DIFFERENTIAL REGULATION OF IgG1 AND IgE SYNTHESIS BY INTERLEUKIN 4

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Interleukin 4 (IL-4)/B cell stimulatory factor 1 is a T cell–derived lymphokine that has effects on virtually all cells of hematopoietic origin. In particular, it strikingly enhances the secretion of IgG1 and IgE in vitro and of IgE in vivo (1–10). IL-4 acts on cells that lack surface IgG to cause IgG1 production (1, 11), strongly suggesting that it promotes Ig class switching rather than the selective expansion of previously switched cells.

In the course of studies using rIL-4 concentrations substantially higher than those previously reported, we observed a number of new and interesting properties of IL-4 in the promotion of IgG1 and IgE production by murine B cells stimulated by bacterial LPS in vitro. We report here that the IL-4 dose/IgG1 response curve is bimodal with peaks at 100 and 10,000 U IL-4/ml and with a nadir at 600 U/ml. Furthermore, very substantial amounts of IgE (~1 μg from 2×10^4 cells initially cultured) are produced by B cells stimulated with LPS and 10,000 U/ml of IL-4. Finally, marked suppression of IgM and IgG2a as well as IgG3 and IgG2b (12) occurs with IL-4 concentrations of ~600 U/ml. These stimulatory and suppressive activities cannot be accounted for by changes in viable cell yields or [^{3}H]thymidine incorporation and can be completely reversed by a monoclonal anti–IL-4 antibody. We further demonstrate that IL-4 acts both early and late in the culture period with markedly different effects on IgG1 and IgE secretion. Our results indicate that IL-4 differentially regulates IgG1 and IgE secretion.

Materials and Methods

Animals. Pathogen-free female DBA/2 mice, 8–12 wk old, were obtained from DCT Animal Program, National Cancer Institute (Frederick, MD).

Culture Medium (RPMI). RPMI 1640 (Biofluids, Inc., Rockville, MD) supplemented with 10% FCS (HyClone Laboratories, Inc., Logan, UT), 2 mM L-glutamine, 0.05 mM 2-ME, 50 μg/ml penicillin, and 50 μg/ml streptomycin was used for culturing cells.

Reagents and Monoclonal Antibodies. Percoll was obtained from Pharmacia Fine Chem-

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icals, (Piscataway, NJ). LPS W extracted from *Escherichia coli* 0111:B4 was obtained from Difco Laboratories, Inc. (Detroit, MI) and was used at 20 μg/ml in all experiments. rIL-4, kindly provided by Immunex Corp., Seattle, WA, was obtained from a yeast expression system using the alcohol dehydrogenase 2 promoter and the α-factor gene leader sequence to direct synthesis and secretion. The material was used in the form of a yeast cell supernatant that had an activity of 250,000 U/ml. Unitage was measured, by Immunex, in an anti-IgM costimulation assay in which 1 U of activity was equal to 5 pg of purified IL-4 (13). Purified T cell–derived IL-4, kindly provided by Dr. Junichi Ohara of this laboratory, was used in some experiments. This material was derived from EL-4 cells by induction with PMA and was purified to homogeneity by affinity chromatography and reverse-phase HPLC (14). Monoclonal rat IgG1 anti–IL-4 antibody (11B11) (15) was purified from ascitic fluid by ammonium sulfate precipitation, passage over a DEAE cellulose column, followed by Sephadex G200 gel filtration. To remove any residual mouse IgG1, the anti–IL-4 antibody preparation was adsorbed over a 20.10.9.1 column (monoclonal mouse IgG2a [b allotype] anti–mouse IgG1 [a allotype]) (16). Monoclonal rat IgG1 anti-NP antibody (J4.1) was a kind gift from Dr. R. Coffman from DNAX (Palo Alto, CA) and was similarly purified from ascites and used as a control for 11B11.

Preparation of B Cells. Enriched populations of B cells were obtained from spleen cells from which T cells had been eliminated by the method of Leibson et al. (17). Small, dense B cells were separated by the modified (18) discontinuous Percoll-gradient centrifugation procedure of DeFranco et al. (19). Cells forming a band between 66 and 70% Percoll and having a density of 1.081–1.086 g/ml were considered to be resting B cells and were used in all experiments.

