 days, the suppression was not so clear. These results suggest that more than 1 or 2 days' contact with IL 4 is required for the suppression of CTL induction.

Effect of IL 4 on the production of IL 1 and IL 2 and the expression of IL 2 receptors The development of CTL is regulated by several kinds of soluble factors produced by macrophages and lymphocytes.[21] So, we next studied the effect of IL 4 on the production of soluble factors. Peripheral blood monocytes were cultured with LPS in the presence or absence of IL 4 for 48 h and the IL 1 activity in the culture supernatant was assayed using murine thymocytes. As shown in Table VI, monocytes without LPS-stimulation scarcely produced IL 1, but the addition of LPS markedly induced the production of IL 1 by monocytes. The IL 1 activity produced by LPS-stimulated monocytes was suppressed by IL 4 in a concentration-dependent manner. This proliferative response was completely abolished by rabbit anti-human IL 1β antibody, but not by anti-IL 1α antibody, suggesting that we can detect IL 1β activity in this experimental system (data not shown).[22] Furthermore, IL 4 did not

Table IX. Effect of IL 4 on LAK Cell Induction

Responder cells	Cultured with	IL 4 (ng/ml)	Exp. I			Exp. II		
			% cell recovery[b]	% killing		% cell recovery	% killing	
				40:1	20:1		40:1	20:1
PBL	IL 2	0	58.3	57.7	40.7	66.7	42.2	25.8
		1	41.7	40.1	23.7	72.2	19.5	12.0
		10	66.7	22.6	14.4	47.8	19.3	8.3
		100	66.7	12.5	5.8	53.3	16.7	6.3

a) PBL (6×10^6) were cultured with IL 2 (100 ng/ml) in the presence of various concentrations of IL 4 for 3 days to induce LAK cells. The induced LAK cell activity was measured by culturing with ^{51}Cr-labeled Raji cells as the target for 5 h. The results are expressed as % specific killing of Raji cells.
b) Cell recovery is expressed as the % of the original cell number cultured.

suppress the murine thymocyte proliferative responses induced with IL 1 (data not shown). Next, PBL were cultured with MT 2 cells in the presence or absence of IL 4 for 48 h and the IL 2 activity in the culture supernatant was assayed using CTLL. As shown in Table VII, PBL alone produced little IL 2, but the stimulation of PBL with MT 2 induced the production of IL 2. The production of IL 2 by PBL was suppressed by IL 4 in a concentration-dependent manner. IL 4 did not suppress the proliferative responses of CTLL induced by IL 2 (data not shown). We further studied the effect of IL 4 on the expression of IL 2 receptors. PBL were cultured with MT 2 cells in the presence or absence of IL 4 for 48 h and the expression of IL 2 receptors on T cells was detected with a flow cytofluorometer using fluorescein-conjugated anti-IL 2 receptor monoclonal antibody (CD 25). As shown in Table VIII, IL 2 receptors on PBL were induced by the stimulation with MT 2. The expression of IL 2 receptors on PBL was suppressed by IL 4 in a concentration-dependent manner. All these results suggest that IL 4 has a suppressive activity on the production of IL 1 and IL 2 and the expression of IL 2 receptors.

Effect of IL 4 on LAK cells The *in vitro* culture of PBL with IL 2 for 3 days induces LAK cell activity which nonspecifically kills natural killer (NK) cell-resistant Raji cells. However, the addition of IL 4 to this culture suppressed the induction of LAK cells in a concentration-dependent manner as shown in Table IX. IL 4 did not have any significant effect on the cell recovery of the PBL cultured for 3 days.

