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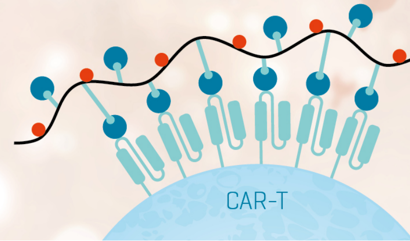

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SUPPRESSION BY IL-2 OF IgE PRODUCTION BY B CELLS STIMULATED BY IL-4[1]

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IgE production was obtained from B cells of BALB/c or nude mice when these cells were cultured with IL-4 plus LPS. IL-2 added to these cultures at the start (day 0), 1 or 2 days later completely suppressed the production of IgE. The production of IgG1 was also inhibited, but only if IL-2 was added on day 0. The production of other isotypes (IgM, IgG2a, IgG2b) was only slightly decreased by addition of IL-2. No suppression of IgE or IgG1 production was observed if monoclonal anti-IL-2 was added, whereas anti-IFN-γ had no effect on the suppression of the production of these isotypes. The expression of CD23 on the third day of culture on B cells stimulated with LPS and IL-4 was markedly decreased when IL-2 was added to the cultures on day 0. Addition of monoclonal anti-IL-2 suppressed all effects produced by IL-2, whereas addition of anti-IFN-γ had no effect. These results show that the suppression by IL-2, at least for the first signaling processes, are different from the suppression produced by IFN-γ.

It is well known that IgE production is enhanced in certain helminthic infections (1–3) and suppression of anti-hapten IgE antibodies have been obtained in adoptive transfers. This suppression was shown to be the result of interaction of T cells (4, 5). Recently it has been shown that the population of Th cells is not as uniform as it was thought years ago. In cloned murine T cells two subpopulations L3T4 (CD4) T cells have been described, the most important difference is that Th1 secretes IL-2 and IFN-γ, but not IL-4 and IL-5 whereas Th2 secretes IL-4 and IL-5, but not IL-2 and IFN-γ (6, 7). It has also been shown, that IL-4 promotes the secretion of IgE and also of IgG1 (8). IL-4 secreted by a subpopulation of T cells or rIL-4 could induce the secretion of IgE from LPS stimulated B cells from different strains of mice and even from B cells from nude mice (9).

Recently Coffman and Carty (10) showed the IgE and IgG1 production by LPS-stimulated B cells was completely inhibited by low concentration of IFN-γ in vitro. Moreover in certain experiments IL-2 and IL-4 had been shown to act in a synergistic manner (11, 12) whereas in others, these lymphokines seem to inhibit each others action (13).

In these experiments we examined the action of IL-2 on IgE production obtained by IL-4 on LPS-stimulated B cells. Using monoclonal anti-IL-2 and anti-IFN-γ antibodies we showed that only anti-IL-2 but not anti-IFN-γ could neutralize the inhibitory action of IL-2 on the IgE-promoting activity of IL-4.

MATERIALS AND METHODS

Mice. Female 6 to 8-wk-old BALB/c mice were purchased from Ohmura Experimental Animal Co. (Sagamihara, Kanagawa, Japan). Female 6 to 8-wk-old BALB/c nu/nu mice were obtained from Shizuoka Agriculture Cooperative Laboratory Animals (Hamamatsu, Shizuoka, Japan).

mAb. Rat mAb 6HD5 and HMK-12, specific for murine IgE, established and characterized as described (14), were purified from ascites (obtained in nude mice) by ion-exchange chromatography. A rat mAb to murine IL-2 (S4B6) was a generous gift of Dr. T. R. Mosmann (Department of Immunology, DNAX, Palo Alto, CA). A murine mAb to human IL-2 (DMS-1) was a generous gift of Dr. F. Melchers (Basel Institute for Immunology, Basel, Switzerland), this mAb cross-reacts with murine IL-2 (15). A rat hybridoma secreting rat IgG1 mAb to murine IFN-γ (RA-6A2) was obtained from the American Type Culture Collection (Rockville, MD). A rat anti-CD23 (FcRε) secreting hybridoma was a generous gift of Dr. D. Conrad (Johns Hopkins University, Baltimore, MD). mAb against murine IgM, IgG1, IgG2a, IgG2b, were purchased from Zymed Co. (San Francisco, CA). Anti-murine L3T4 mAb was obtained from Biosys S.A. (Compiegne, France). Anti-Mac-1 mAb was obtained from Hybritech. Co. (San Diego, CA). Anti-murine Lyt-2.2 mAb and Thy-1.2 mAb were obtained from Meiji Co. (Tokyo, Japan)

