IgE-ANTIGEN COMPLEXES ENHANCE FcεR AND
Ia EXPRESSION BY MURINE B LYMPHOCYTES

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Receptors for the Fc portion of Igs were first identified on lymphocytes in 1970 (1), and later differentiated on the basis of their selective affinity for various Ig classes. Fc receptors for IgE (FcεR) are found on a variety of human (2, 3) and rodent (4) cells and can be categorized on the basis of their affinity for IgE. High-affinity FcεR (FcεR-I) found on mast cells and basophils (5) clearly mediate allergic degranulation. However, the moderate-affinity FcεR (FcεR-II) present on macrophage/monocytes (6), eosinophils (7), and lymphocytes (2, 8) is less well characterized. The role that lymphocyte FcεR-II plays in the regulation of the immune system is the object of much recent study (9–22). Current evidence supports a model whereby the interaction between IgE and its receptor initiates a cascade of events that give rise to increased FcεR expression and, ultimately, control of IgE production.

However, little is known of the consequences of IgE-FcεR interaction as they apply to events that are not directly involved with IgE regulation, such as modulation of other surface molecules on FcεR-bearing cells. Indeed, the surprisingly high numbers of FcεR$^+$ lymphocytes, including at least 50% of B cells from BALB/c mouse spleen (23), suggest that the IgE system might participate in immune regulation outside of the limited role to which it has been traditionally ascribed. Additionally, little attention has been directed to the influence of antigen-mediated crosslinking of FcεR-bound IgE molecules particularly as it relates to the role(s) of FcεR$^+$ B cells in an immune response. One report on murine macrophages showed that immune complexes of IgG or IgE inhibit IFN-γ-mediated induction of class II antigens of the MHC (24).

Herein we present data that demonstrate that the efficiency with which monoclonal IgE binds to and enhances the expression of B cell FcεR is substantially increased when IgE is complexed with specific antigen. More importantly, we report for the first time that soluble IgE-antigen complexes potently stimulate the cell surface expression of B cell class II MHC (Ia) antigens, while IgE alone (in the absence of antigen) has little effect. This enhancement of Ia expression suggests that IgE-antigen complexes may play a more generalized role in immune responses than previously assumed.

Materials and Methods

Animals.   All assays were performed with spleen cells from male BALB/c mice, aged 8–12 wk. Hybridoma ascites were obtained from 12–16-wk-old male (BALB/c × A/J)F₁ (CAF₁) mice. Both mouse strains were obtained from the breeding colonies of the Medical Biology Institute.

Reagents.   BSA, Ficoll (Histopaque), 2-ME, and propidium iodide were obtained from Sigma Chemical Co. (St. Louis, MO). NP-40 was purchased from Bethesda Research Laboratories (Rockville, MD). FITC-avidin conjugates and OVA were purchased from Miles Laboratories, Inc. (Elkhart, IN). The conjugates of 2,4-dinitrophenyl-ovalbumin (DNP₁₅-OVA) and DNP₁₁-BSA were prepared as described (25). CNBr-activated Sepharose 4B was acquired from Pharmacia Fine Chemicals (Uppsala, Sweden). Guinea pig complement (Pel-Freeze Biologicals, Rogers, AR) was adsorbed at 4°C with BALB/c spleen cells before use. [³H]TdR was purchased from New England Nuclear (Boston, MA).

Antibodies.   FITC-conjugated and unconjugated goat anti-mouse IgG were obtained from Cappel Laboratories (Malvern, PA). FITC–goat anti-mouse IgM was purchased from Fisher Scientific Co. (Orangeburg, NY). Anti-DNP IgE and anti-DNP IgG mAbs were affinity purified from mouse ascites according to the methods described by Liu et al. (25). Iodination of IgE was accomplished using the chloramine-T method. Monoclonal anti-Thy-1.2 was derived from F7D5e7 hybridoma clone (26), obtained as ascites, and clarified by centrifugation. Anti-I-Aᵏ, anti-I-Aᵈ, and anti-I-E antibodies were obtained from 10.3.6, MKD6, and 14.4.4 hybridomas, respectively, and purified from mouse ascites. Biotinylated 10.3.6, MKD6, and 14.4.4 antibodies were kindly provided by Dr. Carol Cowing, Medical Biology Institute. Anti-class I antibodies (anti-KᵈDᵈ, 34-1-2S; anti-Dᵈ, 34-5-8S) were provided as culture supernatants by Dr. Matthew Mescher, Medical Biology Institute. Goat anti-mouse IgE (GAME)[1] antibodies were prepared and purified as described previously (25). Goat anti-mouse IgM antibody and biotinylated anti-IgMᵇ (mouse IgG₁, AF6-78.25) were gifts from Dr. Donald Mosier, Medical Biology Institute.

Induction of FcεR and Surface Ia (sIa) in Lymphoid Cells.   BALB/c mouse spleen cells were dispersed in MEM (MA Bioproducts, Walkersville, MD) using a tissue grinder, and washed twice in MEM. Red blood cells were eliminated by treating the dispersed spleen cells with ammonium chloride solution for 5 min followed by two washes in MEM. T cell depletion was performed by incubating the cells with anti-Thy-1.2 mAb, followed by guinea pig complement, as described (27). Macrophage depletion was accomplished by passage of T cell–depleted lymphocytes over Sephadex G-10 (28). Lymphocytes were cultured with the indicated inducing substance in DME (MA Bioproducts), 10% FCS (HyClone Laboratories, Logan, UT), 100 U/ml penicillin, and 100 μg/ml streptomycin (DME-FCS) at a concentration of 10⁷/ml in 0.5 ml aliquots in 24-well culture plates (Costar, Cambridge, MA). Incubations were carried out in a humidified 10% CO₂ enviroment maintained at 37°C for 18–24 h, unless indicated otherwise.

