

## INDUCTION OF IgE SYNTHESIS IN NORMAL HUMAN B CELLS

### Sequential Requirements for Activation by an Alloreactive T Cell Clone and IgE-Potentiating Factors

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Studies of the human IgE antibody response have been limited by the lack of reproducible in vitro models for the induction of IgE synthesis by B cells from normal individuals (1-7). Polyclonal B cell activators, such as PWM or EBV have failed to induce IgE synthesis in peripheral blood B cells of both nonatopic and allergic donors (4, 6-7). Several laboratories, however, have found that culture supernatants of unfractionated T cells from patients with extremely high serum IgE levels e.g. hyper-IgE syndrome (HIE)<sup>1</sup> induce significant IgE synthesis by normal B cells, whereas supernatants from the T cells of normal subjects do not induce IgE synthesis by normal B cells (4, 8, 9). These results suggested that T cells from HIE patients but not normal donors secreted helper factors that were required for the induction of IgE synthesis.

Recently (10), we have established  $Fc_\epsilon R^+$  and  $Fc_\epsilon R^-$  T cell lines from patients with HIE. Supernatants from the  $Fc_\epsilon R^+$  T cell lines secreted IgE binding factors that specifically potentiated IgE production by B cells from patients with perennial allergic rhinitis but not B cells from normal nonatopic donors (10). These observations suggest the need for additional T cell-derived signals that bring normal B cells to a level of activation that is responsive to IgE binding factors.

Several laboratories, including our own, have shown that human alloreactive helper T cell clones could activate B cells from normal donors to produce high levels of IgM, IgG, IgA, and IgE (11-13). In this study, we report an alloreactive helper T cell clone that was capable of inducing normal B cells to synthesize IgG, but not IgE. However, addition of T cell-derived IgE potentiating factors to normal B cells preactivated with this alloreactive helper T cell clone resulted in the induction of IgE synthesis. These experiments further define the sequential

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<sup>1</sup>*Abbreviations used in this paper:* BCDF, B cell differentiating factor; BCGF, B cell growth factor;  $Fc_\epsilon R$ , IgE Fc receptor; HS, horse serum; HIE, hyper-IgE syndrome; HS, horse-serum; IgE-PF, IgE-potentiating factor; IgE-BF, IgE-binding factor; IL-2 Sup, IL-2-containing supernatants; PBMC, peripheral blood mononuclear cells.

requirements for differentiation of normal B cells into IgE-secreting plasma B cells.

### Materials and Methods

*Isolation of Mononuclear Cell Subpopulations.* Peripheral blood mononuclear cells (PBMC) were isolated from heparinized peripheral blood by Ficoll-Hypaque density gradient centrifugation. The isolated PBMC were washed three times in HBSS and suspended in RPMI 1640 medium containing 10% AB<sup>+</sup> serum. Suspensions of PBMC were depleted of adherent cells by allowing them to incubate for 1 h at 37°C in plastic petri dishes ( $5 \times 10^6$  cells/ml). Cell suspensions enriched in B cells were prepared by rosetting nonadherent mononuclear cells with sheep erythrocytes (E) pretreated with neuraminidase, and recovering the non-rosette forming cells by centrifugation over Ficoll-Hypaque as previously described (14). After repeating the rosetting procedure a second time, the non-rosette forming cells were collected. They contained 70–85% surface Ig-positive cells, and <2% E<sup>+</sup> cells.

*Preparation of Monocytes.* PBMC were incubated overnight in petri dishes at 37°C in media containing 10% AB<sup>+</sup> serum. Nonadherent cells were then removed and the remaining adherent cells were washed with warm media. Ice cold 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 ( $2 \times 10^4$  cells/well).