Polyclonal antibodies. Affinity purified rabbit polyclonal anti-murine IgG1 or IgG2a antibodies were made in our laboratories by immunizing rabbits with myeloma protein MOPC 21 or UPC 10, respectively. Affinity purified rabbit polyclonal antibodies against murine IgM and IgG2b were also purchased from Zymed Co. Rabbit anti-asialo GM1 (GA1) antibodies were obtained from Wako Co. (Tokyo, Japan). FITC-labeled anti-Thy-1.2 antibody was obtained from Cederlane Co. Phycoerythrin labeled anti-B220 antibody was from our laboratory. Rabbit anti-BAT[4] was a generous gift of Dr. Y. Asano (Department of Immunology, Tokyo University, Tokyo, Japan), this antibody reacts with murine T cells, as described (16).

Myeloma proteins. The following myeloma proteins: IgG1 (MOPC 21), IgG2a (UPC 10), IgG2b (MOPC 195), IgM (MOPC 104E) were purchased from Litton Bionetics Inc. (Charleston, SC). The myeloma proteins were used for immunizations and standard curve determinations of Ig concentrations.

Cytokines. Culture supernatants containing murine rIL-2 or murine rIL-4 were obtained from transformants of X63Ag8-653 as described (17). In addition, rIL-2 and rIL-4 were also purchased from Genzyme Co. (Boston, MA). Mouse IFN-γ was a generous gift from

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[4] Abbreviation used in this paper: BAT, brain-associated T cell Ag antibody.

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Shionogi Pharmaceutical Co. (Tokyo, Japan). (18).

IL-2 and IL-4 activity. IL-2 activity was measured by the proliferation ([^{3}H]thymidine uptake) of IL-2-dependent CTLL cells. Serial dilutions of IL-2 were incubated with 5000 CTLL cells in a volume of 200 μl for 2 h, followed by a 6-h pulse of 1 μCi = 37 bq of [^{3}H] thymidine (Radio Chemical Center, Amersham, GB). One unit of activity was defined as the amount of IL-2 that induced 50% maximal proliferation of CTLL cells in 200 μl culture. Activity of IL-4 was determined by the same method as for IL-2 except that 5×10^3 HT-2 cells in 200 μl medium were used as indicator cells. IL-4 activity on HT-2 cells were measured by the same method as for IL-2.

Complement. Rabbit complement was purchased from Cederlane Co. (Ontario, Canada) and maintained in 2-ml aliquots at −80°C, until use.

Medium. RPMI 1640 (GIBCO, Grand Island, NY) was supplemented with 2 mM L-glutamin, 50 U penicillin/ml, 100 μg streptmycin/ml, and 10% FCS (GIBCO). NaN_3 (Fisher Scientific) was added to a final concentration of 0.01% and this solution was used as staining buffer. Salmonella enteridis LPS was purchased from Difco Laboratories (Detroit, MI). The 96-well, flat-bottom, sterile plastic plates were purchased from Corning (Corning, NY).

Purification of mAb. Rat anti-murine IgE mAb, rat anti-murine IL-2 antibodies were purified from ascitic fluids of nude mice (previously inoculated i.p. with the respective hybridoma cells), on Sephacel columns, as described (19). Briefly, 5 ml of ascitic fluid was precipitated at 4°C with equal amount of saturated $(NH_4)_2SO_4$, the precipitate dissolved in 5 ml PBS (pH 7.2 and 0.03 M PBS), then dialyzed against PBS until no sulfate was present, and applied to DEAE-Sephacel (Pharmacia Fine Chemicals, Piscataway, NJ) columns (bed volume 2 ± 6 cm^3) and the first peak of OD at 280 nm was collected. The purity was confirmed by SDS-PAGE.