FcεR Labeling Assay.   In previous studies we have used a rosette assay to detect nascent and induced FcεR molecules on mouse lymphocytes (19). In this study, a more quantitative, less cumbersome assay has been developed using ¹²⁵IgE to label FcεR sites, thus permitting characterization of the ligand-receptor interaction. Nascent FcεR molecules expressed on freshly isolated BALB/c spleen cells were quantitated by exposing splenocytes to cold monomeric IgE (in varying concentrations) containing ¹²⁵I-labeled IgE as tracer in the absence or presence of varying quantities of DNP-OVA. All labeling exposures were carried out at 4°C in PBS containing 0.1% NaN₃ plus 0.1% BSA (PAB) in a final volume of 200 μl (5 × 10⁶ cells) for 2 h. When quantitating FcεR on lymphocytes harvested from induction cultures, cell-bound IgE on unfractionated or T lymphocyte-depleted spleen cells was first removed by acid elution (see Results) before labeling. After the 2-h incubation at 4°C, labeled cells were centrifuged over 85% dibutyl phthalate/15% mineral oil and radioactivity of the pellet was quantitated. The cpm of cell-associated ¹²⁵I-IgE was determined in the presence

of 200 μg/ml of unlabeled IgE and subtracted from all experimental values. All samples were tested in duplicate or triplicate, and the experiments were repeated with similar results. The data were most reproducible when ^{125}I-IgE tracer was coincubated with near-saturating concentrations of unlabeled IgE (5–40 μg/ml). This minimized rebinding of the radiolabel and other kinetic idiosyncracies that might have distorted interpretation of the results. The results obtained with this method were qualitatively comparable to the rosette assay.

Measurement of Cell Surface I-A and I-E Antigens. Preincubated B cells were washed in MEM, reconstituted in 3 ml DME-FCS and centrifuged over Ficoll (specific gravity 1.090) at 2,000 rpm for 20 min. Cells at the interface were collected, washed twice and reconstituted in HBSS (MA Bioproducts) plus 0.1% NaN$_3$ and 0.1% BSA. 10^6 cells (in 100 μl) were incubated with 2 μg biotinylated anti-I-A^d (MKD6) or anti-I-E^d (14.4.4) antibodies for 30 min on ice. After washing, samples were incubated for an additional 30 min with 2 μg FITC-avidin or Texas Red–avidin (0°C), washed twice, reconstituted in HBSS/NaN$_3$/BSA (0°C) and the fluorescence was measured on a flow cytometer (Epics 753; Coulter Electronics, Hialeah, FL). The fluorescence intensities of 10,000 cells were determined for each sample. Viable lymphocytes were selected by gating on forward angle and 90°C light scatter. Hence, mean fluorescence intensity (MFI) represents the average fluorescence value for viable lymphocytes in a sample.

Other Cell Surface Molecules. A similar double-labeling approach was used for measuring class I MHC antigens on the flow cytometer. After incubation and isolation of viable cells, samples were cleared of surface Fc receptor–bound Ig by consecutive 1-min washes in DNP-saturated MEM and pH 4 MEM (see Results). Each sample of 10^6 cells was then probed with anti-class I antibody (anti-K^d D^d, 34-1-2S or anti-D^d, 34-5-8S) for 30 min on ice. The second incubation was carried out with FITC-goat anti-mouse IgG followed by measurment on the flow cytometer. Measurement of sIgM was accomplished with a single 30-min incubation with a FITC-goat anti-mouse IgM antibody. All antibodies used in flow cytometry experiments were airfuged before use to remove aggregated materials.

Cell Cycle Characterization by Flow Cytometry. Propidium iodide was used to stain the DNA of intact nuclei from hypotonically lysed cells, similar to the assay reported by DeFranco et al. (29). B lymphocytes from BALB/c mouse spleen were incubated for 48 h in 24-well culture plates (5 × 10^6 cells/ml). Colcemid (30 ng/ml) was added at 24 h to prevent completion of M phase. The cells were washed, centrifuged over Ficoll (specific gravity 1.090), removed from the interface, and washed twice. To each sample of 10^6 cells, 50 μg/ml propidium iodide, 0.1% sodium citrate, and 0.1% NP-40 were added followed by flow cytometric analysis. Cell populations were categorized according to whether DNA was diploid (G$_0$ or G$_1$ phases), intermediate (S phase), or tetraploid (G$_2$ or M phases).

Thymidine Incorporation. Lymphocyte cultures were established in 96-well plates; 10^5 cells in 200 μl of DME/5% FCS containing 0.05 mM 2-ME. After 36 h, 50 μl [^{3}H]TdR (20 μCi/ml MEM) was added to each well and allowed to incubate an additional 12–16 h before harvesting (Titertek cell harvester).

Results

Binding Characteristics of IgE-Antigen Complexes vs. Monomeric IgE to Lymphocyte FcεR

IgE-Antigen Complexes Resist Acid Elution From FcεR. Before quantitating FcεR sites by ^{125}I-IgE labeling in vitro, it was necessary to remove IgE from the lymphocyte surface. Cell-bound monomeric Ig is sensitive to removal by acid (30, 31) and exposure of lymphocytes to low pH solutions does not appear to affect subsequent IgE binding to FcεR (32). Moreover, we have observed no differences in specific ^{125}I-IgE binding to B cells previously exposed to either pH 4 or pH 7 solutions.

Greater than 95% of monomeric IgE was removed from BALB/c spleen cells by a 1-min wash in pH 4 MEM (Fig. 1). However, if anti-DNP IgE was complexed with DNP-OVA, ~80% of the IgE remained bound to lymphocyte surfaces after the acid wash at pH 4.0, and even solutions of lower pH did not completely remove the IgE-antigen complexes. This resistance to acid elution was dependent on an-
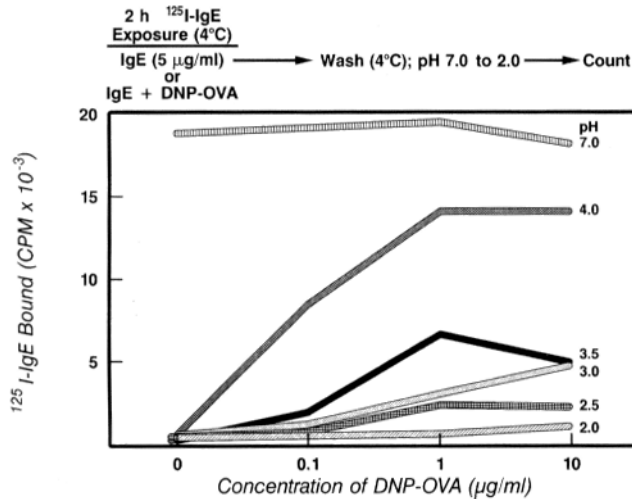
FIGURE 1. IgE-antigen complexes resist acid elution from FcεR. 5×10^6 BALB/c spleen cells were incubated in PAB with 5 μg/ml of IgE plus ^{125}I-IgE in the absence or presence of varying concentrations of DNP-OVA. After 2 h, all samples were washed in PAB (4°C), incubated for 1 min in 0.5 ml MEM (4°C) of varying pH, washed in 4 ml MEM (pH 7.0), and centrifuged over oil. Data points represent means of duplicate samples. As for all ^{125}I-IgE– FcεR binding experiments, nonspecific cell–associated radioactivity (cpm of ^{125}I-IgE bound in the presence of 200 μg/ml IgE) was subtracted from all values.

