Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 7556–7560, October 1989
Immunology

Low-affinity IgE receptor (CD23) function on mouse B cells: Role in IgE-dependent antigen focusing

(antigen presentation/T-cell activation/low-affinity Fcε receptor)

MARILYN R. KEHRY AND LESLIE C. YAMASHITA

Department of Immunology, DNAX Research Institute of Molecular and Cellular Biology, Inc., 901 California Avenue, Palo Alto, CA 94304-1104

Communicated by Ray D. Owen, July 3, 1989 (received for review May 5, 1989)

ABSTRACT **B-cell surface immunoglobulin very efficiently focuses specific protein antigens for presentation to T cells. We have demonstrated a similar role in antigen focusing for the low-affinity Fcε receptor (FcεRII) on mouse B lymphocytes. B cells treated with an IgE monoclonal antibody to 2,4,6-trinitrophenyl (TNP) (IgE–B cells) were 100-fold more effective than were untreated B cells in presenting low concentrations of TNP–antigen to T cells. Blocking the binding of IgE to FcεRII on IgE–B cells with a monoclonal antibody to FcεRII completely eliminated this increased effectiveness. Preformed complexes of IgE anti-TNP and TNP–antigen were more effectively presented (≈100-fold) than TNP–antigen in the presence of nonspecific IgE. In contrast, complexes of IgG1 anti-TNP and TNP–antigen, capable of binding to Fcγ receptors on B cells, were presented less effectively than TNP–antigen in the presence of nonspecific IgG1. Antigens focused by means of FcεRII or by means of B-cell surface immunoglobulin receptors were presented at comparably low concentrations. For several reasons, FcεRII on B lymphocytes seems to be particularly effective in efficiently focusing IgE–antigen complexes.**

The low-affinity Fc receptor for IgE (FcεRII) is expressed on several types of bone marrow-derived cells, including B lymphocytes, monocytes, and eosinophils (1, 2). Although FcεRII molecules from B lymphocytes have been biochemically characterized, their function has heretofore remained unclear. Mouse (ref. 3; S. O. Gollnick, M. Trounstine, L.C.Y., K. W. Moore, and M.R.K., unpublished data) and human (4–7) FcεRII are single polypeptide chains (M_r, 46,000–49,000) that are members of a family of carbohydrate-binding proteins that includes hepatic lectin, mannose-binding protein, and asialoglycoprotein receptor. FcεRII is a type-2 transmembrane protein, with the COOH terminus oriented extracellularly and with the short NH_2 terminus oriented intracellularly (refs. 4–6; S. O. Gollnick, M. Trounstine, L.C.Y., K. W. Moore, and M.R.K., unpublished data). We (8) and others (9, 10) have found that FcεRII is expressed on mature, surface IgD^+/IgM^+ B lymphocytes, and expression is upregulated on activated B lymphocytes in the presence of interleukin 4 (4, 11–14). FcεRII is not expressed on pre-B cells or on mature antibody-secreting plasma cells (8). The stage-specific expression and interleukin 4-dependent upregulation of FcεRII closely parallels that of class II major histocompatibility complex (MHC) molecules (15, 16).

Antigen-specific T helper lymphocytes primarily recognize protein antigens as peptides bound to class II MHC molecules on the surfaces of different antigen-presenting cells. Therefore, by internalizing and degrading protein antigens to small peptides, antigen-presenting cells initiate T-cell activation. Recent studies have shown that protein antigens that specifically bind to surface immunoglobulin on B lymphocytes are presented very efficiently to T cells at low concentrations (17–22). In contrast, antigens that do not bind to the surface of antigen-presenting cells are only effectively internalized, processed, and presented at very high concentrations (17–22).

In the present study we propose a possible role for B-lymphocyte FcεRII in antigen internalization. We report that low concentrations of antigen are efficiently focused into the degradative pathway by means of FcεRII occupied by antigen-specific IgE. Antigens internalized by binding to FcεRII seem to be presented to T cells with an efficiency comparable to antigens focused by B-cell surface immunoglobulin.

MATERIALS AND METHODS

T Cells and Mice. BALB/cByJ mice were purchased from The Jackson Laboratory. D1.1, a type-1 helper T-cell clone specific for rabbit IgG, was a gift of A. Abbas (Harvard Medical School and Brigham and Women's Hospital) and R. Coffman (DNAX). HDK1, a type-1 helper T-cell clone specific for keyhole limpet hemocyanin (KLH) (23) was a gift of N. Street and T. Mosmann (DNAX).