ELISA. For measurement of different Ig classes ELISA was used with the different antibodies, listed above as described (20, 21). As standards, the appropriate myeloma proteins were included in each assay. Previously, we ascertained that there was no crossreaction with any of the antibodies and that they were strictly monospecific. The sensitivity of the assay was 5 ng/ml for IgM, 10 ng/ml for IgG1, IgG2a, IgG2b and 2 ng/ml for IgE.

Cell preparation for in vitro culture. Spleen cells were separated in sterile condition into single cell suspensions in the medium described above, on ice. The 1×10^6 cells were treated with a mixture of mAb to Thy-1.2, L3T4, Lyt-2.2, MAC-1, and asialo GM1 (GA1) (to lyse pre-T cells (22)) or anti-BAT antibody for 45 min on ice. The final dilution of these mAb was 1/100 and total volume was 30 ml. The cells were then centrifuged for 10 min at 1200 rpm and then suspended at 37°C for 1 h in rabbit complement diluted 1/10 in medium, in a total volume of 30 ml. Small, dense B cells were separated by Percoll (Pharmacia) gradient (density 1.081), as described (23). The cells were then washed in medium and centrifuged for 10 min, as described above, checked by immunofluorescence using FITC-labeled anti-B220 and anti-Thy-1.2 antibodies (see below) and called purified B cells (Fig. 1). The 5×10^5 or 2×10^5 B cells were seeded with or without LPS (final concentration 10 μg/ml) in a final volume of 0.2 ml complete medium in 96-well flat-bottom plates and cultured at 37°C, 5% CO_2 for 24 h. Where indicated, rIL-2 (Genzyme) 50 or 100 U/ml of medium, anti-IL-2 antibody, or anti-IFN antibody and 100 U/ml of IL-4 were added (Table II). The cells were cultured at 37°C (95% CO_2 humidified atmosphere) for 9 days. Antibody production in the supernatant was detected by ELISA.

Immunofluorescent staining. The 1×10^6 spleen cells before and after depletion by the antibody mixture or anti-BAT antibody were stained with FITC-labeled anti-B220 or anti-Thy-1.2 mAb and analyzed on the FACStar (Becton Dikinson, Mountain View, CA). For staining, the cells were suspended in a 1/50 dilution of antibody in medium containing 10% FCS, in a total volume of 0.1 ml for 30 min at 4°C, then washed three times with staining buffer. For CD23 staining a similar procedure was used, using FITC-labeled anti-CD23 antibody.

RESULTS

Establishment of purified B cells from whole spleen. Spleen cells were treated with cytotoxic mAb to Thy-1.2, Lyt-2.2, L3T4, MAC-1, and asialo GM1 (GA 1) or polyclonal rabbit anti-BAT antibody. After treatment with complement and removal of dead cells, the cells were stained with fluorescent antibody to the B cell marker B220 and analyzed by the FACStar. Purified fractions were more than 97.5% B cells and less than 1% T cell (Fig. 1).

IgE production by IL-4 of LPS-stimulated purified B cells and its suppression by IL-2. IgE production in vitro by purified B cells from BALB/c or nude mice was studied with a combination of LPS, IL-4, and IL-2. The results are presented in Table I. When 5×10^5 or 2×10^5 purified B cells from BALB/c or nude mice were cultured with 10 μg/ml of LPS from the start (day 0) and 100 U/ml of IL-4 was added 1 day later (day 1) substantial amount of IgE was produced on day 7. However, when 50 U/ml of IL-2 was added on day 0 no detectable IgE production was observed. With the same amounts of LPS alone, IL-4 alone, or IL-2 alone no detectable amount of IgE could be obtained (Table I). These results confirm our previous studies on IgE production of LPS-stimulated B cells by IL-4 (9). Viability checked by die-exclusion was the same with or without IL-2.