*Preparation of T Cell Clones.* Alloreactive T cell clones were obtained as previously described (11, 15).  $10^7$  PBMC from a nonatopic HLA-DR3,4 donor were stimulated with  $10^7$  irradiated (5,000 rad) PBMC from a nonatopic HLA-DR5,6 donor in 10 ml of RPMI 1640 (M. A. Bioproducts, Walkersville, MD) containing penicillin (100 U/ml), streptomycin (50 µg/ml), and 10% heat-inactivated FCS (complete medium). After incubation at 37°C in 5% CO<sub>2</sub> for 6 d, the cultures were harvested, and dividing cells were enriched by centrifugation over a Percoll (Pharmacia Fine Chemicals, Upsala, Sweden) discontinuous (30, 50, 70, 90%) gradient at 1,500 g for 45 min. Cells at the 30/50% interface were resuspended in complete medium supplemented with 25% IL-2-containing supernatants (IL-2-Sup) that contained irradiated (5,000 rad) allogeneic PBMC. The mixture was distributed in the wells of 96-well trays (Nunc, Roskilde, Denmark). Each 0.2-µl well contained an average of 0.3 T cell blast and  $10^5$  irradiated allogeneic PBMC. Cultures were fed twice weekly: once with IL-2 Sup (25% vol/vol), and once with IL-2 Sup (25% vol/vol) and irradiated allogeneic PBMC ( $10^5$  cells). After 14–21 d of culture, the contents of wells exhibiting cell growth were transferred to the wells of 24-well trays (Linbro Scientific Co., Hamden, CT) and expanded with IL-2 Sup in the presence of stimulator cells until a sufficient cell number was available for experiments. The clones so obtained were tested for their capacity to proliferate in response to the original stimulator in the absence of exogenous growth factors.

*IL-2 Sup.* PBMC were obtained from normal 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 with 1,000 rads. Cells were suspended at  $10^6$  cells/ml in RPMI 1640 with 2% FCS containing PHA (1 µg/ml, Wellcome Labs., Research Triangle Park, NC), and supernatants were harvested after 48 h.

*Proliferation Assays.*  $4 \times 10^4$  cloned T cells were added to triplicate cultures in 96-well flat-bottom microtiter plates to irradiated (2,500 rad) adherent monocytes ( $2 \times 10^4$  cells/well). Thymidine incorporation was assessed by pulsing with 0.8 µCi methyl-[<sup>3</sup>H]-thymidine (New England Nuclear, Boston, MA) during the last 18 h of a 96-h culture.

*Establishment of T Cell Lines with Receptors for Human IgE (Fc<sub>ε</sub>R).* The isolation and establishment of Fc<sub>ε</sub>R<sup>+</sup> T lymphocyte lines from patients with HIE has been described in detail elsewhere (10). Briefly, E-rosetted T lymphocytes from patients with HIE were incubated with 10 µg/ml of human myeloma IgE P.S. (kindly provided by Dr. K. Ishizaka, Johns Hopkins University, Baltimore, MD). After a 1-h incubation at 4°C, the cells were washed twice and treated with an immunosorbent-purified FITC-goat anti-human IgE.

After a 45-min incubation at 4°C, the cells were washed twice, analyzed, and sorted for Fc<sub>γ</sub>R<sup>+</sup> and Fc<sub>γ</sub>R<sup>-</sup> cells on the FACS IV cell sorter. Background staining was assessed with a control group of cells incubated with 10 μg/ml of human myeloma IgG instead of IgE.

The Fc<sub>γ</sub>R<sup>+</sup> and Fc<sub>γ</sub>R<sup>-</sup> T cell fractions obtained from the FACS IV were suspended in growth medium and seeded into the wells of a microtiter plate at 0.5 × 10<sup>6</sup> cells/ml, 0.2 ml/well. The growth medium consisted of RPMI 1640 and ITS medium (Collaborative Research Inc., Lexington, MA) supplemented with 10% heat-inactivated FCS, with penicillin (100 U/ml), streptomycin (50 μg/ml), L-glutamine (2 mM), and with 20% IL-2 Sup. RPMI 1640/ITS medium contained 5 μg/ml insulin, 5 μg/ml transferrin, and 5 ng/ml selenium.

