INDUCTION OF HUMAN IgE SYNTHESIS BY CD4+ T CELL CLONES

Requirement for Interleukin 4 and Low Molecular Weight B Cell Growth Factor

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Although it is known that T cell help is required for the induction of IgE synthesis (1), the precise requirements for IgE synthesis have not been fully delineated. It is known that IL-4 is important for IgE synthesis, since murine B cells stimulated with LPS will synthesize IgE and IgG1 in the presence of IL-4 (2–7). In addition, murine Th2 clones, which produce IL-4 and IL-5, but not Th1 clones, which produce IL-2 and IFN-γ, have been shown to induce IgE synthesis when cultured directly with B cells (8). Furthermore, in vivo administration of anti-IL-4 mAb inhibits murine polyclonal IgE responses, confirming the requirement of IL-4 for IgE synthesis (9, 10). Although other lymphokines that may be involved in IgE synthesis have been purified and cloned, the exact roles of these molecules have been difficult to define in part because many can stimulate both B cells and T cells, often delivering multiple signals to the same lymphocyte (11).

Since LPS does not elicit potent activation signals in human B cells, much of our understanding of the requirements for human IgE synthesis has been generated by examination of IgE synthesis induced by culturing B cell–enriched populations in the presence of IL-4 (12–15). Although IL-4 appears necessary for human IgE synthesis (16), the role of other B cell growth or differentiation factors, or the exact role of subsets of human CD4+ T cells in the induction of IgE synthesis, has not been fully determined.

The purpose of our current studies was to examine the precise requirements for the induction of human IgE synthesis using a panel of CD4+ T cell clones and several different experimental approaches. First, we compared the capacity of several well-characterized alloantigen-specific CD4+ T cell clones, which expressed different

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profiles of lymphokine secretion, to induce IgE synthesis in purified B cells under cognate conditions in which the B cells expressed alloantigens recognized by the cloned T cells. Under such conditions, which mimic MHC-restricted B-T cell interactions (17-19), we demonstrated that not all T cell clones that produce IL-4 are capable of inducing IgE synthesis, indicating that factors in addition to IL-4 are required. We further determined that low molecular weight B cell growth factor (LMW-BCGF)[1] played a significant role in IgE synthesis, since it restored the capacity of some "IgE-nonhelper" T cell clones to induce IgE synthesis. In addition, we examined IgE synthesis induced by cloned T cells pretreated with cyclosporin A to inhibit lymphokine secretion. Using cyclosporin A-pretreated T cell clones, we determined that restoration of the capacity of such T cells to induce IgE synthesis required the addition of both IL-4 and LMW-BCGF. IL-2, but only when added with IL-4 and LMW-BCGF, also had an enhancing effect on IgE synthesis in this system. Collectively with these different experimental approaches, we have demonstrated that IL-4, although required for human IgE synthesis, is by itself not sufficient. Optimal induction of IgE synthesis required exposure of B cells to a particular complex of signals, which included signals provided by the cognate interaction between B cell and T cell, by IL-4, LMW-BCGF, and by IL-2, all of which synergized to stimulate the synthesis of large quantities of IgE.

Materials and Methods

Monoclonal Antibodies. Anti-Leu-1 mAb was purchased from Becton Dickinson & Co. (Mountain View, CA). mAbs against human IgE (numbers 8 and 9) were obtained from Dr. Ruben Siraganian (National Institute of Dental Health, Bethesda, MD). Rat anti-human IL4 mAb, 25D2, was prepared by immunizing a Lewis rat with purified Cos7-expressed human IL-4, and was capable of neutralizing the T cell growth factor bioactivity of rIL-4 on PBMC (20).

Lymphokines. Human rIL-2 and IFN-γ were obtained from Amgen Corp., Thousand Oaks, CA. Human rIL-4 was purchased from Genzyme, Boston, MA and from Amgen Corp. Human rIL-5 (Cos supernatants) were generously provided by Dr. Steven Clark, Genetics Institute (Boston, MA) and by Dr. Frank Lee, DNAX Research Institute. Purified LMW-BCGF was purchased from Cellular Products, Buffalo, NY. Human rTNF-α and human recombinant lymphotoxin (TNF-β) were obtained from Genentech, Inc., S. San Francisco, CA.

Preparation of Peripheral Blood B Cells. PBMC were isolated from the blood of normal adult donors by flotation over Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ). PBMC were then washed three times with PBS, and rosetted with aminoethylisothiouronium-treated sheep cells. The erythrocyte rosette-negative cells were enriched by flotation over Ficoll-Hypaque, and were further purified by treatment with Leu-1 mAb and baby rabbit complement to remove residual T cells (21). The resulting B cell population contained 40-50% monocytes but <1% T cells.

Percoll Gradients. 5-10 $\times$ 10^6 E-rosette (-) cells were placed on discontinous Percoll gradients as described (21), and centrifuged at 1,280 *g* for 15 min at 4°C. Low density and high density B cells were recovered in the fractions between 40/50% and 60/70%, respectively.

Preparation of T Cell Clones. Alloantigen-specific $CD4^+$ T lymphocytes clones were generated by limiting dilution as previously described (22, 23). Briefly, PBMC were stimulated with irradiated allogeneic PBMC for 6 d. Dividing cells were enriched by centrifugation over discontinous gradients of Percoll (Pharmacia Fine Chemicals) at 1,500 rpm for 45 min. Cells at the 30-50% interface were cultured in DME at limiting dilution (0.3 cell/well) with irradi-

[1] *Abbreviations used in this paper:* LMW-BCGF, low molecular weight B cell growth factor; SAC, Staphylococcus A, Cowan strain.

ated PBMC added every 7 d. These $CD3^+$, $CD4^+$, $CD8^-$ clones have been subcloned and maintained in long-term culture in DME containing 10% FCS (Sterile Systems, Inc., Logan, UT) by repeated stimulation with irradiated allogeneic PBMC and IL-2-containing supernatants.

