DIFFERENTIAL REQUIREMENTS OF B CELLS FROM NORMAL AND ALLERGIC SUBJECTS FOR THE INDUCTION OF IgE SYNTHESIS BY AN ALLOREACTIVE T CELL CLONE

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The application of immunologic advances regarding B cell activation to the understanding of the human IgE antibody response has been hampered by the lack of reproducible in vitro models for the induction of IgE synthesis (1–3). Recently, Lanzavecchia (4) has reported that human alloreactive T cell clones can induce IgE secretion by human B cells. However, their study did not characterize the allergic status of the B cell donors. B cells from allergic donors differ from normal B cells in that they spontaneously secrete IgE (1–3, 5, 6). Because of this, B cells from allergic vs. normal subjects are likely to differ in their requirements for the induction of IgE synthesis in vitro. The delineation of these differences has important implications for the understanding of the human allergic response.

In the present study we report that an alloreactive T cell clone can induce IgE synthesis in B cells from both allergic and nonallergic individuals. It was found that induction of IgE synthesis in B cells from nonallergic donors occurred only under conditions of cognate interaction with T cells, in which the B cells expressed the alloantigen recognized by the T cells. In contrast, IgE synthesis in B cells from allergic donors did not necessarily require direct interaction with T cells. It occurred under conditions of cognate interaction with T cells as well as under bystander conditions in which the B cells did not express the alloantigen recognized by the T cell clones. These differential requirements for induction of IgE synthesis in B cells from normals and allergic subjects may reflect differences in the state of activation of in vivo circulating IgE B cells in these donors.

Materials and Methods

B Cell Donors. Six healthy adult nonallergic subjects were selected on the basis of a negative history for allergies and on the basis of a serum IgE level <75 IU/ml (as

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determined by a PRIST assay; Pharmacia, Inc., Uppsala, Sweden). Three adult allergic subjects with positive histories of allergic disease (e.g., seasonal allergic rhinitis, extrinsic asthma, and eczema) and with elevated serum IgE levels (>400 IU/ml) were also studied. Written consent was obtained from all subjects before the study. Peripheral blood mononuclear cells (PBMC)[1] from donors were HLA-DR typed in a standard microcytotoxicity assay by the Histocompatibility Lab at the Dana Farber Cancer Institute, Boston.

Isolation of Mononuclear Cell Subpopulations. PBMC were isolated from the blood of healthy donors by flotation over Ficoll-Hypaque, and washed three times with Hanks' balanced salt solution.

B Cells. T cells were depleted from PBMC by rosette formation with aminoethylisothioronium (AET)-treated sheep cells. E rosette–negative cells were enriched by flotation over Ficoll-Hypaque. In some experiments, E rosette–negative cells were further purified by treatment with OKT3 (Ortho Pharmaceutical, Raritan, NJ) and complement (Pel-Freeze Biologicals, Rogers, AR) to remove residual T cells.

Preparation of Monocytes. PBMC were incubated overnight in petri dishes at 37°C in media containing 10% AB+ serum. In some experiments the PBMC were pulsed overnight with tetanus toxoid, 50 μg/ml. Nonadherent cells were then removed, and the remaining adherent cells were washed with warm media. Ice-cold phosphate-buffered saline (PBS) was added to the dishes, and the monocytes were aspirated and resuspended in RPMI 1640. Monocytes were irradiated (2,500 rad) before being added to 96-well plates (2×10^4 cells per well).