Antibodies and Antigens. B3B4, a rat IgG2a anti-mouse FcεRII, was a gift from D. Conrad (Medical College of Virginia) (24). Monoclonal rat IgE (IR162) (25) and mouse monoclonal IgE anti-TNP (IGEL a2) (26) were purified as described (27). Rabbit anti-mouse immunoglobulin was a gift from D. Lebman and R. Coffman (DNAX). Normal rabbit IgG (rIgG) was purified on a protein A–Sepharose column (28). $F(ab')_2$ fragments of rIgG [$rF(ab')_2$] were made by digestion with pepsin (29) and purification on protein A–Sepharose. TNP_{15}–rIgG and $TNP_{7\text{-}9}$–$rF(ab')_2$ were made as described (27). G11/3.3.3 hybridoma cells producing rat IgG2a anti-phosphorylcholine were a gift from G. Gutman (University of California, Irvine); IgG2a was purified as described (28). Culture supernatant from M5/114.15.2 cells (rat IgG2b anti-$I\text{-}A^{b,d,q}/I\text{-}E^{d,k}$) (30) was a gift from A. Zlotnik (DNAX). M5/114.15.2 supernatant was titrated by staining BALB/c spleen cells and was used in proliferation assays at the concentration that gave maximum fluorescence (1:20). Culture supernatant from GL1.21.1 cells, (rat IgG2b anti-β-galactosidase) was a gift from J. Abrams (DNAX). A hybridoma producing mouse IgG1 anti-TNP (U7.6) was obtained from Z. Eshhar (Weizmann Institute); antibody was purified as described (28). Mouse IgG1 (MOPC21) was purchased from Litton Bionetics. KLH was obtained from N. Street (23) and was derivatized with TNP as described (27) to a ratio of 17 TNP molecules per M_r 100,000. All reagents were dialyzed twice against RPMI 1640 medium/10 mM Hepes,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: FcεRII, low-affinity IgE receptor (CD23); MHC, major histocompatibility complex; TNP, 2,4,6-trinitrophenyl; rIgG, normal rabbit IgG; KLH, keyhole limpet hemocyanin; IgE–B, B cells incubated with an IgE monoclonal antibody to TNP; $rF(ab')_2$ fragment, rabbit $F(ab')_2$ fragment.

pH 7.3 (GIBCO) and sterile filtered (Spin-X, Costar) before use.

Complexes of anti-TNP antibody and TNP–rF(ab')$_2$ were formed by incubating TNP–rF(ab')$_2$ (20 μg/ml) for 1 hr at 0°C with three different concentrations of antibody [200 μg/ml, 10:1 (wt/wt); 60 μg/ml, 3:1 (wt/wt); 20 μg/ml, 1:1 (wt/wt)]. The approximate molar ratios, based on molecular weight were, for IgG1, 8:1, 2.4:1, and 0.8:1, respectively, and for IgE, 6:1, 1.8:1, and 0.6:1, respectively. These complexes were titrated as antigen in T-cell proliferation assays.

B-Cell Preparation. T-cell-depleted spleen cells were prepared from BALB/cByJ mice essentially as described (8) with the exception that Low Tox guinea pig complement (Cedarlane) was used. Viable B cells isolated on Ficoll were incubated for 40 min (37°C in the dark) with mitomycin C (Sigma) (50 μg/ml) to inhibit B-cell proliferation. Cytophilic IgE was removed by acid stripping the cells (31). The portion of B cells to be IgE-coated (IgE–B cells) was then incubated for 1 hr at 0°C with a2 IgE (50 μg/ml). Cells were centrifuged and resuspended in B-cell culture medium (8) for use as antigen-presenting cells.

T-Cell Proliferation Assay. T cells (2×10^4 cells per well), B cells (1×10^5 cells per well), and various concentrations of antigen in B-cell culture medium were cultured for 48 hr at 37°C. [*methyl*,1',2'-^{3}H]Thymidine (120 Ci/mmol, 1 Ci = 37 GBq; Amersham) was added (1 μCi per well) during the last 4 hr of culture. Cultures were automatically harvested with either a Cambridge PHD cell harvester or an LKB cell harvester.

RESULTS

Presentation of Low Concentrations of TNP-Antigen Is Enhanced by IgE Anti-TNP Treatment of B Cells. The IgE-dependent focusing of specific antigen through FcεRII on B cells was assessed by antigen presentation to an rIgG-specific type-1 helper T-cell clone, D1.1 (32). T-cell activation was assayed by proliferation. Initially, lymphokine (interferon γ) secretion was measured in parallel with proliferation and gave equivalent results.