2–3 wk after initiation of the culture, cells growing in microtiter wells were transferred to 24-well Linbro plates (Linbro Inc., New Haven, CT). Feeder cells (0.5–1.0 × 10<sup>6</sup>/well) consisted of autologous PBL irradiated with 2,500 rad. T cells in 24-well plates were fed every 3–4 d with IL-2, and every 1–2 wk with IL-2 and feeder cells. Immunofluorescence studies on Fc<sub>γ</sub>R<sup>+</sup> T cell lines have revealed a surface phenotype consisting predominantly of Fc<sub>γ</sub>R<sup>+</sup>, T3<sup>+</sup>, T4<sup>+</sup>, Ia<sup>+</sup> T cells with <2% M1<sup>+</sup> cells, and no detectable B1<sup>+</sup> cells (10).

*Preparation of IgE Potentiating Factor(s) (IgE-PF).* Supernatants from Fc<sub>γ</sub>R<sup>+</sup> and Fc<sub>γ</sub>R<sup>-</sup> T cell lines were collected and passed through Centriflo CF50A cones (Millipore, Bedford, MA) to remove any IgE carried over from the initial incubation with 10 μg/ml of IgE done before separation of Fc<sub>γ</sub>R<sup>+</sup> cells from Fc<sub>γ</sub>R<sup>-</sup> cells or from IgE released by irradiated autologous feeder cells. The IgE-free filtrates were then passed through a 0.45-μm Millipore filter and stored at -20°C until tested for their capacity to enhance IgE synthesis.

In previous studies (10), we showed that supernatants from Fc<sub>γ</sub>R<sup>+</sup> T cell lines but not from Fc<sub>γ</sub>R<sup>-</sup> T cell lines contained IgE binding factors (IgE-BF) that selectively enhanced IgE synthesis in cultures of B cells obtained from patients with allergic rhinitis but not from normal nonatopic subjects (10). Purified IgE-BF from Fc<sub>γ</sub>R<sup>+</sup> T cell supernatants were isolated by absorption with IgE immobilized on Sepharose and recovery of IgE-BF in the acid eluates from these beads, as previously described (10).

*Ig Synthesis in Cell Cultures.* Cells were suspended in RPMI 1640 medium containing 10% FCS and grown in 96-well microtiter plates in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C. Each microtiter well contained: 10<sup>5</sup> cell-enriched E-cells, 2 × 10<sup>4</sup> irradiated (2,500 rad) monocytes (to activate the T cell clones) and, when indicated, 4 × 10<sup>4</sup> irradiated (2,500 rad) cloned T cells. After 4 d of culture, the cells were washed twice with culture medium to remove IgE released during the first 4 d of culture. In previous studies (11) we showed that this uniformly reduced the amount of IgE associated with the cell pellet derived from nonatopic donors to <150 pg/ml. Fresh medium was added and cultures were incubated for an additional 8 d. Supernatants were harvested from triplicate cultures and assayed for IgE and IgG content. IgE associated with the cell pellet was determined by acid treatment of B cells on day 4 of culture, as described by Turner et al. (6). De novo or net IgE synthesis was calculated by subtracting the values for IgE obtained in day 4 acid-treated cell pellets, i.e., preformed IgE, from IgE values of day 12 supernatants plus day 12 cell pellets.

*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 (Cooke Laboratory Products, Alexandria, VA). The wells were filled with 0.1 ml of a 10 μg/ml solution of a 1:1 mixture of two anti-human IgE Fc-specific mAb. These two antibodies (mAb 8 and 9) were the kind gift of Dr. Siraganian, National Institutes of Health, Bethesda, MD. 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, 0.1 ml of culture supernatant or of IgE standard was added to each of the triplicate wells and incubated for 16 h in a humidified chamber at room temperature. The wells were then washed twice with PBS plus 1% HS containing 0.5% Tween 20, and twice with PBS plus 1% HS, then 0.1 ml of Phadebas RAST <sup>125</sup>I-anti-human IgE (ND) (sp act, 12 μCi/μg; Pharmacia Fine Chemicals) was added to each well. 6 h later, the radiolabeled anti-IgE was removed. Wells were then washed three times with PBS and 1% HS, and eight times under running distilled water. The wells were cut