Preparation of T Cell Clones. T lymphocyte clones with specificity for tetanus toxoid (TT) and/or alloantigens were produced as previously described (7). Two clones were used. Clone F6 is a TT-specific clone that is restricted by HLA-DR3 and shows limited alloreactivity (7). G8 is a TT-specific clone restricted by HLA-DR5. These clones were derived from an HLA-DR3,5 donor as follows. 10×10^6 PBMC were suspended in 10 ml RPMI 1640 (M. A. Bioproducts, Walkersville, MD) containing 10% AB+ serum (complete medium) and stimulated with TT at 30 μg/ml. After incubation at 37°C in 5% CO_2 for 6 or 7 d, the cultures were harvested and dividing cells were enriched by centrifugation over a Percoll (Pharmacia, Inc.) discontinuous gradient at 1,500 rpm for 45 min. Cells at the 30–50% interface were resuspended in complete medium supplemented with 25% interleukin 2–containing supernatants (IL-2 SN) that contained irradiated (2,500 rad) autologous PBMC plus TT and were distributed in 96-well trays (Nunc, Roskilde, Denmark). Each 0.2 ml well contained an average of 0.3 blast and 10^5 irradiated PBMC. After 14–21 d of culture, cells were transferred to 24-well trays (Linbro Scientific Co., Hamden, CT). Clones have been maintained in culture for >1 yr with repeated stimulation with antigen (TT and autologous PBMC) and IL-2 SN. T cell clones were extensively subcloned by limiting dilution at 0.3 cell per well.

IL-2 SN. PBMC were obtained from donors previously screened for their capacity to generate high activity IL-2. PBMC were depleted of monocytes by adherence to plastic petri dishes and the nonadherent cells were irradiated (1,000 rad). Cells were suspended at 10^6 cells/ml in RPMI 1640 with 2% AB+ serum containing phytohemagglutinin (1 μg/ml; Burroughs Wellcome Co., Research Triangle Park, NC), and harvested after 48 h.

Immunoglobulin Synthesis in Cell Cultures. The cell culture system used for the induction of immunoglobulin synthesis was performed in 96-well microtiter plates, in RPMI 1640 medium containing 10% fetal calf serum in a humidified atmosphere of 5% CO_2 in air. Each microtiter well contained the following: (*a*) E rosette–negative cells (B cells), 1×10^5/well; (*b*) irradiated (2,500 rad) monocytes (to activate the T cell clones), 2×10^4/well; (*c*) irradiated (2,500 rad) cloned T cells, 4×10^4/well, when indicated. After 96 h of culture the cells were washed twice with culture medium. This washing step was done to remove IgE released in the first 4 d of culture. Fresh medium was added, and cultures were incubated for an additional 8 d in the presence or absence of the protein synthesis

[1] *Abbreviations used in this paper:* ELISA, enzyme-linked immunosorbent assay; HS, horse serum; IL-2, interleukin 2; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PWM, pokeweed mitogen; RIA, radioimmunoassay; SN, supernatant; TT, tetanus toxoid.

inhibitor cycloheximide (100 μg/ml). SN were harvested from duplicate cultures and assayed for immunoglobulin (IgE, IgG, IgA, IgM) content.

Acid Treatment of Cultured Cells. To determine the amount of IgE associated with the cells, we acid-treated the cells at days 0 and 4 after washing, as described by Turner et al. (8). 5×10^5 E rosette–negative B cells with 10^5 irradiated adherent cells were pelleted in 5-ml tubes. The cells were resuspended in 0.4 ml of 0.01 M glycine buffer, pH 2, for 1 min. 0.6 ml of 0.15 M PBS, pH 7.4, was added to obtain a final pH of 7 and a final volume of 1 ml. The SN were then collected and assayed for IgE.

Radioimmunoassay (RIA) for IgE. Duplicate samples from each culture were analyzed for their IgE content. The RIA for IgE was performed in flexible flat-bottom microtiter plates (Becton, Dickinson & Co., Oxnard, CA). The wells were filled with 0.1 ml of a 10 μg/ml solution containing a 1:1 mixture of two monoclonal anti–human IgE, Fc-specific antibodies (monoclonal antibodies 8 and 9). After incubation for 16 h, the coating solution was removed, and the wells were washed and blocked with 10% horse serum (HS) in PBS for 2 h. After washing three times with PBS containing 1% HS, we added 0.1 ml of culture SN or of IgE standard to each of the triplicate wells and incubated them for 16 h in a humidified chamber at room temperature. The wells were then washed twice with PBS, 1% HS containing 0.5% Tween 20 and twice with PBS, 1% HS alone; then 0.1 ml of Phadebas RAST ^{125}I-anti–human IgE (ND) (Pharmacia, Inc.; sp act, 12 μCi/μg) was added to each well. 6 h later the radiolabeled anti-IgE was removed and wells were washed three times with PBS, 1% HS and eight times under running distilled water. The wells were cut out and counted in a gamma spectrometer (TM Analytic, Inc., Elk Grove, IL). Standard curves were constructed using dilutions of the IgE standards obtained from Pharmacia, Inc. The concentration of IgE in the SN was read from the standard curve. Results were expressed as the mean of duplicate determinations of duplicate cultures. The lower limit of sensitivity of this assay was 100–200 pg/ml.