IL-2 Assay. Human IL-2 activity was determined by using the murine IL-2-responsive T cell line HT2 (generously provided by Dr Sam Strober, Stanford University). HT2 cells were added to triplicate wells (5,000 cells/well) of a 96-well plate with or without IL-2-containing supernatants or human rIL-2. mAbs to the mouse IL-2 receptor (7D4 + 3C7 + PC61.53), were added to some wells to specifically inhibit IL-2-induced proliferation. Proliferation of these cells was assessed by the incorporation of [^{3}H]thymidine over the last 7 h of a 24-h culture period. Specificity of the IL-2 assay was assured by the fact that human IL-4 is species specific and does not bind to mouse T cells, by the failure of anti-mouse IL-2 receptor mAb to inhibit proliferation induced with mouse IL-4, and by the failure of mAb 11B11 (anti-mouse IL-4) to inhibit proliferation induced by rIL-2 (24, 25).

Solid-Phase Immunoassay for IL-4. IL-4 activity in T cell clone supernatants was assayed in an ELISA assay, using rabbit anti-human IL-4 IgG and a rat monoclonal anti IL-4 antibody (20). In this assay for human IL-4, no signal was generated by IL-1, IL-2, IL-3, IL-5, IFN-γ or IFN-α, granulocyte/macrophage CSF, granulocyte CSF, B cell stimulatory factor 2/IL-6, or TNF-α and TNF-β.

TNF-α and IFN-γ. TNF-α and IFN-γ levels were determined by ELISAs specific for each cytokine. The assay for human TNF-α detects 50 pg/ml (2.5 U/ml), and the assay for human IFN-γ detects 4 pg/ml (4 U/ml). In these assays, human rTNF-α has a specific activity of 5×10^7 U/mg, and rIFN-γ has a specific activity of 1×10^7 U/mg (26).

TNF-β. TNF-β was assayed using an L-M bioassay with a detection limit of 20 pg/ml (4 U/ml) (27). To determine the concentration of TNF-β, all TNF-α activity was first neutralized by adding a fivefold excess of a rabbit polyclonal anti-human rTNF-α antibody, for at least 30 min before assay at 37°C. This antiserum has a neutralizing titer of $\sim 3 \times 10^4$ L-M units and shows no neutralizing activity against TNF-β. In the L-M bioassay, human rTNF-α has a specific activity of 5×10^7 U/mg of protein, and recombinant human TNF-β has a specific activity of 2×10^8 U/mg of protein (27).

Supernatants. Supernatants were generated from T cell clones (10^6 cells/ml) by stimulation with alloantigen on appropriate stimulator cells (10^6 monocytes/ml) for 24 h. For TNF-α, IFN-γ, and lymphotoxin determinations, supernatants were generated by stimulation with PHA-P (Difco Laboratories, Detroit, MI) for 24 h.

Induction of Ig Synthesis. Cultures were performed in 96-well microtiter plates in DME containing 10% FCS. E-rosette-negative cells (1×10^5 per well) were cultured with 4×10^4 cloned T cells and 2×10^4 irradiated (2,500 rad) monocytes in 200 μl of complete media. Supernatants from triplicate wells were combined after 9 d of culture and assayed in duplicate. ELISAs of supernatants for IgG and IgM were performed as described (22). ELISA for IgE was performed in 96-well polystyrene plates coated with two anti-human IgE mAbs (1 μg/ml). After washing with PBS with 0.1% Tween 20, the plates were blocked with a 0.1% BSA solution for 1 h and washed, and 0.1 ml of appropriately diluted supernatants was added to the wells. Afer 18 h, the plates were again washed, and biotinylated goat anti-IgE (Tago Inc., Burlingame, CA) was added for 60 min. The plates were washed and horse radish peroxidase-labeled streptavidin (Zymed Laboratories, San Francisco, CA) was added for 60 min. Bound enzyme was quantitated using the substrate *o*-phenylene diamine (Sigma Chemical Co., St. Louis, MO) for 30 min, and the absorbance at 414 nm was read on a Bio-Rad Laboratories (Richmond, CA) automatic ELISA reader. Standard curves for IgE were performed using purified IgE (Pharmacia Fine Chemicals). The lower limit of this assay is 0.15 ng/ml. Specificity has been demonstrated by adding human IgG or IgM antibody (up to 500 μg/ml) as sample, resulting in an absorbance no higher than an absorbance equivalent to <0.15 ng/ml.

Cyclosporin A Pretreatment of T Cell Clones. Stock solutions of cyclosporin A (generously provided by Dr. David Winter, Sandoz Research Institute, E. Hanover, NJ) were prepared by dissolving 1 mg in 0.1 ml ethanol, adding 0.02 ml Tween 80, and mixing. RPMI 1630 was added dropwise to a final volume of 1.0 ml. T cell clones were incubated overnight at 1×10^6 cells/ml in 3–6 μg/ml of cyclosporin A at 37°C for 24 hr. Viable cells were recovered, washed, and cultured with purified B cells, as described by Krusemeir and Snow (28).

Results

Study of human IgE synthesis has been hampered not only by the inability of polyclonal B cell activators to induce IgE synthesis but also by the complexity of the different cytokine profiles expressed by cloned human $CD4^+$ T cells, compared with that in the mouse. We initiated our study of induction of IgE synthesis by generating alloantigen-specific $CD4^+$ helper T cell clones that were heterogeneous with regard to their lymphokine profiles. Table I lists the alloantigen-specific $CD4^+$ T cell clones that were used in our studies. Clone H8 secretes IL-2 but not IL-4. Clone D12 secretes IL-4, but not IL-2 or IFN-γ. Clone H9 secretes IL-2, IL-4, and IFN-γ, while clone D11 secretes TNF-α and lymphotoxin, but neither IL-2 nor IL-4. These alloantigen-specific clones have been maintained in long-term culture by repeated stimulation with irradiated feeder cells and have retained their lymphokine profiles for >6 mo. We used this panel of T cell clones to determine the lymphokine requirements for the induction of IgE synthesis.

