

## BINDING THE LOW AFFINITY Fc $\epsilon$ R ON B CELLS SUPPRESSES ONGOING HUMAN IgE SYNTHESIS<sup>1</sup>

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Our results support the hypothesis that binding the low affinity Fc $\epsilon$ R (Fc $\epsilon$ R-II, CD23) on IgE-secreting B cells, directly suppresses IgE production. IgE production from AF-10/U266 (a human IgE plasmacytoma) decreased upon incubation with anti-IgE mAb or IgE:anti-IgE immune complexes (IgE-IC). Synthesis was suppressed a maximum of 51% with 10  $\mu$ g/ml of IgE-IC after a 24-h incubation. Spontaneous *in vitro* IgE synthesis from the B cells of highly atopic individuals was also inhibited in a similar fashion. This effect was isotype specific as IgA or IgG immune complexes did not alter IgE production from AF-10 nor did IgE-IC affect IgA or IgG synthesis from lymphoblastoid cell lines making IgG (GM1500 and RPMI 8866) or IgA (GM1056). U266/AF-10 cells displayed both membrane IgE (>90%) and Fc $\epsilon$ R-II (23%). To evaluate the role of these membrane proteins in the observed suppression of IgE synthesis, we treated U266/AF-10 cells with IgE-IC that bound Fc $\epsilon$ R-II but could not bind membrane IgE, as the mAb used was directed against an idiotype determinant on the myeloma IgE (PS) used to make the IgE-IC. Suppression was maximal (>50%) with these complexes at 0.1  $\mu$ g/ml and at a 1/1 ratio of mAb anti-IgE to human myeloma IgE. When IgE-IC were used that were constructed with heat denatured IgE or F(ab')<sub>2</sub> fragments of IgE, suppression was abrogated indicating IgE-Fc $\epsilon$ R binding was required. Neither PS IgE nor mAb 5.1 (the components of IgE-IC) alone affected IgE synthesis. Furthermore, a mAb binding directly to CD23 suppressed IgE synthesis from AF-10 up to 60%. Using limiting dilution analysis, we determined that IgE production per AF-10 cell was constant (0.9 pg/cell/24 h), independent of cell density and cells incubated with IgE-IC were uniformly suppressed. To clarify the mechanism of IgE-IC-induced suppression on AF-10 cells, we assessed both the proliferative rate and cell cycle distribution upon incubation with IgE-IC. There was

no correlation between IgE production and [<sup>3</sup>H]TdR incorporation by AF-10 cells incubated with IgE-IC or anti-CD23 mAb. The distribution of cells within the cell cycle was unaffected by these treatments, with 60% of the cells in G1. These results define a direct role for the Fc $\epsilon$ R-II on B cells in the regulation of ongoing IgE synthesis.

The ability to inhibit ongoing Ig synthesis may be critical for the down regulation of IgE antibody responses. After exposure to an Ag such as bee venom or penicillin, allergic individuals characteristically show protracted serum IgE responses which persist for many years without re-exposure to the Ag (1). Using irradiated reconstituted mice, Okudaira and Ishizaka (2) have shown that radio-resistant long-lived B cells producing specific IgE may account for this occurrence. Whereas numerous approaches in animals have been successful in inhibiting the induction of such IgE responses (3), it has been much more difficult to inhibit or down-regulate such responses once they have been established (4). Uede et al. (5) have shown that their IgE suppressive factor can suppress a plasma cell-enriched fraction from the spleen of IgE-responding mice. We have previously shown that IgE-IC can induce T cells to actively inhibit ongoing IgE production (6). Preliminary data we reported demonstrated that anti-IgE antibodies could directly inhibit ongoing IgE production (7). All of these studies raise the question as to how such suppression of ongoing IgE synthesis would be communicated at the B cell surface. Several laboratories have demonstrated that cross-linking of integral membrane Ig and the Fc $\gamma$ R will subsequently inhibit a resting (non-Ig secreting) B cell from being stimulated to secrete Ig (8, 9). However, these cells once stimulated for 24 h *in vitro*, become refractory to this suppressive signal. Ongoing Ig production is not inhibited by this approach, suggesting perhaps that different mechanisms of suppression are used as the B cell progresses through distinct functional developmental stages (10, 11).

We now demonstrate that IgE-IC<sup>4</sup> as well as anti-IgE antibodies can inhibit ongoing IgE production from a human plasmacytoma cell line (U266/AF-10). Furthermore, by constructing immune complexes with limited binding permutations, we show that IgE-IC mediate their effect by binding the Fc $\epsilon$ R-II (CD23) on the AF-10 cells (12, 13). In this system, binding to membrane integral IgE is not involved. Thus our data elucidates a mecha-

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<sup>4</sup> Abbreviations used in this manuscript: IgE-IC, anti-IgE: IgE immune complexes; IgG-IC, anti-IgG: IgG immune complexes; IgA-IC, anti-IgA: IgA immune complexes; Fc $\epsilon$ R-II, low affinity Fc $\epsilon$ R, cRPMI, complete RPMI medium.

nism in which isotype specific suppression of ongoing Ig synthesis may be affected for human B cells.

#### MATERIALS AND METHODS

**Cell lines and fresh cells.** The cell lines U266/AF-10, GM1056, GM1500, and RPMI 8866 are human B cell lines previously described in detail (6). Briefly AF-10 is a cloned mycoplasma-free variant developed in our laboratory from the human plasma cell line U266 described and kindly provided by Dr. K. Nilsson (Department of Pathology, University Hospital, Uppsala, Sweden) (6, 14, 15). This clone has plasmacytoid morphology and has the advantage of consistently producing IgE- $\lambda$  protein rather than losing  $\epsilon$ -chain production or gaining  $\alpha$ -chain production. The IgE produced by U266 and its clones such as AF-10 is the paraprotein ND. Cell lines GM1056 and GM1500 are human EBV transformed cell lines obtained from the National Institute of General Medical Sciences (Camden, NJ). These EBV transformed lymphoblastoid human cell lines produce IgA and IgG, respectively. These cell lines were maintained at a density between 0.25 and 1.0 million cells/ml in continuous culture in cRPMI which contains 10% FCS, glutamine 2 mM, penicillin 50 U/ml, and streptomycin 50  $\mu$ g/ml. Cells were always fed the day before experiments were performed.

Fresh human B cells spontaneously producing IgE were obtained from the blood of patients with known allergic respiratory and cutaneous diseases or the hyper-IgE syndrome who had been documented previously to have elevated circulating levels of IgE (>2500 ng/ml). A B cell-enriched fraction was derived by AET (2-aminoethylisothio-uronium bromide hydrobromide)-enhanced rosette and adherent cell depletion of Ficoll-Hypaque isolated mononuclear cells as described (16). None of the patients was receiving corticosteroids at the time the blood was obtained.

**Immune complexes and mAb.** mAb Cia-E-4.15 (4.15) and CIA-E-7.12 (7.12) are human  $\epsilon$ -chain Fc region-specific mAb produced in our laboratory and which bind the  $\epsilon$ -CH2 domain (amino acids 218-301, H. Gould, personal communication). mAb CIA-I-5.1 (5.1) is a mAb directed against a V region determinant on the L chain of the IgE paraprotein PS. This mAb is not directed against a private PS L chain epitope as it binds to a number of other lambda paraproteins as well as to an occasional  $\kappa$ -paraprotein (17). At the same time, we have shown that it does bind to the Fab region of the PS  $\lambda$  L chain and does not bind to other IgE paraproteins, in particular ND (the paraprotein produced by U266/AF-10), or several other IgE paraproteins (unpublished observation, Saxon, A., Chapman, M.). These mAb (7.12, 4.15, and 5.1) are available free to interested research investigators. The IgE  $\kappa$ -paraprotein, Bedora, was the kind gift of Dr. Hans Spiegelberg, Scripps Clinic and Research Foundation, La Jolla, CA. Anti-Human IgG mAb was purified by  $(\text{NH}_4)_2\text{SO}_4$  precipitation of ascites from the cell line HB-60 American Type Culture Collection, Rockville, MD.