tigen concentration. In the presence of 5 μg/ml of IgE, optimal DNP-OVA concentrations were 1–10 μg/ml, thus approximating 1:1 molar concentrations of IgE and antigen. Comparable results were obtained with 10 μg/ml of IgE.

Reversal of Acid Resistance of FcεR-bound IgE-Antigen Complexes by Competitive Elution with Free DNP.   To remove IgE-antigen complexes from lymphocyte FcεR while avoiding the potentially harmful effects of very low pH solutions, we adapted a competitive elution approach using saturated solutions of dinitrophenol. As illustrated in Fig. 2, exposure to free DNP in MEM had little effect on the binding of monomeric IgE to nascent FcεR on lymphocytes or the ability to elute the IgE at acid pH (group II vs. group I). On the other hand, the acid resistance of FcεR-bound
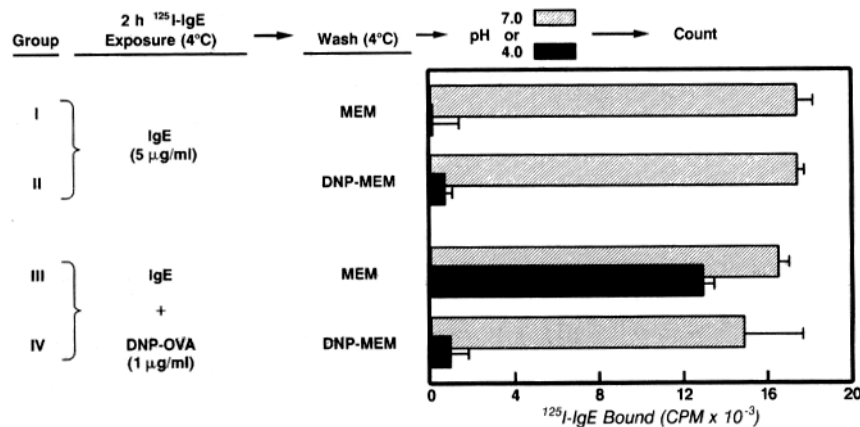

FIGURE 2.   Reversal of acid resistance of FcεR-bound IgE-antigen complexes by competitive elution with free DNP. BALB/c spleen cells were treated in a similar manner to those of Fig. 1 except that groups II and IV also were washed for 1 min in DNP-saturated MEM (4°C) immediately before the 1-min incubation in 0.5 ml MEM (final pH, 4 or 7) and wash in 3 ml MEM (pH 7.0). The data reflect means (±SE) of triplicate samples.
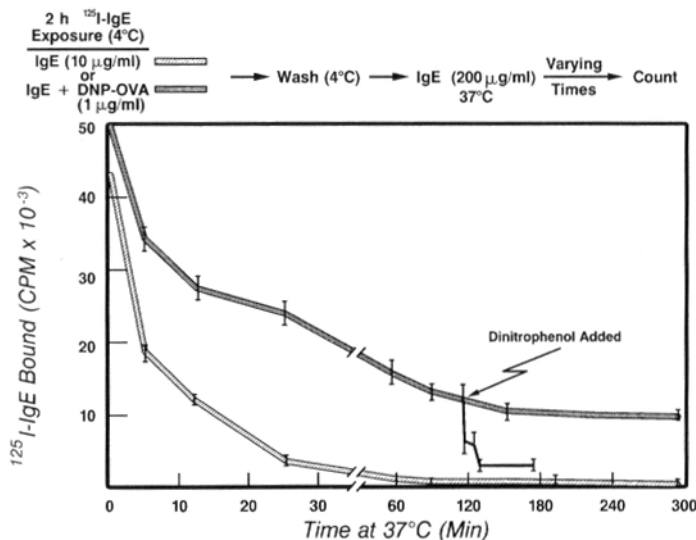
FIGURE 3. IgE-antigen complexes dissociate from lymphocyte FcεR more slowly than monomeric IgE. Unfractionated BALB/c spleen cells were exposed to 10 µg/ml IgE plus ^{125}I-IgE alone or in combination with 1 µg/ml of DNP-OVA for 2 h in PAB (4°C). After a 5 min, 4°C wash, samples were immediately reconstituted in PAB (37°C) containing 200 µg/ml unlabeled IgE and centrifuged over oil at varying times thereafter. Minimal dissociation of ^{125}I-IgE occurred during the wash. After 120 min of dissociation, excess free DNP was added to several samples that were initially exposed to IgE plus DNP-OVA. Sampling was performed in triplicate and the means (±SE) are shown.

IgE-antigen complexes (group III) was completely reversed by exposure to free DNP (group IV), indicating that such exposure succeeded in competitively eluting the antigen from the cell-bound IgE. These results were confirmed in another experiment in which cell-bound IgE was quantitated with ^{125}I-GAME instead of ^{125}I-IgE (not shown).