Titration of inhibitory activity of IL-2 during entire culture period. The 5×10^5 or 2×10^5 purified B cells from BALB/c mice were stimulated with 10 μg/ml of LPS on day 0 and 100 U/ml of IL-4 was added on day 1, IL-2 was added on day 0. IgE production was checked in the supernatants on days 3, 5, 7, and 9. The results are presented in Figure 2. No IgE could be detected before day 7. However, 81 ng/ml (5×10^5 B cells) or 40 ng/ml (2×10^5 B cells) of IgE was detected on day 7 and 98 ng/ml (5×10^5 B cells) or 60 ng/ml (2×10^5 B cells) on day 9 in the controls, without IL-2. When 100 U/ml of IL-2 was added no IgE was detected in the supernatants even on day 9 and with as little as 50 U/ml only on day 9 could IgE be detected; the amount was less than 40 ng/ml (5×10^5 B cells) or 20 ng/ml (2×10^5 B cells), which is a great inhibition when compared to the controls without IL-2 (more than 90 ng/ml (5×10^5 B cells) or 60 ng/ml (2×10^5 B cells)).

Neutralization of suppressive effect of IL-2 on IgE production by IL-4 with monoclonal anti-IL-2, but not with monoclonal anti-IFN-γ. Similar cultures were made to those presented in Table I and anti-IL-2 or anti-IFN-γ mAb were added at the start (day 0). The amount of IgE was determined on day 7 (Table II). The suppression produced by rIL-2 was greatly diminished by addition of anti-IL-2 mAb (rows 4 to 6), but not by addition of anti-IFN-γ mAb (rows 7 to 8). A total of 200 ng/ml of anti-IL-2 restored 87% (5×10^5 B cells), or 81% (2×10^5 B cells) of the production of IgE, whereas anti-IFN-γ mAb was without any effect. This anti-IFN-γ mAb was very effective in restoring the suppression produced by IFN-γ (rows 9 to 12 in Table II).

Effect of IL-4 and IL-2 on production of different Ig isotypes. The production of different isotypes was studied in spleen cells of BALB/c or nude mice using the same system, 5×10^5 or 2×10^5 purified B cells stimulated with 10 μg/ml of LPS on day 0 and 100 U/ml of IL-4 on day 1 and 100 U/ml of IL-2 were added on day 0, 1, or 2. The amounts of the different isotypes in the supernatants was determined on day 7, as described in *Materials and Methods,* by ELISA. The results are presented in Table III. The strongest effect on IgE suppression was obtained when IL-2 was added on day 0, but even when it was added on day 2, had still strong suppressive effects. However, the suppression of IgG1 production was never absolute and it was seen only when IL-2 was added on day 0. On the other isotypes IL-4 or IL-2 had no great effects, excepted on the production of IgG2b, where IL-4

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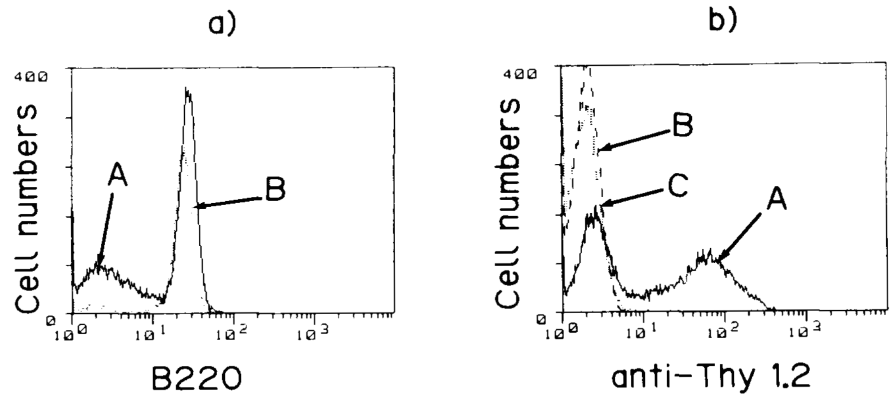

Figure 1. Establishment of purified B cells from whole spleen. *A*, Whole spleen cells; *B*, purified B cells (treated with mixture of mAb); and *C*, purified B cells (treated with anti-BAT antibody).