IC were formed by incubating at 37°C for 60 min the IgE paraproteins or their F(ab')<sub>2</sub> fragments with the various mAb at the ratios indicated. IC were standardized for their nomenclature as to the amount of total protein added. Heat-inactivated IgE-IC were formed between PS IgE which had been heated to 56°C for 3 h and the indicated mAb. We have previously shown that mAb 4.15 and 7.12 bind to such heat-treated IgE though heat inactivated IgE fails to bind to Fc $\epsilon$ R (18, 19). F(ab')<sub>2</sub> fragments of PS IgE were produced by pepsin digestion and column chromatography as described (20). To test the purity of these fragments, the pooled fractions were tested for the presence of intact IgE molecules in a previously described IgE RIA (16). The radioactive counts registered were below background (80 pg/ml) of the control curve when as much as 1  $\mu$ g/ml of IgE F(ab')<sub>2</sub> fragments were added, indicating a contamination of less than 0.01% with complete IgE molecules. F(ab')<sub>2</sub> fragments of mAb 7.12 generated as above but further purified by being passed over an affinity column of goat anti-mouse IgG that was Fc specific (Tago Inc., Burlingame, CA). IgA-IC were formed by adding a polyvalent specific goat anti-IgA antiserum (Tago) to purified human IgA paraprotein (N. L. Cappel Laboratories, Cochranville, PA) whereas IgG-IC were formed between a mAb anti-human IgG MH-60 and purified human IgG (N. L. Cappel).

mAb against the Fc $\epsilon$ R-II (CD23) were the kind gift of Dr. Guy Delespesse, Montreal, Canada (mAb 135, an IgG1) and Dr. Tadimitsu Kishimoto, Osaka, Japan (mAb 8-30, an IgM and 3-5, an IgG1).

**Measurement of secreted Ig.** The amount of IgE, IgG, and IgA in culture supernatants was measured at days 1 through 3 by isotype specific ELISA assays as described (21). In cell cultures involving less than 1000 cells, an enhanced ELISA was used to measure IgE production. Details of this assay have recently been published (22). Polystyrene microtiter plates (Immulon 2-Microelisa Plates, Dynatech Labs, Alexandria, VA) were coated with 150  $\mu$ l/well of mAb

anti-human IgE 7.12 and 4.15 at a 1  $\mu$ g/ml each in borate-buffered saline at room temperature in a humidified box for 2 h to overnight. The coating materials were discarded and the wells were washed three times with 300  $\mu$ l/well of PBS/Tween. The wells were then blocked with 150  $\mu$ l of 1% BSA in PBS for 0.5 h. The BSA was discarded and 100  $\mu$ l of culture supernatant to be assayed were added to each well. The plate was incubated in a humidified chamber for at least 2 h at room temperature, the contents of the wells were discarded, and the wells were washed four times with 300  $\mu$ l/well PBS-Tween. Each well was then filled with 100  $\mu$ l of a 1/3000 dilution of the developing antibody (rabbit anti-human IgE Fab' fragments coupled to alkaline phosphatase, the kind gift of Dr. M. Kemeny, Guys Hospital, London, England) diluted in 1% BSA. This antiserum was allowed to incubate humidified from 2 h at room temperature. The developing antibody was then discarded, the plates washed three times with 300  $\mu$ l PBS-Tween and four times with 300  $\mu$ l/Tris-NaCl. A substrate solution (50  $\mu$ l/well) containing NADP (Enhanced ELISA kit, BRL, Gaithersburg, MD) was added and the plates were allowed to incubate (23°C) for 30 to 45 min. The longer this incubation the more NAD<sup>+</sup> generated and thus the greater the signal produced by this amplification step. Next, 50  $\mu$ l/well of the amplified solution containing NAD dehydrogenase, alcohol dehydrogenase, ethanol, and iodinitroretetrazolium blue in a buffer was added (Enhanced ELISA kit, BRL). The plates were then repetitively scanned in an ELISA reader until the O.D. of the highest wells on the standard curve, containing 1000 pg of IgE, registered more than 1.75. The wells are typically saturated in this IgE assay with about 1 ng of IgE. Complete color development generally occurs within 15 min at 20°C. This assay has been reproducibly shown to be sensitive to between 5 and 10 pg of IgE added to the assay well in 100  $\mu$ l, equivalent to 10 to 20 pg of IgE secreted in the 0.2-ml microculture well. In all assays, cycloheximide-treated (50  $\mu$ g/ml) cultures were used to determine the amount of preformed IgE in these cells at the initiation of culture and this amount was subtracted from the amount produced in active cultures. We have previously found the cycloheximide treatment of the U266/AF-10 cells is equivalent to multiple freeze-thawing of these cells in their ability to accurately measure preformed IgE (16). Cytoplasmic IgE staining was performed using mAb 7.12 and 4.15 in indirect immunohistochemical staining as described previously (18).

**Northern blot analysis of RNA.** Cytoplasmic RNA was isolated from AF-10 or IG7 (an EBV transformed B cell line known to express high levels of surface CD23) by the Nonidet P-40 lysis method (23). Samples of RNA (10  $\mu$ g each) were denatured with glyoxal, electrophoresed on a 1% agarose gel using a 10 mM sodium phosphate, pH 7.0, running buffer, and transferred to a nitrocellulose membrane (Millipore Corporation, Bedford, Mass) using standard procedures (24). After blotting overnight, the filter was baked for 90 min at 80°C in a vacuum oven, and then prehybridized overnight at 42°C in 50% formamide, 5 $\times$  SSC, 50 mM sodium phosphate, pH 7.0, 5 $\times$  Denhardtts solution, 250  $\mu$ g/ml denatured salmon sperm DNA, 0.1% SDS. The filter was hybridized at 42°C with a [<sup>32</sup>P]dATP-labeled probe generated by oligo-labeling (25) plasmid pFc $\epsilon$ R-1 (26), which was the kind gift of Dr. T. Kishimoto, Osaka, Japan. Heat denatured probe was added directly to the pre-hybridization mixture. After overnight hybridization, the blot was washed under high stringency conditions (0.1 $\times$  SSC, 0.1% SDS, 60°C) and exposed to Kodak XAR-5 X-ray film with an intensifying screen at -80°C.

**Culture conditions.** To induce suppression, cells were incubated at 5  $\times$  10<sup>4</sup>/ml in triplicate polystyrene culture tubes (Falcon, Oxnard, CA) overnight in cRPMI with various concentrations of anti-IgE or IgE-IC as indicated. The next day the cells from replicate tubes were combined, washed at least three times in large volumes of MEM, resuspended in cRPMI, and the total number and viable cells were carefully counted. A total of 1000 to 5000 cells were then plated in flat or round bottomed microtitre wells, cultured for 1 to 3 days, and the amount of Ig in the culture supernatants measured. As IgE-IC carried over into the IgE-producing cultures would significantly cloud our results, cultures were tested for levels of mouse Ig and or PS IgE (using mAb 5.1). No evidence for IgE-IC could be detected in the AF-10 supernatants immediately after washing or when levels were measured at 24-h intervals.

In some experiments, cells were stimulated with rIL-4 (kindly provided by Dr. T. Yokota and Ms. A. V. Kimmenade, DNAX Inc., Palo Alto, CA) for 24 to 48 h and the expression of CD23 and membrane IgE on the cells determined by flow cytometry as described below.

**Cell surface analysis.** Cells were stained with unlabeled mAb to human IgE (7.12 and 4.15) and binding of these unlabeled antibodies was detected with purified FITC labeled goat-anti-mouse Ig (Tago). The low affinity Fc $\epsilon$ R (CD23) was detected using a mAb anti-Fc $\epsilon$ R (mAb 135) which was biotinylated by Vector Laboratories, Burlingame, CA. The Fc $\gamma$ R was detected by direct staining with anti-Leu

11 antibody (Becton Dickinson, Mountain View, CA) or by staining with IgG-IC. Stained cells were analyzed using an EPICS C (EPICS Division of Coulter Electronics, Hialeah, FL) calibrated daily to standardize fluorescence intensity using 10- $\mu$ m diameter fluorescent beads (Ortho Diagnostics Systems Inc, Raritan, NJ), as described previously (27).

**Cell proliferation and cell cycle.** Cell proliferation was measured by [ $^3$ H]TdR incorporation as described (16). Cells (1000 to 5000) were pulsed with 1  $\mu$ Ci of [ $^3$ H]TdR and 8 to 16 h later, harvested on a minishaker, and the amount of [ $^3$ H]TdR measured. Cell viability was measured by trypan blue exclusion. For cell cycle analysis, cells were treated with IgE-IC or mAb anti-CD23 overnight and then cultured for 24 to 72 h. The supernatants were harvested for IgE measurement and the cells were disrupted by detergent and their nuclei stained with propidium iodide and analysed for DNA content using an Epic C flow cytometer.

**Analysis of data.** Statistical analysis was performed using the Student's two-sided *t*-test to evaluate the difference between means and distributions. Critical values were estimated with a table for probabilities of 0.5, 0.8, 0.9, 0.95, 0.98, and 0.99. All tests used  $\geq 4$  degrees of freedom. The enhanced ELISA data were processed on a Macintosh II computer using the ELISA Master program written by Richard Deem, UCLA Department of Microbiology and Immunology.