out and counted in a Gamma spectrometer (Tracor Analytic, Elk Grove Village, IL). Standard curves were constructed using dilutions of the IgE standards obtained from Pharmacia Fine Chemicals. The concentration of IgE in the supernatants was read from the standard curve. The lower limit of sensitivity of this assay varied from 150–300 pg/ml.

The specificity and sensitivity of our IgE RIA was confirmed in a recently completed multiinstitutional study coordinated by the Mayo Clinic (Helm, Buckley, Adkinson, Squillace, Gleich, and Yungingen, manuscript submitted for publication). Coded samples with varying quantities of polyclonal IgE in the presence of other Ig isotypes sent to our laboratory were evaluated using our IgE RIA. In no test sample were falsely elevated IgE values measured in our laboratory, thus ruling out the possibility of crossreactivity with other isotypes. Furthermore, values of IgE >300 pg/ml were measured accurately with a mean deviation from the expected values of <20%.

*ELISA of Supernatants for IgG.* 96-well polystyrene plates were coated with immunoabsorbent-purified goat anti-IgG (Tago, Inc., Burlingame, CA) (10 µg/ml). After washing with PBS containing 0.1% Tween 20, the plates were incubated with a solution of 0.1% gelatin for 1 h. The plates were washed, and 0.1 ml of appropriately diluted supernatants were added to the wells. After 18 h, the plates were again washed and horse radish peroxidase-conjugated anti-IgG (Tago, Inc.) was added (1:300). The plates were incubated for 1 h at 37°C, washed, and the bound antibody conjugate 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, Austen, TX). The specificity of the conjugated antisera was ascertained by testing them in a direct ELISA against purified human myeloma IgG, IgM, IgA, and κ and λ Bence-Jones proteins.

*Mouse mAb.* Antibodies to human HLA-DR and to human HLA-A,B framework antigens were obtained from Becton Dickinson Monoclonal Center (Mountain View, CA).

## Results

*Some Alloreactive T Cell Clones Induce Synthesis of IgG But Not IgE.* After the initial cloning of T cell blasts from a nonatopic (serum IgE, 20 IU/ml) HLA-DR3,4 donor stimulated with irradiated PBMC from a nonatopic HLA-DR5,6 donor, we obtained two alloreactive T cell clones: 2H6 and 3A3. Each of these clones was IL-2-dependent and gave a strong proliferative response to irradiated PBMC from the original stimulator, but not to irradiated autologous PBMC (Table I). Furthermore, the alloreactive response of these T cell clones to irradiated stimulator PBMC was blocked by mAb against HLA-DR antigen but not by anti-HLA-A,B (Table I). The surface phenotype of these clones was T3<sup>+</sup>, T4<sup>+</sup>, HLA-DR<sup>+</sup>, and T8<sup>-</sup>.

Clones 2H6 and 3A3 were examined for their capacity to induce IgG and IgE synthesis in B cells from the donor of the original stimulator PBMC. As shown in Table II, the B cells from this nonatopic donor made low levels of IgG but undetectable levels of IgE. Coculture of these B cells with the two T cell clones resulted in high levels of IgG synthesis, but failed to stimulate detectable levels of IgE synthesis.

*Induction of IgE Synthesis by Clone 2H6 Requires IgE-PF.* In this series of experiments, we explored the possibility that IgE-PF(s) derived from HIE Fc<sub>γ</sub>R<sup>+</sup> T cell lines were required for the successful induction of IgE synthesis by the alloreactive helper T cell clones as shown in Table II. Thus, B cells from the clone stimulator were cultured with T cell clones in the presence or absence of culture supernatants from the HIE Fc<sub>γ</sub>R<sup>+</sup> and Fc<sub>γ</sub>R<sup>-</sup> T cell lines, and the B cells were examined for their capacity to produce IgE.