IgE-Antigen Complexes Dissociate from Lymphocyte FcεR More Slowly than Monomeric IgE. The apparent increased avidity of IgE-antigen complexes for FcεR is further illustrated by the comparative ^{125}I-IgE dissociation curves in the absence and presence of antigen (Fig. 3). The dissociation half-times at 37°C from unfractionated BALB/c splenic lymphocytes, taken from the terminal linear portion of the curve (mean of three experiments), were 440 min for IgE-antigen complexes vs. 8 min for monomeric IgE. Dissociation half-times from isolated B cells were 325 and 7 min, respectively (two experiments; not shown). A similar pattern was observed when the experiment was performed at 4°C, except that the dissociation rates were commensurately longer for both monomeric and complexed IgE. Competitive elution of antigen from the complexed ^{125}I-IgE by addition of DNP after 120 min markedly increased the dissociation rate, approaching that of the monomeric form (Fig. 3). This further demonstrates the reversibility of the binding phenomenon and eliminates internalization of the IgE-antigen complex as an explanation for its exceedingly slow dissociation rate. Ligand/receptor internalization, however, might account for the residual 5% of the original cell-associated radioactivity that remained 1 h after addition of DNP.
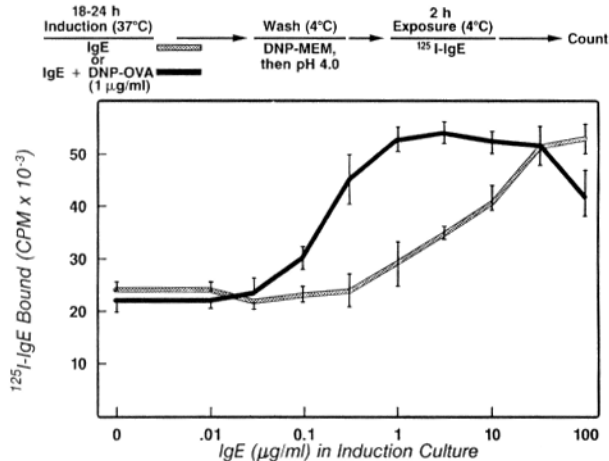
FIGURE 4. FcεR expression by mouse lymphocytes is more efficiently induced by IgE-antigen complexes. B cell–enriched splenocytes were incubated for 18–24 h in medium alone or together with IgE (10 μg/ml) without or with 1 μg/ml DNP-OVA. After removal of IgE by a free DNP/acid wash, FcεR were quantitated by exposure to 10 μg/ml monomeric IgE plus ^{125}I-IgE for 2 h at 4°C. Each data point represents the mean (±SE) of triplicate samples. This experiment was performed two times with both B cell–enriched and unfractionated spleen cell populations with similar results.

IgE-antigen complexes did not appear to bind to additional sites since lymphocyte-bound ^{125}I-IgE was essentially equivalent in the presence or absence of antigen as determined at the end of the initial 2-h exposure. However, presumably because of multiple crosslinked ^{125}I-IgE–antigen monomers, cell bound radioactivity was slightly higher when the molar concentrations of IgE and DNP-OVA were equivalent (not shown). Removal of these complexes by addition of free DNP just before measurement of radioactivity reduced cell-associated cpm to the level of those samples incubated in the absence of DNP-OVA.

Modulation of Cell Surface Molecules By IgE-Antigen Complexes

FcεR Expression by Mouse Lymphocytes Is More Efficiently Induced by IgE-Antigen Complexes. In addition to increasing the binding avidity of IgE to its receptor, formation of complexes with antigen increased the efficiency with which IgE induced the expression of FcεR molecules on lymphocytes (Fig. 4). Thus, ∼30-fold less antigen-complexed IgE than free IgE induced equivalent peak levels of FcεR expression by cultured lymphocytes. This increased efficiency of IgE-antigen complexes vs. free IgE (Fig. 4) was consistently observed in three experiments with either isolated B cells or unfractionated lymphocytes. Significantly, exposure of cells to DNP-specific
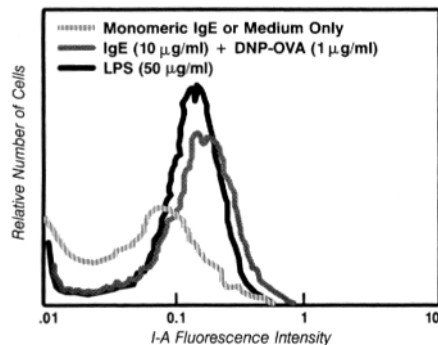

FIGURE 5. IgE-antigen complexes, but not monomeric IgE, induce Ia antigen expression by BALB/c B cells. After an 18 h incubation (37°C) with either medium alone, LPS (50 μg/ml), monomeric IgE (10 μg/ml), or IgE-DNP-OVA complexes, B cells were washed and stained for I-A antigens. The latter was accomplished by successive 30-min, 4°C exposures to biotin-MKD6 (anti-I-A^d) antibody and Texas red–avidin separated by a single wash. The change in flow cytometric measurements of I-A antigen density is depicted in a typical histogram.

TABLE I

IgE-Antigen Complexes Stimulate B Lymphocyte Ia Expression in the Absence of T Cells and Macrophages

18-24 h induction (37°C) by:	Surface I-A expression (percent increase MFI)			
	Unfractionated spleen cells	T cell–depleted spleen cells	T cell– and macrophage-depleted spleen cells	M12.4.5 cells
IgE (10 µg/ml)	5	1	17	2
IgE (10 µg/ml) + DNP-OVA (1 µg/ml)	11	143	145	– 1
LPS (50 µg/ml)	80	110	277	35

BALB/c mouse spleen cells (unfractionated, T cell-depleted, or T cell- and macrophage-depleted) or murine lymphoma cells (M12.4.5) were induced with IgE, IgE-antigen complexes, or LPS for 18-24 h at 37°C. Cells were stained for sI-A using the approach outlined in Materials and Methods and Fig 5. For each sample, 10,000 cells were analyzed.

IgG₁, IgA, or IgM mAbs either in monomeric or antigen-complexed form did not affect the expression of FcεR by cultured spleen cells.