## RESULTS

**Anti-IgE and I-IC suppress ongoing IgE synthesis.** When AF-10 cells were incubated with mAb anti-human IgE (1 to 10  $\mu$ g/ml) overnight, washed extensively in 1 $\times$  MEM to remove the exogenous antibody, counted carefully, and subsequently cultured in cRPMI for an additional 24 to 72 h, the cells treated with anti-IgE (4.15) demonstrated a decrease in IgE production relative to concurrent control cells (Fig. 1). Maximum suppression ( $51 \pm 5\%$ ) was observed when AF-10 cells were treated with 10  $\mu$ g/ml of anti-IgE and cultured for 24 h ( $p < 0.01$ ). Significant suppression was not observed at 0.1  $\mu$ g/ml or

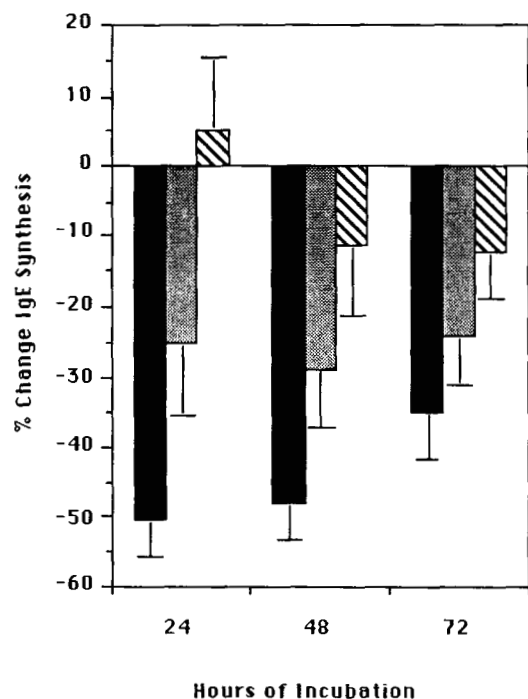


**Figure 1.** Inhibition of ongoing IgE synthesis by pretreatment with anti-IgE antibody. U266/AF-10 cells (50,000/ml) were incubated overnight (16 h) in cRPMI with the indicated antibody. The cells were then washed extensively and 10,000 cells were seeded in 200- $\mu$ l wells. Culture supernatants were harvested from individual wells at 24, 48, and 72 h and the IgE was measured in duplicate for each well. The values displayed represent the mean and SD of quadruplicate cultures as a percent of control treated cells: cells treated with anti-IgE mAb 4.15, 10  $\mu$ g/ml (■) 4.15, 1.0  $\mu$ g/ml (□), or cells treated with 10  $\mu$ g/ml of polyclonal goat anti-IgA (▨). Control cells made  $22.8 \pm 2$  ng/ml at 24 h,  $50.5 \pm 3.5$  ng at 48 h, and  $91.0 \pm 12$  ng at 72 h.

at 100  $\mu$ g/ml ( $p > 0.2$ ). These results confirmed our preliminary findings (7). Suppression was also observed when IgE-IC (PS IgE:mAb 4.15, 1:1 ratio) were used in place of anti-IgE alone. The range of effective concentrations and the time course of suppression for the IC were similar to that for the mAb alone (data not shown). Inhibition of IgE production was isotype specific: anti-IgA antibodies did not suppress AF-10 IgE production and anti-IgE did not inhibit IgA or IgG synthesis from cell lines GM1056 (IgA), GM1500 (IgG), or RPMI 8866 (IgG). These three lines are all  $>75\%$  FcεR-II (CD23) positive (19) (unpublished observations, Saxon, A., Kagan, J. Brown, A.).

**Anti-IgE and IgE-IC suppress in vitro IgE production from B cells of atopic individuals.** AF-10, an IgE myeloma that has been maintained in long term culture, may be susceptible to in vitro manipulations that have no in vivo correlate. To provide further evidence for a potential physiologic role of the observed suppression, we used fresh purified B cells obtained from highly atopic patients, as such cells have been shown to be capable of spontaneous in vitro IgE synthesis believed to represent in vivo development (28, 29). Similar to the protocol for the cell lines, the B cells were incubated overnight with mAb anti-IgE, washed thoroughly, and then cultured at  $10^5$  cells/well for 3, 5, or 7 days. Using the mAb 7.12 at 10  $\mu$ g/ml produced maximum suppression, 83% (Fig. 2). Unlike the results with the AF-10 cells, the suppression did not diminish as the cultures were extended to 7 days.

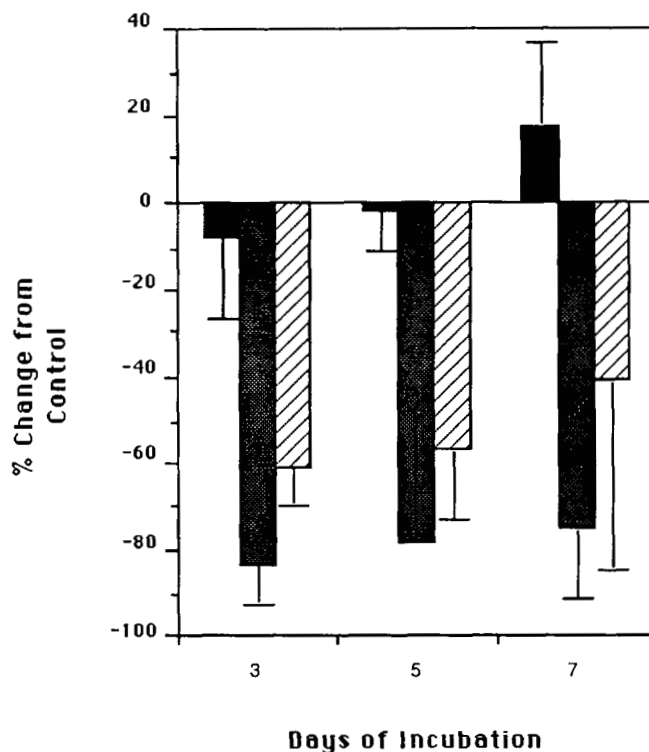


**Figure 2.** Inhibition of IgE synthesis from patient's spontaneously IgE-secreting B cells. B cells from an individual with the hyper-IgE syndrome (serum IgE  $> 20,000$  IU/ml) were cultured overnight with anti-IgE, 10  $\mu$ g/ml (■), anti-IgE mAb 7.12, 10  $\mu$ g/ml (□), mAb 7.12, 1.0  $\mu$ g/ml (▨), or medium alone. The cells were then washed and recultured in triplicate in cRPMI at 100,000 cells/well and harvested separately at 3, 5, and 7 days. Cycloheximide- (50  $\mu$ g/ml) treated controls were run in parallel with each group. Values are expressed as percentages of IgE compared to untreated cells which made  $406 \pm 93$  pg/ml at 3 days,  $854 \pm 246$  pg/ml at 5 days, and  $1079 \pm 186$  pg/ml at 7 days. Cycloheximide values were consistently less than 80 pg/ml at all time points.

This may correlate with previous results demonstrating that the majority of spontaneous *in vitro* IgE synthesis from atopic B cells occurs in the first 3 days of culture (28). Neither anti-IgG nor anti-IgA altered IgE production from the patients' cells (Fig. 2). We have previously shown that spontaneous IgA and IgG production from peripheral blood B cells is not altered by initial incubation with anti-IgE (6). To specifically address the role of CD23, spontaneous IgE-secreting peripheral blood cells from a patient with hyper-IgE immunodeficiency were treated overnight with mAb 135 (1.0  $\mu\text{g}/\text{ml}$ ) or IgE-IC (0.1  $\mu\text{g}/\text{ml}$ , mAb 5.1:IgE at 1/1 ratio), washed, and cultured for 7 days. In two separate experiments, there was a 33 and 57% suppression ( $p < 0.05$ ) in IgE compared to control untreated cultures which produced was  $>3000$   $\mu\text{g}/\text{ml}$  (total IgE at 7 days minus IgE present in cycloheximide-treated controls).

**Mechanisms for suppression of ongoing IgE production.** The suppression of ongoing IgE we observed is open to several testable hypotheses as to possible mechanisms. Both the IgE-IC and the mAb anti-IgE could bind to membrane IgE and/or to FcεR-II. IgE-IC have the ability to bind directly both membrane IgE and FcεR-II. mAb anti- $\epsilon$  can bind directly to membrane IgE or indirectly to an FcεR by first forming an IgE-IC with secreted IgE in the cultures or by binding IgE that is already on the cells FcεR-II (18, 29). Binding of these two membrane structures could create three potential combinations to mediate the observed suppression (diagrammatically depicted in Fig. 3): 1, anti-IgE binding membrane IgE. 2, IgE molecules, formed into a complex by anti-IgE, binding FcεR, or 3, both membrane IgE and FcεR are cross-linked by anti-IgE as a result of its binding membrane IgE and a secreted IgE molecule that occupies an FcεR-II.

