INDUCTION OF HUMAN IgE SYNTHESIS REQUIRES INTERLEUKIN 4 AND T/B CELL INTERACTIONS INVOLVING THE T CELL RECEPTOR/CD3 COMPLEX AND MHC CLASS II ANTIGENS

By DONATA VERCELLI, HAIFA H. JABARA, KEN-ICHI ARAI,* AND RAIF S. GEHA

*From the Division of Immunology, The Children's Hospital, and the Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115; and the *Department of Molecular Biology, DNAX Research Institute of Cellular and Molecular Biology, Palo Alto, California 94304*

The synthesis of IgE is regulated by isotype-specific mechanisms. Powerful polyclonal B cell activators, such as PWM, *Staphylococcus aureus* Cowan I (SAC),[1] and EBV, consistently failed to induce IgE synthesis by normal B cells (1, 2). The latter can, however, be induced to produce IgE upon cognate interaction with selected alloreactive or autoreactive T cell clones (3–5). Furthermore, T cell clones activated by anti-CD3 mAb or PHA induced B cells to synthesize IgE in the apparent absence of alloantigen recognition (6). These results indicated that normal B cell precursors can be induced to differentiate into IgE-secreting cells by appropriate stimuli.

It has been recently shown that the T cell–derived lymphokine B cell stimulatory factor 1 (BSF-1)/IL-4 plays a central role in the regulation of IgE synthesis. In mice, IL-4 induces polyclonal IgE and IgG1 production in vitro by LPS-stimulated B cell blasts (7, 8), and the in vivo administration of an anti-IL-4 antibody inhibits the IgE response (9, 10). In humans, we and others have recently shown that rIL-4 induces IgE synthesis by normal peripheral blood lymphocytes (11–15). Furthermore, supernatants from T cell clones secreting IL-4 possess an IgE-inducing ability that can be completely abolished by an anti-IL-4 antibody (12, 13, 16).

The cellular and lymphokine requirements for the IL-4-dependent induction of IgE synthesis are quite complex. We have recently shown (17) that the induction of IgE by human rIL-4 is strictly T cell dependent, is optimal in the presence of monocytes, and is amplified by IL-5 and IL-6. Furthermore, endogenous IL-6 plays an obligatory role in IL-4-induced IgE synthesis, since an antibody to IL-6 strongly inhibited IL-4-induced IgE production. However, under no condition of lymphokine stimulation could purified B cells be induced to synthesize IgE. Thus, soluble signals delivered by recombinant lymphokines in various combinations could not replace T cells and monocytes in the IL-4-dependent induction of IgE synthesis. This strongly

This work was supported by U. S. Public Health Service grants AI-22058 and PO1 CA-39352. Address correspondence to Raif S. Geha, Division of Immunology, The Children's Hospital, 300 Longwood Ave., Boston, MA 02115.

[1] *Abbreviations used in this paper:* AET, 2-aminoethylisothiouronium; BSF, B cell stimulatory factor; CAM, cell adhesion molecule; HS, horse serum; IF, immunofluorescence; SAC, *Staphylococcus aureus* Cowan I.

suggested that cell-cell interactions might also be required. In the present study, we show that cognate T/B cell recognition via MHC class II antigens and the TCR/CD3 complex is necessary for IL-4-dependent IgE synthesis to proceed. Accessory adhesion molecules expressed on B and/or T cells also contribute to the cellular interactions required for IgE synthesis.

Materials and Methods

Interleukins and Antibodies. Human rIL-4 was used in a purified form (specific activity: 1.2×10^7 U/mg). Human rIL-6 (specific activity: 5×10^6 U/mg) was a kind gift of T. Kishimoto and T. Hirano (Osaka University, Osaka, Japan). mAb TS1/22, a murine IgG1 specific for the α chain of the LFA-1 molecule, was a kind gift of Drs. Steven J. Burakoff and Barbara E. Bierer (Harvard Medical School, Boston, MA). Anti-HLA-DR (clone L243, IgG2a specific for a nonpolymorphic HLA-DR epitope), anti-Leu-5b (IgG2a anti-CD2), anti-Leu-4 (IgG1 anti-CD3), anti-Leu-3a (IgG1 anti-CD4), anti-Leu-2a (IgG1 anti-CD8), anti-Leu-9 (IgG2a anti-CD7), anti-Leu-M3 (IgG2b anti-CD14), TCR-1 (WT31, an IgG1 mAb specific for a framework determinant of the TCR-α/β heterodimer) mAbs, as well as the appropriate isotype controls, were obtained from Becton Dickinson & Co. (Mountain View, CA). OKT3 (IgG2a anti-CD3) and OKIa1 (IgG2 anti-HLA-DR) mAbs were obtained from Ortho Diagnostic Systems Inc. (Westwood, MA). mAb B1 (IgG2a anti-CD20) and W6-32 (IgG2a anti-MHC class I) were obtained from Coulter Immunology (Hialeah, FL) and American Type Culture Collection (Rockville, MD), respectively. A polyclonal rabbit antibody to IL-4 was a kind gift of Dr. J. de Vries (UNICET, Dardilly, France).

Preparation of T Cell Clones. TT- and alloantigen-specific helper T cell clones were obtained by limiting dilution as previously described (3, 18, 19). Briefly, PBMC suspended in RPMI 1640 (M. A. Bioproducts, Walkersville, MD) and 10% AB^+ serum (Hazleton Research Products, Lenexa, KA) were stimulated with TT or irradiated allogeneic PBMC for 6 d and the blasts were enriched by centrifugation over a discontinuous Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) gradient. Cells at the 30–50% interface were then cultured at limiting dilutions (0.3 cells/well) in 96-well plates in the presence of irradiated autologous PBMC plus TT, and IL-2-containing supernatants. The $CD3^+$, $CD4^+$, $4B4^+$, $CD8^-$ T cell clones thus obtained have been maintained in long-term culture by repeated addition of irradiated autologous PBMC plus TT, or irradiated allogeneic PBMC and IL-2-containing supernatants.

