

NONCOGNATE CONTACT-DEPENDENT B CELL ACTIVATION CAN PROMOTE IL-4-DEPENDENT IN VITRO HUMAN IgE SYNTHESIS¹

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We have previously shown that IL-4 is an essential mediator for the synthesis of human IgE in vitro. In this study we demonstrate that prior physical contact with T cells is required by B cells to synthesize IgE in response to IL-4. Both autologous and allogeneic freshly prepared T cells were consistently able to support IL-4-dependent IgE synthesis, provided that they were added to B cells together with, or before, the addition of IL-4. In addition, most CD4⁺, as well as a proportion of CD8⁺, PHA-induced T cell clones (TCC) established from two HLA-DR incompatible donors, supported, in the presence of exogenous IL-4, the synthesis of IgE in B cells from the majority of individuals tested including both donors of cloned T cells. An alloreactive TCC able to produce IL-4 in response to HLA-DR4⁺ B cells and to induce HLA-DR4⁺ B cells to synthesize IgE, acquired the ability to support IgE synthesis by B cells lacking the appropriate alloantigen provided that exogenous IL-4 was added. Although the ability of freshly prepared T cells to support IgE synthesis was consistently abrogated by fixation with paraformaldehyde (PF), such a treatment variably affected the IgE-inducing ability of TCC. Preactivation with anti-CD3 before treatment with PF maintained or even enhanced the ability of TCC to support IL-4-dependent IgE synthesis. More importantly, preactivation with anti-CD3, followed by fixation with PF, enabled TCC, apparently devoid of IgE-inducing activity in unfixed condition, to support IL-4-dependent IgE synthesis. Taken together these data suggest that at least two signals are involved in the triggering of human B cells to IgE production: the first is delivered by a T-B cell contact and the second by IL-4. The physical signal delivered by T cells does not necessarily consist of cognate interaction. Noncognate contact-dependent induction of B cells to IgE synthesis in response to IL-4 appears to be related to molecule(s) distinct from the TCR/CD3 com-

plex, but fully expressed on the membrane of TCR/CD3-activated T cells.

It has recently been shown that IL-4 is an essential mediator for the synthesis of IgE. In mice, IL-4 stimulates in vitro production of IgE by LPS-activated splenic B cells (1) and in vivo injection of an anti-IL-4 antibody inhibits both polyclonal and Ag-specific IgE responses (2). In humans, we have recently shown that only TCC³ able to produce IL-4 upon appropriate stimulation, as well as their IL-4-containing supernatants, are able to induce IgE synthesis in vitro. Such a capability could be abrogated by an anti-IL-4 antibody (3). In addition, we and others have shown that rIL-4 is able to induce IgE synthesis by normal PB or tonsil MNC (3, 4), whereas highly purified B cells were not induced to produce IgE by rIL-4 (3). In subsequent experiments, we have found that responsiveness of T cell-depleted MNC suspensions to IL-4 was restored by the addition of autologous or allogeneic T cells (5). Recently, it has been shown that the induction of human IgE synthesis by rIL-4 necessarily requires cognate interaction between TCR/CD3 complex on T cells and MHC class II Ag on B cells, suggesting that IL-4 contributes to the enhancement of the specific conjugate between Th cell and B cell APC (6).

In the present study, we show that physical contact between T and B cells has to precede the activity of IL-4 and that direct T/B cell recognition via MHC class II Ag and TCR/CD3 complex is not necessarily required for IL-4-dependent IgE synthesis. A nonspecific interaction with activated T cells can also enable B cells to synthesize IgE in response to IL-4.

MATERIALS AND METHODS

Reagents. PHA and PWM were purchased by GIBCO (Grand Island, NY). rIL-1 β rIFN- γ , and rIL-2 were kindly provided by Glaxo (Geneva, Switzerland), rIL-4 was kindly provided by Unicet (Dardilly, France) and Immunex (Seattle, WA). rTNF- α and rIL-6 were purchased by Genzyme (Boston, MA). OKT3 (IgG2a anti-CD3), OKT4 (IgG2a anti-CD4) and OKT8 (IgG2a anti-CD8) mAb were purchased by Ortho Pharmaceutical Corp. (Raritan, NJ), anti-Leu-M3 (IgG2b anti-CD14) mAb from Becton Dickinson (Mountain View, CA), and mAb B1 (IgG2a anti-CD20) from Kontron (Zurich, Switzerland). The production and characterization of E45 (IgG2a anti-human ϵ -chain) mAb and of rabbit antibody specific for the human ϵ -chain have been detailed in previous papers (3, 4, 7).