Cell Cultures. Functional assays were carried out in 96-well flat-bottom plates (Costar, Cambridge, MA) in a 200-μl final volume. Plates were incubated at 37°C in a humidified 6% CO_2 atmosphere. DNA synthesis was determined by [^{3}H]thymidine uptake (2 μCi/well; 6.7 Ci/nmol; 1 mCi = 37GBq; ICN K & K Laboratories Inc., Irvine, CA) over a 6-h period. Cells were harvested onto glass filter paper and [^{3}H]thymidine incorporation was determined by liquid scintillation spectrometry. For preincubation experiments, resting B cells were cultured in 25-cm^2 tissue culture flasks (Corning Glass Works, Corning, NY) at 2.5 × 10^5 cells/ml in the presence of LPS and IL-4 (600 or 10,000 U/ml) for 48 h. Cells were then washed three times in cold HBSS, counted, and replated into 96-well flat-bottom plates (Costar) for further analysis.

Measurement of Ig Isotype Concentrations in Culture Supernatants. Ig isotype concentrations were measured by an ELISA using Immulon 2, 96-well flat-bottomed ELISA plates (Dynatech Laboratories, Inc., Alexandria, VA) and has been described by us in detail elsewhere (11). Briefly, a fluorescent product, generated from cleavage of 4-methyl umbilliferyl phosphate (Sigma Chemical Co., St. Louis, MO) by specifically bound alkaline phosphatase–conjugated antibodies, was quantitated on a fluorometer (Allergenetics, a division of Axonics, Inc., Mountain View, CA). Fluorescence units were converted to Ig concentrations by extrapolation from standard curves, determined using purified myeloma proteins of known concentration. Each assay system showed no significant cross-reactivity or interference from the concentration of other isotypes (IgM, IgD, IgG3, IgG1, IgG2b, IgG2a, IgE, IgA) found in the culture supernatants. For some IgG1 determinations in which high concentrations of anti–IL-4 (11B11) and anti-NP (J4.1) antibodies were present in culture supernatants, ELISA wells were coated with the monoclonal anti-IgG1 antibody, 20.10.9.1. The 11B11 and J4.1 preparations had been adsorbed on a 20.10.9.1 column. After addition of culture supernatants and standards and washing, polyclonal rabbit anti–mouse IgG1 (20) was added followed by alkaline phosphatase goat anti–rabbit IgG (21). In this assay, 1 mg/ml of either 11B11 or J4.1 scored as 100 ng/ml of IgG1 (0.01%) which was too low to influence the measurements of IgG1 concentrations in the culture supernatants.

Results

IL-4 Causes Bimodal Stimulation of IgG1 Production, Secretion of Large Amounts of IgE, and Profound Suppression of Other Ig Isotypes. Purified, resting B cells

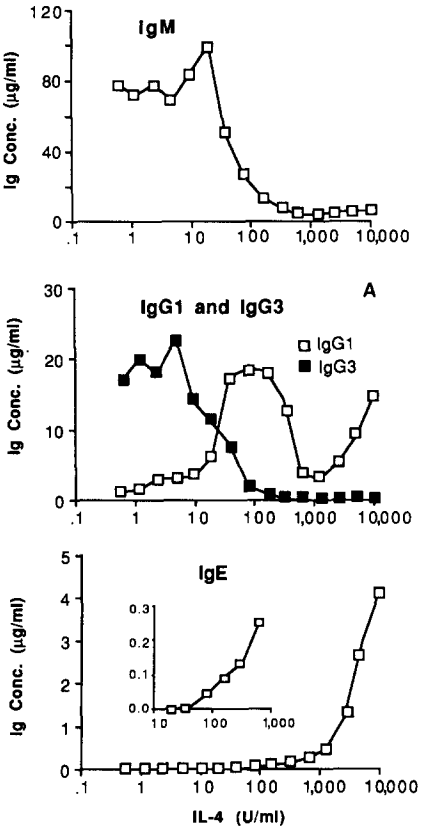

FIGURE 1. IL-4 modulates Ig isotype secretion by LPS-stimulated murine B cells. Purified, resting B cells were distributed into 96-well plates at 2×10^4 cells/0.2 ml and stimulated with 20 μg/ml LPS. Increasing concentrations of IL-4 were added and Ig isotypes were measured in culture supernatants by ELISA at the end of 6 d.

were stimulated with LPS and increasing concentrations of rIL-4. Production of Ig of various isotypes was measured on the sixth day of culture. The IL-4 dose/IgG1 response curve was strikingly bimodal (Fig. 1). IgG1 levels peak at IL-4 concentrations between 40 and 160 U/ml to levels of ~18.5 μg/ml compared with 1 μg/ml in cells stimulated with LPS alone. However, with increasing IL-4 concentrations, IgG1 levels progressively decline reaching a nadir at ~1,000 U/ml (3.0 μg/ml). A further increase in IL-4 concentration to 10,000 U/ml results in a progressive rise in the level of IgG1 to 15.0 μg/ml.