$CD4^+$ T Cell Clones with Different Lymphokine Profiles Have Differing Capacities to Induce IgE Synthesis. Our first approach in studying the requirements for human IgE synthesis was to examine the induction of IgE synthesis using the $CD4^+$ clones with different lymphokine profiles, and to correlate the capacity to induce IgE with the secretion of various lymphokines. Each T cell clone was cultured with purified B cells expressing the alloantigen recognized by the particular T cell clone. Such conditions mimic normal T cell–B cell MHC-restricted interactions where the B and T cells are bridged via alloantigen, and have been shown to be very effective at inducing IgE synthesis (22, 29, 30). Table II shows the results using a noncytolytic alloantigen-specific $CD4^+$ clone, H9, which secretes both IL-2 and IL-4. Clone H9 was capable of inducing large quantities of all isotypes, including IgG, IgM, IgA, and IgE. Addition of IL-2, or IL-4, or IFN-γ had little effect on Ig synthesis. These results indicate that the clone H9 produced factors important for the synthesis of all Ig isotypes, including IgE, and that providing excess quantities of IL-2, IL-4, or IFN-γ was of little value.

Optimal Induction of IgE Synthesis by Small Dense B Cells Requires the Presence of Cloned T Cells. In the previous experiments, the B cells expressed alloantigens recognized by clone H9 and therefore could be directly stimulated by the T cell clone. However,

TABLE I

The $CD4^+$ T Cell Clones Used in These Studies

Clone	IL-2	IL-4	IFN-γ	TNF-α	Lymphotoxin
	cpm	*pg/ml*	*pg/ml*	*pg/ml*	*pg/ml*
H8	93,580	181	2,000	637	1,740
D12	756	8,145	<400	<50	205
H9	98,905	5,327	2,600	355	90
D11	121	282	4,800	908	2,355
Control	98	<100	<400	<50	<20

Supernatants tested for IL-2 and IL-4 activity were generated by stimulating the T cell clones with purified irradiated monocytes expressing the appropriate stimulatory alloantigens, while supernatants tested for IFN-γ, TNF-α, and lymphotoxin were generated by stimulation with PHA-P (Difco Laboratories), 1:200. Each cytokine assay is described in Materials and Methods.

TABLE II

Induction of Ig Synthesis by a T Cell Clone Secreting Both IL-2 and IL-4

Stimulus	IgG	IgM	IgA	IgE
		ng/ml		
H9	39,232	130,735	42,284	77.28
H9 + IL-2	28,653	71,705	28,659	81.88
H9 + IL-4	35,903	78,955	33,425	109.56
H9 + IFN-γ	30,438	110,445	42,889	92.72
None	94	<58	238	1.95
IL-2	133	<58	351	2.76
IL-4	158	<58	364	3.41

Purified B cells (10^5 cells/well) were cultured for 9 d with and without cloned T cells (4×10^4 cells/well) under cognate T-B cell conditions and the indicated lymphokine. Supernatants were harvested and tested by ELISA for Ig content. IL-2 was used at 2 U/ml, IL-4 at 1,000 U/ml, and IFN-γ at 20 U/ml. In other experiments, addition of larger amounts of IL-2 (up to 20 U/ml), or of IL-4 (up to 2,000 U/ml) gave similar results. Addition of larger amounts of IFN-γ (250–2,500 U/ml) resulted in a 50% decrease in IgE synthesis.

since small quantities of IgE were secreted in the absence of cloned T cells (Table II, lines 5, 6, and 7), it was important to determine the precise role of the T cell clone, and whether some or all of the B cells could respond to soluble lymphokines in the absence of T cells. E-rosette-negative cells were therefore separated on Percoll density gradients into large (low density) and small (high density, resting) B cells. Table III shows that low density B cells secreted IgE spontaneously, and secretion doubled in response to soluble IL-2 plus IL-4 in the absence of T cells. In contrast, small dense B cells did not secrete IgE spontaneously, and addition of IL-2 and IL-4 (Table III, line 4) or of supernatants from clone H9 (data not shown) to purified small dense B cells had no effect on IgE synthesis in the absence of T cells. Moreover, addition of clone H9 to the small B cells resulted in the synthesis of IgG, IgM, and IgE. These results indicated that large B cells secrete IgE spontaneously and can respond to soluble lymphokines. On the other hand, small resting B cells do not

TABLE III

Small Resting B Cells Require the Presence of T Cells for the Generation of Ig Responses

B cells	Clone H9	IgG	IgM	IgE
			ng/ml	
Large (low density)	–	381	194	9.69
Large + IL2 + IL4	–	773	172	17.97
Small (high density)	–	<58	191	<0.31
Small + IL2 + IL4	–	105	345	<0.31
Small	+	103,165	125,275	41.12

Purified B cells were separated on Percoll density gradients into small dense and large partially activated B cells. Such B cells (10^5 cells/well) were cultured for 9 d with and without clone H9 (secreting IL-2, IL-4, and IFN-γ; 4×10^4 cells/well) under cognate conditions and with the indicated lymphokines. IL-2 was used at 4 U/ml and IL-4 was used at 1,000 U/ml.

respond to soluble lymphokines, but require the presence of T cells to stimulate de novo IgE synthesis. In subsequent experiments, therefore, since we wished to study the effect of cloned T cells on the induction of Ig synthesis, we used purified B cell populations that did not respond to soluble lymphokines in the absence of T cells.