Cell Preparations. PBMC were isolated from heparinized venous blood of normal nonallergic donors by density gradient centrifugation on Ficoll-Hypaque, washed three times in HBSS (Microbiological Associates, Bethesda, MD) and resuspended in RPMI 1640/10% heat-inactivated FCS (HyClone Laboratories, Logan, UT) supplemented with 2 mM L-glutamine, 50 μg/ml streptomycin and 100 U/ml penicillin (complete medium). To obtain purified B cells, T cells were removed by rosetting twice with 2-aminoethylisothiouronium bromide (AET)-treated SRBC. Further T cell depletion was obtained by two cycles of lysis with anti-CD3 mAb + rabbit C (Pel-Freeze Biologicals, Inc., Rogers, AR). To remove monocytes, non-T cells in RPMI 1640/10% AB^+ serum were adhered twice in plastic petri dishes. The resulting B cell populations contained <6% $CD14^+$ cells and <1% $CD3^+$ cells, as determined by immunofluorescence (IF). In addition, these B cell preparations gave no proliferative response to Con A or PHA (10 μg/ml), while they strongly proliferated upon stimulation with PMA (25 ng/ml; Sigma Chemical Co., St. Louis, MO) and insolubilized anti-μ antibody (Immunobead rabbit anti-human IgM; 1 μg/ml; Bio-Rad Laboratories, Richmond, CA). Cell viability, as assessed by trypan blue exclusion, was always >95%.

T cells were purified by rosetting twice with AET-SRBC. The SRBC were then removed by NH_4Cl lysis and the cells were resuspended in RPMI 1640/10% AB^+ serum and adhered twice in plastic petri dishes. The nonadherent cells thus obtained contained >97% $CD3^+$ cells, as detected by IF.

Cell Cultures for IgE Induction. Unfractionated PBMC or purified B cells (1.5×10^6 cells/ml) in complete medium were cultured at 37°C in a 5% CO_2 humidified atmosphere, in the presence of rIL-4 (100 U/ml) and the various mAbs, as indicated for each experiment

in Results. In some experiments, purified B cells were incubated with supernatants from cultures of T cells or T+B cells (1.5×10^6 cells/ml, 2:1 ratio) stimulated for 48 h with rIL-4 (100 U/ml). Insolubilized anti-μ antibodies (1-10 μg/ml) or SAC (0.1%; The Enzyme Center Inc., Malden, MA) were added to cultures of purified B cells in other experiments. Autologous T/B cell mixtures (1.5×10^6 cells/ml) were cultured under the same conditions. In some experiments, autologous purified T and B cells were separated during culture by a 0.4 μm membrane using well inserts (Millicell-HA, Millipore Corp., Bedford, MA). After 9 d, the culture supernatants were harvested and assessed by RIA for their IgE content. Control cultures for the evaluation of preformed IgE were set up in the presence of cycloheximide (100 μg/ml; Sigma Chemical Co.). Net IgE synthesis was evaluated by subtracting the IgE concentrations detected in cycloheximide-treated cultures from the IgE values found in untreated cultures.

In some experiments, purified B cells (10^6/ml) from selected donors were cultured for 10 d with autologous monocytes (2×10^5/ml) and irradiated (2,500 rad), cloned T cells (0.4×10^6/ml), in the presence or absence of exogenous IL-4 (100 U/ml). Control cultures were set up with cycloheximide. Culture supernatants were then harvested and assessed by RIA for their IgE content.

RIA for IgE. The assay was performed in flexible flat-bottomed microtiter plates (Cooke Laboratory Products, Alexandria, VA) at room temperature as previously described (3). The wells were coated with 0.1 ml of a 1:1 mixture of purified anti-Fcϵ mAbs (7.12 and 4.15; a kind gift of A. Saxon, University of California Los Angeles, Los Angeles, CA), 2 μg/ml in carbonate-bicarbonate buffer, pH 9.6. After a 16-h incubation, the wells were washed, blocked with PBS/10% horse serum (HS) for 2 h, and subsequently washed three times with PBS/1% HS. 0.1 ml of culture supernatant or different dilutions of IgE standard (Pharmacia Fine Chemicals) were then added to the wells in triplicate and incubated for 16 h in a humidified chamber. The wells were then washed one time with PBS/1% HS containing 0.05% Tween 20, twice with PBS/1% HS, and finally incubated with 0.1 ml of Phadebas RAST ^{125}I-anti-human IgE (ND) (Pharmacia Fine Chemicals) for 6 h. The wells were then washed three times with PBS/1% HS/0.05% Tween 20, eight times under running distilled water, cut out, and counted in a gamma spectrometer (Tracor Analytic, Elk Grove Village, IL). The concentrations of IgE in the supernatants were read from the standard curve. The lower limit of sensitivity of this assay is 150 pg/ml. This assay was validated in a recent multicenter collaborative assessment of the variability of IgE measurement in cell culture supernatants (20).