TABLE I

Suppression by IL-2 of IgE production by B cells stimulated by IL-4

Cell Nos. of Purified B Cells	Cultured with			IgE Levels (ng/ml) in Supernatants at Day 7 as Determined	
	LPS[a]	IL-4[b]	IL-2[c]	BALB/c	Nude
5×10^5	+	−	−	<2.0	<2.0
	−	+	−	<2.0	<2.0
	−	−	+	<2.0	<2.0
	+	+	−	89.8	95.1
	+	+	+	<2.0	<2.0
2×10^5	+	−	−	<2.0	<2.0
	−	+	−	<2.0	<2.0
	−	−	+	<2.0	<2.0
	+	+	−	36.1	42.3
	+	+	+	<2.0	<2.0

[a] Added on day 0, final concentration 10 μg/ml.
[b] Added on day 1, final concentration 100 U/ml.
[c] Added on day 0, final concentration 50 U/ml.

TABLE II

Neutralization by anti-IL-2 mAb of suppression of IgE production obtained by IL-4 from LPS-stimulated B cells

Cell Nos. of Purified B Cells	LPS[a]	IL-4[b]	IL-2[c]	mAb (ng/ml)	IgE Levels (ng/ml) in Supernatant on Day 7
5×10^5	1) +	−	+		<2.0
	2) +	+	−		94.3
	3) +	+	+		<2.0
	4) +	+	+	200 anti-IL-2[d]	82.5
	5) +	+	+	100	58.2
	6) +	+	+	50	26.2
	7) +	+	+	200 anti-IFN-γ[e]	<2.0
	8) +	+	+	100	<2.0
			IFN-γ[f]		
	9) +	+	−		93.1
	10) +	+	+		<2.0
	11) +	+	+	200 anti-IFN-γ[e]	69.9
	12) +	+	+	100	46.0
2×10^5	1) +	−	+		<2.0
	2) +	+	−		33.0
	3) +	+	+		<2.0
	4) +	+	+	200 anti-IL-2[d]	27.2
	5) +	+	+	100	20.1
	6) +	+	+	50	21.3
	7) +	+	+	200 anti-IFN-γ[e]	<2.0
	8) +	+	+	100	<2.0
			IFN-γ[f]		
	9) +	+	−		35.3
	10) +	+	+		<2.0
	11) +	+	+	200 anti-IFN-γ[e]	36.2
	12) +	+	+	100	23.7

[a] 10 μg/ml LPS added on day 0.
[b] IL-4 100 U/ml added on day 1.
[c] IL-2 50 U/ml added on day 0 (rows 1, 3 to 8).
[d] Anti-IL-2 (DMS-1) added on day 0.
[e] Anti-IFN-γ (RA-6A2) added on day 0.
[f] IFN-γ 400 U/ml added on day 1 (rows 10 to 12).

was suppressive, but IL-2 had no effects.

Neutralization of IgE and IgG1 suppression by IL-2 with anti-IL-2 mAb. The 5×10^5 or 2×10^5 purified B cells from BALB/c mice were stimulated as in the previous experiments, with 10 μg/ml of LPS on day 0 and IL-4 at final concentration of 100 U/ml was added on day 1. When indicated, IL-2 at a final concentration of 50 U/ml was added on day 0. Also, when indicated, the monoclonal murine IgG1 anti-human IL-2 (DMS-1), the rat mAb to murine IL-2 (S4B6), or the monoclonal anti-IFN-γ (RA-6A2) were added on day 0. The results in Table IV show that IL-2 produced a complete suppression of IgE and a partial suppression of IgG1. The suppression of IgE production was abrogated with both mAb to IL-2. The suppression of IgG1 production could not be verified with the mAb DMS-1, as this anti-human IL-2 antibody is of murine origin and the class of this antibody is IgG1. With the other mAb (S4B6) the suppression of IgG1 was nearly completely abrogated. Excepted IgG2b (the production of which was reduced by IL-4) no other changes were observed either with IL-2 or the mAb on the production of other isotypes.