TABLE I  
*Specificity of Alloreactive T Cell Clones*

| Stimulus             | mAb added    | <sup>3</sup> H]Thymidine incorporation (cpm) by clone: |        |
|----------------------|--------------|--|--------|
|                      |              | 2H6  | 3A3    |
| Medium               | —            | 2,755  | 444    |
| IL-2 Sup             | —            | 81,369   | 48,428 |
| Autologous monocytes | —            | 4,010  | 795    |
| Stimulator monocytes | —            | 89,237   | 55,232 |
| Stimulator monocytes | Anti-HLA-A,B | 100,870  | 54,244 |
| Stimulator monocytes | Anti-HLA-DR  | 9,050  | 831    |

The capacity of IL-2, x-irradiated (2,500 rad) autologous and allogeneic (stimulator) monocytes to stimulate the proliferation of clones 2H6 or 3A3 over a 4-d culture period was studied. Cultures did not have IL-2 unless specified. Proliferative response is expressed as cpm [<sup>3</sup>H]thymidine incorporated into cultures. Values represent the mean of triplicate cultures; all SD were <10%. Irradiated monocytes incorporated <300 cpm.

TABLE II  
*Some Alloreactive T Cell Clones Do Not Induce IgE Synthesis*

| Exp. | Cells in culture |              | Ig produced         |                     |
|------|------------------|--------------|---------------------|---------------------|
|      | B cells          | T cell clone | IgG<br><i>ng/ml</i> | IgE<br><i>pg/ml</i> |
| 1    | +                | 2H6          | 4,655               | <150                |
|      | +                | 3A3          | 3,270               | <150                |
|      | +                | —            | 155                 | <150                |
| 2    | +                | 2H6          | 5,140               | <150                |
|      | +                | 3A3          | 4,935               | <150                |
|      | +                | —            | 127                 | <150                |
| 3    | +                | 2H6          | 4,220               | <150                |
|      | +                | 3A3          | 3,870               | <150                |
|      | +                | —            | 95                  | <150                |

All cultures contained  $10^5$  E-rosette-negative cells (B cells). Cultures stimulated with T cell clones received  $4 \times 10^4$  irradiated (2,500 rad) T cell clones. All cultures were washed on day 4, and supernatants from day 4 to day 12 were collected and assayed for Ig content. Preformed IgE values on day 4 of culture were <150 pg IgE per milliliter. The results represent the mean Ig determinations of triplicate cultures. SD were <10% of the mean.

The results in Table III show that B cells from this nonatopic donor failed to synthesize IgE in the presence of culture supernatants from either the  $Fc_\epsilon R^+$  or  $Fc_\epsilon R^-$  T cell lines. However, when the same B cells were cocultured with clone 2H6 in the presence of  $Fc_\epsilon R^+$  T cell supernatants containing IgE-PF, a high level of IgE synthesis was observed. In contrast,  $Fc_\epsilon R^-$  T cell supernatants failed to activate IgE synthesis by B cells cocultured with clone 2H6. The capacity of clone 2H6 to induce IgE synthesis in the presence of IgE-PF appears to be a unique feature of this T cell clone, because T cell clone 3A3 (Table III), as well

TABLE III  
*Induction of IgE Synthesis by Clone IHH6 Requires Fc<sub>c</sub>R<sup>+</sup>  
 T Cell Supernatant*

| Cells in culture |               | Supernatant added               | Ig Produced |       |
|------------------|---------------|---------------------------------|-------------|-------|
| B cells          | T cell clones |                                 | IgG         | IgE   |
|                  |               |                                 | ng/ml       | pg/ml |
| +                | —             | Fc <sub>c</sub> -R <sup>-</sup> | 90          | <150  |
| +                | —             | Fc <sub>c</sub> -R <sup>+</sup> | 95          | <150  |
| +                | IHH6          | Fc <sub>c</sub> -R <sup>-</sup> | 3,900       | <150  |
| +                | IHH6          | Fc <sub>c</sub> -R <sup>+</sup> | 4,100       | 2,500 |
| +                | IHA3          | Fc <sub>c</sub> -R <sup>-</sup> | 4,650       | <150  |
| +                | IHA3          | Fc <sub>c</sub> -R <sup>+</sup> | 4,900       | <150  |