Results

Requirements for Cellular Interactions in IL-4-dependent IgE Synthesis. Fig. 1 shows a dose-response curve for the effect of rIL-4 on IgE synthesis by human PBMC. We have recently shown that IL-4-induced IgE synthesis is T cell dependent and op-

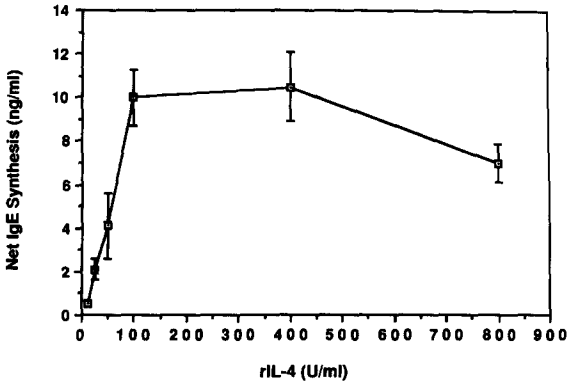

FIGURE 1 Dose-response curve for the effect of rIL-4 on IgE synthesis by human PBMC. Different concentrations of purified human rIL-4 were added to normal PBMC (1.5×10^6 cells/ml). After 9 d the culture supernatants were harvested and assessed by RIA for their IgE content. The data represent the mean ± SE of three experiments.

timal in the presence of monocytes. IL-5 and IL-6 strongly amplify IgE synthesis. More importantly, endogenous IL-6 is required for IL-4-induced IgE synthesis (17). Table I shows that a combination of these lymphokines could not induce IgE production by highly purified B cells, even in the presence of powerful polyclonal B cell activators, such as insolubilized anti-μ antibodies and SAC. B cells, however, became responsive to IL-4 upon readdition of T cells. In five experiments with different normal donors, the B/T cell ratio required for substantial induction of IgE synthesis ranged from 10:1 to 1:2. The addition of T cells at a 1:4 B/T cell ratio consistently suppressed IL-4-dependent IgE synthesis (data not shown). These results indicate that soluble signals cannot replace T cells in the IL-4-dependent induction of IgE synthesis. Furthermore, purified normal B cells did not produce IgE in response to supernatants from T cells or T/B cell mixtures incubated for 48 h in the presence of IL-4 (100 U/ml) (data not shown). This ruled out the possibility that the induction of IgE synthesis by purified B cells requires unidentified labile short-range mediator(s) released by the T and/or B cells upon IL-4 stimulation.

Taken together, these data indicated that the requirement for T cells could not be bypassed by soluble T cell products and suggested that physical interactions between T and B cells might be required for IgE synthesis. This hypothesis was further supported by experiments in which autologous purified T and B cells were cultured in the presence of rIL-4 (100 U/ml), either in the same well or separated by a 0.4-μm membrane permeable to protein macromolecules. As shown in Table II, vigorous IgE synthesis was only induced in the cultures in which T and B cells were allowed to come in contact. These data indicate that disruption of cell-cell contact results in the inhibition of the IgE-inducing activity of IL-4.

IL-4 Dependent IgE Synthesis is Inhibited by mAbs Specific for Cell Adhesion Molecules. A number of surface molecules have been recently characterized which enhance antigen-independent adhesion between T cells and APCs or target cells, and stabilize cell-cell contacts. These structures are collectively known as cell adhesion molecules (CAMs) (21, 22). Since the previous experiments had indicated that IL-4 induced IgE syn-

TABLE I

IL-4 and IL-6 Do Not Induce IgE Synthesis by Highly Purified B Cells

Cell populations	Net IgE synthesis	
	+ medium	+ rIL-4 + rIL6
	pg/ml	
PBMC, unfractionated	<150	4,350
Purified B cells, unstimulated	<150	230
+ anti-μ antibody	<150	200
+ SAC	<150	230
+ T cells (1:2 ratio)	390	3,410

Cell populations (PBMC, purified B cells, or reconstituted autologous T/B cell mixtures: 1.5 $\times$ 10^6 cells/ml) from normal donors were incubated with medium or rIL-4 + rIL-6 (100 U/ml). Purified B cells were stimulated with insolubilized anti-μ antibody (1–10 μg/ml) or SAC (0.1%). Culture supernatants were harvested after 9 d and assessed by RIA for their IgE concentration. Control cultures were set up in the presence of cycloheximide (100 μg/ml). Net IgE synthesis was determined by subtracting IgE concentrations in cycloheximide-treated cultures from IgE concentrations in untreated cultures.

TABLE II
Physical Separation of B from T Cells Prevents IgE Synthesis

Culture	Cells	Net IgE synthesis Exp. 1	Exp. 2
		pg/ml	
Well:	B + rIL-4	<150	<150
Insert:	T + rIL-4	<150	<150
Well:	B + T + rIL-4	11,000	1,500

B cells (0.6×10^6 cells/culture) from normal donors in complete medium were cultured in 0.7 ml wells. T cells (1.2×10^6 cells/culture) were cultured either in wells with the B cells or in inserts, separated from B cells by a 0.4-μm membrane. rIL-4 was added at 100 U/ml. After 9 d the supernatants from wells and inserts were separately harvested and assessed for their IgE content. Net IgE synthesis was evaluated as described in the legend to Table I.

thesis requires physical T-B cell interactions, we next asked whether CAMs play a role in such interactions. To this purpose, PBMC from normal donors were incubated with optimal concentrations of rIL-4 (100 U/ml) in the presence of mAbs specific for various CAMs expressed by B cells and/or T cells. As shown in Table III, anti-CD2 and anti-CD4 mAbs strongly and consistently inhibited IL-4-induced IgE synthesis. Anti-LFA-1 (α chain) mAb had a more variable effect, although in most experiments it inhibited IL-4-induced IgE synthesis. By contrast, no inhibition was observed using anti-CD8 mAb or the pan-T anti-CD7 mAb.

