

Fcε RECEPTOR, A SPECIFIC DIFFERENTIATION MARKER TRANSIENTLY EXPRESSED ON MATURE B CELLS BEFORE ISOTYPE SWITCHING

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B lymphocytes originate from pluripotent hematopoietic stem cells and differentiate into antibody-secreting cells through multistep differentiation stages, such as pre-B cells, immature B cells with surface IgM, and mature B cells with surface IgM and IgD. A number of human B cell antigens have been defined by mAbs (1–10). However, most B cell-specific mAbs, except for antibodies against plasma cells (5) and activated B cells (9, 10), are known to recognize cells of the B lineage at a wide range of differentiation stages from pre-B cells to mature B cells. It has therefore been difficult to identify B cells at a specific differentiation stage by using such B cell-specific mAbs.

Several previous studies (11–14) have shown the expression of FcεR on a certain percentage (1–5%) of circulating B or T cells using the rosette method with IgE-conjugated ox red blood cells (ORBC).¹ Our previous study (15) with FcεR-specific mAbs with several different epitope specificities showed that >50% of circulating B cells express FcεR on their surface, while T cells do not display this receptor, even with T cells from patients with hyper-IgE syndrome. The discrepancy in the results may be due to the difference in the sensitivity of the assay system and the antibodies used. In the present study, the distribution of FcεR⁺ cells was studied in various lymphoid tissues, and the results show that FcεR is a B cell-specific phenotype marker, the expression of which is strictly correlated with the differentiation stage of B cells, especially with the isotype switching in B cells.

Materials and Methods

Antibodies. Human IgE(PS) was the same preparation as described previously (16). Human IgG1 myeloma protein was kindly given by Dr. A. Shimizu (Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan). FITC-conjugated

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¹ *Abbreviations used in this paper:* AT, ataxia teleangiectasia; B-BCGF, B cell-derived B cell growth factor; BCDF, B cell differentiation factor; BSF-1, B cell stimulatory factor 1; CVI, common variable immunodeficiency; MNC, mononuclear cell; ORBC, ox red blood cell; PC, phycocyanin; PHA-sup, supernatant from cultures of T cells stimulated with PHA; PI, propidium iodide; TR, Texas red.

F(ab)₂' fragment of goat anti-human IgD and FITC-F(ab)₂' fragment of goat anti-human IgA were purchased from Tago Inc. (Burlingame, CA). FITC-anti-B1 was from Coulter Immunology (Hialeah, FL). An anti-human IgM mAb was a kind gift from Dr. M. Sugi (Yamasa Shoyu Co., Ltd, Tokyo, Japan). An anti-human IgE mAb was the same preparation as described previously (17). The anti-FcεR mAbs 1-7, 3-5, and 8-30, which are of IgG2b, IgG1, and IgM, respectively, were produced by hybridization of P3U1 myeloma with spleen cells from BALB/c mice immunized with RPMI-8866 cells (15). Two antibodies (1-7 and 8-30) recognized the epitope close to IgE-binding site of FcεR and blocked IgE binding to 8866 lymphoblastoid cells. The antibody 3-5 recognized a different epitope on FcεR, and could not block IgE binding to 8866 cells. These antibodies precipitated 25,000 and 46,000 M_r polypeptides under reducing and nonreducing conditions. The mAbs were purified from ascites by 50% saturated ammonium sulfate precipitation followed by gel filtration using Sepharose 6 B (Pharmacia Fine Chemicals, Uppsala, Sweden) for IgM class, or by ion-exchange chromatography using QAE-Sephadex (Pharmacia Fine Chemicals) for IgG1 and IgG2b classes. Some of the antibodies were conjugated with biotin or FITC as described (18, 19). Some of the antibodies were also labeled with phycocyanin (PC) by crosslinking with sulfosuccinimydyl 4-(*p*-maleimido-phenyl) butylate (Pierce Chemical Co., Rockford, IL) as described (20).

Factor Preparations. As conventional T cell factors, partially purified culture supernatants from PHA-stimulated tonsillar T cells were used (21). Briefly, tonsillar T cells were cultured at 10⁶ cells/ml with 0.1% PHA (Difco Laboratories, Detroit, MI) for 48 h and the supernatant was collected. The concentrated supernatant was fractionated by HPLC gel filtration, and a fraction with an M_r of ~20,000 was used as a source of T cell factor(s). This preparation contains B cell stimulatory factor 1 (BSF-1), B cell differentiation factor (BCDF or BSF-2), and IL-2 activities. BCDF has been purified to homogeneity as described (22). Purified BCDF induced Ig secretion in *Staphylococcus aureus* Cowan I-stimulated B blast cells at 1 pM concentration, but did not have growth activity (22). B cell-derived B cell growth factor (B-BCGF) was obtained from the culture supernatant of RPMI-1788 lymphoblastoid cells (23). The B-BCGF induced the proliferation in anti-μ-activated human B cells at a concentration of 10% (vol/vol).