The amount of IgE appearing in these cultures rises progressively with increasing IL-4 concentrations reaching a concentration of 4.2 μg/ml at 10,000 U/ml compared with <0.001 μg/ml in cells stimulated with LPS alone (Fig. 1). At IL-4 concentrations at which IgG1 secretion reaches its first peak (40–160 U/ml), IgE levels, although substantially above those obtained with LPS alone, are only 2% of maximal. No suppression of IgE production at intermediate IL-4 concentrations is observed. The amount of IL-4 needed for optimal IgE secretion is similar to that required for the second peak for IgG1 production and is 100-fold higher than that needed for the first peak of IgG1 production.

As IL-4 concentrations are increased, IgM and IgG3 (Fig. 1) and IgG2b and IgG2a production (Table I) show progressive, marked declines over amounts obtained with LPS stimulation only. Significant degrees of suppression of these

TABLE I
IL-4 Suppresses IgG2b and IgG2a Secretion by LPS-stimulated B Cells

Treatment	Ig concentration	
	IgG2b	IgG2a
	ng/ml	
LPS	950	70
LPS + IL-4 (100 U/ml)	550	50
LPS + IL-4 (500 U/ml)	55	6
LPS + IL-4 (2,500 U/ml)	20	6

Purified, resting B cells were distributed into 96-well plates at 2×10^4 cells/0.2 ml and stimulated with LPS (20 μg/ml). 100, 500, or 2,500 U/ml IL-4 was added to selected wells and culture supernatants were removed on day 6 for determination of IgG2b and IgG2a concentrations by ELISA.

isotypes are generally seen at IL-4 levels at which the initial peak IgG1 secretion is obtained (40–160 U/ml). Maximal degrees of suppression, ranging from 95 to 98% for these isotypes, were observed at 300–600 U IL-4/ml. Concentrations of IL-4 up to 10,000 U/ml fail to enhance either IgA or IgD secretion over the undetectable levels seen with LPS stimulation only (data not shown).

We also studied Ig production by B cells stimulated with LPS and purified T cell–derived IL-4, in concentrations from 10 to 1,000 U/ml. We observed both the initial peak and nadir of IgG1 production, stimulation in IgE production, and suppression of IgM, IgG3, IgG2b, and IgG2a (data not shown), confirming that both recombinant and T cell–derived IL-4 have similar functions.

Anti–IL-4 Antibody Reverses Both the Enhancement and Suppression of Ig Isotype Secretion by IL-4. Monoclonal anti–IL-4 antibody was used to assess whether the dramatic changes in Ig isotype secretion induced by IL-4 in LPS-stimulated B cell cultures was an effect of IL-4 itself and not of some possible contaminant. An isotype-matched mAb (J4.1) was used as a control. The cells were stimulated with LPS and 600 U/ml of IL-4, a concentration which yields a nadir in the IgG1 response curve. Addition of increasing concentrations of anti–IL-4 Ab to cultures stimulated with LPS and 600 U IL-4 initially produced a rise in IgG1 levels and then a progressive decline to levels similar to those obtained in cultures to which LPS but no IL-4 had been added (Fig. 2). Addition of J4.1 had no effect. This result indicates that both the first peak and the nadir in the IL-4 dose/IgG1 response curve is due to IL-4 and not to some possible contaminant in the IL-4 preparation.

The addition of 600 U IL-4/ml caused a 94% diminution in IgM secretion. Addition of increasing amounts of anti–IL-4 produced a steady rise in IgM concentrations approaching that observed in response to LPS alone indicating that the suppressive effect of IL-4 on IgM production is also an effect of IL-4.