IgE-Antigen Complexes, but not Monomeric IgE, Induce Ia Antigen Expression by BALB/c B Cells. The enhanced expression of FcεR induced by IgE-antigen complexes inspired an examination of other cell surface antigens. As shown in Fig. 5, IgE-antigen complexes induced increased expression of class II MHC (Ia) antigens on the surface of BALB/c B lymphocytes. Thus, I-A^d antigen expression was enhanced by exposure to IgE-antigen complexes, and to a similar magnitude occurring in response to 50 µg/ml of LPS. Conversely, exposure to monomeric IgE did not increase Ia antigen expression. Maximum increases of Ia-antigen expression were observed after 12-44 h of incubation with IgE-antigen complexes (data not shown). A 4-h incubation with IgE-antigen complexes resulted in a negligible change in surface Ia, whereas significant induction of surface Ia occurred after 4 h incubation with LPS and this increased progressively through 44 h.

IgE-Antigen Complexes Stimulate B Lymphocyte Ia Expression in the Absence of T Cells and Macrophages. IgE-antigen complexes, in contrast to IgE alone, produced a modest increase of I-A expression in unfractionated splenic lymphocytes, and to a much
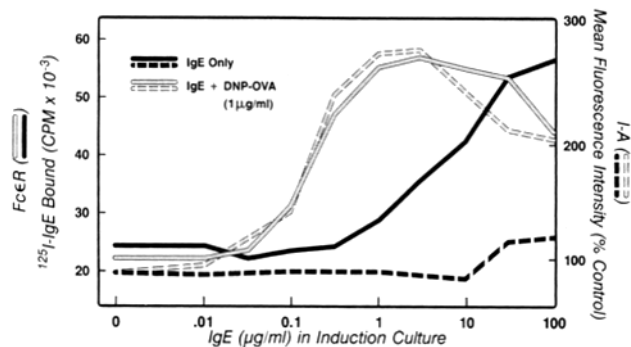

FIGURE 6. Both FcεR and I-A expression by mouse B lymphocytes are induced by IgE-antigen complexes of similar composition. The in vitro response of FcεR and I-A antigen expression to monomeric IgE or IgE-DNP-OVA complexes are compared in B lymphocytes. The data for FcεR expression are reproduced from Fig. 4. Cell surface I-A were measured as outlined in the legend for Fig. 5. Incubations were carried out for 18-24 h.
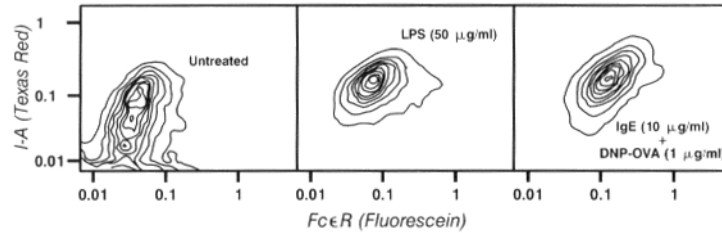
FIGURE 7.   Murine B cells expressing a high density of FcεR in response to IgE-antigen complexes also express a high density of Ia antigens. After an 18-h incubation period, B cells were washed in free DNP/acid to remove surface Ig. Lymphocytes (~95% B cells) were then exposed to IgE for 1 h (4°C) while biotinylated MKD6 antibody was included during the last 30 min of the first labeling period. After washing, the cells were exposed to GAME and Texas red-avidin for an additional 30 min, washed, and analyzed by flow cytometry. Crossreaction of FITC-GAME with other cell surface molecules (i.e., in the absence of IgE) was significant but did not present a major interference in this experiment.

greater extent in B cell–enriched populations (Table I). This lower level of I-A expression in unfractionated spleen cells also was observed when gating the analysis on the more strongly I-A$^+$ cells. Macrophages did not contribute to the effects of IgE-antigen complexes on B cell surface Ia expression since splenic lymphocytes depleted of both T cells and macrophages displayed similar increases of I-A expression as observed for the T cell–depleted lymphocytes (Table I). I-A expression was enhanced by LPS in all spleen cell populations. Interestingly, the FcεR-bearing murine lymphoma cell line, M12.4.5, did not display increased Ia expression in the presence of IgE-antigen complexes, whereas LPS induced a modest response (Table I).

Both FcεR and Ia Expression by Mouse B Lymphocytes Are Induced by IgE-Antigen Complexes of Similar Composition.   Induction of class II MHC antigen expression more vividly demonstrates the discrepancy between the effects of monomeric and complexed IgE (Fig. 6). Although half-maximal stimulation of B cell FcεR expression required 3 µg/ml of monomeric IgE, a concentration far exceeding that normally found in vivo, surface I-A expression was only modestly increased by as much as 100 µg/ml of monomeric IgE. Conversely, in the form of IgE-antigen complexes, the IgE concentration-response curve for surface Ia expression paralleled that of FcεR expression (Fig. 6).

Murine B Cells Expressing a High Density of FcεR in Response to IgE-Antigen Complexes Also Express a High Density of Ia Antigens.   Contour plots of cells stained for FcεR and surface Ia antigens (Fig. 7) show that while LPS stimulates only sIa expression, IgE-antigen complexes induced expression of both cell surface markers and, moreover, B cells that exhibited higher levels of FcεR also expressed more surface Ia.

Antigen-complexed IgE, but not IgG₁, Selectively Modulates Ia Expression Regardless of Antigen Specificity.   DNP-specific IgE was equally effective in stimulating I-A expression when complexed with either DNP-OVA or DNP-BSA (Table II). Moreover, induction of I-A antigen expression was not limited to IgE of DNP specificity or any other unique quality of that particular mAb. Thus, ragweed-specific IgE induced surface Ia expression on B cells when complexed with 0.5 to 5 µg/ml of ragweed antigen, but not in its monomeric form or in the presence of DNP-protein.

Table II

Antigen-complexed IgE, But not IgG1, Selectively Modulates Ia Expression Regardless of Antigen Specificity

18–24 h induction (37°C) by:		Surface Molecule Expression (percent increase MFI)		
Antibody (μg/ml)	Antigen (μg/ml)	I-A	H-2^d	IgM^d
IgE anti-DNP (10)	None	0	0	– 8
	DNP-OVA (1)	158	– 4	– 13
	DNP-BSA (1)	157		
IgE anti-ragweed (5)	None	0		
	Ragweed (5)	76		
IgG1 anti-DNP (10)	None	27		
	DNP-OVA (1)	19		
IgG1 anti-DNP (1,000)	None	5		
	DNP-OVA (10)	8		
LPS (50 μg/ml)		110	14	– 77

BALB/c splenic B cells were induced with antibody, antigen-antibody complexes, or LPS for 18–24 h at 37°C. Cells were stained for sI-A using the approach outlined in Materials and Methods and Fig. 5. Using a similar procedure, class I MHC antigens were labeled by exposing the cells to anti-D^d or anti-K^{d}D^d antibodies, washing the cells, and exposing them to FITC-GAMG antibody. FITC-anti-IgM was used to label sIgM. For each sample, 10,000 cells were analyzed.