These results suggested that CAMs play an important role in the cellular interactions required for IL-4-dependent IgE synthesis.

mAbs Specific for the TCR/CD3 Complex and MHC Class II Antigens Inhibit IL-4-induced IgE Synthesis. We next asked whether the TCR/CD3 complex also plays a role in T/B cell interactions required for IL-4-induced IgE synthesis. To this purpose, we

TABLE III
Anti-CAM mAbs Inhibit IgE Synthesis Induced by rIL-4

Stimulus	mAb	Net IgE synthesis Exp. 1	Exp. 2	Exp. 3
			pg/ml	
Nil	–	<150	<150	<150
rIL-4	–	20,300	2,700	8,200
rIL-4	Anti-LFA-1	8,900	2,600	150
rIL-4	Anti-CD2	300	<150	<150
rIL-4	Anti-CD4	4,850	650	1,600
rIL-4	Anti-CD8	22,000	4,000	9,400
rIL-4	Anti-CD7	19,300	3,100	9,000

Unfractionated normal PBMC (1.5×10^6 cells/ml) were cultured with rIL-4 (100 U/ml), in the presence or absence of mAbs to LFA-1 (10μg/ml), CD2 (1 μg/ml), CD4 (2.5 μg/ml, CD8 (1 μg/ml), CD7 (3 μg/ml). After 9 d the culture supernatants were harvested and assessed by RIA for their IgE content. Net IgE synthesis was evaluated as described in the legend to Table I. The mAbs by themselves had no effect on IgE synthesis.

TABLE IV

mAbs to the TCR/CD3 Complex Inhibit IgE Synthesis Induced by rIL-4

Stimulus	mAb	Net IgE synthesis		
		Exp. 1	Exp. 2	Exp. 3
			pg/ml	
Nil	–	<150	<150	<150
rIL-4	–	5,900	2,450	1,750
rIL-4	Anti-CD3	850	<150	550
rIL-4	Anti-Leu-4	ND	<150	300
rIL-4	WT-31	<150	ND	<150

Unfractionated normal PBMC (1.5×10^6 cells/ml) were cultured with rIL-4 (100 U/ml), in the presence or absence of anti-CD3 (0.1 μg/ml), anti-Leu-4 (1 μg/ml), or WT-31 (1 μg/ml) mAbs. After 9 d the culture supernatants were harvested and assessed by RIA for their IgE content. Net IgE synthesis was evaluated as described in the legend to Table I.

studied the effect of anti-CD3 mAbs on IgE production induced by rIL-4 in normal PBMC. Both OKT3 and anti-Leu-4 inhibited IL-4-dependent IgE synthesis (Table IV, Exps. 1 and 2), although they induced a strong proliferative response (data not shown). Inhibition of IgE synthesis by anti-Leu-4 was also observed in the virtual absence of proliferation using PBMC from a "Leu-4 nonresponder" (23) whose monocytes do not express Fc receptors for mouse IgG1 (Table IV, Exp. 3). Thus, anti-CD3 mAbs inhibited IL-4-induced IgE synthesis by PBMC regardless of their effect on cell proliferation. Furthermore, IgE synthesis was inhibited by WT31, a mAb specific for a framework determinant of the TCR-α/β heterodimer (24), which also induced strong cell proliferation (data not shown).

In parallel, we assessed the role of MHC class II antigens in cell interactions required for IL-4-induced IgE synthesis. As shown in Table V, anti MHC class II (HLA-DR) mAb (L243) completely inhibited IgE synthesis induced by rIL-4 (100

TABLE V

Anti-MHC Class II (HLA-DR) mAb Inhibits IgE Synthesis Induced by rIL-4 ± rIL-6

Stimulus	mAb	Net IgE synthesis		
		Exp. 1	Exp. 2	Exp. 3
			pg/ml	
Nil	–	<150	<150	<150
rIL-4	–	16,200	2,500	2,200
rIL-4	Anti-MHC class II	<150	<150	<150
rIL-4	Anti-MHC class I	13,800	3,950	2,500
rIL-4	Anti-CD20	17,000	2,200	ND
rIL-4 + rIL-6	–	57,000	18,500	9,900
rIL-4 + rIL-6	Anti MHC class II	<150	250	<150

Unfractionated normal PBMC (1.5×10^6 cells/ml) were cultured with rIL-4 (100 U/ml) ± rIL-6 (100 U/ml), in the presence or absence of anti-MHC class II (HLA-DR: 2.5 μg/ml), anti-MHC class I (1 μg/ml), or anti-CD20 (1 μg/ml) mAbs. After 9 d the culture supernatants were harvested and assessed by RIA for their IgE content. Net IgE synthesis was evaluated as described in the legend to Table I.

U/ml). Comparable results were obtained using an anti-HLA-DR mAb (OKIa1) from a different source (data not shown). By contrast, mAb specific for MHC class I antigens or the pan-B cell antigen CD20 had no effect. Furthermore, anti-HLA-DR mAb strongly inhibited high-rate IgE synthesis induced by a combination of IL-4+IL-6. Interestingly, anti-HLA-DR mAb inhibited PBMC proliferation induced by rIL-4, but not by rIL-2 (data not shown). This suggests that inhibition by anti-HLA-DR mAb did not result from cell toxicity.

These results show that the cellular interactions required for IL-4-dependent IgE synthesis are mediated by the TCR/CD3 complex on T cells and MHC class II antigens on B cells, and suggest that cognate T/B cell interactions may be involved in IL-4-driven induction of IgE synthesis.