Preparation of T cells and TCC. Fresh T cells were obtained from MNC suspensions of PB or tonsils by double E-rosetting procedure with neuraminidase-treated SRBC, as previously described (8). In some experiments, CD4⁺-enriched T cell suspensions were prepared

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³ Abbreviations used in this paper: TCC, T cell clone; PB, peripheral blood; MNC, mononuclear cell; ToB, tonsillar B; LMW-BCGF, low m.w. B cell growth factor; PF, paraformaldehyde.

by removing CD8⁺ T cells by cytotoxicity with anti-CD8 mAb plus fresh rabbit serum (Sigma, Chemical Co.) as source of C' (8).

PHA-induced TCC were established according to the technique detailed elsewhere (9). Briefly, purified T cells were seeded in limiting numbers in round-bottomed microwells (0.3 cell/well) containing 10⁵ feeder cells and PHA (1% v/v) in a final volume of 0.2 ml RPMI 1640 medium (Flow Laboratories, McLean, VA) supplemented with 10% heat-inactivated FCS (HyClone, Logan, UT), 2 mM L-glutamine (GIBCO, Grand Island, NY), 50 µg/ml streptomycin, and 100 U/ml penicillin (complete medium). Spleen cell suspensions to be used as a source of feeder cells were irradiated with 5000 rad. Microcultures were supplemented after 48 h with rIL-2 (20 U/ml) and, at weekly intervals, with 10⁵ irradiated feeder cells and rIL-2 (20 U/ml). Growing microcultures were then expanded by repeated addition of feeder cells and rIL-2. The phenotype distribution of TCC was examined by flow microfluorimetry (Facstar, Becton Dickinson, Mountain View, CA) analysis.

An alloreactive TCC (TR46) was obtained by a cloning technique as reported in a previous paper (10). Briefly, T blasts derived from a secondary mixed lymphocyte reaction and enriched by centrifugation over a discontinuous Percoll (Pharmacia Fine Chemicals) gradient were seeded at 0.3 cells/well in the presence of irradiated (5,000 rad) allogeneic stimulator cells followed by rIL-2. TCC so obtained were further expanded with rIL-2 and screened for their ability to proliferate in response to the original stimulator (HLA-DR) (4, 5) non-T cells in the absence of exogenous growth factors. In order to maintain cell growth and function for long time periods, such clones were periodically restimulated with irradiated (5,000 rad) non-T cells from the original donor. The clone TR46 (CD3⁺, CD4⁺, CD8⁺) used in the present study, recognized HLA-DR4 Ag and was selected because of its ability to produce high levels of IL-4 and no IFN-γ in response to PHA (1% v/v) or to the original mytomycin C-treated stimulatory cells. Before use, every TCC was routinely screened for lack of Mycoplasma contamination, as described by Kaplan et al. (11).

Fixation of T cells and T blasts with PF. In some experiments, freshly prepared T cells or cloned T blasts were fixed with 0.05% PF (Sigma, Chemical Co.) (1 ml for 3 × 10⁶ cells for 90 s), extensively washed, and incubated for 24 h in medium alone, as originally reported (12). Fixed lymphocytes were then washed before adding to B cell cultures. After these treatments, fixed T cells or T blasts were apparently devoid of toxic effects (due to leakage of fixative) as shown by their inability to affect Ig synthesis when cocultured with PWM-stimulated MNC. In some experiments, before fixation with PF, T cells and T blasts were preactivated under capping conditions (overnight at 37°C) with soluble anti-CD3 mAb (1 µg/ml). After treatment with anti-CD3 and PF fixation, T cells and T blasts were reactive neither with FITC-conjugated anti-mouse Ig nor with FITC-conjugated anti-CD3 mAb, as assessed by flow microfluorimetry analysis. In addition, fixed T cells or T blasts were consistently unable to proliferate in response to PHA (1% v/v), as assessed by [³H] thymidine incorporation assay (13), as well as to secrete minimal amounts of IL-2, IL-4, or IFN-γ, as those sufficient to induce proliferation in cocultured murine CTLL-2 cells or to be detectable by appropriate immunoassays, respectively.

Quantitation of IFN-γ and IL-4. For the quantitative measurement of IFN-γ in the supernatant of TCC, a specific RIA (Centocor Inc., Malvern, PA) was used according to the manufacturer's instructions (3, 4). The quantitative determination of human IL-4 by ELISA has been reported elsewhere (3, 4). Values of the two cytokines 5 SD, over those of control supernatants derived from irradiated feeder cells alone, were regarded as positive.