Induction of IgE Synthesis in the Presence of mAb against IL-4. Since IL-4 has been shown to be necessary for the induction of IgE (2–9, 12–16), we attempted to selectively inhibit induction of IgE synthesis by clone H9 with the addition of a neutralizing mAb against IL-4. In all experiments (n = 5), using clone H9 and B cells under cognate conditions, the anti-IL-4 mAb failed to inhibit IgE synthesis (data not shown). This is consistent with experiments with murine T cell clones, in which the presence of mAb to IL-4 had little effect on Ig synthesis (31). This was thought to be due to the close physical interaction between T and B cells (32), such that IL-4 is transferred between the cells in a manner that is refractory to antibody blockade. In contrast to these experiments with B cells stimulated under cognate conditions, Fig. 1 shows that using clone H9 under noncognate conditions with large B cells from an allergic donor, the anti-IL-4 mAb was very effective in inhibiting IgE synthesis. These results confirmed that IL-4 is required for the induction of IgE synthesis, in partially activated B cells under noncognate conditions.

Induction of IgE Synthesis Requires the Presence of IL-4. We next examined the ability of a clone that did not produce detectable levels of IL-2 or IL-4 to induce IgE synthesis, to determine if IgE synthesis could occur in the absence of IL-4. Table IV shows that culture of clone D11 with purified B cells resulted in synthesis of some IgG, IgM, and IgA, and these levels dramatically increased with the addition of IL-2. In contrast, IgE synthesis was negligible even in the presence of IL-2, but increased substantially with the addition of IL-4. Spontaneous IgE synthesis by the B cells was minimal, and almost no IgE synthesis occurred with IL-4 in the absence of cloned T cells. Therefore, few partially activated B cells were present in these cultures, and the presence of T cells was required for IgE synthesis. Since addition

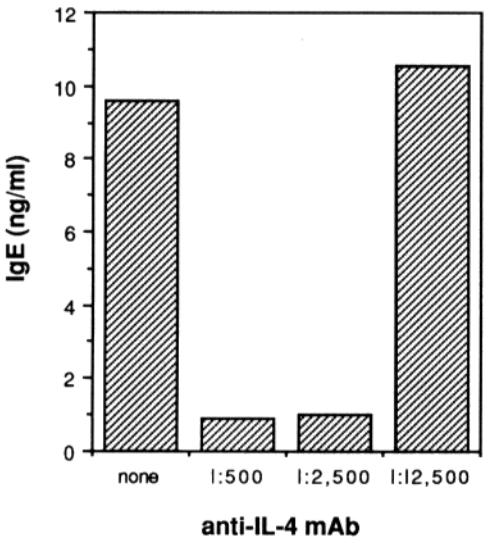

FIGURE 1. IgE antibody synthesis is inhibited by anti-IL-4 mAb under noncognate B-T cell conditions. Anti-IL-4 mAb was added at the indicated dilution to cultures of B cells and clone H9 (secreting IL-2, IL-4, and IFN-γ). The appropriate irradiated monocytes were added to activate clone H9, since the B cells did not express alloantigens recognized by the clone.

TABLE IV
Ig Synthesis Induced by a T Cell Clone that Does Not Secrete IL-4 is Selectively Enhanced by IL-2 or IL-4

Stimulus	IgG	IgM	IgA	IgE
		ng/ml		
D11	34,332	3,233	9,511	1.94
D11 + IL-2	188,620	40,746	58,846	1.96
D11 + IL-4	21,792	10,576	11,877	25.06
None	<39	<58	<270	1.30
IL-2	44	<58	214	<0.31
IL-4	<39	<58	239	0.44

Purified B cells were cultured as described in Table I, with clone D11, which does not secrete detectable levels of IL-2 or IL-4. IL-2 was added at 2 U/ml, and IL-4 was added at 1,000 U/ml. Similar results were obtained in two other experiments.

of IL-2 with clone D11 to B cells selectively increased IgG but not IgE synthesis, it was unlikely that IL-2 affected Ig synthesis indirectly by enhancing T cell survival. Similarly, since addition of IL-4 with clone D11 selectively enhanced IgE synthesis, it was unlikely that IL-4 affected Ig synthesis by enhancing T cell survival. Rather these results suggest that IL-2 and IL-4 have differential but direct effects on the B cell, that IL-2 is a potent B cell differentiation factor (especially for IgG synthesis), and support the idea that while IgG synthesis does not require IL-4, IgE synthesis is absolutely dependent on the presence of IL-4.

An IL-2-producing T Cell Clone that Suppresses IgE Synthesis. We next examined the capacity of another clone, H8, which produced IL-2 but not IL-4, to induce IgE synthesis. Like mouse Th1 clones, this clone was unable to induce IgE synthesis when cultured with B cells expressing the appropriate alloantigen (Fig. 2). Addition of IL-2 or of IL-4 did not enhance the synthesis of IgE (data not shown). In fact, in coculture experiments, clone H8 inhibited synthesis of IgG, IgM, IgA, and IgE induced by clone H9 (Fig. 2). Thus this T cell clone had an inhibitory role on IgE synthesis, similar to that which has been reported in the past for IgG synthesis with both human and murine T cell clones (23, 33).

IL-4 Is Required for the Induction of IgE Synthesis But Is Not Sufficient Alone. Because IL-4 appeared necessary for the induction of IgE, we expected that clone D12, which produces IL-4 but not IL-2, would be very effective in inducing IgE synthesis when cultured with B cells. However, clone D12 induced only modest amounts of IgG or IgE when cultured with B cells expressing the target alloantigen (Table V). Addition of IL-2 to these cultures reversed the failure of clone D12 to induce IgG, but had only a marginal effect on IgE synthesis. In contrast, addition of IL-4 to these cultures did not affect IgE or IgG synthesis. These results again indicated that while IgE synthesis required the presence of IL-4, other factors that were not provided by clone D12 were also necessary for IgE synthesis to occur.