The B-cell surface molecule that most efficiently focuses antigen is surface immunoglobulin (22). To focus antigen through surface immunoglobulin, B cells or B cells incubated with IgE anti-TNP (IgE–B cells) were used to present rabbit anti-mouse immunoglobulin to D1.1 T cells. B cells (data not shown) and IgE–B cells were comparable in their ability to present low concentrations of rabbit anti-mouse immunoglobulin (Fig. 1*A*). To examine antigen focusing by FcεRII, B cells were incubated with or without IgE anti-TNP and their ability to present TNP–rF(ab')$_2$, TNP–rIgG, or rIgG was compared. IgE–B cells presented TNP–rF(ab')$_2$ at ≈10^2-fold lower concentrations than did B cells not treated with IgE anti-TNP (Fig. 1*A*). A ≈10^2-fold difference in antigen dose–response curves was also seen when the antigen was TNP–rIgG (Fig. 1*B*). No difference between IgE–B cells and B cells was seen in the presentation of rIgG (Fig. 1*C*) or rF(ab')$_2$ (data not shown). Thus, the presence of "cytophilic" anti-TNP IgE specifically enhanced presentation of TNP–antigen by ≈10^2-fold. The results in Fig. 1*A* also show that IgE–B cells presented rabbit anti-mouse immunoglobulin and TNP–rF(ab')$_2$ to D1.1 T cells with similar efficiencies. Thus, FcεRII occupied by antigen-specific IgE seemed to focus antigen as efficiently as did membrane-bound immunoglobulin.

Reproducibly, TNP–antigen was presented by B cells to T cells at ≈10-fold lower concentrations than was unconjugated antigen (Fig. 1 *B* and *C*). This enhanced presentation may be due to the existence of a low frequency (<1%) of TNP-specific B cells in BALB/c spleen, which would efficiently internalize TNP–antigen bound to surface immunoglobulin.

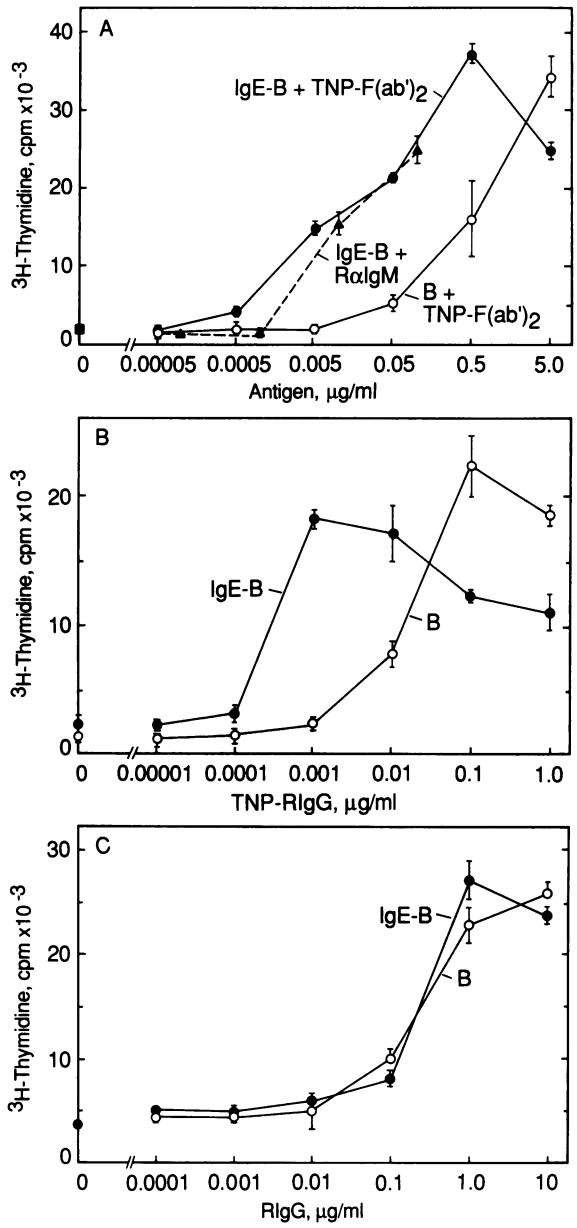

FIG. 1. Presentation of TNP–antigen is enhanced by IgE anti-TNP treatment of B cells. Proliferation of D1.1 T cells was measured at 48 hr as described. (*A*) Mitomycin C-treated B cells (○) or IgE–B cells (●, ▲) (1×10^5) were cultured with various concentrations of TNP–rF(ab')$_2$ (○, ●) or rabbit anti-mouse immunoglobulin (▲) and D1.1 T cells (2×10^4). (*B*) Mitomycin C-treated B cells (○) or IgE–B cells (●) were cultured with various concentrations of TNP–rIgG and D1.1 T cells. (*C*) Mitomycin C-treated B cells (○) or IgE–B cells (●) were cultured with various concentrations of rIgG and D1.1 T cells.