Preparation of B cells. Purified tonsillar B-cell suspensions, derived from five nonallergic children who underwent tonsillectomy, were prepared as previously described (8). They usually consisted of 85 to 96% B cells, 1 to 2% monocytes, and less than 1% T cells, as judged by the use of anti-CD20, anti-CD14, and anti-CD3 mAb, respectively. They will be referred to as ToB lymphocytes for simplicity.

Twenty adult nonallergic subjects (laboratory personnel and students) were selected as donors of PB B cells. They had a negative history for allergies and low serum IgE level (<20 IU/ml, as determined by the IgE RIA-Pharmacia Inc.). PB B cells were obtained by removal of adherent cells on plastic surfaces and depletion of T cells by a double-step of rosetting with SRBC followed by C'-dependent cytotoxicity with anti-CD3 mAb plus fresh rabbit serum (8). They usually consisted of 55 to 75% B cells, 3 to 10% monocytes, and less than 1% T cells. They will be referred to as PB B cells.

Cell cultures and measurement of IgE synthesis. The cell culture system used for the induction of IgE synthesis was performed in duplicate tubes containing 5 × 10⁵ B (ToB or PB B) cells and 1 × 10⁵ freshly prepared T cells or clonal T blasts (extensively washed to remove residual IL-2) in 1 ml in the presence or in the absence of

rIL-4 (200 U/ml), if not otherwise stated. In some experiments, autologous purified T and B cells were cultured in compartments separated by a 0.4-µm membrane with well inserts (Transwell 3412, Costar, Cambridge, MA). After 10 days of culture at 37°C in a 5% CO humidified atmosphere, supernatants were collected and assayed for their IgE content. The procedure for the measurement of IgE has been described in detail elsewhere (3, 4, 8). Briefly, triplicate samples for each duplicate culture were analyzed for their IgE content by a RIA performed in polyvinyl microtiter plates (Dynatech Laboratories, Alexandria, VA). Wells were coated with the anti-ε-chain E45 mAb (5 µg/well) and 0.2 ml culture supernatant or standard IgE (WHO IgE reference preparation 69/204) added to each of triplicate wells. After 24-h incubation followed by washings, 0.2 ml ¹²⁵I-anti-human IgE (PS/ADZ) was added for an additional 16 h. After extensive washings, the individual wells were cut apart and bound radioactivity was determined in a gamma counter. The lower limit of sensitivity of the test was 0.2 ng/ml and the mean intra-assay coefficient of variation was 17%. This assay was validated in a multicenter collaborative assessment of the variability of IgE measurement in cell culture supernatants (14). De novo or net IgE synthesis was calculated by subtracting the values for IgE obtained in parallel cultures containing cycloheximide (100 µg/ml; Sigma Chemical Co.) or treated with 1 N HCl from IgE values found in SN of untreated cultures, as previously reported (3, 4, 7, 8).

RESULTS

Prior interaction with T cells is required by B cells in order to synthesize IgE in response to IL-4. We have recently shown that in vitro IL-4-dependent IgE synthesis does not occur in absence of T lymphocytes (3). In this study, we asked whether T cells exerted their helper activity on B cells through a physical interaction or by release of lymphokines other than IL-4. Table I shows that rIL-1β, rTNF-α, rIFN-γ, rIL-2, or rIL-6 or combinations of them (data not shown) could not induce highly purified ToB cells to synthesize IgE in response to IL-4. Likewise, culture supernatants of T cells stimulated with allogeneic non-T cells consistently failed to induce IL-4-dependent IgE synthesis in ToB cells. In contrast, IgE synthesis consistently occurred in the presence of either autologous or allogeneic T cells (1:5 optimal ratio). Furthermore, when autologous purified T and B cells were cultured in the presence of IL-4, either in the same chamber or separated by a 0.4-µm membrane permeable to protein molecules, IgE synthesis was detected only in

TABLE I
Both autologous and allogeneic T cells, but neither recombinant cytokines nor supernatant of activated T cells, enable highly purified B cells to synthesize IgE in response to IL-4

Cytokine Added	IgE Synthesis (ng/ml) ^a	
	Medium	IL-4 (200 U/ml)
None	<0.2	<0.2
IL-1β (10 U/ml)	<0.2	<0.2
TNF-α (10 U/ml)	<0.2	<0.2
IFN-γ (100 U/ml)	<0.2	<0.2
IL-2 (10 U/ml)	<0.2	<0.2
IL-6 (100 U/ml)	<0.2	<0.2
Allo-activated T cell Sup ^b	<0.2	<0.2
Autologous T cells	<0.2	3.5
Allogeneic T cells		
A	<0.2	4.9
B	<0.2	3.7
C	<0.2	2.8

^a Highly purified tonsillar B cells (5 × 10⁵) were cultured with or without human rIL-4 (200 U/ml) in the presence of medium alone, different cytokines at concentrations ranging between 1 and 100 U/ml or combinations of them (data not shown), supernatants (Sup) of allo-activated T cells (10 to 50% final dilution), as well as in the presence of 10⁵ autologous or allogeneic (donor A, B, and C) PB T cells. After 10 days, culture supernatants were assayed for their IgE content.