Influence of IL-2 on number of cells expressing CD23. In view of the fact that 2×10^5 cells/well gave similar results to those obtained by culturing 5×10^5 cells/well in the experiments presented above, the only difference being quantitative and we did not observe any effect of overcrowding in the cultures with 5×10^5 cells/well we cultured 5×10^5 cells for 3 days without or with IL-2 for investigating the effect of IL-2 on CD23 expression. Purified B cells from BALB/c or nude mice were cultured with LPS added on day 0 and IL-4 added on day 1. The cells were stained on day 3 with fluorescein-labeled anti-CD23 antibody. A substantial number of cells were stained (Fig. 3). If IL-2 was added on day 0 to these cultures there was a decrease of the number of cells stained by the anti-CD23 antibody (24). In these cultures fractions of $CD23^+$ B cells without IL-2 was 38.4% ± 3.2 (BALB/c) or 48.2% ± 4.1 (nude), whereas that of B cells with IL-2 was 16.4% ± 5.1 (BALB/c) or 15.2% ± 4.3 (nude). The mean fluorescence of CD 23 positive B cells with IL-2 (BALB/c: 68.8 ± 5.0, nude: 23.2 ± 4.3) was less than without IL-2 (BALB/c: 80.5 ± 4.0, nude: 100.2 ± 4.2).

DISCUSSION

IL-2 and IL-4 are lymphokines secreted by CD4 cells. In cloned lymphocytes IL-2 is secreted by the subset Th1, and IL-4 by the subset Th2 (6, 7). Both lymphokines are

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TABLE III
Effects of IL-2 and IL-4 on antibody production of Ig classes

Cell Nos. of Purified B Cells	Cultured with			Antibody in ng/ml				
	LPS[a]	IL-4[b]	IL-2[c]	IgE	IgG1	IgG2a	IgG2b	IgM
				BALB/c				
5 × 10^5	+	+		<2.0	1562	786	1484	49980
	+	+		60.1	8870	553	417	75300
	+	+	Day 0	<2.0	3570	766	378	40280
	+	+	Day 1	22.3	12040	600	456	39640
	+	+	Day 2	33.9	11370	583	446	63090
				Nude				
	+	−		<2.0	597	883	1290	60140
	+	+		62.0	9040	689	563	69930
	+	+	Day 0	18.4	3650	708	572	75740
	+	+	Day 1	21.3	10970	552	475	56190
	+	+	Day 2	25.2	12760	621	537	77900
				BALB/c				
2 × 10^5	+	−		<2.0	1020	480	960	34280
	+	+		35.1	5130	317	222	32010
	+	+	Day 0	<2.0	2430	470	195	27500
	+	+	Day 1	7.0	6720	350	250	27040
	+	+	Day 2	16.3	6260	440	242	43460
				Nude				
	+	−		<2.0	350	448	830	41300
	+	+		36.4	5250	412	324	48250
	+	+	Day 0	6.2	1860	326	330	42320
	+	+	Day 1	8.0	6980	316	263	38600
	+	+	Day 2	10.6	7232	365	306	53830

[a] LPS 10 μg/ml added on day 0.
[b] IL-4 100 U/ml added on day 1.
[c] IL-2 100 U/ml added on day 0, 1, and 2. Antibody content determined by ELISA on day 7.

TABLE IV
Neutralization of IgE and IgG1 suppression of LPS-stimulated B cells by IL-2 with anti-IL-2 mAb