Cognate T/B Cell Interaction Is Required for IL-4-dependent IgE Synthesis. Although the results of the previous experiments strongly pointed at a role of cognate T/B cell interactions in IL-4-dependent IgE synthesis, we had to consider the possibility that inhibition of IgE production by mAbs to CAMs, MHC class II antigens, and TCR/CD3 complex may result from direct negative signaling to the T and/or B cells. We therefore examined the requirement for T/B cell interactions for IgE induction by alloreactive human T cell clones. To this purpose, we compared the IgE inducing ability of two human IL-4-producing alloreactive T cell clones, F6 and A1, which have different MHC recognition properties.

Clone F6 is a TT-specific T cell clone, which also recognizes HLA-DR4 and produces IL-4, as well as IL-2 and IFN-γ (3, 19). As shown in Table VI, clone F6 strongly

TABLE VI

Cognate T/B Cell Interaction Is Required for IL-4-dependent IgE Synthesis

B cell donor	Cognate interaction	Clone	rIL-4	Net IgE synthesis
				pg/ml
DL	Yes	–	–	<150
		–	+	<150
		F6	–	20,500
		F6	– + anti-IL4	600
		F6	+	23,000
RC	No	–	–	<150
		–	+	<150
		F6	–	500
		F6	+	<150
DL	Yes*	–	–	<150
		–	+	<150
		A1	–	<150
		A1	+	<150

Purified B cells (10^6/ml) from selected normal donors were cultured for 10 d with autologous monocytes (2×10^5/ml) and irradiated (2,500 rad), cloned T cells (0.4×10^6/ml), in the presence or absence of exogenous IL-4 (100 U/ml). A polyclonal anti-IL-4 antibody was added at 1:500. Control cultures were set up with cycloheximide. Culture supernatants were then harvested and assessed by RIA for their IgE content. Net IgE synthesis was evaluated as described in the legend to Table I. Tissue typing of the B cell donors was kindly performed by the HLA laboratory at the Dana Farber Cancer Institute, Boston, MA. Clone F6 and A1 showed intense proliferation upon incubation with B cells from donor DL (HLA-DR4$^+$), but not with cells from donor RC (HLA-DR5$^+$).

* Clone A1 recognizes an HLA-DP-associated epitope on monocytes, but not on B cells (26).

induced IgE synthesis by purified normal B cells from an HLA-DR4$^+$ allostimulator (DL). IgE production was not further significantly increased by the addition of exogenous IL-4, but was IL-4 dependent, since it was blocked by an anti-IL-4 antibody. By contrast, clone F6 did not induce IgE synthesis from HLA-DR5$^+$ allogeneic B cells (RC) or from HLA-DR3, 5$^+$ autologous B cells (SA; data not shown). Exogenous IL-4 at high concentrations (100 U/ml) could not restore the IgE-inducing ability of clone F6 in the latter cultures, while it induced a vigorous IgE response by PBMC from all three B cell donors (data not shown). Thus, the failure of clone F6 to induce IgE production by B cells lacking the relevant alloantigen was not due to the absence of circulating B cells potentially able to differentiate into IgE production, nor to the absence of IL-4.

Clone A1 is the human equivalent of a murine Th2 clone, in that it secretes IL-4 but not IL-2 or IFN-γ (19). Supernatants from clone A1 induce IgE synthesis by normal PBMC and FcϵR2/CD23 expression on monocytes. Both effects are IL-4 dependent, since they can be inhibited by an anti-IL-4 antibody (12, 25). Interestingly, clone A1 recognizes an HLA-DP-associated antigen expressed on monocytes, but not on B cells (26). We therefore incubated clone A1 with monocytes (as stimulator cells) and B cells (as bystander effector cells) from a donor (DL) bearing the appropriate alloantigen. As shown in Table VI, clone A1 did not induce IgE synthesis, although under such conditions the clone strongly proliferates and secretes large amounts of IL-4 (19, 26). Furthermore, addition of exogenous IL-4 to cultures of clone A1 and B cells from donor DL did not result in IgE synthesis. As mentioned above, PBMC from donor DL vigorously synthesized IgE upon stimulation with rIL-4, and B cells from the same donor were induced to synthesize IgE in the presence of clone F6. These results conclusively show that the signal delivered by IL-4 is not sufficient to induce B cells to synthesize IgE; cognate T/B cell recognition is necessary, as well.

Discussion

We have recently shown that the induction of IgE synthesis by IL-4 requires T cells and monocytes, as well as T cell- and monocyte-derived cytokines (17). Optimal cytokine combinations, however, fail to induce highly purified B cells to secrete IgE, indicating that additional signals are necessary. Our results now show that IL-4-dependent IgE synthesis by circulating B cells requires cognate interaction between the TCR/CD3 complex on T cells and MHC class II antigens on B cells, as well as the contribution of cell adhesion molecules, as determined by the ability of mAbs directed against these structures to block IgE synthesis induced by IL-4, and by the MHC class II-restricted IgE-inducing ability of IL-4-producing alloreactive T cell clones. The observation that anti-MHC class II, but not anti-MHC class I, mAbs inhibited IL-4-dependent IgE synthesis is consistent with the finding that anti-CD4 mAb, but not anti-CD8 mAb, blocked IgE production, and indicates that a CD4$^+$ helper T cell subset is involved in B cell recognition. In future experiments we will attempt a further phenotypic and functional characterization of the T cell population(s) involved in the T cell-dependent induction of IgE synthesis.