The specificity and sensitivity of our IgE RIA was confirmed in a recently completed multi-institutional study coordinated by the Mayo Clinic (Drs. Yunginger, Helm and Gleich; manuscript in preparation). Coded samples with varying quantities of polyclonal IgE in the presence of other immunoglobulin isotypes sent to our laboratory were evaluated using our IgE RIA. In no test sample did we measure falsely elevated IgE values, thus ruling out the possibility of crossreactivity with other isotypes. Furthermore, values of IgE >300 pg/ml were measured accurately within 10% of expected values.

Enzyme-linked Immunosorbent Assays (ELISA) of SN for IgG, IgA, and IgM. 96-well polystyrene plates were coated with immunoabsorbed, purified goat anti-IgG, anti-IgA, or anti-IgM (Tago, Inc., Burlingame, CA) (10 μg/ml). After washing with PBS, 0.1% Tween 20, the plates were incubated with a 0.1% gelatin solution for 1 h and washed, and 0.1 ml of appropriately diluted SN were added to the wells. After 18 h the plates were again washed, and horseradish peroxidase–conjugated anti-IgG, -IgA, or -IgM was added (1:3000). The plates were incubated for 1 h at 37°C and washed, and the bound, tagged antibody was quantitated using the substrate, azino-diethyl-benzthiazoline sulfonic acid. Plates were incubated with the substrate for 30 min, and the absorbance at 414 nm was read on a Titertek Multiskan Spectrophotometer (Flow Laboratories, Inc., McLean, VA). Standard curves were performed using purified Ig (Kallestad Laboratories, Austin, TX). The specificity of the conjugated antisera was ascertained by testing in a direct ELISA against purified human myeloma G,M,A and kappa and lamda Bence Jones proteins.

Results

Induction of IgE Synthesis by Alloreactive T Cell Clones. The characteristics of the T cell clones used in this study are shown in Table I. Both clones were $T4^+$. Clone F6 exhibited both alloreactivity and reactivity to TT antigen (described in detail in reference 7). The alloreactivity of F6 is preferentially but not exclusively directed to HLA-$DR4^+$ cells. F6 proliferated in response to stimulation with 13 of 14 HLA-$DR4^+$ cells and 3 of 18 of HLA-$DR4^-$ cells. F6 also

TABLE I
Characteristics of T Cell Clones

Clone	Surface phenotype	Alloreactivity	Antigen specificity
F6	$T4^+$	HLA-DR4 and others	TT and HLA-DR3
G8	$T4^+$	None	TT and HLA-DR5

TABLE II
Characteristics of B Cell Donors

Donors	Atopic disease	Serum IgE	HLA-DR	Capacity to stimulate F6
		IU/ml		
Allergic subjects				
KR	A.R., asthma, eczema	2,000	3, 5	−
DU	A.R., eczema	1,200	9, −	+
DY	A.R., asthma	488	3, 4	+
Nonallergic subjects				
MS	—	72	1, 7	+
RG	—	63	5, 6	−
NW	—	50	3, −	−
DL	—	36	3, 4	+
RF	—	10	1, 2	−
SY	—	<4	3, 4	+

The capacity of x-irradiated (2,500 rad) PBMC to stimulate the proliferation of clone F6 over a 4 d culture period was studied. Stimulation indices were calculated by dividing counts per minute (cpm) of [^{3}H]thymidine incorporated in these cultures by the cpm of [^{3}H]thymidine incorporated in cultures where F6 was stimulated with autologous irradiated PBMC obtained from donor F6. Irradiated PBMC from DU, DY, MS, DL, and SY, respectively, caused a 81, 89, 35, 80, and 84-fold stimulation of F6 cells. PBMC from all other donors caused less than twofold stimulation of F6 cells. A.R., allergic rhinitis.

proliferated to TT presented by HLA-$DR3^+$ accessory cells. Clone G8 is a TT-specific clone that exhibited no alloreactivity against a panel of 10 randomly chosen donors. G8 proliferated to TT presented by HLA-$DR5^+$ cells (7).