In a separate experiment, we evaluated the capacity of anti–IL-4 to inhibit IgE production in response to LPS and 10,000 U/ml of IL-4. Cells stimulated with LPS only produced <0.001 μg/ml of IgE. Addition of 10,000 U IL-4/ml gave 4 μg IgE/ml. Addition of increasing amounts of anti–IL-4 to these cultures caused a progressive decline in IgE concentrations to 0.009 μg/ml whereas addition of

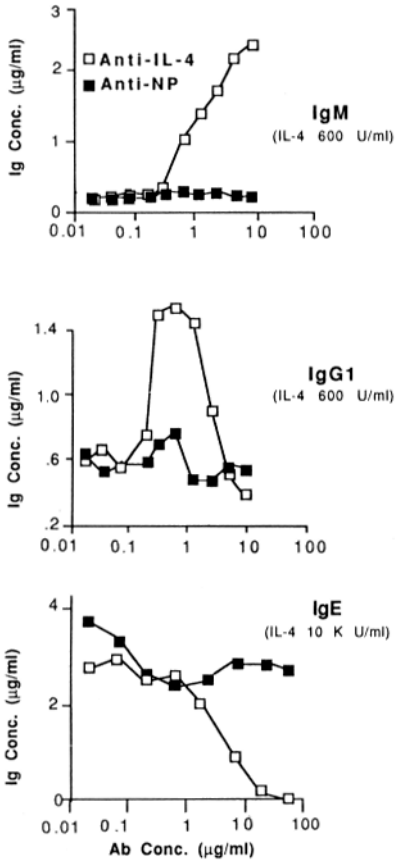

FIGURE 2. Anti–IL-4 antibody reverses the effect of high concentrations of IL-4. Purified, resting B cells were distributed into 96-well plates at 2×10^4 cells/0.2 ml and stimulated with 20 μg/ml LPS and either 600 U/ml (for IgM and IgG1) or 10,000 U/ml (for IgE) IL-4. In addition, increasing amounts of either anti–IL-4 (11B11) or control anti-NP (J4.1) antibody were added (0.03–10 μg/ml for IL-4 600 U/ml or 0.03–70 μg/ml for IL-4 10,000 U/ml). Ig isotypes were measured in culture supernatants by ELISA at the end of 6 d.

J4.1 at similar concentrations had no effect (Fig. 2). In this experiment, we also measured IgG1 production in response to 10,000 U/ml of IL-4, the concentration that causes the second peak of IgG1. Anti–IL-4 inhibited this response whereas J4.1 had no effect (data not shown). These results indicate that even for effects that require very large amounts of the IL-4 preparation, the active entity is IL-4.

Changes in Ig Isotype Secretion Induced by IL-4 Cannot Be Accounted for by Changes in Cell Yield or [^{3}H]Thymidine Incorporation. Resting B cells were stimulated with LPS; IL-4 (125,600, and 10,000 U/ml) was added to selected wells. Viable cell yields and [^{3}H]thymidine incorporation were measured on each of days 2–6 and culture supernatants were tested for Ig isotype concentrations on day 6. The addition of IL-4 at 125 and 600 U/ml caused a modest suppression in viable cell yields on day 3 and 4 (16–22% and 21%, respectively) and [^{3}H]thymidine incorporation (17–34% and 16–31%, respectively) over that observed with LPS stimulation only (Fig. 3 and Table II). The greatest degree of suppression was seen with 10,000 U/ml of IL-4 (44–63% for cell yield and 38–44% for [^{3}H]-thymidine incorporation). Since IgM secretion was inhibited by 95% and IgG3 by 99% at an IL-4 concentration of 600 U/ml, this inhibition could not be ascribed to generalized inhibition of cell growth or survival. Furthermore, IgG1

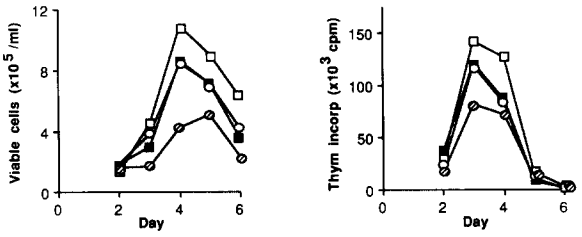

FIGURE 3. IL-4 induces changes in viable cell yields and thymidine incorporation of LPS-stimulated B cells. Purified, resting B cells were distributed into 96-well plates at 2×10^4 cells/0.2 ml and stimulated with 20 μg/ml LPS. IL-4 (0 [□], 125 [○], 600 [■], or 10,000 [⊘] U/ml) was added to selected wells. On each of days 2–6, wells were harvested for determination of viable cell yields and [^{3}H]thymidine incorporation. In addition, culture supernatants were removed at the end of 6 d for determination of Ig isotype concentrations by ELISA (Table II).