To evaluate the possibility of these mechanisms, AF-10 cells were analyzed by flow cytometry for the display of membrane IgE and the FcεR-II (CD23). Using mAb 7.12 as the primary antibody, 88% of AF-10 cells stained positively for membrane IgE (Fig. 4A). Washing the AF-

10 cells in an acetate buffer, pH 5.0, before staining to remove cytophilic IgE from the cultured cells (30) did not decrease the fluorescence. Cells were stained directly for CD23 with a previously defined mAb (the kind gift of Dr. G. Delespesse, Notre Dame Hospital, Montreal, Canada) (31) which was biotin labeled. Of the cells 22% showed positive fluorescence (Fig. 4B) though the number of AF-10 cells that stained positively varied from 5 to 46% in experiments performed over a 1-yr period. As previous reports indicate that IL-4 can increase the display of the FcεR-II on B lymphocytes (32, 33), AF-10 cells were incubated for 24 to 72 h with up to 100 U/ml of rIL-4. The display of membrane IgE and CD23 on AF-10 cells was unchanged.

To obtain corroborating evidence for the CD23-positive phenotype of the AF-10 subclone, we carried out Northern blot analysis to test for the expression of the gene encoding the CD23 protein (Fig. 5). As a positive control for CD23 expression, we used RNA isolated from 1G7 cells, an EBV transformed cell line expressing high levels of CD23 on the cell surface. Samples of cytoplasmic RNA from 1G-7 and AF-10 cells were Northern blotted to nitrocellulose and screened with a nucleic acid probe that would hybridize with the CD23 mRNA. As shown by the autoradiograph in Figure 5, AF-10 cells express the CD23 mRNA with the expected mobility of approximately 1.7 kb (26). Inasmuch as similar amounts of cytoplasmic RNA were electrophoresed for Northern blotting (verified by staining with ethidium bromide), the difference in band intensities for the two samples shown in the autoradiograph is taken to reflect a difference in the level of stable CD23 mRNA within the two cell lines. Hence, the lower surface expression of CD23 on AF-10 cells relative to 1G-7 cells reflects a difference in the abundance of CD23 mRNA in the two cell types.

**Suppression of ongoing IgE production is mediated through FcεR-II.** To distinguish between the possible mechanisms for the observed isotype-specific suppression, we constructed soluble IgE-IC with the human IgE

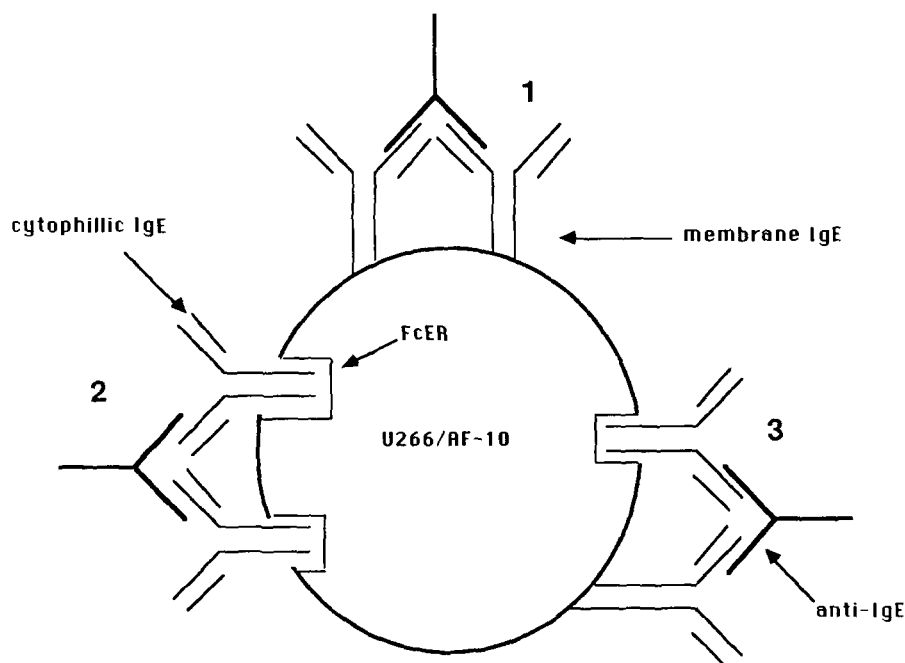


**Figure 3.** Model depicting the binding permutations of anti-IgE or an IgE immune complex to the surface of AF-10 cells. 1, Anti-IgE binding integral membrane IgE. 2, Anti-IgE binding cytophilic IgE that is bound to an FcεR. 3, Anti-IgE binding both membrane and FcεR bound IgE.

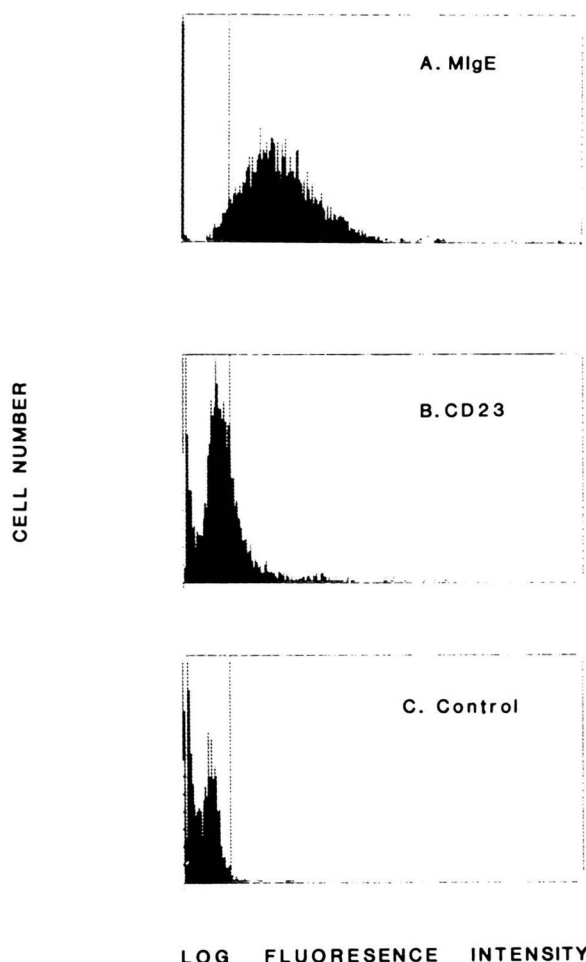


**Figure 4.** AF-10 cells display membrane IgE and FcεR-II (CD-23). AF-10 cells were stained with mAb anti-IgE (7.12) or anti-CD23 (mAb 135) and analyzed by flow cytometry. Histograms of the fluorescence are shown: A, anti-mIgE; B, anti-CD23; and C, control cells stained with IgG1 control antibody. The baseline cursor was set at a channel that yielded less than 2% of the events positive using the IgG1 isotype control.

paraprotein PS and mAb 5.1 which recognizes a determinant on the variable region of the  $\lambda$  L chain of PS IgE (7). Figure 6 demonstrates that 5.1 has a high affinity for PS IgE yet binds negligibly to other IgE paraproteins, including ND IgE, the IgE secreted by AF-10 cells. Thus, IgE-IC formed between mAb 5.1 and PS IgE cannot bind the membrane IgE (ND) of AF-10. This complex suppressed IgE synthesis maximally (49%) at 0.1  $\mu$ g/ml, identifying FcεR as a membrane structure mediating the suppression (Fig. 7). This response was very sensitive to the concentration of the mAb 5.1-PS immune complexes with significant suppression observable only at concentrations of 0.01 to 1.0  $\mu$ g/ml ( $p < 0.05$ ) (Figs. 7 and 8). IgA synthesis by GM-1056 cells was unchanged by this treatment ( $p > 0.5$ ) (Fig. 7). Neither mAb 5.1 nor IgE (up to 10  $\mu$ g/ml) alone significantly inhibited IgE synthesis.

To show that binding FcεR-II on AF-10 is necessary for suppression, we constructed IgE-IC with mAb 5.1 and heat denatured PS IgE (56°C for 3 h). IgE-IC that cannot bind FcεR (19). These IgE-IC did not suppress IgE synthesis at concentrations from 0.001 to 10  $\mu$ g/ml ( $p > 0.2$ ) (Fig. 8). To further confirm the role of the IgE Fc region in mediating suppression, we treated cells with IgE-IC constructed with F(ab')<sub>2</sub> fragments of PS IgE, and again suppression was abrogated by removal of the Fc region (Table I).