I-A-associated MFI was minimally altered by incubation with ≤5 μg/ml of ragweed antigen (data not shown).

The inducible expression of Ia antigens by antigen-complexed antibodies appears to be dependent on IgE. Thus, a modest increase of I-A-associated fluorescence was discernable after a 24-h incubation of B cells with monomeric DNP-specific IgG₁ (20–1,000 μg/ml) but, unlike IgE, further enhancement did not occur in the presence of varying amounts of DNP-OVA (Table II).

The effects of IgE-antigen complexes on other cell surface molecules also were tested by flow cytometry (Table II). LPS (50 μg/ml) reduced the expression of sIgM, while IgE-antigen complexes produced little variation in sIgM density on B cells labeled by a FITC-goat anti-mouse IgM antibody. Class I antigen expression, using anti-K^{d}D^d (34-1-2S) or anti-D^d (34-5-8S) antibodies as the initial probes, revealed that a small enhancement of fluorescence was achieved with LPS but no change occurred in samples treated with IgE-antigen complexes. Similar results were obtained regardless of which anti–class I antibody was used as the initial probe.

The binding of biotinylated anti-IgM^b (IgG₁, AF6–78.25) and anti-I-A^k (IgG₂ₐ, 10.3.6) to T cell–depleted splenocytes was tested by flow cytometry after a 16-h incubation. Neither antibody bound sufficiently to exceed background fluorescence and no change in fluorescence was observed in cells incubated with either IgE-antigen complexes or LPS. These results further demonstrate the specificity of the Ia response and indicate that binding of MKD6 and 14.4.4 antibodies to Fcγ receptors is not responsible for the enhanced fluorescence signal in IgE-antigen–treated cells.
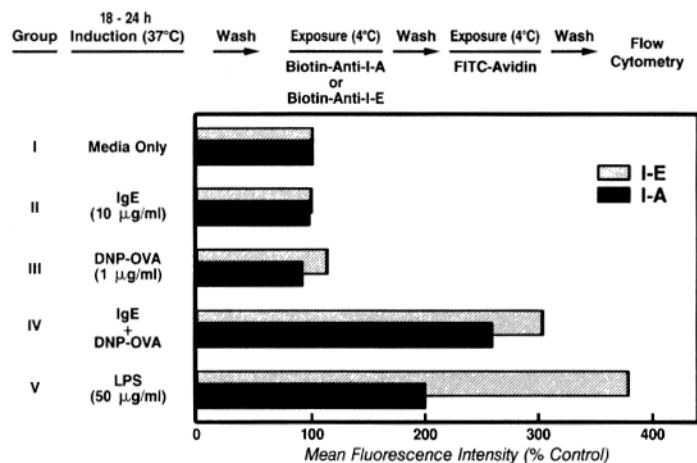
FIGURE 8.   IgE-antigen complexes, but not IgE or antigen alone, induce I-A and I-E antigen expression by B cells. B lymphocytes were incubated with either media alone, monomeric IgE (10 µg/ml), DNP-OVA (1 µg/ml), IgE-DNP-OVA complexes or LPS (50 µg/ml) for 18–24 h at 37°C. Biotinylated MKD6 or 14.4.4 (anti-I-E^d) antibodies were used to label I-A or I-E, respectively, using the approach outlined in Materials and Methods and the legend of Fig. 5. The values are single determinations representing analysis of 10,000 cells each. Similar results were obtained in other experiments.

IgE-Antigen Complexes, But Not IgE or Antigen Alone, Induce I-A and I-E Antigen Expression By B Cells.   As shown in Fig. 8, neither monomeric IgE nor DNP-OVA alone affected class II antigen expression (groups II and III). Conversely, IgE-antigen complexes, like LPS, enhanced the expression of surface I-E antigens in a manner that paralleled surface I-A enhancement (groups IV and V).

IgE-Antigen Complexes Do Not Stimulate DNA Replication.   Because increased expression of surface Ia is often associated with B cell activation, other parameters that might reflect B cell activation processes by IgE-antigen complexes were examined. Increasing cell size is an early indicator of cell activation (33). When monitoring cell volume by either a Channelyzer (Coulter Electronics) or by forward angle light scatter on the flow cytometer, LPS produced significant cell enlargement on both scales, whereas only nominal increases of cell size were detected after overnight incubation with IgE-antigen complexes (data not shown). Cell cycle was determined by labeling B cell nuclear DNA with propidium iodide and the fluorescence measured by flow cytometer. The percentage of cells in either the S (intermediate) or G$_2$/M (tetraploid) phases were combined, representative of cell cycle activation (Fig. 9). No cell activation was observed after 48 h of incubation with monomeric or complexed IgE, while LPS stimulated a large fraction of cells to exceed diploid DNA. Similarly, [^{3}H]TdR incorporation by B lymphocytes was unaffected by incubation with either monomeric or complexed IgE, whereas LPS stimulated substantial increases of [^{3}H]TdR uptake (Fig. 9).