Commercial LMW-BCGF Plays an Important Role in IgE Synthesis. We next attempted to determine what additional factors were needed by clone D12 to induce IgE synthesis. Since commercially available LMW-BCFG has been previously shown to be a potent B cell stimulus (34–36), we asked if LMW-BCGF would have an effect on

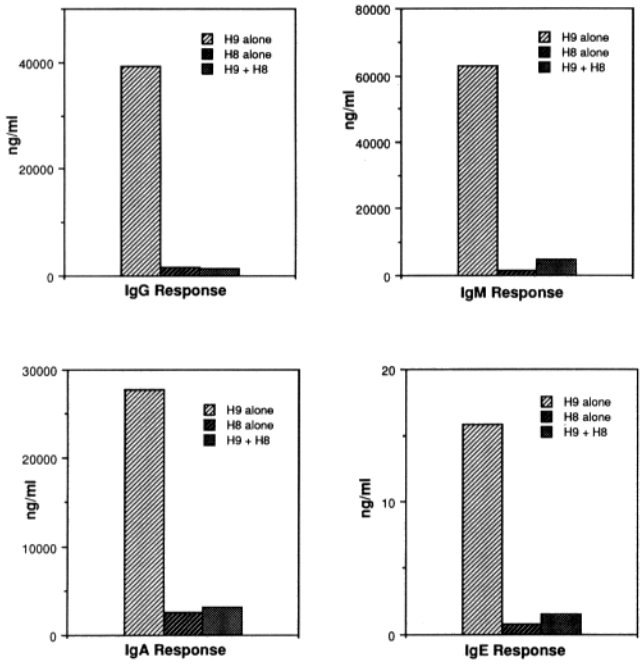

FIGURE 2. IL-4 and LMW-BCGF reconstitute the capacity of clone D12 (secreting IL-4 but not IL-2 or IFN-γ) to induce IgE synthesis. Clone D12 was cultured with purified B cells and the indicated lymphokines: IL-2 at 2, 4, or 10 U/ml, IL-4 at 500 U/ml, and LMW-BCGF at a 1:20 dilution. IgE in culture supernatants was determined after 9 d of culture.

IgE synthesis in our cultures. Fig. 3 shows that addition of both LMW-BCGF and large amounts of IL-4 dramatically increased IgE synthesis. Furthermore, IgE synthesis was optimal with the addition of IL-2, IL-4, and LMW-BCGF. This combination of lymphokines appeared to act directly on the B cell and not by improving the survival of the cloned T cells, since IL-2 by itself had a relatively small effect on the induction of IgE synthesis. However, the possibility that these lymphokines together were enabling clone D12 to more effectively secrete other lymphokines could not be excluded.

Induction of IgE Synthesis with Cyclosporin A-pretreated T Cell Clones. To more precisely evaluate the requirements for IgE synthesis, we adopted a system developed

TABLE V

Ig Synthesis Induced by an IL-4-secreting T Cell Clone Is Enhanced with IL-2

Stimulus	IgG	IgM	IgA	IgE
		ng/ml		
D12	485	678	649	3.98
D12 + IL-2	10,112	13,615	12,803	6.03
D12 + IL-4	614	673	844	4.12
None	49	<58	171	<0.31
IL-2	32	<58	96	<0.31
IL-4	110	105	267	<0.31

Purified B cells were cultured as described in Table IV with clone D12, which secretes IL-4 but not IL-2. IL-2 was added at 10 U/ml, and IL-4 was added at 1,000 U/ml. Similar results were obtained in three other experiments.

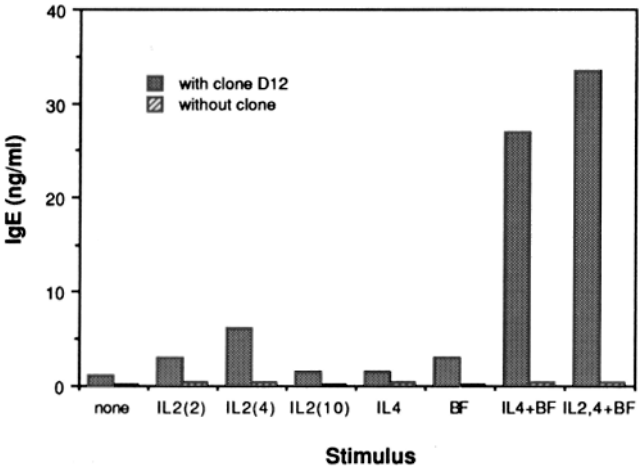

FIGURE 3. Clone H8 (secreting IL-2 and IFN-γ) does not induce IgG, IgM, IgA, or IgE synthesis when cultured with purified B cells, and inhibits Ig synthesis when cocultured with clone H9 (secreting IL-2, IL-4, and IFN-γ) and B cells. B cells that expressed alloantigens recognized by both clones were cultured with clone H8 (40,000 cells/well), with clone H9 (40,000 cells/well), or with both clones H8 and H9. Supernatants were taken after 9 d of culture and examined for IgG, IgM, IgA, and IgE.

by Krusemeier and Snow (28) using $CD4^+$ T cell clones pretreated with cyclosporin A to prevent the release of soluble lymphokines. Table VI shows that treatment of clone H9 with cyclosporin A abolished its capacity to induce IgG, IgM, and IgE in purified B cells. Although cyclosporin A-treated T cells were unable by themselves to induce Ig synthesis (line 2), the interaction of the treated T cells with B cells allowed the B cells to develop the capacity to respond to exogenously added soluble lymphokines. Thus the addition of IL-2, IL-4, and LMW-BCGF to these cultures resulted in the synthesis of IgG, IgM, and >450 ng/ml of IgE. Although cyclosporin A pretreatment may not have completely abolished T cell secretion of all lymphokines, and although cyclosporin A did not affect secretion of factors from monocytes, cyclosporin pretreatment was useful in separating the signals provided by the direct interaction of the T cell with the B cell from signals provided by soluble lymphokines.