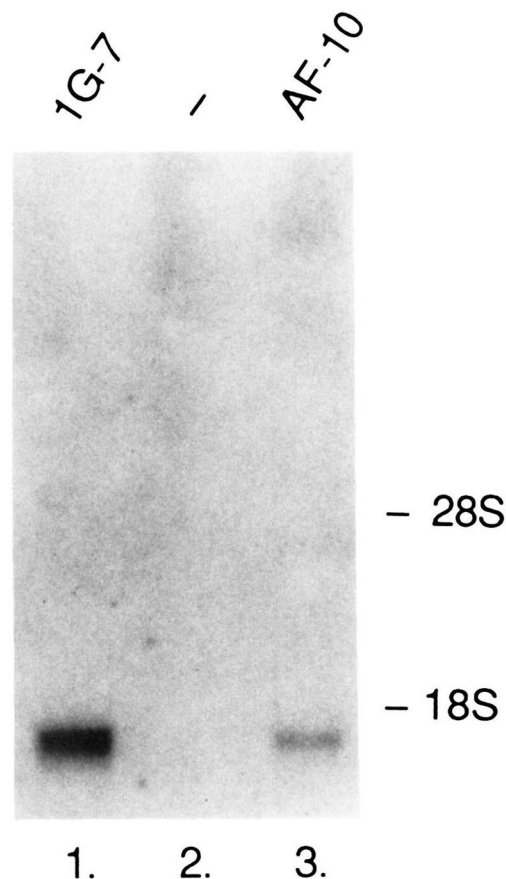


**Figure 5.** Analysis of the FcεR-II (CD23) mRNA in AF-10 cells. Samples (10  $\mu$ g/lane) of cytoplasmic RNA isolated from AF-10 cells (lane 3) and the strongly CD23-positive EBV transformed cell line 1G-7 (lane 1) were Northern blotted and analyzed for the presence of FcεR-II mRNA using a [<sup>32</sup>P]dATP labeled nucleic acid hybridization probe. No RNA was loaded in lane 2. The 1.7-kb band on the autoradiograph identifies the FcεR-II (CD23) mRNA.

These experiments provide strong evidence that cross-linking an FcεR is necessary for suppression. However, although we have shown AF-10 cells to display CD23, the FcεR-II, it is still possible that suppression was mediated through a yet unidentified FcεR. To demonstrate directly that CD23 binding induces IgE suppression, AF-10 cells were incubated with three different anti-CD23 mAb for 16 h, washed extensively, and cultured for a further 48 to 72 h. Two of the mAb, 135 and 3-5, suppressed IgE synthesis at 1.0  $\mu$ g/ml, mimicking the effect of IgE-IC in the two of the same experiments (Table II). The effect of these two antibodies was dose dependent and there was no significant suppression at doses of 0.01  $\mu$ g/ml. Another anti-CD23 mAb 8-30 (which is IgM as opposed to an IgG1) did not reproducibly suppress IgE at doses from 0.001 to 1.0  $\mu$ g/ml, nor did unrelated IgG1 mAb.

Although these experiments show that FcεR binding is involved in the IgE suppression, it was possible that binding of the various mAb (which are murine IgG) via their Fc regions was also a necessary part in the induction of suppression. We tested AF-10 for binding of murine antibodies by flow cytometry using IgG1, IgG2a, and IgM control mAb as well as IgG-IC comprised of human IgG and a mAb anti human IgG; no binding was detected. AF-10 also was negative on staining with Leu 11. IgE-IC formed between F(ab')<sub>2</sub> fragments of anti-IgE (mAb 7.12) and IgE (2/3 ratio) were incubated with AF-10 cells (Fig.

Figure 6. mAb 5.1 binds specifically to IgE paraprotein PS. Three IgE paraproteins, PS (IgE-2) (■), ND (IgE-λ) (□), the IgE secreted by AF-10, and Bedora (IgE-κ) (▨) were applied to polystyrene microtiter plates as Ag in a solid phase RIA. mAb 5.1 was added to the wells in increasing concentration followed by 1 μCi/well of <sup>125</sup>I labeled goat anti-mouse IgG. Values represent mean plus 1 SD. Binding of 5.1 to PS was significantly more ( $p < 0.05$ ) than to the other IgE proteins at all concentrations.

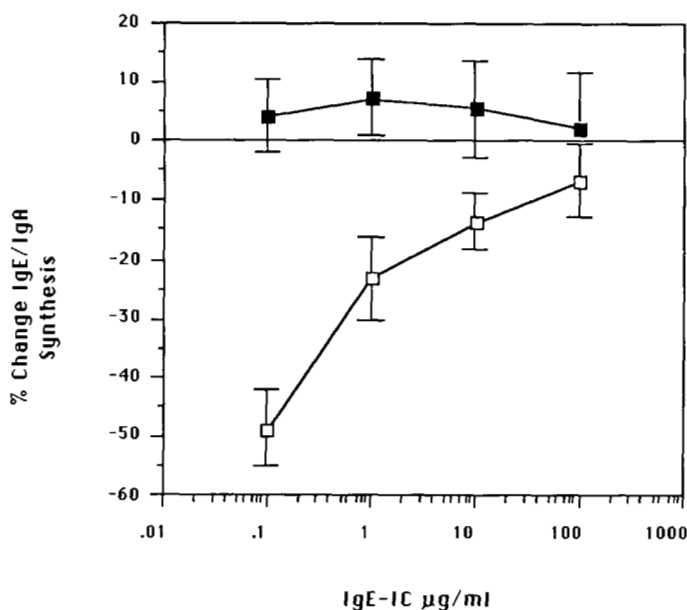
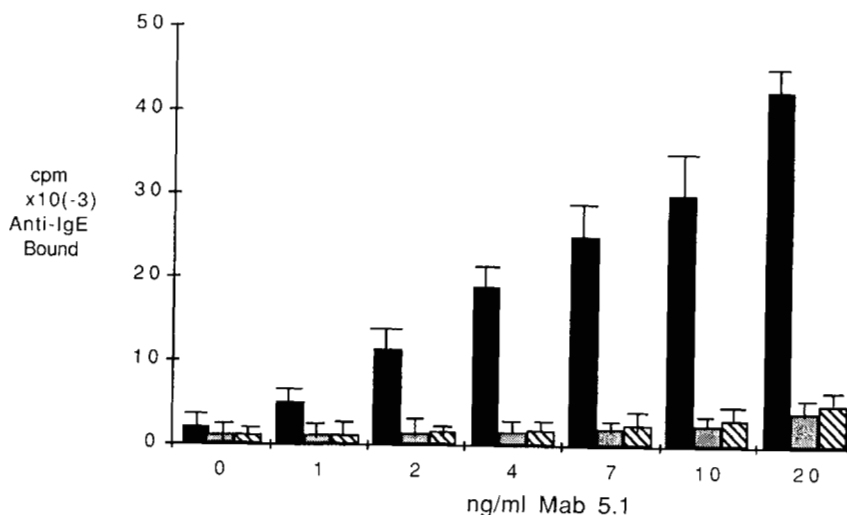


Figure 7. Inhibition of IgE production by IgE-IC formed with mAb 5.1. AF-10 (IgE) (□) and GM1056 (IgA) (■) were incubated with various concentrations of IgE-IC consisting of PS IgE and anti-IgE mAb 5.1 at a 1/1 ratio, washed, and re-cultured for 3 days in triplicate as previously described. Control IgE production was  $90.3 \pm 8.8$  ng/ml and IgA production =  $324 \pm 52$  ng/ml in this experiment. Error bars represent 1 SD. IgA synthesis was unaffected ( $p > 0.5$ ) whereas at an IgE-IC concentration of 1 or 0.1 μg/ml, IgE synthesis was significantly suppressed ( $p < 0.05$ ).

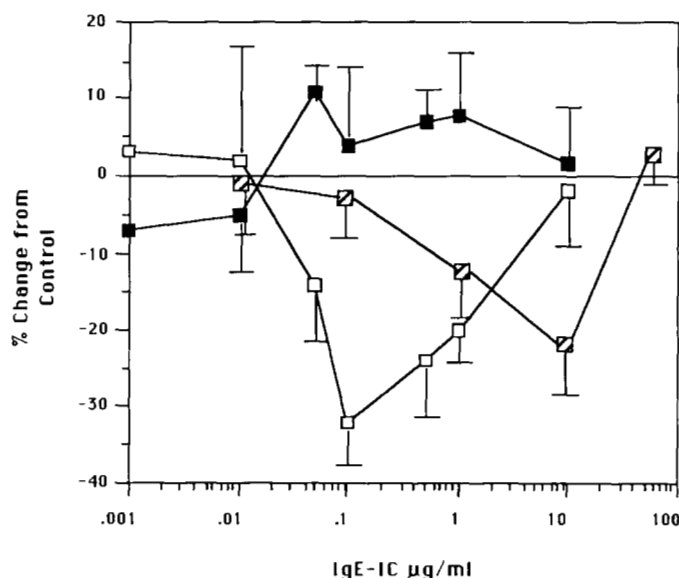


Figure 8. The ability of IgE-IC to mediate suppression is dependent upon FcεR binding. AF-10 cells were pretreated with varying concentrations of IgE-IC (1/1 ratio) formed between mAb 5.1 and either heat denatured (ΔH) (■), native (□) PS IgE, or IgE-IC formed between F(ab')<sub>2</sub> fragments of mAb 7.12 and native PS IgE (●) at a 2/3 (wt/wt) ratio. Cells were then cultured in triplicate for 3 days as described and each supernatant was assayed in duplicate for IgE. Control IgE production was  $175 \pm 13$  ng/ml for the experiment with mAb 5.1 IC and  $123.6 \pm 9$  ng/ml for the experiment using the F(ab')<sub>2</sub> IC. Error bars represent 1 SD.