Cell Nos. of Purified B Cells	LPS[a]	IL-4[b]	IL-2[c]	mAb ng/ml	Antibody in ng/ml				
					IgE	IgG1	IgG2a	IgG2b	IgM
5 × 10^5	+	−	−		<2.0	178	980	970	59410
	+	+	−		61.1	5190	698	584	71060
	+	+	+		<2.0	1160	689	574	61980
	+	+	+	Anti-IL-2[d] 200	52.6	ND	970	572	61080
	+	+	+	100	50.6	ND	873	563	67550
	+	+	+	Anti-IL-2[e] 200	52.5	5120	698	563	62370
	+	+	+	100	43.8	3550	760	582	66930
	+	+	+	Anti-IFN-γ[f] 200	<2.0	1250	610	440	69370
	+	+	+	100	<2.0	1330	718	553	68470
2 × 10^5	+	−	−		<2.0	430	620	610	40880
	+	+	−		35.7	3560	420	340	49040
	+	+	+		<2.0	740	400	330	42690
	+	+	+	Anti-IL-2[d] 200	30.0	ND	610	330	42100
	+	+	+	100	28.4	ND	540	320	46590
	+	+	+	Anti-IL-2[e] 200	25.3	3510	410	350	43000
	+	+	+	100	28.0	2410	460	340	46150
	+	+	+	Anti-IFN-γ[f] 200	<2.0	850	350	230	47900
	+	+	+	100	<2.0	860	430	320	47300

[a] LPS 10 μg/ml added on day 0.
[b] IL-4 100 U/ml added on day 1.
[c] IL-2 50 U/ml added on day 0.
[d] DMS-1 added on day 0.
[e] S4B6 added on day 0.
[f] Anti-IFN-γ added on day 0. Antibody content determined by ELISA on day 7.

pleiotropic, i.e., they have several different action on cells from the lymphocyte lineage.

The action of IL-2 on T cells is well documented (15). The action of IL-2 on B cells was also investigated and it was found that B cells express receptors for, and proliferate in response to IL-2 (25). Recently, Karasuyama et al. (26) demonstrated that IL-2 may induce maturation of resting mouse B lymphocytes and may propagate proliferation of activated B cells blasts.

The action of IL-4 on T cell is also well documented (27). For example it cause survival and proliferation of the IL-2-dependent T cell line CTLL (28). When first identified as B cell stimulatory factor-1, it has been shown that it is a potent factor for IgG1 secretion (29). Soon afterward, it has been shown that B cell stimulatory factor-1 is involved in the production of IgE from LPS-activated lymphocytes (8). It was then called IL-4 (30, 31). In previous experiments we found that when IL-4 is added to B cells, from BALB/c, CB-20, SJL, SJA/9, or even nude mice, stimulated 24 h previously by LPS, Ig molecules of the IgG1 and IgE isotypes were produced (9).

IL-4 has also other interesting action on B cells, it increases the number of Ia molecules on the surface of B cells (32) and it increases the number of CD23 molecules (the low affinity IgER) on resting B cells (33). IL-2 and IL-4, when added together to culture of Th1 and Th2 cells,

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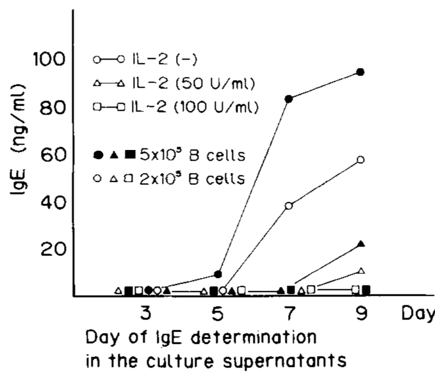

Figure 2. IgE production from LPS-stimulated B cells by IL-4 with or without addition of IL-2. IgE was determined by ELISA as described in *Materials and Methods*.

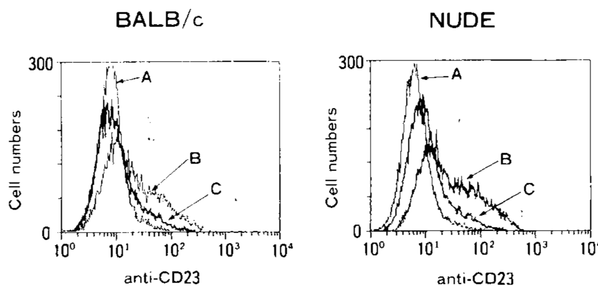

Figure 3. Decreased expressions of CD23⁺ B cells induced by IL-2. The 5×10^5 purified B cells (BALB/c or nude) were cultured in 200 µl with LPS plus IL-4 in the presence of IL-2 (*C*) in the absence of IL-2 (*B*) and stained for CD23 on day 3. *A*, Auto fluorescence. *B* and *C*, B cells stained with monoclonal FITC-labeled anti-CD23 antibody.

may have a synergistic action (34).