However, such a low frequency of TNP-specific B cells would seem unlikely to account for a 10-fold change in dose–response curves. Different dose–response curves were seen for unconjugated and TNP-conjugated forms of different antigens [KLH, rIgG, rF(ab')$_2$] and for different T-cell clones (KLH-specific, HDK1, and rIgG-specific D1.1 and CDC35), suggesting that the enhanced presentation was a nonspecific effect. Thus, a possible explanation is that TNP groups increased overall hydrophobicity of the antigens and promoted a nonspecific adherence to the B-cell surface.

Presentation of TNP–Antigen by IgE–B Cells Is Inhibited by Anti-FcεRII. A single high-affinity monoclonal antibody, B3B4, that binds mouse FcεRII and blocks IgE binding has been produced (24). Including B3B4 antibody in the cultures

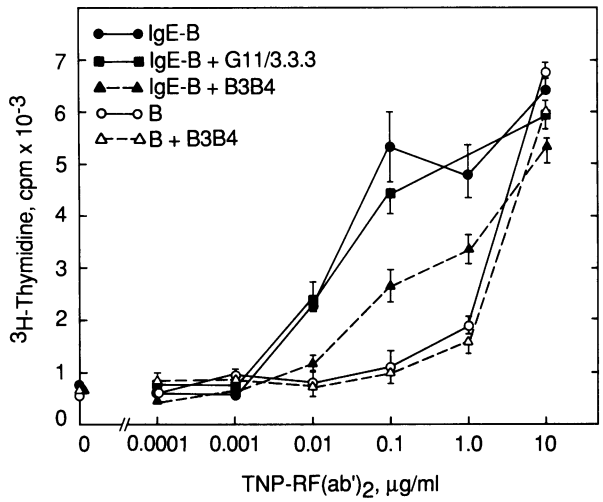

FIG. 2. Presentation of TNP–antigen by IgE–B cells is inhibited by anti-FcεRII. Mitomycin C-treated IgE–B cells (●, ▲, ■) or B cells (○, △) (1×10^5) were cultured with various concentrations of TNP–rF(ab')$_2$ and D1.1 T cells (2×10^4). Anti-FcεRII (B3B4, 15 μg/ml) (△, ▲) or a control rat antibody (G11/3.3.3, 15 μg/ml) (■) was included in the cultures. Proliferation of D1.1 cells was measured at 48 hr.

produced no effect on the presentation of TNP–rF(ab')$_2$ by B cells (Fig. 2). In contrast, presentation of low concentrations of TNP–rF(ab')$_2$ by IgE–B cells was inhibited by B3B4 antibody but not by a control rat monoclonal antibody (G11/3.3.3) (Fig. 2). In three other experiments (data not shown), antigen presentation by IgE–B cells was more completely inhibited by B3B4; this inhibition resulted in antigen dose–response curves similar to those of B cells. The B3B4 antibody does not bind to T cells or macrophages (24). Thus, we can conclude that FcεRII on the B-cell surface, occupied by antigen-specific IgE, gives enhanced TNP-antigen presentation by IgE–B cells.

Antigen Presentation by IgE–B and B Cells Is Inhibited by Anti-Class II MHC Antibody. Presentation of TNP–rIgG by IgE–B cells was inhibited ≈10^3-fold by a rat monoclonal antibody, M5/114.15.2, that recognizes I-A^d and I-E^d class II MHC molecules but was not inhibited by a control antibody, GL1.21.1 (Fig. 3). Anti-I-A^d/I-E^d inhibited the presentation of TNP–rIgG by B cells ≈10-fold (data not shown). With anti-I-A^d/I-E^d, the TNP–rIgG dose–response curves of B cells and IgE–B cells were virtually identical (Fig. 3, data not shown for B cells). Thus, presentation of low TNP–antigen concentrations by IgE–B cells was also class II MHC-restricted, and FcεRII occupied by IgE did not seem to promote non-class II MHC-restricted B-cell/T-cell interactions.

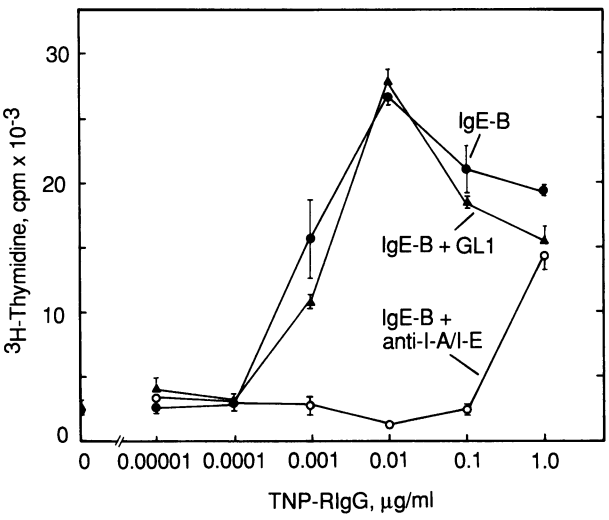

FIG. 3. Presentation of TNP–antigen is inhibited by anti-I-A^d/I-E^d. Mitomycin C-treated IgE–B cells (1×10^5) were cultured with various concentrations of TNP–rIgG and D1.1 T cells (2×10^4) (●). Culture supernatant from M5/114 cells (anti-I-A^{b,d,s}/I-E^{dk}) (○) or GL1.21.1 cells (anti-β-galactosidase) (▲) was included in the cultures as described.