We considered the possibility that inhibition of IL-4-induced IgE synthesis by the various antibodies used in this study resulted from direct negative signaling to

the B and/or T cells, rather than from interference with T/B cell recognition. Anti-HLA-DR mAbs, including L243, are known to directly inhibit B cell activation, particularly in its early steps (27, 28). At the T cell level, antibodies to CD2, CD4, and LFA-1 can inhibit signal transduction, thus blocking T cell functions (29–32). It is, however, unlikely that the inhibition of IgE synthesis results from direct negative signals to the T and/or B cells, because inhibition of IgE synthesis by anti-TCR and anti-CD3 mAbs occurred in spite of the induction of vigorous T cell proliferation.

To completely rule out the negative-signal hypothesis, we used an experimental system in which normal B cells are stimulated to produce IgE by IL-4-producing alloreactive T cell clones. Such a system, in which no external stimuli can deliver direct negative signals to the T and/or B cells, is only constrained by T/B cell recognition. Clone F6, which recognizes MHC class II antigens expressed on both B cells and monocytes (26), induced IL-4-dependent IgE synthesis in B cells bearing the relevant alloantigen, but not in HLA-mismatched B cells. By contrast, clone A1, which recognizes MHC class II antigens only on monocytes (26), did not induce IgE synthesis when incubated with HLA-matched B cells and monocytes, in spite of a strong proliferative and lymphokine response. A comparison of the results obtained with clone A1 and F6 suggests that the failure of clone A1 to induce IgE synthesis is likely to result from the lack of direct T/B cell recognition. Previous studies with clone F6 (3) and with clone A1 (19) indicated that bystander B cells are induced to synthesize IgG in cultures containing the cloned T cells and monocytes bearing the stimulator alloantigen. This indicates that the requirements for the synthesis of these two isotypes are not identical.

Interestingly, clone F6 induced IgE synthesis by B cells in spite of its ability to secrete high amounts of IFN-γ. These results are in apparent contrast with previous reports describing the inhibitory effect on human IgE production by IFN-γ contained in the supernatant of alloreactive T cell clones producing both IFN-γ and IL-4 (13, 16). In those reports, however, the responding B cells were not engaged in direct cognate interaction with the T cell clones. Since cognate interaction has been shown to play a crucial role in focusing the release of T cell–derived lymphokines (and in particular, IL-4) at the area in which the TCR is crosslinked by the antigen (33, 34), direct T/B cell recognition, in the presence of endogenously secreted IL-4, may deliver to the B cells an activating signal sufficient (and necessary) to bypass the inhibitory effect of IFN-γ. Furthermore, experiments in the murine system (in which IL-4 typically induces IgE and IgG1 [7]) have shown that the addition of IL-4 to cultures containing a TNP-specific Th1 clone (producing IFN-γ but not IL-4) resulted in the production of IgG1 anti-TNP by TNP-specific B cells (35). These data indicate that IL-4 responsiveness can be retained in the presence of IFN-γ in systems that involve T/B cognate interaction.

Taken together, our results suggest that at least two signals are involved in the T cell–dependent triggering of IgE production by B cells: one signal is delivered by T cells recognizing MHC class II antigens on B cells, the other by T cell–derived IL-4. These two signals are likely to synergize in the activation of B cells into IgE production. IL-4 stimulation of B cells, which bear IL-4 receptors even in their resting state (36, 37), increases MHC class II antigens density by 5- to 10-fold (38). An increased density of MHC class II antigens on B cells may enhance their capacity to activate a subset of T cells recognizing autologous MHC class II antigens + self

peptides (5, 39), or + foreign peptides. In our experimental system, foreign peptides may be derived from the bovine serum. The interaction between TCR and their targets described here seems to have a low affinity, since it is critically dependent on accessory molecules (40).

Thus IL-4 would, on the one hand enhance T/B cognate recognition, and on the other hand, drive the B cell response towards the IgE isotype, acting as an IgE-switching factor (41–43). If T/B cell recognition in the presence of IL-4 is necessary and sufficient to induce IgE synthesis, we would predict that IL-4-dependent IgE production should occur also in allogeneic T/B cell mixtures, as a result of the activation of a subset of T cells able to recognize MHC class II antigens expressed on the allogeneic B cells. Experiments currently in progress in our laboratory indicate that this is indeed the case (Vercelli, D., H. H. Jabara, and R. S. Geha, unpublished observation).

Summary

The induction of IgE synthesis by IL-4 requires T cells and monocytes, as well as T cell- and monocyte-derived cytokines. Optimal cytokine combinations, however, fail to induce highly purified B cells to secrete IgE, indicating that additional signals are required. We show herein that the induction of human IgE synthesis by rIL-4 requires cognate interaction between the T cell receptor/CD3 complex on T cells and MHC class II antigens on B cells: mAbs directed against these molecules completely blocked IL-4-dependent IgE induction. mAbs against cell adhesion molecules (CD2, CD4, LFA-1) also inhibited IgE synthesis induced by IL-4, confirming that cell–cell contact is necessary for IgE induction. The requirement for cognate T/B cell interaction was further shown by comparing the IgE-inducing ability of two human IL-4-producing alloreactive T cell clones: F6, which recognizes MHC class II antigens on both B cells and monocytes, and A1, which recognizes an HLA-DP-associated epitope expressed on monocytes, but not on B cells. When incubated with B cells and monocytes from a normal donor bearing the appropriate alloantigen, clone F6, but not clone A1, induced vigorous IgE synthesis, although both clones proliferated and secreted IL-4. Taken together, our results suggest that at least two, possibly synergizing, signals are required for the T cell-dependent induction of IgE synthesis by B cells: one signal is delivered by cognate T/B cell interaction, the other by T cell-derived IL-4.