TABLE II

Ig Isotype Concentrations as Shown by ELISA

Treatment	IL-4	Ig concentration			
		IgM	IgG3	IgG1	IgE
	U/ml	*μg/ml*			
LPS	0	128.0	10.0	4.0	<.001
LPS + IL-4	125	21.2	1.3	28.1	0.12
LPS + IL-4	600	6.5	0.1	5.1	0.22
LPS + IL-4	10,000	7.0	0.1	16.2	1.9

Culture supernatants were removed at the end of 6 d for determination of Ig isotype concentrations. See Fig. 3 for details.

levels decreased from 28.1–5.1 μg/ml (82% suppression) as IL-4 was increased from 125–600 U/ml, despite no change in cell yield or [^{3}H]thymidine incorporation. Finally, the highest IgE levels (1.9 μg/ml) and the second IgG1 peak (16.2 μg/ml) occurred at an IL-4 concentration of 10,000 U/ml where cell yield and [^{3}H]thymidine incorporation were at their lowest levels. Hence, the degree of suppression observed for IgM, IgG3, and IgG1, and the level of enhancement for IgE and the second rise in IgG1 cannot be explained simply by an overall change in cell numbers or [^{3}H]thymidine incorporation.

Short-term Exposure of B Cells to IL-4 Causes Maximal Production of IgG1. B cells were cultured with LPS and either 100 or 600 U/ml of IL-4. To terminate the action of the lymphokine, anti–IL-4 was added on various days of culture. All supernatants were removed 6 d after the initiation of culture for determination of Ig isotype concentrations. Addition of anti–IL-4 at the outset of culture completely blocks the stimulation of IgG1 (Fig. 4) and of IgE and the inhibition of IgM (data not shown).

Delaying the addition of anti–IL-4 by 2 d results in IgG1 production in response to 100 U/ml of IL-4 equivalent to that obtained if IL-4 was present for the entire culture period. By contrast, stimulating B cells with 600 U/ml of IL-4 for only the first 2 d caused greater production of IgG1 than was observed with either 600 or 100 U/ml of IL-4 present for the entire culture (Fig. 4).

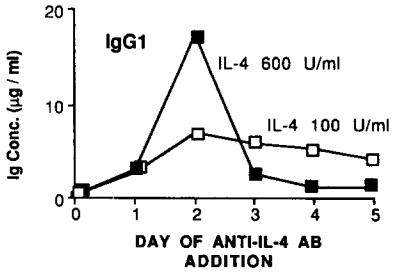

FIGURE 4. Duration of IL-4 exposure influences levels of secreted IgG1. Purified, resting B cells were distributed into 96-well plates at 2×10^4 cells/0.2 ml and stimulated with 20 μg/ml LPS. Either 100 (□) or 600 (■) U/ml IL-4 was added at the beginning of culture. On each of days 0–5, 20 μg/ml anti–IL-4 Ab was added to different wells (sufficient to completely terminate the action of IL-4). Ig isotypes were measured in culture supernatants at the end of 6 d by ELISA.

Indeed, the presence of 600 U/ml of IL-4 for the entire 6-d culture period consistently leads to the lowest levels of IgG1 in the bimodal response to the lymphokine while 100 U/ml represents one of the two maxima of the IL-4 dose/IgG1 response curve. This "hyperstimulatory" effect of 600 U/ml of IL-4 is lost if the addition of anti–IL-4 antibody is delayed until day 3; the presence of this concentration of IL-4 for an additional day causes a marked diminution in the amount of IgG1 produced, to levels below that stimulated by 100 U/ml IL-4. This suggests that short-term exposure of B cells to IL-4 leads to heightened stimulation for IgG1 production and that longer exposure to high concentrations is suppressive.

Distinctive IL-4 Regulation of IgG1 and IgE Production. The striking diminution in IgG1 production observed when the neutralization of 600 U/ml of IL-4 was delayed from day 2 to 3 of culture indicates that the presence of high concentrations of IL-4 "late" in culture is suppressive for IgG1 production. To examine this in greater detail both for IgG1 and IgE production, B cells were cultured for 2 d with LPS and either 600 or 10,000 U/ml of IL-4 (Fig. 5). The cells were then washed and replated at 3×10^4 cells/0.2 ml with LPS and varying IL-4 concentrations. Culture supernatants were removed for measurement of Ig isotype concentrations after an additional 4 d in culture. B cells cultured with LPS and 600 U/ml of IL-4 for 2 d produced large amounts of IgG1 when cultured for the remaining 4 d in LPS alone. The addition of up to 37.5 U/ml of IL-4 at day 2 had no effect on IgG1 production. Higher concentrations of IL-4 suppressed IgG1 production; the greatest degree of suppression was obtained at 600 U/ml. 10,000 U/ml, although still suppressive, nonetheless led to the production of considerably more IgG1 than that obtained with 600 U/ml during the second phase of culture. This indicates that very high concentrations of IL-4 are less suppressive than intermediate concentrations during the final 4 d of culture.