IL-4 plus LMW-BCGF with Cyclosporin A-treated T Cells Selectively Enhances IgE Synthesis. We next attempted to find a combination of added lymphokines that would selec-

TABLE VI

Induction of IgE Synthesis with Clone H9 Pretreated with Cyclosporin A

B cells cultured with:				
T cells	Lymphokines	IgG	IgM	IgE
			ng/ml	
$H9^{untreated}$	None	56,250	49,000	23.00
$H9^{csa}$	None	903	<195	<0.31
$H9^{csa}$	IL-2 + IL-4 + BCGF	22,224	42,300	463.00
None	None	437	<195	<0.31
None	IL-2 + IL-4 + BCGF	161	<195	3.70

Purified B cells were cultured for 9 d under cognate conditions with clone H9 (secretes IL-2, IL-4, and IFN-γ), which was pretreated with cyclosporin A (3 μg/ml for 16 h) to inhibit lymphokine release. Supernatants from B cell/cloned T cell cultures were harvested and tested for Ig content by ELISA. IL-2 was used at 4 U/ml, IL-4 at 500 U/ml, and LMW-BCGF at 1:20 dilution.

tively induce IgE but not IgG synthesis. Table VII shows that addition of IL-4 with LMW-BCGF selectively enhanced IgE synthesis (Table VII, lines 7 and 15). The IgE-enhancing effect of LMW-BCGF and IL-4 was specific for IgE synthesis since addition of LMW-BCGF with IL-2 or of IL-4 with IL-2 resulted in synthesis of only small amounts of IgE. Addition of IL-4 alone (Table VII, line 3), even with IL-4 from two different sources (Genzyme and Amgen) and with doses up to 300 ng/ml (data not shown), had no effect on the induction of IgE. This again indicated that while IL-4 was important for IgE synthesis, it was not sufficient. Addition of IL-5 alone, or of LMW-BCGF alone (Table VII, lines 4 and 5) also had no effect on IgE or on IgG synthesis.

In contrast, addition of IL-2 (line 2), or of IL-2 + LMW-BCGF (line 14), to cultures of purified B cells and cyclosporin A–pretreated clone H9, resulted in the synthesis of IgG, IgM, and IgA, but only minimal amounts of IgE. This confirmed the importance of IL-2 in the induction of IgG and IgM, and suggested that IL-2 was also important in the synthesis of IgA. Since IgG but not IgE synthesis occurred when IL-2 or IL-2 + LMW-BCGF were added, and since irradiation of the cyclosporin-treated T cell clone had no effect on these results (data not shown), it was unlikely that IL-2 was simply acting to improve the survival of the T cell clone or to improve lymphokine secretion from the T cell clone. Although IL-2 by itself had little effect on IgE synthesis, the combination of IL-2 with IL-4 and LMW-

TABLE VII

Induction of Ig Synthesis with Clone H9 Pretreated with Cyclosporin A

Exp.	B cells cultured with:	IgG	IgM	IgA	IgE
			ng/ml		
1	$H9^{csa}$	227	<200	260	<0.31
	$H9^{csa}$ + IL-2	5,791	10,470	4,900	0.53
	$H9^{csa}$ + IL-4	297	<200	915	0.49
	$H9^{csa}$ + IL-5	252	<200	238	<0.31
	$H9^{csa}$ + BCGF	276	<200	594	<0.31
	$H9^{csa}$ + IL-4 + IL-5	330	<200	212	0.62
	$H9^{csa}$ + IL-4 + BCGF	479	<200	697	42.30
	$H9^{csa}$ + IL-2 + IL-4	8,983	11,184	7,219	2.00
	$H9^{csa}$ + IL-2 + IL-4 + BCGF	7,387	10,600	2,633	123.10
	IL-2 + BCGF	373	<200	607	0.35
	IL-4 + BCGF	314	<200	590	0.80
	IL-2 + IL-4 + BCGF	968	320	771	2.50
2	$H9^{cca}$	<30	184	<20	<0.31
	$H9^{csa}$ + IL-2 + BCGF	8,003	16,576	3,631	3.41
	$H9^{csa}$ + IL-4 + BCGF	710	1,341	2,094	33.20
	$H9^{csa}$ + IL-2 + IL-4 + BCGF	5,291	7,189	3,928	97.00
	$H9^{csa}$ + IL-2 + IL-4 + IL-5	5,752	12,828	2,011	1.17
	IL-2 + BCGF	414	696	801	<0.31
	IL-4 + BCGF	127	264	640	2.1
	IL-2 + IL-4 + BCGF	960	248	671	2.63
	IL-2 + IL-4 + IL-5	815	320	379	0.45

Purified B cells were cultured as described in Table VI. IL-2 was used at 4 U/ml, IL-4 (from Genzyme) at 500 U/ml, IL-5 (from Genetics Institute) at 1:500, and LMW-BCGF at 1:20 dilution.

BCGF resulted in a significant increase in IgE synthesis. This suggested that IL-2 was important in combination with other lymphokines for the induction of IgE synthesis, or that IL-2 had beneficial effects on the survival or function of the T cell clones.