We thank Drs. S. J. Burakoff, B. E. Bierer, and J. E. de Vries for the kind gift of the antibodies, and Mr. T. Bonjorno and Mrs. G. Washington for excellent secretarial assistance.

Received for publication 10 October 1988 and in revised form 22 December 1988.

References

1. Saryan, J. A., D. Y. M. Leung, and R. S. Geha. 1983. Induction of human IgE synthesis by a factor derived from T cells of patients with hyper-IgE states. *J. Immunol.* 130:242.
2. Spiegelberg, H. L., J. M. Plummer, J. Ruedi, L. Thompson, M. H. Mellon, and R. S. Zeiger. 1983. Lack of pokeweed mitogen-induced IgE formation in vitro by human peripheral blood mononuclear cells: detection of cross-reacting idiotypic determinants on polyclonal Ig by antibodies to a single IgE myeloma protein. *J. Immunol.* 131:3001.
3. Umetsu, D. T., D. Y. M. Leung, R. Siraganian, H. H. Jabara, and R. S. Geha. 1985.

Differential requirements of B cells from normal and allergic subjects for the induction of IgE synthesis by an alloreactive T cell clone. *J. Exp. Med.* 162:202.

4. Lanzavecchia, A., and B. Parodi. 1984. In vitro stimulation of IgE production at a single precursor level by human alloreactive T-helper clones. *Clin. Exp. Immunol.* 55:197.
5. Leung, D. Y. M., M. C. Young, and R. S. Geha. 1986. Induction of IgG and IgE synthesis in normal B cells by autoreactive T cell clones. *J. Immunol.* 136:2851.
6. Romagnani, S., G. F. Del Prete, E. Maggi, and M. Ricci. 1987. Activation through CD3 molecule leads a number of human T cell clones to induce IgE synthesis in vitro by B cells from allergic and nonallergic individuals. *J. Immunol.* 138:1744.
7. Coffman, R. L., J. Ohara, M. W. Bond, J. Carty, A. Zlotnik, and W. E. Paul. 1986. B cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide-activated B cells. *J. Immunol.* 136:4538.
8. Coffman, R. L., and J. Carty. 1986. A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-γ. *J. Immunol.* 136:949.
9. Finkelman, F. D., I. M. Katona, J. F. Urban, J. Holmes, J. Ohara, A. S. Tung, J. v. Sample, and W. E. Paul. 1988. IL-4 is required to generate and sustain in vivo IgE responses. *J. Immunol.* 141:2335.
10. Finkelman, F. D., I. M. Katona, J. F. Urban, C. M. Snapper, J. Ohara, and W. E. Paul. 1986. Suppression of in vivo polyclonal IgE responses by monoclonal antibody to the lymphokine B-cell stimulatory factor 1. *Proc. Natl. Acad. Sci. USA.* 83:9675.
11. Jabara, H. H., R. S. Geha, and D. Vercelli. 1988. Induction of IgE synthesis by human recombinant IL-4. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 2:6652.
12. Jabara, H. H., S. J. Ackerman, D. Vercelli, T. Yokota, K. Arai, J. Abrams, A. M. Dvorak, M. C. Lavigne, J. Banchereau, J. deVries, D. Y. M. Leung, and R. S. Geha. 1988. Induction of interleukin-4 dependent IgE synthesis and interleukin-5 dependent eosinophil differentiation by supernatants of a human helper T cell clone. *J. Clin. Immunol.* 8:437.
13. Del Prete, G. F., E. Maggi, P. Parronchi, I. Chretien, A. Tiri, D. Macchia, M. Ricci, J. Banchereau, J. deVries, and S. Romagnani. 1988. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *J. Immunol.* 140:4193.
14. Pene, J., F. Rousset, F. Briere, I. Chretien, J. Y. Bonnefoy, H. Spits, T. Yokota, N. Arai, K. Arai, J. Banchereau, and J. deVries. 1988. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons γ and α and prostaglandin E_2. *Proc. Natl. Acad. Sci. USA.* 85:6880.
15. Sarfati, M., and G. Delespesse. 1988. Possible role of human lymphocyte receptor for IgE (CD23) or its soluble fragments in the in vitro synthesis of human IgE. *J. Immunol.* 141:2195.
16. Pene, J., F. Rousset, F. Briere, I. Chretien, X. Paliard, J. Banchereau, H. Spits, and J. E. deVries. 1988. IgE production by normal human B cells induced by alloreactive T cell clones is mediated by IL-4 and suppressed by IFN-γ. *J. Immunol.* 141:1218.
17. Vercelli, D., H. H. Jabara, K. Arai, T. Yokota, and R. S. Geha. 1989. Endogenous IL-6 plays an obligatory role in IL-4 induced human IgE synthesis. *Eur. J. Immunol.* In press.
18. Umetsu, D. T., E. J. Yunis, Y. Matsui, H. H. Jabara, and R. S. Geha. 1985. HLA-DR4 associated alloreactivity of an HLA-DR3 restricted human tetanus toxoid specific T cell clone: inhibition of both reactivities by an alloantiserum. *Eur. J. Immunol.* 15:356.
19. Umetsu, D. T., H. H. Jabara, R. DeKruyff, A. K. Abbas, J. S. Abrams, and R. S. Geha. 1988. Functional heterogeneity among human inducer T cell clones. *J. Immunol.* 140:4211.
20. Helm, R. M., R. H. Buckley, N. F. Adkinson, D. L. Squillace, G. J. Gleich, and J. W. Yunginger. 1986. Variability of IgE protein measurement in cell-culture supernatants: results from a multicenter collaborative study. *J. Allergy Clin. Immunol.* 77:880.
21. Bierer, B. E., and S. J. Burakoff. 1988. T cell adhesion molecules. *FASEB (Fed. Am. Soc.*

Exp. Biol.) J. 2:2584.