The characteristics of the nine adult donors whose B cells were used in this study are shown in Table II. These characteristics include allergic status, serum IgE, HLA-DR phenotype, and their cells' ability to stimulate F6.

Table III depicts an experiment in which the alloreactive clone F6 was used to induce Ig synthesis in B cells obtained from two donors, one allergic and one nonallergic. Cells from both donors were known to be capable of stimulating the F6 proliferation. In the presence of irradiated, cloned F6 cells, polyclonal Ig, including IgE, synthesis was induced in B cells of both donors (Table III). Spontaneous IgE synthesis could be detected only in cultures of B cells from the allergic and not the nonallergic subject. Pokeweed mitogen (PWM) (Sigma Chemical Co., St. Louis, MO), in the presence of added autologous T cells, failed to induce IgE synthesis in B cell cultures of both subjects in spite of PWM's capacity to cause vigorous synthesis of IgM, IgA, and IgG isotypes.

TABLE III

Induction of Antibody Synthesis by Alloreactive T Cell Clone F6

B cell donor			Ig produced			
Donor	Allergic status	Stimulus	IgG	IgM	IgA	IgE
			ng/ml	*ng/ml*	*ng/ml*	*pg/ml*
DL	Nonallergic	—	200	200	90	<150
		F6	6,250	8,000	543	2,530
		PWM	4,440	10,800	3,150	<150
DU	Allergic	—	200	200	90	670
		F6	39,000	20,250	840	4,657
		PWM	4,760	3,200	1,380	<150

All cultures contained 10^5 E rosette-negative cells (B cells). Cultures stimulated with F6 received 4 × 10^4 irradiated (2,500 rad) F6 cells. Cultures stimulated with PWM received autologous T cells (T/B ratio, 2:1) and 1 μg/ml of PWM. All cultures were washed on day 4 and SN were collected from day 4 to day 12 and assayed for Ig content. The results represent the mean of duplicate Ig determinations of duplicate cultures. Similar results were obtained in four other experiments.

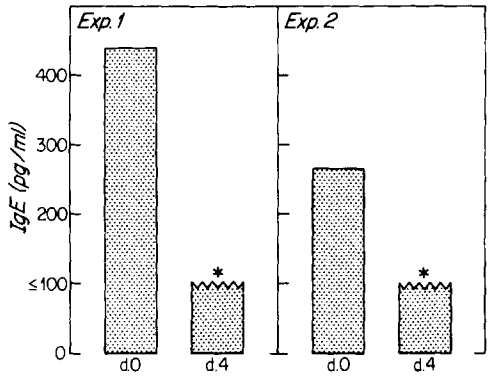

FIGURE 1. IgE associated with the cell pellet as determined by acid elution from the cell pellets. IgE associated with the cell pellet on day 0 was released into the culture supernatant by day 4 of culture. (*) Undetectable.

Calculation of IgE Synthesis. In the present experiments we have minimized the contribution of preformed IgE to our measurement of IgE synthesis. Cultures were washed on day 4 to remove IgE released into the SN in the first 4 d in culture. Acid elution of the cell pellets at days 0 and 4 showed that, by day 4, IgE associated with the cell pellet had become undetectable (Fig. 1) presumably because it was released into the culture supernatant. The day-4 washed cells were then incubated for eight more days in the presence or absence of cycloheximide. Fig. 2 shows that cycloheximide-treated cultures contained no detectable IgE. In subsequent tables results are presented as net IgE synthesis, obtained by subtracting IgE values of cell pellets at day 4 from IgE values in SN of day 4–12 cultures.