IL-5 Cannot Substitute for LMW-BCGF in the Induction of IgE Synthesis with Cyclosporin-pretreated with T Cell Clones. Since IL-5 enhances the secretion of IgG and IgA (but not of IgE) in mice (8, 37–39), and under suboptimal conditions enhances secretion of IgE in man (40), we asked if the addition of recombinant IL-5 enhanced IgE synthesis using our system with cyclosporin A pretreated T cell clones. Results using rIL-5 from Genetics Institute, shown in Table VII (lines 4, 6, and 17), indicate that rIL-5 alone or in combination with IL-4, or with IL-2 had little effect on IgE synthesis even in the presence of the T cell clone. Similar results were obtained with rIL-5 from DNAX (data not shown). In contrast, the combination of IL-2, IL-4, and LMW-BCGF in the presence of the T cell clone resulted in the synthesis of >100 ng/ml of IgE. Collectively, these results indicated that neither IL-4 nor IL-5 could substitute for LMW-BCGF, and therefore, the IgE-enhancing effect in the LMW-BCGF preparation was unlikely to be due to a contaminant such as IL-4 or IL-5.

Discussion

There is general agreement that IL-4 is required for the synthesis of both human and murine IgE (2–9, 12–16). In man, however, the results of studying IgE synthesis have been less clear cut than in the mouse. Polyclonal B cell activators such as LPS, Staphylococcus A, Cowan strain (SAC), anti-Ig, PWM, or EBV do not induce IgE synthesis, even in the presence of IL-4 (41–43). More recently, several investigators have shown that when IL-4 or antigen *non*specific human T cell clones producing IL-4, were added to unfractionated PBMC or to B cell-enriched populations, IgE synthesis was induced (12–16). The results of these experiments indicated that IL-4 was an important lymphokine in the induction of human IgE synthesis, and that IFN-γ had an inhibitory effect on IgE synthesis. However, IgE synthesis in these systems required the presence of sizable numbers of peripheral blood T cells, therefore making it difficult to know what signals were provided by IL-4 and what signals were provided via the stimulation of the contaminating T cells that produced a second required factor(s). Moreover, although induction of IgE correlated positively with the presence of T cell clones producing IL-4 and negatively with clones producing IFN-γ, some clones that made IL-4 did not induce IgE, and some clones that made IFN-γ were potent inducers of IgE (12, 13, 16). This indicated that lymphokines or factors in addition to IL-4 were important in the induction of IgE synthesis, and that the mechanisms for the induction of IgE synthesis were more complex than suggested by data generated in the mouse with LPS.

In the current article we studied the induction of IgE synthesis in severely T cell-depleted B cell populations with a number of well-characterized antigen-specific helper $CD4^+$ T cell clones, each with a different profile of lymphokine synthesis, to determine what signals in addition to IL-4 were required for the induction of IgE synthesis. Our system with T cell clones, differs from previous studies (12, 13, 16), that showed the requirement of IL-4, in that we used purified B cell populations with antigen-specific clones under cognate T-B cell conditions, which mimic physiologic T-B cell MHC-restricted interactions (17–19, 22). Although we did not routinely measure serum IgE levels in our donors to assess their allergic status, and although

some of our purified B cell populations contained some large preactivated B cells that secreted small amounts of IgE spontaneously, the response induced with our T cell clones in our unfractionated T cell-depleted B cells was 40–100 times that of this spontaneous background. Finally, using cyclosporin A-pretreated clones, we were able to functionally dissect the signals provided by the direct interaction of the T cell with the B cell from signals provided by lymphokines.

Our results using these different experimental approaches showed that optimal induction of IgE synthesis required exposure of B cells to a particular complex of signals, that included signals provided by (*a*) Th-B cell interaction that primed B cells to receive additional signals from soluble lymphokines, (*b*) by IL-4, (*c*) by LMW-BCGF, and (*d*) by IL-2. Therefore, the requirements for IgE synthesis appear to be different from those for IgG or IgM, and this may allow for the selective regulation of IgE synthesis.

Although the precise characteristics of subsets of human $CD4^+$ T cell clones are not yet clear (16, 23, 44–46), we have generated antigen-specific human $CD4^+$ T cell clones with different profiles of lymphokine synthesis and confirmed that clones that differ in their lymphokine profiles have differing capacities to induce IgE synthesis. Our detailed analysis of each clone indicated that of the clones that did not produce IL-4, none could induce IgE, and one (clone H8) was inhibitory for IgE synthesis. The inhibitory effect of clone H8 on IgE synthesis was unlikely to be solely due to synthesis of IFN-γ, since clone H8 also inhibited synthesis of Ig of all other isotypes. Another IL-4 nonproducing clone (D11) could be made to induce IgE synthesis by the addition of IL-4. On the other hand, using cyclosporin A-pretreated T cell clones with purified B cells, addition of IL-4 alone (even in concentrations up to 300 ng/ml) (Table VII), or of IL-4 with IL-2 or IL-5 (Table VII), did not result in IgE synthesis. Thus, using several different clones and several different approaches, we have confirmed the idea that IL-4 is required for the induction of IgE synthesis, but we have clearly demonstrated that IL-4 by itself is not sufficient.

Although clones that did not produce IL-4 could not induce IgE synthesis, some of the clones that produced IL-4 could not induce IgE, again indicating that factors in addition to IL-4 were important for the synthesis of IgE. IL-4-secreting clones may not proliferate to antigen presented by B cells, as has been shown in mouse (47) and in man (48), but this is unlikely to account for the failure of some IL-4-secreting clones to induce IgE, since mouse Th2 clones, which do not proliferate well to antigen presented by B cells, do secrete lymphokines after encountering B cells and are clearly able to induce Ig secretion (8, 31, 49). Moreover, our observation that LMW-BCGF restored the ability of the IL-4 secreting "nonhelper" clone D12 to induce IgE (Fig. 2), and our observation with cyclosporin-treated T cell clones that addition of IL-4 plus LMW-BCGF resulted specifically in IgE but not IgG synthesis, indicated that LMW-BCGF and IL-4, in combination, are lymphokines of major importance in the induction of IgE synthesis. LMW-BCGF was likely to have a direct effect on B cells rather than an indirect one via the T cell clone, since LMW-BCGF had no effect on proliferation of T cell clones (data not shown), and since the effect was also seen using irradiated T cell clones. It is possible, however, that LMW-BCGF or IL-2 increased the survival or enhanced the secretion of other lymphokines from the cyclosporin A-treated T cell clone.