^b Unfractionated peripheral blood T cells (10⁶/ml) were cocultured with irradiated allogeneic non-T cells (10⁶/ml). After 48 h, supernatant was collected and tested on highly purified B cells. The effect of one representative out of five T cell Sup tested is reported.

cultures containing both T and B cells (Table II). These data suggest that soluble signals cannot replace T cells in supporting IL-4-dependent IgE synthesis and that disruption of T-B cell contact inhibits the ability of IL-4 to induce IgE synthesis.

We then asked which of the two signals (contact with T cells and interaction with IL-4) was required first by B cells for IgE synthesis. To this end, IL-4 and freshly prepared T cells were sequentially added to autologous purified B cells and IgE synthesis obtained under these different experimental conditions was evaluated. As shown in Figure 1, when IL-4 was added at the beginning of culture and T cells were added later, a strong reduction or abrogation of IgE synthesis was observed if compared with that obtained in cultures where IL-4 and T cells were added together on day 0. In contrast, when freshly prepared T cells were added on day 0 and IL-4 after 24 h, IgE synthesis was still achieved and it was even higher when addition of IL-4 was delayed until day 3. These data suggest that contact with T cells delivers the first signal to B cells, whereas the second one is provided by IL-4.

Physical contact between T and B cells does not necessarily involve cognate interaction. We then asked whether the T-B interaction required for IL-4-dependent IgE synthesis necessarily involved cognate interaction between TCR/CD3 complex on T cells and MHC class II Ag on B cells. In the experimental conditions in which IgE synthesis by B cells was induced by exogenous IL-4 in the presence of autologous T cells, cognate interaction may result from recognition by T cells of MHC class II Ag or MHC class II Ag associated with some foreign peptides (for example the ones contained in bovine serum). On the other hand, when B cells were induced to synthesize IgE by exogenous IL-4 in the presence of allogeneic T cells, cognate interaction may result from recognition of B cell alloantigens by some T cells. To challenge both these possibilities, a high number of TCC established from two DR-incompatible subjects were assessed for their ability to support IL-4-dependent IgE synthesis in B cells from both donors. As shown in Table III, in the presence of exogenous IL-4, most CD4⁺, and even a proportion of CD8⁺, TCC were able to support IgE synthesis in purified B cells from both individuals, although they were apparently devoid of either auto- or alloreactivity, as judged by their failure to proliferate in response to non-T cells of the same donors (data not shown).

The possibility that cognate interaction is not necessarily involved in IL-4-dependent IgE synthesis was further supported by experiments performed with an alloreactive TCC. This clone (TR46) recognized HLA-DR4 and produced IL-4, but not IFN- γ , upon stimulation with mytomicin C-treated non-T cells from the appropriate donor. Clone TR46 supported the synthesis of IgE by purified B cells from a HLA-DR4 allostimulator in the absence of exogenously added IL-4, whereas it was unable to support the synthesis of IgE by purified B cells from PB (six) or tonsil (three) of nine different donors lacking HLA-DR4 Ag (Table IV). However, after addition of appropriate concentrations of IL-4 (200 U/ml), B cells from all nine donors lacking the stimulating alloantigen synthesized detectable amounts of IgE in the presence of TR46 clone (Table IV). Taken together, these data strongly argue against the possibility that cognate interaction is necessarily required for IL-4-dependent IgE synthesis.

Activated T cells can provide noncognate contact-dependent help for IgE synthesis even after fixation with PF. The above data suggest that a noncognate contact-dependent activation can prepare B cells to produce IgE in response to IL-4. To further investigate this point, we asked whether the IgE helper activity of freshly prepared T cells, mainly consisting of resting lymphocytes, differed in some way from that of cloned (activated) T cells. When active, CD4⁺ T blasts usually appeared to be more potent than freshly prepared unfractionated or CD4⁺-enriched T cells in supporting IL-4-dependent IgE synthesis by autologous B cells (Table V). However, if freshly prepared CD4⁺-enriched T cells (or T blasts) and IL-4 were sequentially added in culture, the former appeared to be maximally active if IL-4 addition was delayed until day 3 (see also Fig. 1). In contrast, T blasts strongly supported IgE synthesis when IL-4 was added 24 h later, but virtually lost their activity when IL-4 addition was delayed until day 3. Freshly prepared T cells and T blasts from the same donors were then compared for their ability to support IL-4-dependent IgE synthesis after fixation of the T cell membrane by treatment with 0.05% PF. As shown in Table VI, treatment with PF virtually abrogated the IgE-inducing ability of freshly prepared T cells, but variably affected that of TCC, although it consistently abolished their ability to proliferate in response to PHA and/or IL-2, or to secrete lymphokines (data not shown). Moreover, fixed TCC consistently failed to induce detectable B cell proliferation in the presence of rIL-4, as