Discussion

The principal findings of the experiments described herein are: (*a*) the existence of differences between monomeric and complexed IgE with regard to their binding
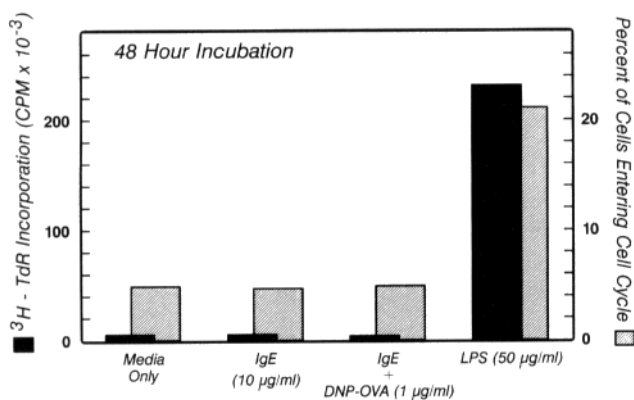
FIGURE 9. IgE-antigen complexes do not stimulate DNA replication. B cells were isolated from BALB/c mouse spleen and incubated with either media only, 10 μg/ml IgE, IgE-DNP-OVA, or 50 μg/ml LPS for 48 h at 37°C. Incorporation of thymidine was measured by pulsing the cells with [³H]TdR (20 μCi/ml) during the last 12 h of incubation before harvesting. Cell cycle was determined by quantitating propidium iodide incorporation into nuclear DNA on the flow cytometer. Cells that contained more than diploid DNA were regarded as entering the cell cycle. Both experiments were repeated, resulting in similar findings.

to, and induction of, FcεR sites on mouse spleen lymphocytes and (*b*) the potent stimulation of B cell surface Ia antigen expression caused by IgE-antigen complexes in vitro.

Monoclonal IgE bound to splenic lymphocytes with greater avidity when complexed with its specific antigen. This is illustrated by the resistance of IgE-antigen complexes to elution at acid pH and by the reversal of such acid resistance by competitive elution of antigen from the complexes by DNP-saturated MEM (Figs. 1 and 2). This marked difference in apparent binding affinity of monomeric and oligomeric IgE can be explained, at least in part, by differing dissociation rates (Fig. 3). However, direct comparisons of these rates are distorted because dissociation of both monomeric and complexed IgE was at least biphasic. Such behavior previously has been noted for monomeric IgE. Vander-Mallie et al. (23) reported that after addition of excess unlabeled IgE, 25% of monomeric ^{125}I-IgE immediately dissociated from lymphocytes followed by a slower dissociation half-time of ∼67 min at 0°C. Our results for monomeric IgE dissociation at 4°C were similar to these findings (data not shown). The reason for the biphasic decline of monomeric IgE is unknown but possibly represents FcεR heterogeneity, cooperative binding events, or a combination thereof. The fact that lymphocyte-bound IgE molecules are not displacable by moderate concentrations of γ_{1-}, γ_{2a-}, γ_{2b-} or μ-Ig (11, 23) tends to rule out IgE binding to other FcR as the cause for its biphasic dissociation.

In the presence of specific antigen, IgE dissociated from lymphocyte FcεR at a more highly variable rate, ranging from an immediate loss of ^{125}I-IgE much like the monomer, to a substantially slower rate after 90 min ($T_{1/2}$ >400 min at 37°C). An additional explanation for this multiphasic dissociation of complexed IgE might be the variable IgE valence of the IgE-antigen complexes. An oligomer containing several IgE molecules capable of binding to lymphocyte FcεR would be expected to bind with greater avidity than an IgE-antigen complex comprising a single IgE molecule. Segal and Hurwitz (34) reported that the avidity of crosslinked IgG oligomers to FcγR on lymphocytes increased with the size of the IgG complex. However, the

decrease in free energy accompanying binding of the monomer was only slightly less than for the dimer, and so on for larger complexes. Such behavior was explained by steric hindrance, negative cooperativity, and/or a loss of entropy associated with unfavorable spatial arrangements of the oligomeric Ig in relation to the receptor. Hence, because of these idiosyncratic events, the increased avidity of FcεR binding of IgE-antigen complexes cannot be quantitatively predicted. Nonetheless, using antibody-antigen complexes that more closely approximate natural conditions than the affinity crosslinked oligomers used by Segal and Hurwitz (34), we obtained similar apparent increases of FcεR avidity. It should be noted that these effects of IgE-antigen complexes could reflect either true increases of binding avidity by FcεR or stabilization of FcεR molecules on the cell surface due to crosslinking, or combinations thereof.

In addition to their effects on direct binding to nascent FcεR, IgE-antigen complexes displayed significantly different properties than monomeric IgE in terms of inducing new FcεR and other cell surface molecules on lymphocytes. Thus, IgE-antigen complexes are ~30-fold more efficient than monomeric IgE in inducing FcεR expression, although maximum FcεR levels are equivalent in both cases (Fig. 4). The increased potency of IgE-antigen complexes for stimulating FcεR could be explained by its increased binding avidity and consquential interference with FcεR recycling. Indeed, from experiments with murine B cell hybridoma lines, Lee et al. (35) reported that by binding to the receptor, monomeric IgE blocks FcεR cleavage and subsequent release from the cells. Alternatively, by prolonged association with FcεR, IgE-antigen complexes might provide a stronger signal for inducing new receptor synthesis. Previous reports that protein synthesis inhibitors block IgE-induced FcεR expression (19, 36) are consistent with this possibility. Nonetheless, the available data do not permit a distinction between these possibilities as yet.

The novel, and unexpected, observation in these studies was the striking inducibility of B cell surface class II MHC molecules by IgE-antigen complexes. Previous descriptions of a direct influence of IgE-antigen complexes on B lymphocyte Ia antigens are unknown to us. Virgin et al. (24) examined the effect of immune complexes of various Ig isotypes on IFN-γ-induced Ia expression on peritoneal macrophages and found that complexes containing either IgG or IgE inhibited such induction in a concentration-dependent manner, provided such complexes were attached to a solid phase support. Complexes of IgA or IgM had no such effects. However, possible direct effects of either monomeric or complexed IgE, in the absence of IFN-γ, on Ia expression by peritoneal macrophages were not reported. In the present studies, we found that IgE-antigen complexes induced Ia expression predominantly on B cells in splenic lymphocyte populations; macrophages did not appear to participate (Table I). However, we have not performed similar analyses with peritoneal macrophages. Nonetheless, there is no evidence to suggest that FcεR must serve the same function or be affected in the same ways in different cell types. Indeed, IFN-γ, which induces Ia expression on peritoneal macrophages, inhibits IL-4-induced expression of Ia on murine B cells (37). IgE-antigen complexes did not block the enhanced Ia expression caused by supernatants obtained from PMA-stimulated EL4 cell cultures (not shown) and soluble IgG complexes had no effect on Ia antigen expression by B cells (Table II). Hence, the findings of Virgin et al. (24) regarding IgE effects

on peritoneal macrophages seem to bear little relationship to our results with B lymphocytes.