Cultures contained  $10^5$  E-rosette-negative cells (B cells), and where indicated,  $4 \times 10^4$  irradiated T cell clones and a 1:10 dilution of Fc<sub>c</sub>-R<sup>-</sup> or Fc<sub>c</sub>-R<sup>+</sup> T cell supernatants. All cultures were washed on day 4, replenished with fresh medium containing Fc<sub>c</sub>-R<sup>+</sup> or Fc<sub>c</sub>-R<sup>-</sup> T cell supernatant, and culture medium was collected from day 4 to day 12 for Ig determination. Preformed IgE values on day 4 of culture were <150 pg IgE per milliliter. The results represent the mean Ig determinations of triplicate cultures. Similar results were obtained in two other experiments.

TABLE IV  
*Induction of IgE Synthesis by Clone IHH6 Requires  
 IgE Binding Factor(s)*

| Cells in culture |            | Supernatant added               | Ig produced |       |
|------------------|------------|---------------------------------|-------------|-------|
| B cells          | Clone IHH6 |                                 | IgG         | IgE   |
|                  |            |                                 | ng/ml       | pg/ml |
| +                | -          | Medium                          | 80          | <150  |
| +                | +          | Medium                          | 5,200       | <150  |
| +                | +          | Fc <sub>c</sub> -R <sup>+</sup> | 5,350       | 3,520 |
| +                | +          | IgE-Sepharose effluent          | 5,250       | <150  |
| +                | +          | IgE-Sepharose eluate            | 5,100       | 2,700 |
| +                | +          | IgE-Sepharose effluent          | 5,100       | 4,050 |
| +                | +          | IgG-Sepharose eluate            | 5,300       | <150  |

Culture conditions are similar to those described in Table III. Preformed IgE values determined on day 4 of culture were <150 pg IgE per milliliter.

as three other alloreactive T cell clones tested directed against two different donors failed to induce IgE synthesis in B cells of stimulator origin in the presence of either Fc<sub>c</sub>-R<sup>+</sup> or Fc<sub>c</sub>-R<sup>-</sup> T cell supernatants (results not shown).

We next examined whether the IgE-potentiating activity of supernatants from Fc<sub>c</sub>-R<sup>+</sup> T cells was contained within the IgE binding fraction. Supernatants were passed over Sepharose 4B conjugated to human myeloma IgE or to human myeloma IgG, and the effluents and eluates from these columns were assayed for their capacity to enhance IgE synthesis when the B cells were cocultured with clone 2H6. Table IV shows that the effluent from the IgE-Sepharose column

TABLE V  
*Sequential Requirements for Induction of IgE Synthesis by Clone I1H6 and IgE Potentiating Factor (IgE-PF)*

| Treatment of B cells on: |                     | Ig synthesis |       |
|--------------------------|---------------------|--------------|-------|
| Day 1                    | Day 4               | IgG          | IgE   |
|                          |                     | ng/ml        | pg/ml |
| —                        | —                   | 120          | <150  |
| IgE-PF                   | IgE-PF              | 150          | <150  |
| Clone I1H6               | Clone I1H6          | 4,500        | <150  |
| Clone I1H6 + IgE-PF      | Clone I1H6 + IgE-PF | 4,700        | 4,750 |
| Clone I1H6               | OKT3 lysis + IgE-PF | 1,200        | 5,267 |
| IgE-PF                   | Clone I1H6          | 3,800        | 375   |