TABLE II
Physical contact between T and B cells is required for the IL-4-dependent IgE synthesis

Culture Conditions ^a		IgE Synthesis (ng/ml) in Expt.:			
Compartment	Cells and/or cytokine	1	2	3	4
Upper	IL-4	<0.2	0.4	<0.2	<0.2
Lower	B				
Upper	T	0.4	0.8	0.7	0.3
Lower	B + IL-4				
Upper	B	<0.2	0.7	0.5	<0.2
Lower	T + IL-4				
Upper	IL-4	6.5	10.7	2.4	4.5
Lower	B + T				

^a PB B cells (1.5×10^6 /culture) were cultured in the presence of IL-4 (200 U/ml) either in the lower or in the upper compartment of double chamber wells. Autologous T cells (0.3×10^6 /culture) were cocultured with B cells either in the same compartment or separated from B cells by a $0.4\text{-}\mu\text{m}$ microporous membrane. After 10 days, supernatants from both compartments of the same well were pooled (4 ml final volume) and assayed for IgE content.

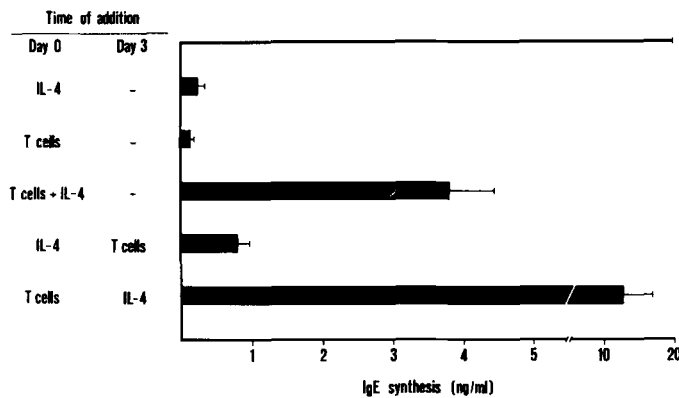


Figure 1. Effect on IgE synthesis of the sequential addition of autologous T cells and IL-4. Highly purified ToB cells (5×10^5 /ml) were cultured in the presence of autologous T cells (1×10^5 /ml) and rIL-4 (200 U/ml) added together on day 0, or separately on day 0 and on day 3, as indicated. After 10 days, culture supernatants were assayed for their IgE content. Results represent the mean ($\pm$ SE) IgE concentrations of four separate experiments.

assessed by [^{3} H]thymidine incorporation on both day 3 and day 6. In addition, if TCC were preincubated overnight at 37°C with anti-CD3 mAb and then fixed with PF, their IgE-inducing activity was usually preserved or even enhanced. More importantly, preincubation with anti-CD3 mAb, followed by fixation with PF, enabled TCC, apparently devoid of IgE helper activity in unfixed condition, to provide helper function for IL-4-dependent IgE synthesis (Fig. 2).

DISCUSSION

We and others have shown that IL-4, either secreted by TCC or in the recombinant form, is essential for the induction of human IgE synthesis in vitro (3, 15). However, in our previous experiments highly purified normal B cells could not be induced to produce IgE by rIL-4 or IL-4-containing TCC supernatants (3). Our results now show that prior physical interaction between T and B cells is required for IL-4-dependent IgE synthesis. First, mixtures of different concentrations of IL, such as IL-1, IFN- γ , TNF- α , IL-2, and IL-6, as well as culture supernatant of activated T Cells, could not replace T cells in the IL-4-

dependent induction of IgE synthesis. More importantly, mixtures of autologous T and B cells produced IgE upon stimulation with IL-4 only when cultured in the same well, but not when they were separated by a semipermeable membrane. Finally, experiments performed by sequential addition of IL-4 and T cells suggest that the first effective signal to B cells was delivered by a physical contact with T cells, whereas the second one was provided by IL-4. Recently, it has been suggested that, in addition to T-B cell contact and IL-4, LMW-BCGF is also required for induction of in vitro human IgE synthesis, suggesting that LMW-BCGF may induce expansion of partially activated B cells and cause them to be receptive to differentiation signals from IL-4 (16). In our experiments, no detectable proliferation was found in B cell cultures showing remarkable IgE synthesis after stimulation with PF-fixed TCC and rIL-4. These data, however, do not exclude that selective expansion of a small B cell subset inducible by IL-4 to IgE synthesis can occur at undetectable level. On the other hand, inasmuch as DeKruyff et al. (16) used commercial LMW-GCGF, which is contaminated by several cytokines, no definite conclusion on the role of LMW-BCGF in IL-4-dependent IgE synthesis can be drawn until the activity of the recombinant molecule is assessed.