Induction of IgE Synthesis by T Cell Clones in B Cells Under Conditions of Cognate and Noncognate Interaction. We next asked if induction of IgE synthesis in B cells by the alloreactive clone F6 could proceed under conditions of noncognate interaction as well as under conditions of cognate interaction. Under conditions

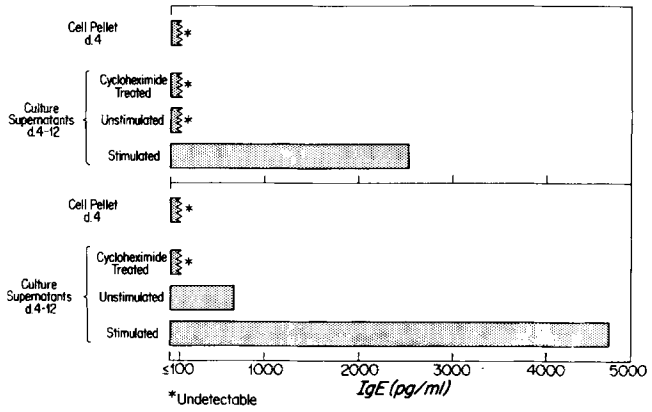

FIGURE 2. IgE values of cultures using B cells from a nonallergic donor (*top*) and B cells from an allergic donor (*bottom*). Net IgE synthesis in stimulated cultures for tables was derived by subtracting IgE associated with cell pellet on day 4 from total IgE in supernatants of stimulated cultures.

TABLE IV

Alloreactive Clone F6 Induces B Cells from Allergic Subjects to Secrete IgE Under Conditions of Cognate and Noncognate Interaction

B-T cell interaction	Donor of B cells	Clone F6	Ig produced	
			IgE	IgG
			pg/ml	*ng/ml*
Cognate	DU	−	600	75
		+	9,600	10,000
	DY	−	355	50
		+	1,465	4,250
Noncognate	KR	−	695	62
		+	1,490	1,963

Cultures contained 10^5 E rosette–negative cells (B cells), and, where indicated, 4×10^4 irradiated F6 cells. Under conditions of cognate interaction, 2×10^4 additional irradiated monocytes from the donor of the B cells were added to cultures. Under conditions of noncognate interaction, 2×10^4 irradiated monocytes from third-party donors whose cells stimulated F6 were added. All B cell donors had serum IgE levels >400 IU/ml.

of cognate interaction the B cells expressed alloantigens recognized by F6 as was seen in Table III. Under conditions of noncognate interaction, the B cells lacked these alloantigens, and irradiated third-party monocytes known to be allostimulators of F6 were added to the cultures. Table IV depicts the results obtained with B cells from allergic subjects stimulated by F6 under cognate or noncognate conditions. IgE synthesis was induced in these cells under conditions of cognate interaction as well as noncognate interaction in which B cells were bystanders. Similar findings were observed for the induction of IgG synthesis.

Table V shows that, in contrast to B cells from allergic donors, B cells from normal donors were induced to synthesize IgE only under conditions of cognate interaction with F6 (donors DL, MS, SY). Bystander normal B cells that did not

express alloantigens capable of stimulating F6 synthesized no IgE in the presence of F6 and third-party allostimulator monocytes (Table V, donors RG, RF, NW). In the same cultures, the normal bystander B cells from these three donors were induced to synthesize IgG. Thus, the failure of normal B cells to synthesize IgE under conditions of noncognate interaction was IgE isotype specific and was not due to lack of activation of F6. Failure of bystander normal B cells to synthesize IgE occurred even when the donor of B cells shared a common HLA-DR determinant (HLA-DR3 or HLA-DR5) with the donor of the T cell clone. Thus, mere sharing of HLA-DR determinants by the clone and by B cells was not sufficient to activate the B cells into IgE synthesis.

The induction of IgE in bystander B cells from allergic donors but not from normal donors was not restricted to alloreactive clones. Table VI shows that similar data was obtained when B cells were cultured with irradiated T cells from the TT-specific clone G8 in the presence of TT-pulsed, HLA-DR5$^+$ third-party monocytes known to activate G8. IgE synthesis in these cultures was induced in B cells from allergic donors, but not in B cells from nonallergic donors, whereas IgG synthesis was induced in B cells from all donors.