Antigen Focusing by FcεRII Does Not Enhance Bystander Antigen Presentation. When IgE–B cells bind TNP–antigen, FcεRII may become crosslinked on the cell surface. This crosslinking may induce the IgE–B cells to be better antigen-presenting cells. In this situation, IgE–B cells that have focused TNP–antigen would efficiently present that antigen to a specific T-cell clone but might also be able to efficiently present a different, unconjugated antigen (bystander antigen) to T cells specific for that antigen. To test this possibility we assessed whether IgE–B cells treated with various concentrations of TNP-conjugated antigen (TNP–KLH) could give enhanced presentation of low doses of unconjugated antigen (rIgG). Fig. 4 shows that the T-cell activation was antigen-specific because IgE–B cells did not present TNP–KLH to the rIgG-specific T-cell clone D1.1. IgE–B cells efficiently presented low concentrations of TNP–KLH to a KLH-specific T-cell clone, HDK1 (23). When suboptimal concentrations of bystander antigen (rIgG at 0.1 μg/ml or 0.01 μg/ml) (data not shown for 0.01 μg/ml) were added to IgE–B-cell cultures, the presence of focused TNP–KLH did not enhance this bystander antigen presentation (i.e., D1.1 T-cell proliferation was not increased above background, Fig. 4). Thus, enhanced presentation of TNP–antigen was specific for antigen focused by means of FcεRII and was not a general increase in antigen-presenting ability of the B cells.

Binding of IgG1–Antigen Complexes to B Cells Does Not Enhance Antigen Presentation. A comparison was made between the efficiencies of FcεRII and Fcγ receptor-dependent antigen focusing by B cells. Because monomeric IgG1 does not readily bind to Fcγ receptors, IgG1–antigen complexes were formed at different ratios of IgG1 to antigen, as described, and then titrated in the T-cell proliferation assay. For comparison, IgE anti-TNP–TNP–rF(ab')$_2$ complexes were

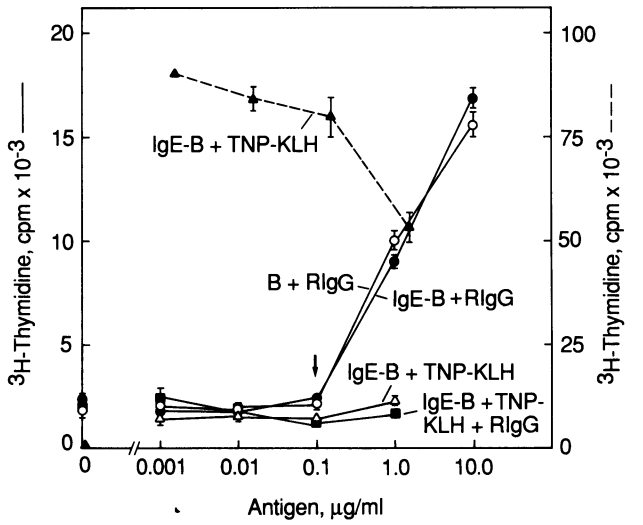

FIG. 4. Antigen focusing by FcεRII does not enhance bystander antigen presentation. Mitomycin C-treated IgE–B cells (●, ▲, △, ■) or B cells (○) (1×10^5) were cultured with T cells (2×10^4) and various concentrations of antigens as follows: TNP–KLH and HDK1 T cells (▲), TNP–KLH and D1.1 T cells (△), rIgG and D1.1 T cells (●, ○), or TNP–KLH and rIgG (0.1 μg/ml throughout) and D1.1 T cells (■). T-cell proliferation was measured at 48 hr as described.