Culture of B cells with LPS and 10,000 U/ml of IL-4 for the initial 2 d of culture results in the production of IgG1 in amounts similar to that observed for cells cultured with 600 U/ml for the first 2 d. Furthermore, cells initially cultured with 10,000 U/ml are as sensitive to subsequent suppression by IL-4 as those initially cultured with 600 U/ml of IL-4. From this experiment, we conclude

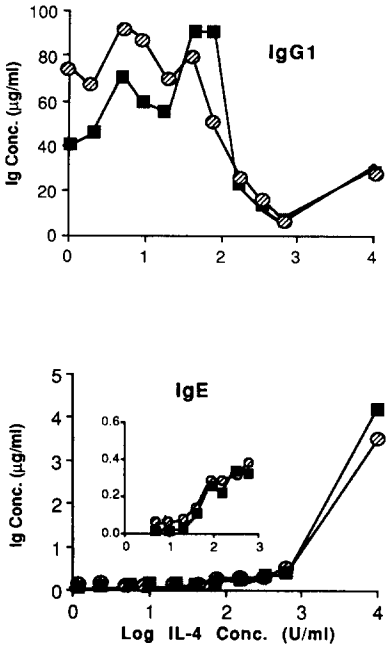

FIGURE 5. 48-hour preincubation with LPS and high doses of IL-4. Purified, resting B cells were cultured in 25-cm^2 tissue culture flasks at 2.5 × 10^5 cells/ml in the presence of LPS and either 600 (▨) or 10,000 (■) U/ml IL-4 for 48 h. Cells were then washed three times in cold HBSS and replated into 96-well plates at 2 × 10^4 viable cells/0.2 ml in the presence of LPS and increasing concentrations of IL-4. Culture supernatants were harvested 4 d after replating into 96-well plates for determination of Ig isotypes by ELISA.

that the difference in IgG1 production in response to 600 and 10,000 U/ml for the entire culture period is due to differences in the late "suppressive" period rather than in the initial "preparatory" period of culture.

Examination of IgE production in this system revealed that cells prepared with IL-4 and LPS for 2 d produced very small amounts of IgE (30–50 ng/ml) at 6 d when cultured in LPS alone for the final 4 d of culture. The presence of IL-4 during the final 4 d of culture stimulated additional IgE production in a dose-dependent manner. In contrast to results for IgG1, no suppression is observed; the amount of IgE produced rises as the concentration of IL-4 present during the final 4 d of culture rises, reaching a maximum (>3.5 μg/ml) at 10,000 U/ml. If no IL-4 is present during the initial 2 d of culture, IgE is not produced in response to addition of even very large amounts of IL-4 during the final 4 d of culture (data not shown). Thus, the presence of IL-4 with LPS during the initial 2 d of culture is essential for IgE production. Furthermore, 600 U/ml of IL-4 is as effective as 10,000 U/ml for this preparatory effect, indicating that the requirement of very high concentrations of IL-4 to obtain substantial IgE production reflects a need for such concentrations in the late part of the culture.

Discussion

IL-4 is a T cell–derived lymphokine that had been previously shown to selectively enhance IgG1 and IgE production and to suppress IgG3 and IgG2b secretion by murine B lymphocytes stimulated by LPS (1–6, 12). Initial studies, while contributing greatly to our knowledge of IL-4–induced regulation of Ig isotype secretion, were limited by the use of crude IL-4–containing supernatants. More recent studies have used purified T cell–derived or rIL-4 but at relatively low concentrations. In the current study, we examined the effects of IL-4 over

a much wider concentration range than previously used (up to 10,000 U/ml [~50 ng/ml]). This has allowed us to observe several properties of IL-4 in the regulation of Ig isotype secretion by LPS-stimulated B cells that had not been previously noted and that may be of considerable physiologic significance. Among these are (*a*) the IL-4–induced increase in IgG1 secretion shows a bimodal dose–response relationship; (*b*) increasing IL-4 concentrations to 10,000 U/ml causes a progressive, marked increase in IgE secretion (>5,000-fold enhancement) to levels >25-fold higher than previously reported; and (*c*) IL-4 causes a marked suppression of IgM and IgG2a secretion as well as of IgG3 and IgG2b production. Measurement of cell yields and of [^{3}H]thymidine uptake indicates that both stimulation of IgG1 and IgE and inhibition of Ig of other isotypes cannot be explained by any general effects on cell proliferation.