The nature of T-B interaction able to provide the first signal for IL-4-dependent IgE synthesis was then investigated. Recently, it has been shown that induction of human IgE synthesis by rIL-4 necessarily requires cognate interaction between TCR/CD3 complex on T cells and MHC class II Ag on B cells (6). In our experiments as well, the IgE synthesis induced by rIL-4 in the presence of autologous or allogeneic T cells was strongly and consistently inhibited by addition in culture of mAb specific for CD3 or MHC class II Ag, supporting the view that cognate interaction between the TCR/CD3 complex on T cells and MHC class II Ag on B cells might be the event required. However, mAb recognizing other T and/or B cell surface molecules, such as CD2, CD4, CD20, and CD23, can also inhibit IL-4-dependent IgE synthesis, even if less consistently (data not shown). We considered, therefore, the possibility that inhibition of IL-4-dependent IgE

TABLE III
Most TCC from two DR-incompatible donors can reciprocally induce IgE synthesis in B cells in the presence of exogenous IL-4

TCC Added ^a	Phenotype	IgE Synthesis (ng/ml) by B Cells from: ^b			
		P.P. (DR 1, 5)		S.T. (DR 3, 7)	
		None	IL-4	None	IL-4
None		<0.2	<0.2	<0.2	<0.2
P.P.					
TCC 9	CD4	<0.2	3.1	<0.2	3.8
TCC 17	CD4	<0.2	7.6	<0.2	4.0
TCC 23	CD4	0.2	4.5	0.3	10.9
TCC 38	CD4	<0.2	5.2	<0.2	4.4
TCC 18	CD8	<0.2	2.9	<0.2	3.5
S.T.					
TCC 1	CD4	<0.2	4.8	<0.2	7.5
TCC 25	CD4	<0.2	3.7	<0.2	1.9
TCC 27	CD4	<0.2	10.7	<0.2	3.4
TCC 34	CD4	<0.2	6.6	<0.2	8.3
TCC 7	CD8	<0.2	3.5	<0.2	2.7

^a TCC were established by PHA stimulation of single PB T cells from donors P.P. and S.T. Thirty-eight TCC (27 CD4⁺ and 11 CD8⁺) from P.P. and 42 TCC (30 CD4⁺ and 12 CD8⁺) from S.T. were obtained and all tested for their ability to support the IgE synthesis in B cells from both P.P. and S.T. in the presence of exogenous IL-4 (200 U/ml). The activity of some representative TCC is shown.

^b For culture conditions, see the legend to Table I.

TABLE IV
Exogenous IL-4 enables the TR46 alloreactive TCC to induce IgE synthesis in B cells from all nine donors lacking the relevant (DR 4) alloantigen

HLA-DR Haplotype of B Cells	IgE Synthesis (ng/ml) ^a			
	Medium	IL-4	TR46	TR46 + IL-4
DR 4, 5	<0.2	<0.2	7.7	20.2
DR 1, 5	0.3	0.5	0.6	6.4
DR 2, 7	<0.2	<0.2	0.2	16.2
DR 3, 7	<0.2	<0.2	<0.2	8.6
DR 2, 5	0.5	0.6	0.7	5.4
DR 3, 5	<0.2	0.3	0.5	13.3
DR 5, 7	<0.2	<0.2	<0.2	9.4
DR 1, 5 ^b	<0.2	<0.2	<0.2	7.1
DR 5, 5 ^b	<0.2	<0.2	0.4	3.6
DR 2, 5 ^b	<0.2	<0.2	<0.2	10.4

^a B cells (5×10^5) from the original allostimulator (DR 4, 5) or from nine donors lacking DR 4 were cultured in 1 ml in the presence of medium alone, IL-4 (200 U/ml), 10^5 TR46 T blasts, or 10^5 TR46 T blasts plus IL-4 (200 U/ml). After 10 days culture supernatants were assayed for IgE content.

^b Highly purified tonsillar B cells.