Suppression of IgE production with IFN-γ was described by Coffman and Carty (10). Our experiments show that B cells from BALB/c or nude mice cultured in vitro secrete substantial amount of IgE and that this secretion is inhibited by the addition of rIL-2 (Table I; Fig. 2). The inhibition is dependent on the time of addition of rIL-2. As can be seen in Table III, if rIL-2 was added on day 0 no IgE secretion was detected in BALB/c mice and only 30% (5×10^5 B cells) or 17% (2×10^5 B cells) of IgE was produced in nude mice as compared to the controls (without addition of rIL-2). Even if added on day 2, the inhibition was more than 50% (5×10^5 B cells) or 46% (2×10^5 B cells). However, although addition of rIL-2 on day 0 did produce inhibition of IgG1 secretion (the IgG1 produced was 40% (5×10^5 B cells and 2×10^5 B cells), from BALB/c and 16% (5×10^5 B cells), or 19% (2×10^5 B cells) from nude mice compared to controls), no inhibition of IgG1 was observed if the rIL-2 was added on days 1 or 2. The secretion of other isotypes were not modified by addition of the lymphokines excepted that the secretion of IgG2b was reduced by addition of rIL-4. That the inhibition by rIL-2 was due to this lymphokine is shown in Tables II and IV. Addition of anti-IL-2 antibody reduced the inhibition whereas addition of anti-IFN-γ had no action on the reduction produced by IL-2. That the anti-IFN-γ was active was shown by the fact that it reduced the inhibition produced by IFN-γ.

Recently Fernandez-Botran et al. (34) found that IL-2 and IL-4 may act synergistically if added together on Th1 and Th2 cells. However, the present observation of inhibitory action of IL-2 on Ig secretion produced by IL-4 was not yet described. Although the action of IL-2 on resting T and B cells was investigated by many (6–13, 25, 27–32, 34) the action of IL-2 on B cells secreting IgG1 or IgE was not yet investigated. Recently, Finkelman et al. (35) had shown that anti-IL-4 in vivo suppresses selectively anti-TNP-IgE but not IgG1 production.

Addition of IL-2 decreased by 50% or more the number of CD23 molecules stained by fluorescent anti-CD23 antibody on the B cells as shown in the experiment presented in Figure 3 and decreased also the mean fluorescent staining (see *Results*). We had similar results in many other experiments. Why is CD23 decreased on the surface of B cells by IL-2 (Fig. 3) and how does this relate to the inhibition of the secretion of IgE are problems to be investigated in the future.

It is known that IL-2 is an important factor for cell proliferation for T(7) and B(26) cells. It is also well known that proliferating cells do not exercise other activities during proliferation, and that Ig-secreting cells do not proliferate during secretion. The fact that suppression was greater if IL-2 was added on day 0, than later, supports the possibility, that the proliferative action of IL-2, inhibited production of IgE and IgG1.

The suppression of IgE and IgG1 secretion by IL-2 is probably different from that produced by IFN-γ. It is possible that there are several suppressor pathways. The IgE suppression by IL-2 and IFN-γ is an example. We would like to recall, that several years ago we described IgE suppression in SJL mice (4); and could not demonstrate suppressor factor(s) or bona fide suppressor cell(s). It is also possible that suppression of Ig secretion (particularly IgE and even IgG1) might be obtained by the products of T cells that are not "suppressor cells" during all their life span. However, it is not excluded that suppression might be the result of suppressor cell(s) and/or suppressor factor(s). Therefore suppression of Ig secretion might be the end result of several independent pathways. One of these pathways could be the action of IL-2 as mentioned above and another could be the action of IFN-γ. The third could be brought about by the bona fide suppressor cell(s) and/or factor(s).

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