Our analysis of both the amounts of IL-4 required and the duration of IL-4 stimulation indicate that control of IgG1 and IgE production is quite distinct. Optimal IgG1 secretion can be achieved by substantially lower concentrations (~100-fold) and a shorter time of exposure to IL-4 than that required for high level IgE secretion.

In light of striking changes in Ig isotype secretion at very high IL-4 concentrations, it was important to determine whether these effects were due to IL-4 or to some possible contaminant. Experiments using the monoclonal anti–IL-4 antibody (11B11) demonstrate unambiguously that IL-4 was critical to all effects described. Furthermore, using T cell–derived IL-4 purified by affinity chromatography and reverse-phase HPLC, we observe similar suppression of IgM, IgG3, IgG2b, and IgG2a, as well as IgG1 and stimulation of IgE at a dose of 1,000 U/ml. The finding that IL-4 obtained from two distinct sources have similar effects on Ig isotype production and that these effects are blocked by monoclonal anti–IL-4 antibody indicates that the pattern of regulation observed is an intrinsic property of IL-4.

One may question the physiological significance of effects seen at such high doses of IL-4. It is quite probable that IL-4 can be delivered to its specific receptor on the B cell surface through intimate physical contact with an IL-4–secreting T cell in an antigen-specific, histocompatibility-restricted fashion. Kupfer et al. have shown that helper T cells align their microtubular-organizing complex and Golgi apparatus toward the contact surface of a linked antigen-presenting cell (22). Within the confines of the "synaptic junction" which forms between interacting T and B cells, it is conceivable that lymphokines could be "focused" at very high local concentrations at the surface of the antigen-presenting B cell surface.

It is of great interest that the same lymphokine, IL-4, controls the expression of two different Ig isotypes, IgG1 and IgE, but that the dose–response curve and time course for this regulation differ strikingly between the two. IgG1 production in response to LPS and IL-4 displays a bimodal dose–response curve. The first peak of IgG1 production occurs at a concentration of IL-4 ~100-fold lower than that required for maximal IgE secretion; the latter, however, is similar to that required for the second peak of IgG1 production. (The second peak occurs at the highest IL-4 concentrations used in these experiments and is thus not necessarily the maximal level that could be achieved. However, in a limited

number of experiments, 30,000 U/ml caused substantial cell death, precluding analysis of the effect of concentrations above 10,000 U/ml). Furthermore, the inhibition of IgG1 production by the addition of IL-4 to B cells that have been cultured for 2 d with LPS and IL-4 has a complex dose–response curve with intermediate concentrations (>100 U/ml) showing striking inhibition of IgG1 production but with very high concentrations (10,000 U/ml) actually causing less inhibition. Although this may reflect IL-4 regulation of a single population of precursors of IgG1-producing cells, it could also be explained by the existence of two populations of precursors of IgG1-producing cells, one stimulated by a low concentration of IL-4 and inhibited by intermediate to high concentrations and a second with IL-4 concentration requirements identical to the precursors of IgE-producing cells. This putative second population of IgG1 producers may also share with the IgE precursors the requirement for a longer duration of exposure to IL-4 and a failure to be inhibited by late addition of IL-4. Indeed, it is conceivable that there is a common precursor for the "high-dose IgG1 producers" and for the IgE producers.

The finding that 2-d exposure of B cells to LPS and IL-4 prepares them for maximal IgG1 responses and that concentrations of 600 and 10,000 U/ml are equally effective for preparation stands in contrast to the dose-dependent inhibition of IgG1 production when additional IL-4 is added to these cells after 2 d of culture. The mechanism underlying the inhibitory effects of "late" IL-4 addition on IgG1 production are unknown. A "two-phase" control of IgE synthesis is also seen, although it is different from the two-phase control of IgG1 production. Incubation of resting B cells with LPS and either 600 or 10,000 U/ml of IL-4 for 2 d prepares these cells, equally well, to secrete large amounts of IgE if 10,000 U/ml are added to culture after 2 d. Thus, for IgE synthesis, the initial phase is relatively concentration independent, just as the initial phase for IgG1 production; the late phase requires a high concentration of IL-4 for IgE production whereas the late presence of IL-4 is inhibitory for IgG1 production.