TABLE V
B cells require different time of contact with freshly prepared and cloned T cells for optimal IL-4-dependent IgE synthesis

	Time of Addition:			IgE Synthesis (ng/ml) ^a		
	Day 0	Day 1	Day 3	Expt. 1	Expt. 2	Expt. 3
None				<0.2	<0.2	<0.2
T cells ^b				<0.2	<0.2	<0.2
T cells + IL-4				3.8	4.3	2.9
T cells		IL-4		4.4	5.1	3.2
T cells			IL-4	6.9	8.7	5.5
T blasts ^c				<0.2	<0.2	<0.2
T blasts + IL-4				4.9	6.8	8.0
T blasts		IL-4		7.2	12.2	11.4
T blasts			IL-4	1.4	2.3	3.7

^a For culture conditions, see the legend to Table I.

^b Freshly prepared CD4-enriched T cells obtained by cytotoxicity with anti-CD8 mAb plus C'.

^c T cell blasts from CD4⁺ TCC in long term cultures.

TABLE VI
Effect of treatment with PF of freshly prepared T cells or TCC on the IL-4-dependent IgE synthesis

T Cells Added	IgE Synthesis (ng/ml) ^a	
	Untreated	PF-treated ^b
None	<0.2	<0.2
Fresh T cells		
Autologous	2.8	<0.2
Allogeneic		
A	3.7	<0.2
B	4.1	<0.2
C	3.0	<0.2
D	5.4	<0.2
TCC		
PP.3	12.1	5.8
PP.24	3.2	0.3
PP.31	2.9	2.7
ST.8	5.5	0.6
ST.15	7.2	2.4
ST.29	4.8	5.3
DP.10	6.3	0.8
DP.21	11.4	9.2
DP.44	6.4	7.2

^a For culture conditions, see legend to Table I.

^b Freshly prepared T cells or T blasts of TCC (3×10^6) were treated with 1 ml 0.05% PF for 1½ min, washed three times, incubated in complete medium for 12 h at 37°C, washed, and cocultured at 1:5 ratio with 5×10^5 B cells in 1 ml in the presence of IL-4 (200 U/ml).

synthesis by anti-CD3 and anti-MHC class II antibodies could result from direct or indirect negative signaling to B cells rather than from interference with T/B recognition. Addition in culture of anti-MHC class II mAb, as well as their F(ab')₂ fragments, has indeed been shown to inhibit both Ag- and mitogen-derived B cell responses (17, 18), suggesting a role for MHC class II Ag in both specific and nonspecific B cell activation (19). In addition, in experiments not reported in this paper we found that

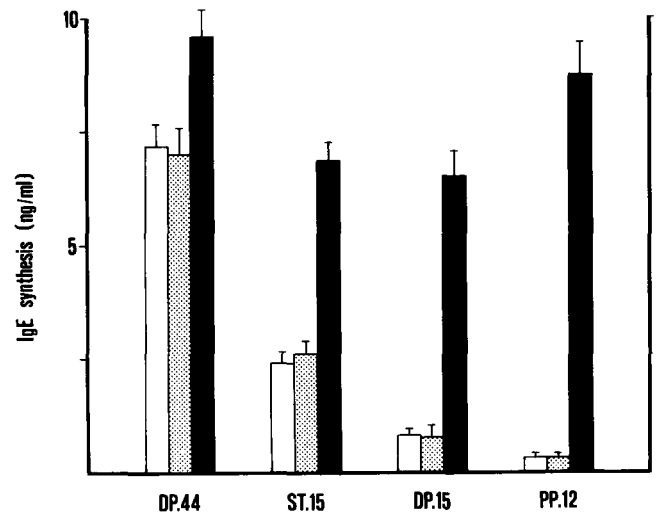


Figure 2. Effect of preincubation with anti-CD3 mAb followed by fixation with PF on the ability of TCCs to provide helper function for IL-4-dependent IgE synthesis. Highly purified ToB cells (5×10^5 /ml) were cultured in the presence of IL-4 (200 U/ml) and PF-fixed T blasts (1×10^5 /ml) preincubated overnight in medium alone (□), or in medium containing anti-CD8 (▨) or anti-CD3 (■) mAb. After 10 days, culture supernatants were assayed for their IgE content. Results represent the mean ($\pm$ SE) of triplicate IgE determinations of duplicate cultures.

the inhibitory effect exerted by anti-CD3 mAb on the IgE helper activity of some TCC could be reverted by addition to cultures of anti-IFN- γ mAb. Because IFN- γ is able to inhibit IL-4-dependent IgE synthesis in both mice (20) and humans (3, 15), this finding suggests that, at least in some experimental conditions, preincubation with anti-CD3 mAb may induce T cells to release IFN- γ , and

possibly other IgE-inhibitory lymphokines, rather than interfere with T/B recognition.