DISCUSSION

It has been reported that IL 4 has a regulatory activity not only on B cells, but also on T cells and macrophages.[2-8] In murine systems, IL 4 has enhancing activity on CTL responses induced by the mixed leukocyte reaction and LAK cell activity induced by IL 2.[9,10] However, in human systems, IL 4 did not induce LAK activity, but suppressed LAK activity induced by IL 2.[11,12] In this study we found that IL 4 also had suppressive activity on the induction of antigen-specific CTL in humans. Namely, the addition of IL 4 to the *in vitro* culture of PBL suppressed CTL responses induced with alloantigens in a concentration-dependent manner; 10–100 ng/ml IL 4 completely suppressed CTL induction. The suppressive effect of IL 4 on CTL induction is not due to a cytotoxic effect of IL 4 on PBL, because the same amounts of IL 4 did not suppress the proliferative responses of PBL induced with alloantigens or mitogens, but slightly enhanced them. Furthermore, the suppressive activity of IL 4 on CTL induction is not due to a kinetic difference of CTL development, because CTL responses assayed on days 4, 6 and 8 of the *in vitro* culture were always suppressed. Since IL 4 has a stimulatory activity on the proliferative responses of PBL, one possibility to explain the suppression of CTL induction by IL 4 may be a relative dilution of CTL populations in the PBL by an increase of non-CTL populations, because we adjusted for the recovered cell number after the *in vitro* culture when we assayed CTL effector function. However, this possibility also seems to be unlikely, because IL 4 did not suppress the CTL induction if we added IL 4 2 days after the initiation of the culture and continued the incubation for 4 days. In this time period, IL 4 enhanced the proliferative responses of PBL. These results are absolutely opposite to the reports of Spits *et al.*, Widmer *et al.*, and Kawakami *et al.*, who reported that IL 4 did not suppress, but sometimes enhanced CTL induction in humans.[11,23,24] The reason for this difference is not clear at present. It is not due to the difference of target cells used in this experiment and their reports. We used three kinds of stimulator and target cells: MT 2, IKD and allogeneic PBL. All CTL responses induced with these cells were suppressed by IL 4. Since these target cells are resistant to NK and

LAK cells, CTL response suppression by IL 4 is not due to the suppression of NK and LAK cell activity. Furthermore, the suppression of CTL responses by IL 4 is not due to the difference of the source of IL 4, because IL 4 purchased from Genzyme Co. (Boston, MA) also suppressed CTL responses (data not shown). Thus, the suppression of CTL induction by IL 4 seems to be a general phenomenon.

Next we studied the mechanisms of the suppression of CTL induction by IL 4. IL 4 expressed its suppressive activity when added at the early period of the CTL induction culture, but not at the later period, suggesting that IL 4 disturbs an early step, possibly the antigen-recognition step, of the CTL induction. Furthermore, IL 4 did not suppress the effector function of CTL to target cells. Since the induction of CTL is regulated by soluble factors produced by macrophages and T cells,[21] we studied the effect of IL 4 on cytokine production. We found that the production of IL 1 by monocytes/macrophages and the production of IL 2 by T cells and the expression of IL 2 receptors were suppressed by IL 4. In these studies, we did not get any evidence to support an enhancing activity of IL 4 on the CTL induction. There are some reports to show that IL 4 suppressed the production of IL 1 by human monocytes/macrophages.[25-27] Our study is consistent with those reports. Since the production of IL 2 and the expression of IL 2 receptors on T cells require the accessory function of macrophages and/or IL 1,[28,29] the suppression of the production of IL 2 and the expression of IL 2 receptors may be due to the suppression of production of IL 1 by monocytes. However, IL 4 also expresses its suppressive activity directly on CTL and LAK precursor cells. The suppression of LAK cells by IL 4 is consistent with the reports of Spits *et al.*, Widmer *et al.* and Kawakami *et al.*[11,23,24] All these results suggest that IL 4 has a suppressive activity on the induction of not only non-specific killer cells, but also antigen-specific CTL, and this suppression seems to be induced by the suppressive activity of IL 4 on cytokine production, cytokine receptor expression and cytokine effector function in humans. In murine systems, IL 4 has an enhancing activity on antigen-specific CTL and LAK cell induction.[9,10,13] The reason for this difference between murine and human systems is not clear at present. There may be a susceptibility difference to the action of IL 4 between murine spleen T cells and human peripheral T cells. In fact, IL 4 has no suppressive activity on T cells 2 days after antigenic stimulation. It was reported that cross-reactivity of IL 4 is not observed between murine and human systems.[30] Thus, this species restriction of IL 4 activity may be a reflection of the functional difference of IL 4 in mice and humans. Originally IL 4 was reported to induce B cell proliferative responses.[1] In human T cells, IL 4 induces proliferative responses, while it suppresses the differentiation to effector cells as shown here. In a preliminary experiment, we also observed that IL 4 stimulated the proliferative responses of monocytes, while it suppressed the differentiation into macrophages. Thus, IL 4 seems to play a negative feedback role for T cell and macrophage lineages in humans.

ACKNOWLEDGMENTS

This work was supported in part by a grant from Fukuoka Cancer Society.

(Received December 21, 1990/Accepted February 15, 1991)

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