Role of Residual T Cells on Induction of IgE Synthesis in B Cells. The induction of IgE synthesis in B cell cultures by cloned T cells was unlikely to have been mediated by residual T cells that contaminated the B cell–enriched populations. B cell–enriched populations from allergic subjects vigorously depleted of residual T cells by treatment with OKT3 and complement (Table VII) synthesized equivalent amounts of IgE as non OKT3 plus C′–treated B cell populations when stimulated with cloned T cells. This was regardless of whether the stimulus was delivered by cognate or noncognate bystander interaction.

Role of Antigen Recognition by T Cells in B Cell Activation. It is important to

TABLE V

Clone F6 Induces B Cells from Nonallergic Donors to Secrete IgE Only Under Conditions of Cognate Alloreactive Recognition

B-T cell interaction	Source of B cells	Clone F6	Ig produced	
			IgE	IgG
			pg/ml	*ng/ml*
Cognate	DL	−	<150	110
		+	8,000	10,000
	MS	−	200	170
		+	7,970	5,000
	SY	−	<150	400
		+	925	33,000
Noncognate	RG	−	<150	20
		+	<150	5,100
	RF	−	<150	20
		+	<150	1,475
	NW	−	<150	75
		+	<150	6,000

Culture conditions are similar to those described in Table IV. All B cell donors had serum IgE levels <75 IU/ml.

TABLE VI

Failure of an Antigen-specific T Cell Clone G8 to Induce IgE Secretion from Nonallergic Subjects

Source of B cells	Clone G8	Ig produced	
		IgE	IgG
		pg/ml	*ng/ml*
Allergic Donors			
DU	−	1,350	120
	+	12,380	2,000
KR	−	700	225
	+	6,275	2,400
Nonallergic Donors			
NW	−	187	140
	+	<150	3,000
MS	−	<150	250
	+	225	3,500
RG	−	<150	20
	+	<150	1,250
DL	−	<150	44
	+	<150	1,335

All cultures contained 10^5 E rosette–negative cells (B cells), and, where indicated, 4×10^4 irradiated G8 cells. Third-party HLA-DR5$^+$, TT-pulsed monocytes (irradiated with 2,500 rad) were added to all cultures to stimulate G8.

TABLE VII

Effect of Residual T Cells on Induction of IgE Synthesis in B Cell Cultures by T Cell Clones

Type of B-T interaction	B cell donor	Stimulating clone	OKT3 plus C′	IgE	IgG
				pg/ml	*ng/ml*
Cognate	Allergic	—	−	235	184
		—	+	500	90
		F6	−	4,600	62,500
		F6	+	4,450	75,000
Noncognate (bystander)	Allergic	—	−	280	230
		—	+	450	100
		G8	−	1,500	2,250
		G8	+	2,080	3,350

Culture conditions are similar to those described in Table IV. E rosette–negative cells were incubated with OKT3 at 0°C as indicated. After 40 min at 0°C, baby rabbit complement was added. After 30 min incubation at 37°C, the cells were washed and then placed in culture (1×10^5/well). Clone F6 was stimulated by alloantigens expressed by the B cell donor. Clone G8 was stimulated by the addition of HLA-DR5$^+$, TT-pulsed monocytes.

emphasize that, in the absence of T cell recognition of antigen, no activation signal was delivered to the B cells. When B cells from a normal subject whose cells did not stimulate clone F6 proliferation were cultured with F6 (Table VIII), neither IgE nor IgG was secreted. Only when HLA-DR4$^+$ third-party monocytes were added to the culture were the normal B cells stimulated to secrete IgG, but

TABLE VIII

Antigen Recognition by Cloned T Cells Is Required for the Induction of Ig Synthesis Under Conditions of Noncognate Bystander Interaction

Source of bystander B cells	Third-party stimulator monocytes	Clone	Ig produced	
			IgE	IgG
			pg/ml	*ng/ml*
Nonallergic	+	F6	<150	1,500
	−	F6	<150	20
Allergic	+	G8	1,105	1,300
	−	G8	<150	55

Culture conditions are similar to those described in Table IV. HLA-DR5$^+$, TT-pulsed monocytes (2,500 rad irradiated) functioned as stimulator cells for G8. HLA-DR4$^+$ monocytes (2,500 rad irradiated) functioned as stimulator cells for F6.

not IgE, via a bystander interaction. When B cells from an allergic subject were cultured with clone G8, neither IgG nor IgE synthesis was induced unless TT-pulsed HLA-DR5$^+$ monocytes were added to activate clone G8.