The finding that IL-4 controls both IgG1 and IgE production is not surprising since they share important biological properties. Both are capable of binding to mast cells and mediating degranulation (23) and both are able to mediate antibody-dependent cellular cytotoxicity by eosinophils for certain parasitic targets (24, 25). IgE, however, is considerably more efficient for mast cell degranulation (23).

In addition, IL-4 is capable of acting as a costimulant for the growth of mast cells (26, 27) and upregulates the receptor for the Fc portion of IgE on B cells (28, 29). It has also been demonstrated that T cell lines, designated T_H2 lines, capable of secreting IL-4 also secrete IL-5 (30, 31; also West, J., F. Carding, R. Rasmeussen, A. Woods, T. Honjo, and K. Bottomly, manuscript submitted for publication). IL-5 has been shown to have growth-promoting properties for eosinophils (32, 33). Finally, it has been recently demonstrated that IL-4 is a potent costimulant for the growth of multiple hematopoietic precursors (34). The selective activation of T_H2 cells by certain parasitic agents, therefore, would allow for a coordinated host response for parasite elimination that would include IL-4 and IL-5 release and the stimulation of IgG1 and IgE secretion. Further-

more, the suppression of secretion of other isotypes may be important in limiting the concentrations of antibodies that compete for antigenic determinants on the "target" but are unable to initiate an appropriate effector function.

On the other hand, the control of IgG1 and IgE are clearly not identical. In vivo, these Igs occur at vastly different concentrations and the frequencies of IgG1- and IgE-secreting cells are enormously different (7). Our results indicate that the regulation of IgG1 and IgE production by IL-4 are not the same. The concentration of IL-4 required to achieve the production of large amounts of IgE is much greater than concentration of IL-4 that is adequate for striking stimulation of IgG1 production.

Monoclonal anti–IL-4 antibody strikingly blocks the increase in serum IgE levels in mice inoculated with the parasitic nematode *Nippostrongylus brasiliensis* and in mice stimulated with anti-IgD antibodies while it has little or no effect on increases in serum IgG1 levels (7). This suggests either that larger amounts of IL-4 are required for IgE than IgG1 production in vivo or that IgE production is exclusively dependent upon IL-4 while IgG1 production may be stimulated by other mechanisms. In either case, the results argue for a differential regulation of IgG1 and IgE production in vivo, just as we have demonstrated for their in vitro regulation. Other examples of such distinctive regulation are the striking induction of IgE in helminth-infected mice with only a moderate increase in IgG1 concentrations (7) and the observation that early in the course of rat schistosomal infections, the predominant protective Ig isotype is the "anaphylactic" rat IgG, IgG2a, while later in the course of the disease, protection is mainly afforded by IgE (35). In view of the very different potencies of murine IgE and IgG1 as anaphylactic antibodies, such distinctive regulation would seem most appropriate.

Summary

IL-4/B cell stimulatory factor-1 is a T cell–derived lymphokine that has been shown to enhance IgG1 and IgE and to suppress IgG3 and IgG2b secretion by B cells stimulated with bacterial LPS. We show here that the stimulation of IgG1 and IgE secretion in response to rIL-4 is differentially regulated. The dose–response curve for IgG1 production is bimodal with peaks at 100 and 10,000 U/ml. IgE production is modest at 100 U/ml and exhibits a progressive enhancement as the IL-4 concentration is increased to 10,000 U/ml, reaching ~1 μg of IgE from an initial cell number of 2×10^4. Both of these effects are reversed by monoclonal anti–IL-4 antibody. Neither the enhancing nor suppressing effects of IL-4 can be explained by changes in viable cell yields or [^{3}H]thymidine incorporation. The production of both IgG1 and IgE is controlled by IL-4 in a two-phase manner. During the initial 2 d of culture with LPS, IL-4 action for both IgG1 and IgE production is relatively concentration independent at doses >600 U/ml. This 2-d treatment leads to maximal IgG1 production at day 6 with no further addition of IL-4. Addition of IL-4 during the final 4 d of culture has no effect at concentrations under 100 U/ml. At higher concentrations, IL-4 is strikingly suppressive for IgG1 production. By contrast, little IgE is produced unless IL-4 is present after 2 d of culture and the response is directly dependent on the concentration of IL-4 during this second phase of culture with maximal responses observed at 10,000 U/ml. These differences in IL-4 requirements for

IgG1 and IgE production, respectively, may have an important role in the regulation of the synthesis of these isotypes in responses to microbial antigens.

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