8); suppression occurred in the same concentration range as with IC formed with intact mAb 7.12 (1 to 10 μg/ml) although, as previously noted, this is at a higher concentration than with IC formed with mAb 5.1. By flow cytometric analysis, AF-10 cells did not bind mAb 5.1 or F(ab')<sub>2</sub> fragments of PS IgE alone, thereby excluding the possibility of an undetected cross-reactivity contributing to the mechanism of suppression.

**IgE synthesis from individual AF-10 cells is suppressed by IgE-IC.** We investigated whether the IgE-IC (5.1-PS) suppressed IgE in the cultures by inhibiting a small subset of high rate secreting cells and whether the secreted IgE was down-regulating its own synthesis and thereby making the cells less susceptible to IgE-IC suppression in the 5000 cell/well cultures. If the latter were true, we would see decreasing amounts of IgE/cell when 5000 vs 50 cells were cultured in a constant volume

TABLE I  
Suppression of IgE production by IgE-IC is abrogated by removal of Igε Fc region<sup>a</sup>

| Treatment                      | IgE (ng/ml) |              |
|--------------------------------|-------------|--------------|
|                                | 48 h        | 72 h         |
| Control cells                  | $76 \pm 7$  | $125 \pm 6$  |
| 5.1:PS IgE                     | $39 \pm 1$  | $80 \pm 12$  |
| 5.1:PS IgE F(ab') <sub>2</sub> | $80 \pm 5$  | $119 \pm 10$ |

<sup>a</sup> Cultures of AF-10 cells ( $5 \times 10^4$ /ml) were established in medium alone or with the indicated IC. Both IgE-IC were added at 0.1 μg/ml at a 1/1 ratio. After 16 h AF-10 cells were combined, washed, and re-cultured as described in *Materials and Methods*. Control cultures were established in cRPMI alone or with cycloheximide (50 μg/ml). Values are expressed as (mean - mean of clx (cycloheximide)-treated cells)  $\pm$  SD of triplicate samples.

of 200 μl. IgE-IC treated or control AF-10 cells were seeded at 50 or 5000 cells/200 μl well in 60 replicates each and cultured for 4, 8, and 24 h. No significant difference was seen in the amount of IgE produced by 50

TABLE II  
IgE production from AF-10 cells is suppressed by anti-CD23 antibodies<sup>a</sup>

| Expt. | Treatment     | Concentration (μg/ml) | ng IgE/ml | % of Control |
|-------|---------------|-----------------------|-----------|--------------|
| 1     | Medium        | 0                     | 42 (5)    | 100          |
| 1     | mAb 135       | 1.0                   | 17 (3)    | 40           |
| 1     | mAb 3-5       | 1.0                   | 33 (2)    | 78           |
| 1     | mAb 8-30      | 1.0                   | 43 (4)    | 102          |
| 1     | IgE-IC (7.12) | 0.1                   | 29 (4)    | 68           |
| 2     | Medium        | 0                     | 77 (4)    | 100          |
| 2     | mAb 135       | 1.0                   | 58 (1)    | 75           |
| 2     | mAb 3-5       | 1.0                   | 56 (3)    | 73           |
| 2     | IgE-IC (4.15) | 0.1                   | 57 (2)    | 74           |
| 3     | Medium        | 0                     | 179 (2)   | 100          |
| 3     | mAb 135       | 1.0                   | 147 (4)   | 82           |
| 3     | mAb 3-5       | 1.0                   | 139 (13)  | 78           |
| 3     | mAb 8-30      | 1.0                   | 171 (8)   | 96           |

<sup>a</sup> AF-10 cells ( $5 \times 10^4$ /ml) were incubated in medium alone or with the indicated anti-CD23 mAb or IgE-IC. After 16 h AF-10 cells were combined, washed, and re-cultured for 2 to 3 days. Values are expressed as the mean of triplicate cultures ( $\pm 1$  SD) and the percent of the medium control for that experiment.

cells/well as compared to 5000/well when normalized for the amount synthesized per cell/24 h ( $p = 0.154$ ). This confirms our results of no suppression when adding exogenous monomeric IgE to the cultures. At 4 h, IgE-IC treatment cells did not induce significant suppression ( $p = 0.601$ ) with treated cell cultures averaging 0.9 pg/cell/24 h. After 8 h, IgE-IC-treated cells did show significant suppression (12% inhibition) compared to controls ( $p = 0.046$ ). IgE-IC-pre-treated cells cultured for 24 h (50 cells/well) showed 30% suppression of the IgE compared to controls ( $p < 0.001$ ).

We were able to estimate that the amount of IgE produced per-cell was at most 0.9 pg/cell/24 h as the described IgE ELISA can detect 10 pg/well and dilutions of 10 cells or less always scored below this level of detection. Furthermore, the observed IgE production was uniform when 24 replicate wells containing 10 cells/well or fewer were assayed. These results suggest that AF-10 cells produce a relatively uniform amount of IgE and that the suppression via the FcεR-II is not limited to a small subpopulation within this cell line.

*IgE suppression is not due to changes in proliferation or cell cycle distribution.* CD23 has been identified as both FcεR-II and a B cell activation antigen (Blast-2) (12, 13, 31). It has been implicated as both a receptor for a 12-kd BCGF (34) and once cleaved from the surface as an autocrine growth factor itself (32). These findings raised the possibility that the observed IgE suppression via FcεR-II binding was a consequence of reduced cell proliferation. We measured the proliferation of AF-10 cells in the presence of IgE-IC both by measuring [<sup>3</sup>H]TdR incorporation and by assessing the number of viable cells. There was no relationship between the amount of IgE produced in IgE-IC or mAb anti-CD23-treated cultures and [<sup>3</sup>H]TdR incorporation when the treatments were considered separately or combined (data not shown). When only conditions showing suppression of IgE (IgE < 100%) were considered, in cultures treated with mAb 135 or 3-5, there was overall significant suppression ( $p < 0.05$ ) of [<sup>3</sup>H]TdR incorporation compared to controls. No difference in AF-10 proliferation ( $p > 0.05$ ) was observed from suppressed cultures resulting from mAb 8-30 or IgE-IC treatment. The number of viable cells were not significantly different ( $p > 0.05$ ) in the IgE-IC vs un-

treated cultures though this is not surprising in view of the short period of total culture (48 to 72 h).

Additionally, as AF-10 cells primarily produce and secrete IgE in the G1 phase of the cell cycle (35), we tested to see if IgE-IC treatment decreased the proportion of cells in G1. There was no change in the relative proportion of cells in the various stages of the cell cycle with 60 to 61% of the cells in G1 under all experimental conditions (data not shown).

## DISCUSSION

We have provided evidence that suppression of IgE synthesis can be mediated through the binding of the B cell FcεR-II (CD23). We initially observed that ongoing production of IgE from both continuously cultured AF-10 and fresh peripheral blood B cells of atopic individuals was decreased upon incubation with mAb anti-IgE or IgE-IC. This response was isotype specific as IgA or IgG synthesis was unaffected. These results suggested that membrane IgE and/or an FcεR were membrane structures mediating this suppression. When analyzed, AF-10 was shown to display by flow cytometry both membrane IgE (>90%) and FcεR-II (26%). Northern analysis revealed that CD23 message was also present. That the FcεR was involved in inhibiting IgE production was shown by demonstrating that 1) IgE-IC (5.1:PS IgE) that could bind FcεR but not membrane IgE suppressed IgE synthesis and that 2) IgE-IC constructed with either heated denatured IgE or F(ab')<sub>2</sub> fragments of PS IgE, so as they could no longer bind to the FcεR-II, were unable to suppress IgE synthesis. To directly implicate the FcεR-II as necessary for suppression, AF-10 cells were treated with mAb anti-CD23 and two of three such antibodies alone induced suppression. The possibility that binding FcγR was also necessary for suppression was excluded by showing that AF-10 cells could not bind IgG-IC, mouse IgG1, IgG2a, did not stain with Leu 11 and that suppression could still be observed with IgE IC formed with F(ab')<sub>2</sub> of the mouse IgG1 anti-human IgE. Using limiting dilution cultures, we assessed whether the IgE-IC FcεR-II mediated suppression affected a small AF-10 subpopulation of high rate IgE secreting cells. IgE production was uniform throughout the cell population (0.9 pg/ml/day) and suppression was also uniform. Furthermore, we found no evidence for secreted IgE providing for feedback inhibition of subsequent IgE production which is also in agreement with the observation that exogenously added monomeric IgE would not substitute for anti-IgE or for IgE-IC. It is likely that cross-linking of FcεR-II is required to see suppression in this system.