Undoubted evidence that IL-4-dependent IgE synthesis did not necessarily require cognate interaction was provided by results of experiments showing that, in the presence of exogenous IL-4, most CD4⁺, and even a proportion of CD8⁺, TCC established from two DR-incompatible individuals, were able to induce the synthesis of IgE in B cells from both donors. It would be surprising indeed if such a high number of clones could recognize autoantigens, as well as alloantigens. In addition, an alloreactive TCC (able to produce IL-4 but not IFN- γ in response to stimulator non-T cells) induced the synthesis of IgE only in B cells possessing the appropriate alloantigen, but, in the presence of exogenous IL-4, stimulated the production of remarkable amounts of IgE by B cells from all nine donors lacking the appropriate alloantigen. Although direct evidence for the formation of stable conjugates between activated TCC and B cells was not provided, the results of this study suggest that a nonspecific interaction with T cells might provide B cells with the signal required to synthesize IgE in response to IL-4. This finding is consistent with recently reported data showing induction of Ag-unrelated and MHC-unrestricted activation of B cells by interaction with intact T cells or even with isolated T cell plasma membranes (21–26). Interestingly, some of these studies have clearly shown that noncognate contact-dependent B cell activation, depending upon formation of conjugates between anti-CD3-activated TCC and B cells, was inhibited, as the cognate one, by addition in culture of anti-MHC class II antibodies (25, 26). Furthermore, IL-4-dependent human IgE synthesis has recently been induced by the use of PMA-activated EL-4 murine thymoma cells instead of human T cells (27), suggesting that even xenogenic activated T cells may express the molecule(s) involved in the noncognate interaction which enables human B cells to synthesize IgE in response to IL-4.

The nature of the molecule(s) involved in the noncognate contact-dependent B cell activation leading to IgE synthesis in the presence of IL-4 is at the present unknown. Although mAb specific for either CD4 or MHC class II Ag may inhibit the T cell- and IL-4-dependent IgE synthesis, an exclusive role for the CD4 molecule is improbable, because a number of CD8⁺ TCC were also able to provide the IgE-inducing signaling to B cells. mAb against CD2 (which interacts with lymphocyte-associated function-3) (28) also exerted, even though less consistently, an inhibitory effect on the IL-4-dependent IgE synthesis, whereas mAb specific for lymphocyte-associated function-1 (which interacts with ICAM-1) (28) were usually not inhibitory. Some of the so-called very late adhesion Ag (29) might be also involved in the T/B interaction. In this study, however, the possible inhibitory effect of mAb directed against very late adhesion Ag was not assessed. In our opinion, inhibition experiments performed with mAb directed against T and/or B cell surface molecules do not allow any definite conclusion, because the possibility that they act via direct or indirect negative signaling to B cells cannot be excluded.

The experiments reported in this paper reasonably suggest that the molecule(s) responsible for the T cell signaling to B cells is fully, even though temporarily, expressed only on activated T cells. First, the signal delivered to B

cells by active TCC was usually more powerful than the one provided by freshly prepared T cells. Second, treatment with PF consistently inhibited the IgE helper activity of freshly prepared T cells, whereas it had a variable effect on the activity of TCC. Finally, preincubation with anti-CD3 mAb, followed by fixation with PF, preserved the IgE-inducing activity of most TCC and conferred it to those apparently devoid of such an activity in unfixed condition. In this regard, one may suggest that transfer of anti-CD3 on the T cell surface may account for the effector function of fixed TCC. This possibility, however, is unlikely, because treatment of TCC with anti-CD3 was carried out under capping conditions. This procedure resulted in a loss of both CD3 and anti-CD3 molecules, which was rendered irreversible by the subsequent treatment of cells with PF.

The in vivo relevance of such in vitro data is noteworthy. Provided that, besides IL-4 and before it, a T cell signaling is required for inducing B cells to synthesize IgE, if such an event exclusively consists of cognate interaction (6), only B cells specific for the triggering Ag would usually be activated. According to this model, it might, therefore, be difficult to explain why in some pathologic conditions, such as parasitic infestations and acute rejection of bone marrow transplantation, strong polyclonal IgE synthesis occurs (30, 31). The in vitro findings reported in this study, showing that activated T cells may provide the first IgE-inducing signal to B cells even irrespective of cognate interaction, suggest that virtually all activated T cells able to produce IL-4 may have the potential to stimulate the production of IgE even in B cells not directly involved in cognate interaction. This would explain why and how the pathologic conditions mentioned above are characterized by a strong production of polyclonal IgE. Obviously, in this context the role exerted in vivo by cytokines other than IL-4, particularly the negative regulatory effect of IFN- γ (3, 15), also needs to be considered.

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