Recombinant Lymphokines. rIL-2 was a kind gift from Dr. J. Hamuro (Ajinomoto Co., Ltd., Tokyo, Japan). rIFN-β and rIFN-γ were kindly provided from Kyowa Hakko Co., Ltd., Tokyo, Japan. rIL-1 was kindly provided from Dr. Y. Hirai, (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan).

Preparation of Human Mononuclear Cells (MNC). PBL were obtained from normal donors and patients with hyper-IgE syndrome or immunodeficiencies. Tonsillar MNC were obtained from patients with chronic tonsillitis. Bone marrow MNC were obtained from the pelves of immunologically normal donors. MNC were prepared by Ficoll-Hypaque (Pharmacia Fine Chemicals) gradient centrifugation. In some experiments, B cells were prepared by rosetting with (2-aminoethyl)isothiuronium bromide (Aldrich Chemical Co., Milwaukee, WI)-treated SRBC.

Patients with Immunodeficiencies or Hyper-IgE Syndrome. Blood samples of patients with common variable immunodeficiency (CVI) or ataxia teleangiectasia (AT) were kindly provided by Drs. K. H. Pyun, H. D. Ochs (University of Washington, Seattle, WA) and Dr. K. Kawa-Ha (Osaka University, Osaka, Japan). Serum Ig levels of these patients were the following; CVI-1 (IgG, 340 mg/dl; IgM, 55 mg/dl; IgA, <7 mg/dl), CVI-2 (IgG, 230 mg/dl; IgM, 18 mg/dl; IgA, 14 mg/dl; IgE, 19 IU/ml), CVI-3 (IgG, 181 mg/dl; IgM, 10 mg/dl; IgA, 10 mg/dl), AT-1 (IgG, 1,350 mg/dl; IgM, 320 mg/dl; IgA, 0 mg/dl), and AT-2 (IgG, 300 mg/dl; IgM, 10 mg/dl; IgA, 0 mg/dl). Blood samples of patients with hyper-IgE syndrome were kindly provided by Drs. K. H. Pyun, H. D. Ochs, and Y. Iikura (National Children Hospital, Tokyo, Japan). These patients showed suppressed neutrophil chemotaxis, recurrent infections, and high serum IgE levels (6,000-104,000 IU/ml).

Two-colour Immunofluorescence Analysis. To remove cytophilic Igs, cells were treated with acetate buffer (0.05 M, pH 4.0) containing 0.085 M NaCl, 0.005 M KCl, and 1% FCS for 1 min on ice (24), and then washed with staining buffer (RPMI 1640 minus biotin, riboflavin, and phenol red; 2% FCS, 10 mM Hepes, 0.1% NaN₃). 10⁶ cells were

incubated with FITC-conjugated or biotinylated antibodies for 20 min in a flexible 96-well microtiter plate in the first step, washed three times with staining buffer, then incubated with Texas red (TR)-avidin for 20 min in the second step and washed three times with staining buffer. Propidium iodide (PI) (10 $\mu\text{g}/\text{ml}$) was included in the last 5 min of the second step to label dead cells. All antibodies except for biotinylated human IgG and IgE were used at the concentrations that gave maximal fluorescence intensities without nonspecific binding. Biotinylated human IgE and IgG were used at 300 $\mu\text{g}/\text{ml}$ because of low binding affinity of the receptors on lymphocytes, moreover, this concentration could not saturate Fc γ and Fc ϵ receptors. Stained cells were analyzed on a FACS 440 equipped with both argon ion laser operating at 488 nm generating forward scatter, fluorescein, PI, and large-angle (obtuse) scatter signals, and a second argon laser pumping an orange dye laser circulating rhodamine 6G tuned to emit 595-nm light to excite TR (25). All fluorescein channels and large-angle scatter were amplified logarithmically with a range of either four decade (for fluorescence) or two decade (obtuse scatter). List-mode data were collected on $20\text{--}50 \times 10^3$ cells for each sample and analyzed on a Vax 11/730 computer using programs originally developed at Stanford University. Histograms represent the relative number of cells as a function of fluorescence intensity within a particular gated population. Contour plots represent the correlated expression of two surface antigens by showing peak lines enclosing equal percentages of cells with the two-parameters distribution.

Induction of Fc ϵ R Expression. Bone marrow MNC, sorted cells, or PBL from a patient with CVI were cultured at 5×10^5 cells/ml in a 96-well microtiter plate for 3 d in the presence or absence of 10 $\mu\text{g}/\text{ml}$ IgE or IgG with or without PHA supernatant (10% vol/vol) or recombinant or purified lymphokines. After 3 d of culture, the cells were stained with FITC-anti-B1 and biotinylated anti-Fc ϵ R antibodies, followed by incubation with TR-avidin, and analyzed by FACS.