Culture conditions are similar to those described in Table III. In the experiment on line 5, B cells were initially cultured for 4 d with clone I1H6. Subsequently the T cell clones were lysed with OKT3 mAb and complement. The remaining B cells were washed and IgE IgE-PF, i.e., Fc<sub>c</sub>R<sup>+</sup> T cell supernatants were added at 1:10 final dilution. In the experiment on line 6, B cells were treated with IgE-PF for 4 d, washed, and  $4 \times 10^4$  irradiated (2,500 rad) I1H6 cells were added. Culture supernatants were collected from day 4 to day 12 for Ig determination. Preformed IgE was <150 pg/ml on day 4 of cultures.

lost its capacity to enhance IgE synthesis. In contrast, the effluent from the IgG-Sepharose column retained its full capacity to enhance IgE synthesis. More importantly, the material that bound to the IgE immunosorbent, and which was eluted with 0.2 M glycine-HCl potentiated IgE synthesis, whereas the material eluted from the IgG immunosorbent did not. The enhancement of IgE synthesis by the material that bound to the IgE column was isotype-specific, because this material did not enhance IgG synthesis.

*Activation of IgE Synthesis Requires Two Sequential Signals.* We studied the sequence of events resulting in the activation of IgE synthesis by normal B cells cultured in the presence of clone 2H6 and IgE-PF(s). We cocultured normal B cells with clone 2H6 for 4 d. T cells were then lysed with OKT3 mAb and complement, and the remaining B cells were incubated for an additional 8 d with IgE-PF. Table V shows that this resulted in a high level of IgE synthesis. In contrast, when B cells were initially cultured with IgE-PF, washed on day 4, and then cocultured with clone 2H6 for an additional 8 d, only a minimal amount of IgE synthesis occurred (Table V). As expected, vigorous IgE synthesis occurred when B cells were cultured with both clone 2H6 and IgE potentiating factor for 4 d, washed, then recultured in the presence of both stimuli (Table V). No IgE synthesis occurred in B cells cultured for the entire 12-d period in the presence of medium, clone 2H6, or IgE-PF alone with a control wash at day 4 (Table V).

## Discussion

Herein, we identify two sequential signals required for the activation of IgE synthesis by normal B cells. The first signal is delivered by the alloreactive T cell clone 2H6, and the second signal was delivered by IgE-BF(s) secreted by Fc-R<sup>+</sup> T cell lines from patients with HIE. This activation of IgE synthesis was isotype-

specific because no changes were observed in IgG synthesis by the same cell cultures.

Current models of B cell differentiation suggest that B cell differentiation factors (BCDF) act on target B cells previously activated by various ligands, such as *Staphylococcus aureus* Cowan I strain or anti-Ig (16, 17). Since unfractionated T cell supernatants from patients with HIE are capable of inducing IgE synthesis in normal B cells (4), but IgE-BFs from the same patients are incapable of doing so, we postulated that T cell-derived factors are required to bring resting B cells to a level of activation that is responsive to IgE-BF. The results obtained from this investigation are consistent with this hypothesis. Indeed, clone 2H6 induced normal B cells to respond to IgE-PF. Although the target of IgE-PFs are activated B cells, the results described herein do not exclude the possibility that BCDF is released from allogeneic T cells, and IgE-PF is an additional factor.

Extensive studies by Ishizaka and coworkers have shown that IgE-BFs secreted from murine T lymphocytes bearing receptors for IgE ( $Fc_\epsilon R^+$ ) have an important role in the isotype-specific regulation of IgE synthesis (18). Rat IgE-binding factors consist of two activities: IgE-PFs (19) and IgE-suppressive factors (20). IgE-PFs are secreted by T cells from rats subjected to immunization regimens that are favorable for the formation of IgE antibodies, whereas IgE-suppressive factors are secreted by T cells from rats immunized under conditions that favor IgG but not IgE responses. Both factors have mol wt  $\sim 15,000$  but differ in their carbohydrate content. The IgE-PF(s) had affinity for lentil lectin and Con A, whereas the IgE-suppressive factor does not bind to the lectins but has affinity for peanut agglutinin. The rat IgE-PF has been shown to mediate its action via antigen-activated IgE-bearing B cells (19). We have previously reported (10) that human IgE-PF also stimulates IgE synthesis via IgE-bearing B cells from patients with allergic rhinitis and recent allergen exposure. The data from our current investigation strongly suggest that human IgE-PF and rodent IgE-PF have similar cell targets i.e. preactivated IgE-bearing B cells.