Now what is LMW-BCGF? LMW-BCGF represents the predominant molecule

that has comitogenic effects on activated human B cells and that is released from normal lectin-activated human T cells (34). It has a molecular weight of 12×10^3, with a major isoelectric point of 6.3–6.6. cDNA for this molecule has been cloned and sequenced by Sharma et al. (50). The commercial preparation of LMW-BCGF that we used contained small amounts of contaminants, but these (IFN-γ, CSF, and lymphotoxin [51]) do not enhance IgE synthesis (13, 16). The IgE-enhancing activity in the commercial preparation was unlikely to be due to IL-4 or IL-5 since two different preparations of rIL-5 and two different preparations of rIL-4 in large quantities were all unable to substitute for LMW-BCGF in our cultures, and since IL-4 was undetectable by immunoassay in the commercial preparation of LMW-BCGF (20). However, the possibility that the IgE-enhancing activity in the commercial preparation of LMW-BCGF was due to a contaminant cannot be formally ruled out until recombinant LMW-BCGF is available.

Nevertheless, LMW-BCGF is known to be a potent B cell growth factor (34), which can act in concert with IL-4 to induce proliferation of anti-IgM preactivated B cells (35, 36). LMW-BCGF by itself does not induce B cell differentiation (34), and may actually inhibit IgG synthesis. Its role then in IgE synthesis may be to induce proliferation and expansion of partially activated B cells, and to cause B cells to be receptive to differentiation signals from IL-4. Since addition of LMW-BCGF alone, IL-2 alone, or IL-4 alone to our cultures of B cells and T cell clones did not result in the synthesis of significant amounts of IgE, we suggest that under physiologic conditions, an optimal IgE response requires the presence of both a growth factor such as LMW-BCGF, as well as a differentiation/isotype switching factor such as IL-4 (7, 52). Although IL-2 is a potent B cell growth factor (53, 54) and is important for induction of IgG and IgM synthesis (Tables IV, V, VII; and references 37, 55–57), LMW-BCGF and not IL-2 is the preferred B cell growth factor for IgE synthesis, since addition of IL-2 + IL-4 to our cultures in the absence of LMW-BCGF did not result in substantial IgE synthesis. Although IL-4 itself can under some circumstances also induce B cell growth (50, 51, 58), in our culture system IL-4 without LMW-BCGF had little effect on IgE synthesis. It is possible, however, that in B cell populations where the frequency of IgE-committed B cells is high, IL-4 alone may induce differentiation resulting in a substantial IgE response.

While our results show the enhancing effect on IgE synthesis of the combination of soluble IL-2, IL-4, and LMW-BCGF, we also demonstrated that IgE synthesis required a signal generated in B cells by the cognate interaction with T cells. Our normal purified B cell populations did not synthesize appreciable amounts of Ig in response to soluble lymphokines, except in the presence of cloned T cells. Furthermore, using cyclosporin A–pretreated T cell clones, we showed that a signal delivered by a T cell can prime or prepare B cells to receive signals provided by soluble lymphokines. This is consistent with the hypothesis of others (28, 31, 59) that regulation of antibody secretion by T helper cells occurs at two levels: one involves Th cell/B cell interaction, and the other involves T cell–derived lymphokines. Our results extend this hypothesis to the regulation of IgE synthesis, and show that optimal induction of an IgE-specific response requires (*a*) a signal involving Th–B cell interaction, (*b*) a specific B cell proliferative signal (LMW-BCGF), and (*c*) a specific B cell differentiation signal (IL-4). Although further analysis of human $CD4^+$ T cells is required to further define subsets of human T cells, to determine which subsets se-

crete LMW-BCGF and IL-4, and to determine if other lymphokines or cell types have important effects on IgE synthesis, our results have refined our understanding of the specific lymphokine requirements for IgE synthesis and of the role of $CD4^+$ T cell clones in IgE synthesis. Such considerations are essential in developing insights into the regulation of IgE synthesis, and are pertinent in devising strategies using immunomodulators in vivo to inhibit human IgE immune responses.

Summary

We have analyzed in detail the precise requirements for the induction of human IgE synthesis using several experimental approaches with purified B cells and well-characterized alloantigen-specific $CD4^+$ T cell clones expressing different profiles of lymphokine secretion. Using these clones under cognate conditions in which the B cells expressed alloantigens recognized by the cloned T cells, we have confirmed that IL-4 is required for the induction of IgE synthesis, but we have clearly demonstrated that IL-4 by itself is not sufficient. With several cloned $CD4^+$ T cell lines, including an IL-4-producing clone that could not induce IgE synthesis, and cloned T cells pretreated with cyclosporin A to inhibit lymphokine synthesis, we showed that Th cell–B cell interactions are necessary for IgE synthesis, and that low molecular weight B cell growth factor (LMW-BCGF) and IL-4, in combination, are lymphokines of major importance in the induction of IgE synthesis. Together our results indicate that optimal induction of an IgE-specific response requires the exposure of B cells to a particular complex of signals that include (*a*) a signal(s) involving Th–B cell interaction that primes B cells to receive additional signals from soluble lymphokines, (*b*) a specific B cell proliferative signal provided by LMW-BCGF, and (*c*) a specific B cell differentiation signal provided by IL-4.

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