Discussion

The present study demonstrates that de novo IgE synthesis can be induced in B cells of allergic as well as normal subjects under conditions of cognate interaction by an alloreactive clone. Furthermore, B cells from allergic but not from normal donors synthesized IgE when present as bystanders in cultures containing alloactivated or antigen-activated cloned T cells.

Until recently, the study of human IgE synthesis has been limited to the spontaneous IgE secretion by B cells from allergic individuals (1–3, 5, 6). In our hands and those of several other investigators (1, 2), polyclonal B cell activators, such as PWM, have failed to induce IgE synthesis in peripheral blood B cells of both normal and allergic donors. The unique ability of T cell clones to induce IgE synthesis in B cells from normal donors may relate to several observations. First, these clones are more powerful polyclonal activators than PWM in that they cause better production of IgG and IgM (Table III) and activate a higher frequency of B cells (4). Second, T cell clones activate both small as well as large B cells (9, 10) whereas PWM appears to activate only large B cells that are thought to represent B cells preactivated in vivo. Finally, helper T cell clones provide helper activity for Ig synthesis in the absence of suppressor T cells. In murine systems, interactions of unfractionated T cells with allogeneic B cells have been reported to induce IgG synthesis and suppress IgE synthesis (11). It is likely that IgE synthesis is uniquely sensitive to suppressor signals generated by unfractionated T cells responding in a mixed lymphocyte reaction. The differential sensitivity of the IgE vs. IgG antibody response to suppressor signals has been well documented (12).

Although, in the present study, we used a single alloreactive T cell clone, F6,

to induce IgE synthesis, this phenomenon is not limited to clone F6. Indeed, Lanzavecchia (4) observed this phenomenon with several alloreactive clones. The successful induction of IgE synthesis in normal nonallergic subjects under conditions of cognate interaction with T cell clones demonstrates the presence of circulating B cells with the potential to differentiate into IgE-secreting cells in normal individuals. It is not known whether the T cell clones activate B cells already committed to express IgE and/or cause switching of some B cells to synthesize IgE. In this regard, Mayer et al. (13) have recently presented evidence on the ability of monoclonal human leukemic T cells to cause an IgM to IgG switch in B cells of patients with the hyper IgM syndrome.

Perhaps the most important finding of the present study is the demonstration of a basic difference between allergic and nonallergic subjects in the IgE response of their B cells under conditions of bystander stimulation by T cell clones. The data demonstrate that normal B cells can be activated only under conditions of cognate interaction by the T cell clones (Table IV). Normal B cells that lacked the stimulatory alloantigens made no IgE in the presence of alloreactive T cells stimulated by third-party monocytes bearing the appropriate alloantigens. This observation makes it likely that the induction of IgE synthesis in normal B cells bearing the stimulating alloantigens actually involves direct recognition of these antigens by the T cell on the B cell surface, rather than bystander activation resulting from recognition of the alloantigens on the surface of monocytes in the B cell–enriched populations. Thus, direct T-B cell interaction via T cell recognition of B cell surface alloantigen appears to be required for IgE induction in normal B cells.

Recently, Nutman et al. (14), using filarial parasite–specific T cell lines or their SN, were able to induce IgE synthesis in B cells from nonatopic B cells. They found, however, that SN from helper T cell lines raised against a nonparasite antigen did not induce IgE production, perhaps indicating that the parasite antigen was critical for the induction of IgE synthesis in their system. The antigen-specific clone used in our study (clone G8) was directed against TT, a nonparasite antigen. Neither this nor other TT-specific clones (data not shown) induced IgE synthesis in normal B cells.