22. Springer, T. A., M. L. Dustin, T. K. Kishimoto, and S. D. Marlin. 1987. The lymphocyte function-associated LFA-1, CD2, and LFA-3 molecules: cell adhesion receptors of the immune system. *Annu. Rev. Immunol.* 5:223.
23. Tax, W. J. M., F. F. M. Hermes, R. W. Willems, P. J. Capel, and R. A. P. Koene. 1984. Receptors for mouse IgG1 on human monocytes: polymorphism and role in antibody-induced T cell proliferation. *J. Immunol.* 133:1185.
24. Spits, H., J. Borst, W. Tax, P. J. A. Capel, C. Terhorst, and J. E. deVries. 1985. Characteristics of a monoclonal antibody (WT-31) that recognizes a common epitope on the human T cell receptor for antigen. *J. Immunol.* 135:1922.
25. Vercelli, D., D. Y. M. Leung, H. H. Jabara, and R. S. Geha. 1989. Interleukin 4 dependent induction of IgE synthesis and CD23 expression by the supernatants of a human helper T cell clone. *Int. Arch. Allergy Appl. Immunol.* In press.
26. Umetsu, D. T., H. H. Jabara, P. Hauschka, and R. S. Geha. 1988. Recognition by a human alloreactive T-cell clone of an HLA-DP-associated epitope on monocytes and fibroblasts but not on B cells. *J. Clin. Immunol.* 8:163.
27. Clement, L. T., T. F. Tedder, and G. L. Gartland. 1986. Antibodies reactive with class II antigens encoded for by the major histocompatibility complex inhibit human B cell activation. *J. Immunol.* 136:2375.
28. Cambier, J. C., and J. T. Ransom. 1987. Molecular mechanisms of transmembrane signaling in B lymphocytes. *Annu. Rev. Immunol.* 5:175.
29. Hünig, T., G. Tiefenthaler, K. H. Meyer zum Büschenfelde, and S. C. Meuer. 1987. Alternative pathway of activation of T cells by binding of CD2 to its cell-surface ligand. *Nature (Lond.).* 326:298.
30. Bank, I., and L. Chess. 1985. Perturbation of the T4 molecule transmits a negative signal to T cells. *J. Exp. Med.* 162:1294.
31. van Noesel, C., F. Miedema, M. Brouwer, M. A. de Rie, L. A. Aarden, and R. A. W. van Lier. 1988. Regulatory properties of LFA-1 α and β chains in human T lymphocyte activation. *Nature (Lond.).* 333:850.
32. Sleckman, B. P., A. Peterson, W. K. Jones, J. A. Foran, J. L. Greenstein, B. Seed, and S. J. Burakoff. 1987. Expression and function of CD4 in a murine T-cell hybridoma. *Nature (Lond.).* 328:351.
33. Sanders, V. M., J. M. Snyder, J. W. Uhr, and E. S. Vitetta. 1986. Characterization of the physical interaction between antigen-specific B and T cells. *J. Immunol.* 137:2395.
34. Poo, W., L. Conrad, and C. A. Janeway. 1988. Receptor-directed focusing of lymphokine release by helper T cells. *Nature (Lond.).* 332:378.
35. Stevens, T. L., A. Bossie, V. M. Sanders, R. Fernandez-Botran, R. L. Coffman, T. R. Mosmann, and E. S. Vitetta. 1988. Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. *Nature (Lond.).* 334:255.
36. Ohara, J., and W. E. Paul. 1987. Receptors for B-cell stimulatory factor-1 expressed on cells of haematopoietic lineage. *Nature (Lond.).* 325:537.
37. Park, L. S., D. Friend, H. M. Sassenfeld, and D. L. Urdal. 1987. Characterization of the human B cell stimulatory factor 1 receptor. *J. Exp. Med.* 166:476.
38. Killar, L., G. MacDonald, J. West, A. Woods, and K. Bottomly. 1987. Cloned, Ia-restricted T cells that do not produce interleukin-4 (IL-4)/B cell stimulatory factor-1 (BSF-1) fail to help antigen-specific B cells. *J. Immunol.* 138:1674.
39. Tilkin, A. F., J. Michon, D. Juy, M. Kayibanda, Y. Henin, G. Sterkers, H. Betuel, and J. P. Levy. 1987. Autoreactive T cell clones of MHC class II specificities are produced during responses against foreign antigens in man. *J. Immunol.* 138:674.
40. Marrack, P., and J. Kappler. 1988. T cells can distinguish between allogeneic major histocompatibility complex products on different cell types. *Nature (Lond.).* 332:840.

41. Lebman, D. A., and R. L. Coffman. 1988. Interleukin 4 causes isotype switching to IgE in T cell-stimulated clonal B cell cultures. *J. Exp. Med.* 168:853.
42. Lutzker, S., P. Rothman, R. Pollock, R. Coffman, and F. W. Alt. 1988. Mitogen- and IL-4-regulated expression of germ-line Ig γ2b transcripts: evidence for directed heavy chain class switching. *Cell.* 53:177.
43. Savelkoul, H. F. J., D. A. Lebman, R. Benner, and R. L. Coffman. 1988. Increase of precursor frequency and clonal size of murine IgE-secreting cells by IL-4. *J. Immunol.* 141:749.