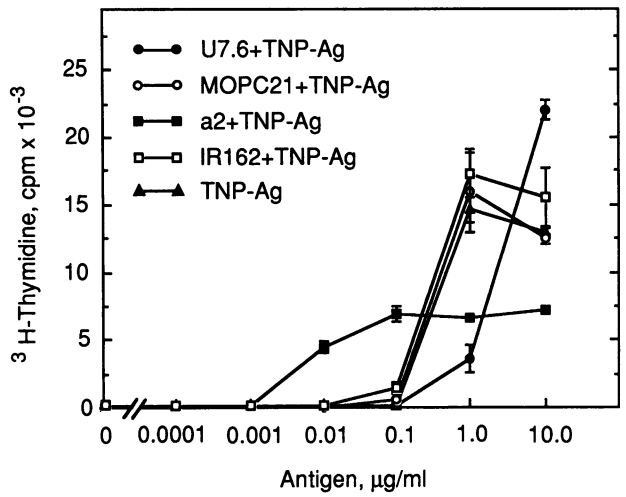

FIG. 5. Presentation of IgG1–antigen complexes and IgE–antigen complexes. Acid stripped and mitomycin C-treated B cells and D1.1 T cells (2×10^4) were incubated with various concentrations of TNP–rF(ab')$_2$ (▲) or preformed complexes of U7.6 (IgG1 anti-TNP)–TNP–rF(ab')$_2$ (●), MOPC 21 (IgG1 control)–TNP–rF(ab')$_2$ (○), a2 (IgE anti-TNP)–TNP–rF(ab')$_2$ (■), or IR162 (IgE control)–TNP–rF(ab')$_2$ (□) at ratios of 3:1 as described. T-cell proliferation was measured at 48 hr. Ag, antigen.

assayed. These IgE-antigen complexes were presented 10^2-fold more efficiently than uncomplexed TNP–rF(ab')$_2$ or nonspecific IgE (IR162):TNP–rF(ab')$_2$ (Fig. 5). In contrast, IgG1 anti-TNP–TNP–rF(ab')$_2$ complexes were presented ≈10-fold less efficiently than were uncomplexed TNP–rF(ab')$_2$ or nonspecific IgG1 (MOPC 21)–TNP–rF(ab')$_2$ (Fig. 5). IgG1–antigen complexes formed at a 1:1 ratio were less inhibitory than complexes formed at a 10:1 or 3:1 ratio (data not shown).

DISCUSSION

Numerous studies have shown that B lymphocytes effectively present antigen to antigen-specific T cells (17–22, 33). The B-cell surface molecule that most efficiently focuses antigen has been the antigen receptor, surface immunoglobulin (17–22). We now demonstrate that FcεRII, occupied by "cytophilic" antigen-specific IgE, on the surface of B cells may also mediate antigen focusing as effectively as surface immunoglobulin. IgE-dependent antigen focusing was IgE- and FcεRII-specific. IgE–B cells presented TNP–antigen at ≈10^2-fold lower concentrations than did non-IgE-treated B cells. A monoclonal anti-FcεRII antibody that reacts only with B lymphocytes inhibited presentation of low antigen concentrations, thus eliminating the possibility that contaminating macrophages contributed to enhanced presentation at low antigen doses. Antigen focusing by B-cell FcεRII occupied by IgE was specific for the focused antigen and did not seem to induce the B cell to more efficiently present a bystander antigen to T cells.

Preformed IgE–TNP–antigen complexes were presented by B cells at least 10^2-fold more efficiently than mixtures of non-TNP-specific IgE and TNP–antigen. The maximum proliferative response of the D1.1 T cells to IgE–TNP–antigen complexes was reproducibly reduced ≈2-fold when compared with the response to TNP–antigen alone. The reason for this difference is not clear but may reflect an altered processing (perhaps biased towards different peptides) of the IgE–TNP–antigen complexes. Complexes formed with higher IgE–TNP–antigen ratios were more efficiently presented (unpublished data). This result may reflect a more efficient binding of larger IgE–TNP–antigen complexes to B-cell surface FcεRII. Additionally, FcεRII crosslinking may specifically promote increased internalization or increased targeting of antigen into a degradative pathway. In contrast to IgE–TNP–antigen complexes, IgG1–TNP–antigen complexes were poorly presented by B cells. Complexes containing high ratios of IgG1 to antigen (10:1 or 3:1) were presented 10-fold less effectively than were mixtures of non-TNP-specific IgG1 and TNP–antigen. Thus, FcεRII, but not Fcγ receptors on B cells, readily promotes focusing of antigens for presentation. These data are supported by the recent studies of Miettinen *et al.* (34) that show the existence of significant differences in ability of the B-cell and the macrophage Fcγ receptor isoforms to be internalized. Although Fcγ receptors on both B cells and macrophages bound IgG–antigen complexes, the B-cell isoform was relatively inefficient in mediating ligand internalization for degradation and failed to accumulate in coated pits (34).

Our TNP–antigens were multivalent and would be expected to induce some crosslinking of FcεRII. It is not clear whether efficient antigen focusing by FcεRII also occurs when FcεRII is occupied by monovalent IgE. The use of hybrid IgE antibodies and monovalent antigens should answer this question.