In contrast to the requirement for cognate interaction for the induction of IgE synthesis in normal B cells, induction of IgG synthesis in the same B cells occurred both under conditions of cognate interaction and bystander activation. Our finding that T cell clones provide major histocompatibility complex–unrestricted help for IgG synthesis by human B cells confirms and extends the reports of others (4, 15). The reason for the differential response of IgG and IgE synthesis to bystander stimulation of normal B cells may relate to the in vivo activation status of B cells committed, respectively, to IgG and IgE synthesis in normal subjects. The lack of spontaneous IgE synthesis by normal circulating B cells suggests that few if any IgE B cells circulate in an activated state in normal nonallergic subjects. On the other hand, B cells from normal individuals spontaneously produce IgG. The majority of the in vivo activated B cells that spontaneously secrete IgG have been shown to reside in the less dense fractions of a density gradient, to be large, and to respond to T cell–dependent, PWM-triggered activation (16) and to T cell–derived factors (17).

In contrast to normal B cells, B cells from allergic subjects responded to bystander stimulation by synthesizing IgE (Table IV). This response is consistent with the presence in these individuals of circulating, activated IgE B cells that synthesize IgE spontaneously. These secreting IgE cells were recently shown (18) to be relatively large and to respond to T cell–derived, IgE-binding factors by enhancing their IgE synthesis.

Several mechanisms may underlie the bystander activation of B cells by alloreactive and antigen-specific T cell clones. It is possible that B cells activated in vivo are selectively capable of capturing shed alloantigens or the immunogenic complex of TT plus HLA-DR, and of presenting these antigens to T cells. This would result in direct T-B cell interaction and subsequent induction of Ig synthesis, and would account for the selective induction of IgE synthesis in bystander B cells from allergic donors, since only those donors possess activated B cells that are committed to IgE synthesis. An alternative explanation for the bystander effect would involve the action of soluble mediators derived from the T cell clones. SN of activated T cell clones have been shown (17) to contain a variety of factors, including B cell growth and differentiation factors (BCGF, BCDF), interleukin 2, and IFN, that enhance expansion and differentiation of partially activated B cells. Furthermore, IgE-binding factors that enhance IgE synthesis (19, 20) can be released by T cells bearing Fc receptors for IgE. All these factors, including IgE-binding factors, appear to exert their action on preactivated B cells rather than on resting B cells (19). Indeed, resting B cells either do not express enough receptors for these factors and/or do not respond to them. Experiments are currently underway to determine whether the bystander effect can be mediated by SN of activated T cells clones.

The results of the present investigation have important implications for the study of the human IgE response. Induction of IgE synthesis by alloreactive T cells may underlie the rise in IgE serum in acute graft-vs.-host disease (21). Bystander activation of B cells into IgE synthesis by antigen-reactive T cells may play a role in the polyclonal IgE response in viral and parasitic infections. Finally, T cell clones should provide a useful tool for detailed study of the regulation of the human IgE response in health and disease.

Summary

Human T cell helper/inducer clones were used to induce IgE synthesis in B cells from both allergic and nonallergic donors. An alloreactive T cell clone, activated by recognition of specific HLA-DR antigens, stimulated peripheral blood B cells from both allergic and nonallergic donors to synthesize IgE antibody. B cells of allergic donors differed from those of nonallergic donors in their requirements for induction of IgE synthesis. Induction of IgE synthesis in B cells from nonallergic individuals occurred only under conditions of cognate interaction, in which the B cells expressed the alloantigen recognized by the T cells. In contrast, IgE synthesis in B cells from allergic donors occurred under conditions of cognate interaction with T cells as well as bystander conditions where the B cells did not express the alloantigen recognized by the T cell clones and where the T cell clones were stimulated by third-party monocytes bearing the relevant alloantigens. Furthermore, bystander stimulation of IgE synthesis

in allergic donors occurred in the presence of tetanus toxoid (TT) antigen-specific T cell clones activated by the appropriate TT-pulsed monocytes.

In contrast to the differing requirements of B cells from normal vs. allergic subjects for the induction of IgE synthesis, these B cells did not differ in their requirements for the induction of IgG synthesis. IgG synthesis was induced in all B cells under conditions of cognate interaction with the T cells as well as under conditions of bystander stimulation. These results suggest that cognate T-B cell interactions may be important in the development of IgE immune responses in the normal host.

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