The nature of the interaction between IgE, antigen, and spleen cells to increase B lymphocyte Ia expression is unknown. Because T cell depletion resulted in an enhanced B cell Ia-associated fluorescence signal (Table I), it is unlikely that T cells or release of T cell–derived lymphokines contributes to the enhanced signal. Thus, as reported for anti-IgM antibody (38), the IgE-antigen complex–stimulated increase of B cell Ia expression is most likely T cell independent. Further, the Ia response to IgE-antigen complexes observed in the T cell–depleted population was maintained when macrophages also were removed. Additionally, indirect evidence points to the autonomy of B cells in responding to IgE-antigen complexes. The population of B cells with the largest Ia antigen response to IgE-antigen complexes also expressed the highest concentration of FcεR (Fig. 7). Moreover, the increased expression of these two cell surface molecules paralleled one another in response to increasing concentrations of IgE in the presence of antigen (Fig. 6). It has been shown that IgE-induced FcεR expression by B lymphocytes requires no accessory cells (19, 37). Hence, in light of the apparent accessory cell–independence of the B cell Ia response, it is interesting to consider the effect of incubating M12.4.5 cells with IgE-antigen complexes (Table I). The M12.4.5 lymphoma cell line has FcεR sites that are inducible by both IgE (39) and IgE-antigen complexes (not shown). However, in spite of a significant Ia response to LPS, IgE-antigen complexes have a negligible effect on M12.4.5 Ia expression. This lack of response might reflect either a need for other cells/factors, the state of activation exhibited by M12.4.5 cells, or a defect in processing the message produced by the IgE-antigen-FcεR interaction. Further study is needed to find more direct evidence for the role of accessory cells in the mediation of IgE-antigen–stimulated B cell Ia expression.

The cellular processes that are ultimately governed by IgE to increase the expression of B cell Ia and FcεR remain to be elucidated. Lee et al. (35) showed that monomeric IgE increases B cell hybridoma FcεR by binding to the receptor and decreasing its turnover. A similar mechanism also might explain the increased expression of Ia antigens. Bonnefoy et al. (40) recently reported that FcεR and HLA-DR antigens are spatially associated on human B cell membranes, based on the binding activity of two mAbs. Further, FcεR and the α and β chains of HLA-DR were found to be complexed noncovalently in the membrane. It follows that if IgE-antigen complexes bind to FcεR, thus stimulating its expression by decreasing its degradation, then Ia antigens would respond in parallel. However, this argument is invalidated by the lack of effect of monomeric IgE on Ia antigen expression. Additionally, if FcεR and Ia antigens were spatially associated, then FcεR-bound IgE-DNP-OVA complexes might be expected to sterically inhibit the binding of anti-I-A and anti-I-E antibodies. Nonetheless, similar increases of Ia-associated MFI were observed whether or not B cell–bound IgE was removed before Ia quantitation. Hence, transmembrane communication likely is required for FcεR-mediated control of Ia antigen expression. IgE-antigen complexes stimulate Ia antigen and FcεR expression in parallel. By extrapolation, if complexation of IgE is required to modulate Ia mRNA, a similar mechanism might be invoked for the transcriptional control of FcεR. These questions currently are under investigation.

The immunological protection attributed to IgE largely has been confined to parasitic infections. IgE suppression in rodents, for example, results in an increased susceptibility to *Shistosoma mansoni* infection (41). Augmenting B cell Ia antigen expression would clearly play an important role in IgE-mediated resistance to these infections. Variation of B cell surface Ia likely would affect interaction with T lymphocytes. It is known that B cells expressing low levels of sIa antigens are less effective targets for helper T cells than B cells expressing more surface Ia (42). Moreover, B cell requirement for Ia-restricted T cell help occurs before B cell activation and entry into the S phase (43); i.e., the stage in which splenic B cells normally reside. Hence, B cell FcεR are maximally expressed at a time when Ia antigens serve an important role in eliciting T cell help. Further, despite the lack of effect on B cell size, cell cycle activation and thymidine incorporation, IgE-antigen complexes might initiate a signal that would ultimately give rise to cell mitogenesis. In the presence of a costimulant, such as a T cell-derived lymphokine, B cells could be induced to mature and replicate. Higher levels of T cell lymphokines might be expected in response to improved T cell interaction with surface Ia-enhanced B cells. The high percentage of FcεR$^+$ cells and the highly potent surface Ia augmentation brought about by IgE-antigen complexes suggest that this Ig system might play a broader role in countering foreign antigens.

Summary

Murine monoclonal IgE interacts with B cells of BALB/c mouse spleen with greater efficiency in the presence of its specific antigen. Complexes of anti-DNP IgE and DNP-OVA not only resist elution from B lymphocytes by acid but have a substantially longer dissociation half-time when compared with monomeric IgE (440 vs. 8 min, respectively). Further, these IgE-antigen complexes induce FcεR expression in lymphoid cells more efficiently than IgE alone. Maximum levels of B cell FcεR were observed after a 24 h incubation with 1 μg/ml IgE in the presence of 1 μg/ml DNP-OVA, while 30 μg/ml monomeric IgE was needed to elicit a similar increase of FcεR expression.

Most importantly, overnight incubation of B cell-enriched BALB/c spleen cells with IgE-antigen complexes resulted in an augmented membrane expression of class II MHC antigens. B cell surface expression of both I-A and I-E antigens responded to a comparable level after incubation with IgE-antigen complexes but did not occur in response to either IgE or antigen alone. The enhanced sIa expression occurred in parallel to IgE-antigen concentrations that gave rise to FcεR hyperexpression. Moreover, double staining for FcεR and surface Ia antigen shows that B cells exhibiting the highest density of FcεR also demonstrated the most surface I-A, suggesting that B lymphocytes are autonomous in their response to IgE-antigen complexes. Changes in class I MHC or sIgM were not observed after overnight incubation with IgE and antigen. These results demonstrate the importance of IgE-antigen complexes for intercellular signaling and further suggest that the IgE system plays a broader role in immune response than it has generally been credited.

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