Mouse and human FcεRII are members of a lectin protein family that includes asialoglycoprotein receptor (refs. 4–6; S. O. Gollnick, M. Trounstine, L.C.Y., K. W. Moore, and M.R.K., unpublished data). Asialoglycoprotein receptor is very efficiently internalized and cycles its ligands through the degradative membrane compartments of cells (35). B-cell FcεRII may cycle its ligand (IgE–antigen complexes) through a similar pathway that would contribute to the efficiency with which FcεRII-bound antigen is processed and presented. In support of this idea is the finding that the Fc portion of IgE is acid labile and, at low pH, readily dissociates from FcεRII (31). This property should allow antigens, regardless of their affinity for IgE, to efficiently dissociate from FcεRII after internalization and be degraded, rather than recycled, by the cell (22). In addition, the cell-surface half life of FcεRII increases after binding of IgE (12) and may provide an increased opportunity for association of specific antigens with FcεRII-bound IgE. In support of this idea, IgE–antigen complexes have been found to dissociate from FcεRII more slowly than monomeric IgE (36), possibly allowing for more efficient internalization. Thus, several properties of FcεRII make the receptor on B cells well-suited for mediating antigen internalization and degradation.

Several recently described cell–cell adhesion molecules (37–40) contain extracellular domains that are homologous to members of the lectin/FcεRII family. Thus, a potential and unexplored role for B-cell FcεRII in cell–cell interaction may exist.

The ability of FcεRII to mediate IgE-dependent antigen focusing implies a role *in vivo* for antigen-specific IgE acting as an "adjuvant" to increase specific T-cell responses to small doses of antigen. This may indicate that antigen-specific IgE participates in augmenting immune responses.

The function of FcεRII on B lymphocytes has been enigmatic. FcεRII has been associated with various phenomena, such as regulation of IgE production in mouse and human (41, 42) and B-cell growth factor activity for human B cells (43–45). The demonstration of an antigen-focusing function for FcεRII is consistent with its stage-specific expression, presence on all mature and activated B cells (8), possible association with class II MHC molecules (36, 46), sequence homology to lectins that mediate protein internalization and degradation (4), and with the acid lability of its ligand, the Fc portion of IgE.

We thank Brian Seymour for growing D1.1 T cells, Dr. T. Mosmann for many stimulating and encouraging discussions on work

in progress, and Drs. T. Mosmann and S. O. Gollnick for comments on the manuscript.