We used AF-10, a mycoplasma free subclone of the human EBV-negative IgE plasmacytoma U266 as our model for an IgE plasma cell. There is evidence to suggest that persistent allergic responses are a result of IgE production from long-lived radio-resistant (non-dividing) plasma cells (2). Thus, in vitro studies may provide insight for therapeutic intervention. However, as an in vitro cell line, AF-10 divides regularly and, as such, manipulations that alter its proliferative rate or the distribution of cells in the cell cycle may affect IgE synthesis in a non-physiologic fashion. In assessing mitogenesis, we observed a small but reproducible decrease in [<sup>3</sup>H]TdR incorporation when AF-10 cells were treated with IgE-IC or various mAb against CD23. However, this change was

present with both suppressive and non-suppressive doses of IC or anti-CD23 whereas the distribution of AF-10 in the cell cycle was unaltered by IgE-IC.

Binding surface Ig and/or FcR on lymphocytes is a common motif in these cells' response to external stimuli. Several investigators have shown that crosslinking mlg and the FcγR on resting B cells will inhibit the normal Ig synthetic response to anti-μ and T cell replacing factors (10, 11). Known initial intracellular events necessary for B cell differentiation (phosphatidyl inositol breakdown and Ca<sup>2+</sup> mobilization) were subsequently shown to be diminished in response to crosslinking mlg and FcγR (36, 37). In our system we have shown that binding the FcεR-II alone is sufficient for the suppression of IgE synthesis; however, we have not excluded the possibility that membrane IgE can also mediate suppression. Further comparison of the mlgM/FcγR system and our work would be premature.

FcεR-II has come under intensive investigation recently and three laboratories have independently sequenced a full length cDNA (26, 38, 39). This work has led to the discovery that the previously described B cell activation antigen, Blast-2, is in fact the FcεR-II (12, 13, 33) which is present on IgM<sup>+</sup> IgD<sup>+</sup> B lymphocytes (40). FcεR-II synthesis can be upregulated by IL-4 or EBNA-2 whereas it is stabilized on the cell surface by binding IgE (32, 41). The FcεR-II appears to be a receptor for a 12-kd BCGF and proteolyzed fragments of the FcεR-II may act as autocrine factors (33, 34, 42). Thus, CD23 may play a pivotal role in B cell development.

FcεR-II has also been studied for its potential role in IgE regulation. In rodents, T cells that secrete IgE binding factors display the FcεR-II (3). Similar human cell lines have recently been established, although the expression of the FcεR-II is low (43, 44). Kasaki et al. (45) have demonstrated that a human hybridoma T cell line, which stains negatively for the FcεR-II using a mAb, will secrete IgE binding factors when incubated with the same antibody. This suggests a low but functional expression of the FcεR-II on these T cells. Expression of the FcεR-II on AF-10 with mAb anti-CD23 has been equally difficult to establish. The histogram we presented here (Fig. 4) did not show a small population of clearly FcεR-II<sup>+</sup> cells but rather a modest increase in intensity evenly throughout the population. Expression of CD23 on EBV transformed or other non-IgE secreting B cell lines is significantly greater and is uniform throughout the population (40) (unpublished observation, Saxon, A., Kagan, J., Brown, A.). Consistent with these observations, the Northern blotting results presented here indicate that the level of accumulated CD23 mRNA is far less in AF-10 cells than that seen in an EBV transformed B cell line 1G-7. These differences in expression may correlate with the different roles suggested for the FcεR-II in overall B cell growth and IgE regulation.

We have used in vitro constructed human IgE: murine anti-human IgE-IC to study the regulation of ongoing IgE synthesis. Such IgE-IC may have in vivo correlates as both anti-idiotypic and anti-isotypic (IgE) antibodies have been detected in the serum of patients with IgE-dependent cutaneous and respiratory diseases (46–48). A recent report demonstrated that anti-isotypic (IgE) antibodies were induced by the administration of syngeneic IgE into A/J mice (49). A separate group reported that injection of

an IgE myeloma into rats with a low IgE responding phenotype induced anti-isotypic antibodies which depleted serum IgE for 8 wk (50). Furthermore, IgE production can be regulated directly at the B cell level by T cell-derived IgE binding factors (3). Although the presumption has been that IgE binding factors function by binding membrane IgE, no evidence other than depletion experiments have established this mechanism (51, 52). We propose that, for IgE-secreting cells such as AF-10, IgE binding factors may work by binding secreted IgE to form a complex and thereby crosslink the FcεR-II, analogous to our in vitro system. Indeed, a recent report identified a unique antigenic determinant on an IgE binding factor that was absent from the FcεR-II, suggesting that they may be distinct molecules (45). This allows for the possibility that an IgE molecule can both bind the FcεR-II and an IgE binding factor, forming an IC that ultimately suppresses ongoing IgE synthesis.

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#### REFERENCES

1. Lichtenstein, L. M., K. Ishizaka, P. S. Norman, A. K. Sobotka, and B. M. Hill. 1973. IgE antibody measurements in ragweed hay fever. Relationship to clinical severity and the results of immunotherapy. *J. Clin. Invest.* 52:472.
2. Okudaira, H., and K. Ishizaka. 1981. Reaginic antibody formation in the mouse. XI. Participation of long-lived antibody-forming cells in persistent antibody formation. *Cell Immunol.* 58:188.
3. Ishizaka, K. 1984. Regulation of IgE synthesis. *Annu. Rev. Immunol.* 2:159.
4. Takatsu, K., and K. Ishizaka. 1976. Reaginic antibody formation in the mouse. VII. Induction of suppressor T cells for IgE and IgG antibody responses. *J. Immunol.* 116:1257.
5. Uede, T., T. F. Huff, and K. Ishizaka. 1984. Suppression of IgE synthesis in mouse plasma cells and B cells by rat IgE-suppressive factor. *J. Immunol.* 133:803.
6. Hassner, A., and A. Saxon. 1984. Isotype-specific human suppressor T cells for IgE synthesis activated by IgE-anti-IgE immune complexes. *J. Immunol.* 132:2844.
7. Saxon, A., A. Hassner, and A. Fisher. 1982. T cells and monoclonal anti-IgE inhibit IgE synthesis by IgE secreting human plasma cells. *J. Allergy Clin. Immunol.* 77:544 (Abstr.).
8. Phillips, N. E., and D. C. Parker. 1983. Fc-dependent inhibition of mouse B cell activation by whole anti-μ antibodies. *J. Immunol.* 130:602.
9. Uher, F., M. C. Lamers, and H. B. Dickler. 1985. Antigen-antibody complexes bound to B-lymphocyte Fc gamma receptors regulate B-lymphocyte differentiation. *Cell. Immunol.* 95:368.
10. Phillips, N. E., and D. C. Parker. 1984. Cross-linking of B lymphocyte Fc gamma receptors and membrane immunoglobulin inhibits anti-immunoglobulin-induced blastogenesis. *J. Immunol.* 132:627.
11. Uher, F., and H. B. Dickler. 1986. Cooperatively between B lymphocyte membrane molecules: independent ligand occupancy and cross-linking of antigen receptors and Fc gamma receptors down-regulates B lymphocyte function. *J. Immunol.* 137:3124.
12. Bonnefoy, J., J. Aubry, C. Peronne, J. Wijdenes, and J. Banchereau. 1987. Production and characterization of a monoclonal antibody specific for the human lymphocyte low affinity receptor for IgE: CD 23 is a low affinity receptor for IgE. *J. Immunol.* 138:2970.
13. Yukawa, K., H. Kikutani, H. Owaki, K. Yamasaki, A. Yokota, H. Nakamura, E. Barsumian, R. Hardy, M. Suemura, and T. Kishimoto. 1987. A B cell-specific differentiation antigen, CD23, is a receptor for IgE (FcεR) on lymphocytes. *J. Immunol.* 138:2576.
14. Nilsson, K., H. Bennich, S. G. O. Johansson, and J. Ponten. 1970. Established immunoglobulin-producing myeloma (IgE) and lymphoblastoid (IgG) cell lines from an IgE myeloma patient. *Clin. Exp. Immunol.* 7:477.
15. Nilsson, K. 1971. Synthesis and secretion of IgE by an established