Recently, several laboratories, including our own (11–13), have shown that some alloreactive T cell clones stimulated B cells from nonatopic donors to synthesize IgE antibody. Such clones presumably provided the full spectrum of signals required for the activation of IgE synthesis by normal B cells. On the other hand, we show herein that there are also alloreactive T cell clones (clone 3A3 and three other clones studied) that can stimulate IgG synthesis, but which are unable to stimulate IgE synthesis even in the presence of IgE-PF. The basis for the differential capacity of alloreactive T cell clones to induce IgE synthesis in normal B cells is currently not known. We postulate, however, that alloreactive T cell clones may vary in their capacity to secrete certain lymphokines essential for the development of the IgE antibody response. Thus, clones such as 3A3, which do not stimulate IgE synthesis even in the presence of IgE-PF may not secrete optimal amounts of other lymphokines (BCDF, B cell growth factor [BCGF], IFN- $\gamma$ , etc.) required for induction of IgE synthesis. This hypothesis is supported by the recent observation by Goldstein et al. (21) of a  $T4^+$ ,  $Leu-8^+$  T cell clone that secreted BCDF but could support IgG production only when exogenous BCGF was added to the cultures.

The basis for the differential response of IgG and IgE synthesis to alloreactive



T cell clones 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 nonatopic subjects. In contrast, B cells from normal individuals spontaneously produce IgG. The majority of the *in vivo* activated B cells that spontaneously secrete IgG are large, and have been shown to reside in the less dense fractions of a density gradient, and to respond to T cell-dependent, PWM-triggered activation (22), and T cell-derived factors (23).

It is not known whether the T cell clones activate B cells already committed to express IgE and/or are also causing isotype switching of B cells to synthesize Ig of the IgE isotype. In this regard, there is evidence that T cell clones can cause B cells to undergo isotype switch. Mayer et al. (24) have reported on the capacity of monoclonal human leukemic T cells to cause an IgM to IgG switch in B cells of patients with the hyper-IgM syndrome. More recently, Strober and coworkers (25) defined a ConA-induced cloned T cell population in Peyer's patches that causes surface IgM-bearing B cells to switch to surface IgA-bearing B cells (26).

In summary, this study identifies two signals required for the activation of IgE synthesis in normal B cells. The initial signal is provided by clone 2H6. This first signal renders B cells responsive to the second signal provided by HIE T cell-derived IgE-BFs. Since B cells from atopic subjects respond to IgE-BFs by increased IgE synthesis, these B cells appear to have been activated *in vivo* by a first signal similar to that delivered by clone 2H6.

### Summary

Two human alloreactive T cell clones were established from a one-way mixed lymphocyte culture involving two nonatopic donors, and were assessed for their capacity to induce IgE synthesis by B cells obtained from the original stimulator. The two alloreactive T cell clones studied induced IgG but not IgE synthesis in normal B cells. However, one of the two clones, clone 2H6, induced IgE synthesis in the presence of supernatants from T cell lines derived from patients with the hyper-IgE syndrome (HIE), and enriched for T cells bearing receptors for IgE. These supernatants by themselves caused no IgE synthesis in nonatopic B cells. The potentiating factors in these supernatants were shown to bind to IgE. Time sequence experiments indicated that interaction of the B cells with the alloreactive clone 2H6 renders them responsive to the action of the IgE-potentiating factors.

These results indicate that induction of IgE synthesis in normal B cells involves at least two sequential T cell derived signals. Furthermore, T cell clones are heterogenous in their capacity to provide these signals.

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