1. Spiegelberg, H. L. (1981) *Immunol. Rev.* **56,** 199–218.
2. Vander-Mallie, R., Ishizaka, T. & Ishizaka, K. (1982) *J. Immunol.* **128,** 2306–2312.
3. Conrad, D. H. & Peterson, L. H. (1984) *J. Immunol.* **132,** 796–803.
4. Kikutani, H., Inui, S., Sato, R., Barsumian, E. L., Owaki, H., Yamasaki, K., Kaisho, T., Uchibayashi, N., Hardy, R. R., Hirano, T., Tsunasawa, S., Sakiyama, F., Suemura, M. & Kishimoto, T. (1986) *Cell* **47,** 657–665.
5. Ikuta, K., Takami, M., Kim, C. W., Honjo, T., Miyoshi, T., Tagaya, Y., Kawabe, T. & Yodoi, J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 819–823.
6. Ludin, C., Hofsetter, H., Sarfati, M., Levy, C. A., Suter, U., Alaimo, D., Kilchherr, E., Frost, H. & Delespesse, G. (1987) *EMBO J.* **6,** 109–114.
7. Meinke, G. C., Magro, A. M., Lawrence, D. A. & Spiegelberg, H. L. (1978) *J. Immunol.* **121,** 1321–1328.
8. Kehry, M. R. & Hudak, S. A. (1989) *Cell. Immunol.* **118,** 504–515.
9. Kikutani, H., Suemura, M., Owaki, H., Nakamura, H., Sato, R., Yamasaki, K., Barsumian, E. L., Hardy, R. R. & Kishimoto, T. (1986) *J. Exp. Med.* **164,** 1455–1469.
10. Waldschmidt, T. J., Conrad, D. H. & Lynch, R. G. (1988) *J. Immunol.* **140,** 2148–2154.
11. Hudak, S. A., Gollnick, S. O., Conrad, D. H. & Kehry, M. R. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 4606–4610.
12. Conrad, D. H., Waldschmidt, T. J., Lee, W. T., Rao, M., Keegan, A. D., Noelle, R. J., Lynch, R. G. & Kehry, M. R. (1987) *J. Immunol.* **139,** 2290–2296.
13. Defrance, T., Aubry, J. P., Rousset, F., Vanbervliet, B., Bonnefoy, J. Y., Arai, N., Takebe, Y., Yokota, T., Lee, F., Arai, K., deVries, J. & Banchereau, J. (1987) *J. Exp. Med.* **165,** 1459–1467.
14. Conrad, D. H., Keegan, A. D., Kalli, K. R., Van Dusen, R., Rao, M. & Levine, A. D. (1988) *J. Immunol.* **141,** 1091–1097.
15. Noelle, R., Krammer, P. H., Ohara, J., Uhr, J. W. & Vitetta, E. S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6149–6153.
16. Roehm, N. W., Leibson, H. J., Zlotnik, A., Kappler, J. W., Marrack, P. & Cambier, J. C. (1984) *J. Exp. Med.* **160,** 679–694.
17. Chesnut, R. W. & Grey, H. M. (1981) *J. Immunol.* **126,** 1075–1079.
18. Tony, H.-P., Phillip, N. E. & Parker, D. C. (1985) *J. Exp. Med.* **162,** 1695–1708.
19. Rock, K. L., Benacerraf, B. & Abbas, A. K. (1984) *J. Exp. Med.* **160,** 1102–1113.
20. Casten, L. A. & Pierce, S. K. (1988) *J. Immunol.* **140,** 404–410.
21. Lanzavecchia, A. (1987) *Immunol. Rev.* **99,** 39–51.
22. Abbas, A. K. (1989) *Semin. Immunol.*, in press.
23. Cher, D. J. & Mosmann, T. R. (1987) *J. Immunol.* **138,** 3688–3694.
24. Rao, M., Lee, W. T. & Conrad, D. H. (1987) *J. Immunol.* **138,** 1845–1851.
25. Bazin, H. P., Querinjean, P., Beckers, A., Heremans, J. F. & Dessy, F. (1974) *Immunology* **26,** 713–723.
26. Rudolph, A. K., Burrow, P. D. & Wabl, M. R. (1981) *Eur. J. Immunol.* **11,** 527–529.
27. Hudak, S. A. & Kehry, M. R. (1985) *J. Immunol. Methods* **84,** 11–24.
28. Hardy, R. R. (1986) in *Handbook of Experimental Immunology*, ed. Weir, D. M. (Blackwell, Palo Alto, CA), Vol. 1, pp. 13.1–13.13.
29. Stanworth, D. R. & Turner, M. W. (1986) in *Handbook of Experimental Immunology*, ed. Weir, D. M. (Blackwell, Palo Alto, CA), Vol. 1, pp. 12.1–12.46.
30. Bhattacharya, A., Dorf, M. E. & Springer, T. (1981) *J. Immunol.* **127,** 2488–2495.
31. Lee, W. T. & Conrad, D. H. (1986) *J. Immunol.* **136,** 4573–4580.
32. Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A. & Coffman, R. L. (1986) *J. Immunol.* **136,** 2348–2357.
33. Ruud, E., Blomhoff, H. K., Funderud, S. & Godal, T. (1986) *Eur. J. Immunol.* **16,** 286–291.
34. Miettinen, H. M., Rose, J. K. & Mellman, I. (1989) *Cell* **58,** 317–327.
35. Geuze, H. J., Slot, J. W., Strous, G. J. A. M., Lodish, H. F. & Schwartz, A. L. (1983) *Cell* **32,** 277–293.
36. Richards, M. L., Marcelletti, J. F. & Katz, D. H. (1988) *J. Exp. Med.* **168,** 571–587.
37. Siegelman, M. H., van de Rijn, M. & Weissman, I. L. (1989) *Science* **243,** 1165–1172.
38. Laskey, L. A., Singer, M. S., Yednock, T. A., Dowbenko, D., Fennie, C., Rodriguez, H., Nguyen, T., Stachel, S. & Rosen, S. D. (1989) *Cell* **56,** 1045–1055.
39. Johnston, G. I., Cook, R. G. & McEver, R. P. (1989) *Cell* **56,** 1033–1044.
40. Bevilacqua, M. P., Stengelin, S., Gimbrone, M. A., Jr., & Seed, B. (1989) *Science* **243,** 1160–1164.
41. Ishizaka, K. (1984) *Annu. Rev. Immunol.* **2,** 159–182.
42. Mathur, A., Kamat, D. M., Van Ness, B. G. & Lynch, R. G. (1987) *J. Immunol.* **139,** 2865–2872.
43. Swendeman, S. & Thorley-Lawson, D. A. (1987) *EMBO J.* **6,** 1637–1642.
44. Gordon, J., Webb, A. J., Walker, L., Guy, G. R. & Rowe, M. (1986) *Eur. J. Immunol.* **16,** 1627–1630.
45. Guy, G. R. & Gordon, J. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6239–6243.
46. Bonnefoy, J. Y., Buillot, O., Spits, H., Blanchard, D., Ishizaka, K. & Banchereau, J. (1988) *J. Exp. Med.* **167,** 57–72.