- human myeloma cell line. *Clin. Exp. Immunol.* 9:785.
16. Sherr, E., L. Stein, H. Dosch, and A. Saxon. 1987. IgE-enhancing activity directly and selectively affects activated B cells: evidence for a human IgE differentiation factor. *J. Immunol.* 138:3836.
  17. Saxon, A. 1986. Detection of V region determinants in IgE: assessment by monoclonal antibodies. *J. Allergy Clin. Immunol.* 77:544.
  18. Kanowitz-Klein, S., and A. Saxon. 1985. Fc epsilon receptors on human cell lines and peripheral blood lymphocytes detected by binding of IgE immune complexes. *J. Clin. Immunol.* 5:38.
  19. Ishizaka, K., T. Ishizaka, and A. Menzel. 1967. Physicochemical properties of reaginic antibody. VI. Effect of heat on gamma E, gamma G and gamma A antibodies in the sera of ragweed sensitive patients. *J. Immunol.* 99:610.
  20. Garbey, J. S., N. E. Cremer, and D. H. Sussdorf. 1979. *Methods in Immunology*, 3rd ed. Benjamin Cummings Publishing Co., Reading, MA, p. 238.
  21. Kanowitz-Klein, S. and A. Saxon. 1986. Regulation of ongoing IgE synthesis by human T-cell supernatants derived from atopic and nonatopic donors. *Int. Arch. Allergy Appl. Immunol.* 80:33.
  22. Macy, E., M. Kemeny, and A. Saxon. 1988. Enhanced ELISA: How to Measure Less than 10 Picograms of a Specific Protein (Immunoglobulin) in Less than 8 Hours. *FASEB J.* 2:3003.
  23. Maniatis, T., F. Fritsch, E. F., and S. Sambrook. 1982. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
  24. Thomas, P. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci.* 77:5201.
  25. Feinberg, A. P., and Vogelstein, B. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Analyt. Biochem.* 132:6.
  26. Kikutani, H., S. Invi, R. Sato, E. L. Barsumian, H. Owaki, K. Yamasaki, T. Kaisho, N. Uenibayashi, R. R. Hardy, T. Hirano S. Tsunasawa, F. Sakiyama, M. Suemura, and T. Kishimoto. 1986. Molecular structure of human lymphocyte receptor for immunoglobulin E. *Cell* 47:657.
  27. Saxon, A., J. V. Giorgi, E. H. Sherr, and J. M. Kagan. In press. 1988. Failure of B cells in Common Variable Immunodeficiency to Transit from Proliferation to Differentiation is Associated with Altered B Cell Surface Molecule Display. *J. Allergy Clin. Immunol.* In press.
  28. Saxon, A., C. Morrow, and R. H. Stevens. 1980. Subpopulations of circulating B cells and regulatory T cells involved in *in vitro* immunoglobulin E production in atopic patients with elevated serum immunoglobulin E. *J. Clin. Invest.* 65:1457.
  29. Romagnani, S., G. F. Del Prete, E. Maggi, R. Troncone, G. M. Giudizi, F. Almerigogna, and M. Ricci. 1980. *In vitro* production of IgE by human peripheral blood mononuclear cells. II. Cells involved in the spontaneous IgE production in atopic patients. *Clin. Exp. Immunol.* 42:579.
  30. Ishizaka, T., and K. Ishizaka. 1974. Mechanisms of passive sensitization. IV. Dissociation of IgE molecules from basophil receptors at acid pH. *J. Immunol.* 112:1078.
  31. Rector, E., T. Nakajima, C. Rocha, D. Duncan, D. Lestourgeon, R. S. Mitchell, J. Fischer, A. Schon, and D. Delespesse. 1985. Detection and characterization of monoclonal antibodies specific to IgE receptors on human lymphocytes by flow cytometry. *Immunology* 55:481.
  32. Defrance, T., J. P. Aubry, F. Rousset, B. Vanbervliet, J. Y. Bonnefoy, N. Arai, Y. Takebe, T. Yokota, F. Lee, K. Arai, J. de Vries, and J. Banchereau. 1987. Human recombinant IL-4 induces Fc epsilon receptors (CD23) on normal human B lymphocytes. *J. Exp. Med.* 165:1459.
  33. Gordon, J., and G. R. Guy. 1987. The molecules controlling B lymphocytes. *Immunol. Today* 8:339.
  34. Swendeman, S., and D. A. Thorley-Lawson. 1987. The activation antigen Blast-2, when shed, is an autocrine BCGF for normal and transformed B cells. *EMBO J.* 6:1637.
  35. Turner, K. J., T. M. Plozza, and P. G. Holt. 1985. Cell cycle-dependent fluctuations in IgE secretion in a human myeloma line. *Clin. Immunol. Immunopathol.* 36:212.
  36. Bijsterbosch, M. K., and G. G. B. Klaus. 1985. Crosslinking of surface immunoglobulin and Fc receptors on B lymphocytes inhibits stimulation of inositol phospholipid breakdown via the antigen receptors. *J. Exp. Med.* 162:1825.
  37. Wilson, H. A., D. Greenblatt, C. W. Taylor, J. W. Putney, R. Y. Tsien, F. D. Finkelman, and T. M. Chused. 1987. The B lymphocyte calcium response to anti-Ig is diminished by membrane immunoglobulin cross-linkage to the Fc gamma receptor. *J. Immunol.* 138:1712.
  38. Ludin, C., H. Hofstetter, M. Sarfati, C. A. Levy, U. Suter, D. Alaimo, E. Kilchherr, H. Frost, and G. Delespesse. 1987. Cloning and expression of the cDNA coding for a human lymphocyte IgE receptor. *EMBO J.* 6:109.
  39. Koichi, I., M. Takami, C. Won Kim, T. Honjo, T. Miyoshi, U. Tagaya, T. Kawabe, and J. Yodoi. 1987. Human lymphocyte Fc receptor for IgE: sequence homology of its cloned cDNA with animal lectins. *Proc. Natl. Acad. Sci. USA* 84:819.
  40. Kikutani, H., M. Suemura, H. Owaki, H. Nakamura, R. Sato, K. Yamasaki, E. L. Barsumian, R. R. Hardy and T. Kishimoto. 1986. Fc epsilon receptor, a specific differentiation marker transiently expressed on mature B cells before isotype switching. *J. Exp. Med.* 164:1455.
  41. Wang, F., C. D. Gregory, M. Rowe, A. B. Rickinson, D. Wang, M. Birkenback, H. Kikutani, T. Kishimoto, and E. Kieff. 1987. Epstein-Barr virus nuclear antigen 2 specifically induces expression of the B-cell activation antigen CD23. *Proc. Natl. Acad. Sci. USA* 84:3452.
  42. Guy, G. R. and J. Gordon. 1987. Coordinated action of IgE and a B-cell-stimulatory factor on the CD23 receptor molecule up-regulates B-lymphocyte growth. *Proc. Natl. Acad. Sci. USA* 84:6239.
  43. Nutman, T. B., G. Delespesse, M. Sarfati, and D. J. Volkman. 1987. T cell-derived IgE-binding factors. I. Cloned and transformed T cells producing IgE-binding factors. *J. Immunol.* 139:4049.
  44. Young, M. C., D. Y. M. Leung, and R. S. Geha. 1984. Production of IgE-potentiating factor in man by T cell lines bearing Fc receptors for IgE. *Eur. J. Immunol.* 14:871.
  45. Kisaki, T., T. F. Huff, D. H. Conrad, J. Yodoi, and K. Ishizaka. 1987. Monoclonal antibody specific for T cell-derived human IgE binding factors. *J. Immunol.* 138:3345.
  46. Johansson, S. G. O. 1986. Anti-IgE antibodies in human serum. *J. Allergy Clin. Immunol.* 77:555.
  47. Quinti, I., C. Brozek, N. Wood, R. S. Geha, and D. Y. M. Leung. 1986. Circulating IgG autoantibodies to IgE in atopic syndromes. *J. Allergy Clin. Immunol.* 77:586.
  48. Nawata, Y., T. Koike, H. Hosokawa, H. Tomioka, and S. Yoshida. 1985. Anti-IgE autoantibody in patients with atopic dermatitis. *J. Immunol.* 135:478.
  49. Haba, S., and A. Nisonoff. 1987. Induction of high titers of anti-IgE by immunization of inbred mice with syngeneic IgE. *Proc. Natl. Acad. Sci. USA* 84:5009.
  50. Marshall, J. S., and E. B. Bell. 1985. Induction of an auto-anti-IgE response in rats. I. Effects of serum IgE concentrations. *Eur. J. Immunol.* 15:272.
  51. Kishimoto, T., Y. Hirai, M. Suemura, K. Naknishi, and Y. Yamamura. 1978. Regulation of antibody response in different immunoglobulin classes. IV. Properties and functions of IgE class-specific suppressive factor(s) related from DNP-mycobacterium-primed T cells. *J. Immunol.* 121:2106.
  52. Hirashima, M., J. Yodoi, and K. Ishizaka. 1980. Regulatory role of IgE binding factors from rat T lymphocytes. III. IgE specific suppressive factor with IgE binding activity. *J. Immunol.* 125:1442.