Results

Expression of Fc ϵ R on Human B Lymphocytes from PBL, Tonsils, and Bone Marrow. Anti-Fc ϵ R mAbs 1-7 (γ_{2b}), 3-5 (γ_1), and 8-30 (μ), have been prepared by using RPMI-8866 cells as immunizing antigen. In an earlier study (15), it was shown that two mAbs (1-7 and 8-30) recognized the same epitope of Fc ϵ R and inhibited the binding of IgE with 8866 cells, while mAb 3-5 recognized a different epitope of Fc ϵ R and did not inhibit the IgE binding. However, all these mAbs could precipitate 25,000 and 46,000 M_r polypeptides under reducing and non-reducing conditions (15). By using these mAbs, the distribution of Fc ϵ R⁺ cells in various lymphoid tissues, such as PBL, tonsils, and bone marrow, was examined. Because the three mAbs showed the same results, mAb 8-30 was used in all the experiments. MNC from various lymphoid tissues were stained with FITC-anti-B1 and biotinylated anti-Fc ϵ R followed by TR-avidin and analyzed by dual-laser FACS. As described in Table I, Fc ϵ R was expressed on most B1⁺ B cells in PBL, and approximately one-half of B1⁺ B cells derived from tonsils. On the other hand, only a small fraction of B1⁺ B cells were positive for the Fc ϵ R expression in bone marrow. It is noteworthy that B1⁻ cells did not express Fc ϵ R in any of the lymphoid tissues tested. The variability in the frequency of Fc ϵ R⁺ cells in PBL, tonsils, and bone marrow suggests that Fc ϵ R may be expressed on B cells at a certain defined differentiation or activation stage.

Correlation of Fc ϵ R Expression and Ig Isotypes on B Cells. Tonsillar MNC were stained with anti-Fc ϵ R and with conjugates directed against different Ig isotypes. As shown in Fig. 1 A, Fc ϵ R was expressed on most μ and δ positive B cells in tonsils. On the other hand, almost all γ - or α -bearing B cells, which were

TABLE I
Expression of FcεR on B Cells from Peripheral Blood, Tonsils, and Bone Marrow

Cell source	FcεR ⁺ cells (%)		
	Total	B1 ⁺ cells*	B1 ⁻ cells*
PBL	11.2 ± 3.1	87 ± 5.2	<0.1
Tonsils	28.4 ± 7.4	51 ± 11.3	<0.1
Bone marrow	0.5 ± 0.2	3.0 ± 1.2	<0.1

PBL from five donors, tonsillar MNC from five donors, and bone marrow MNC from three donors were stained with FITC-anti-B1 and biotinylated anti-FcεR, developed with TR-avidin and analyzed by FACS. Mean percentage of FcεR⁺ cells ± SD.

* Mean percentage of FcεR⁺ cells among B1⁺ or B1⁻ cells, assayed by two-color analysis as described in Materials and Methods.

abundantly observed in tonsils, did not express FcεR on their surface. Other experiments were conducted to study whether FcγR expression was also correlated with the isotype expression as observed with the FcεR. Several reports (26–28), have shown that FcγR and FcεR could be detected by flow-cytometric analysis of cells stained with monomeric IgG or IgE. Since antibodies specific to human FcγR were not available, biotinylated IgE and IgG1 were used for the comparison of the expression of FcγR and FcεR on μ⁺, δ⁺ B cells, and on γ⁺ or α⁺ B cells. As shown in Fig. 1 A and B, the specificity of the binding of biotinylated IgE was almost comparable to that of the anti-FcεR antibody, in fact, the IgE-binding was observed on μ⁺, δ⁺ B cells, but not on γ⁺ or α⁺ B cells. ~50% of μ⁺ or δ⁺ cells could not bind IgE, although they were stained with anti-FcεR antibody. This may be due to the difference in the affinity of IgE and anti-FcεR antibody with FcεR. On the other hand, IgG1-binding cells were observed in μ⁻, δ⁻ B cells as well as μ⁺, δ⁺ B cells. For example, almost all α⁺ B cell could bind IgG1. The results indicate that the FcγR expression was not correlated with Ig isotype expression.

Most peripheral blood B cells were μ⁺, δ⁺, and this may explain why most B1⁺ cells in PBL are positive for the FcεR expression, while only 50% of tonsillar B cells were positive for this marker. It is therefore difficult to analyze the expression of FcεR on γ⁺ or α⁺ B cells in PBL on the contour plot because of the paucity of these cells. Fig. 2 shows the histograms of the FcεR expression on δ⁺, μ⁺, γ⁺, and α⁺ B cells in PBL. Most δ⁺ (Fig. 2 A) or μ⁺ (B) B cells expressed FcεR, as in the case of tonsillar B cells. However, γ⁺ (Fig. 2 C) or α⁺ (D) B cells did not express FcεR, although a small fraction of these cells showed weak expression. The results show that FcεR is preferentially expressed on circulating δ⁺ and μ⁺ B cells, but not on γ⁺ or α⁺ B cells; this may explain why PBL include more FcεR⁺ cells than found in tonsils.

Because of the paucity of ε-bearing B cells in PBL or tonsils, the expression of FcεR on ε⁺ B cells could not be examined. Therefore, PBL from patients with hyper-IgE syndrome were studied. Fig. 3 shows the representative results observed in PBL from two patients with hyper-IgE syndrome. In fact, the FcεR expression was augmented in μ⁺ or δ⁺ B cells in these patients compared to that in normal individuals (Fig. 3, A and B). However, γ⁺ or α⁺ B cells did not express

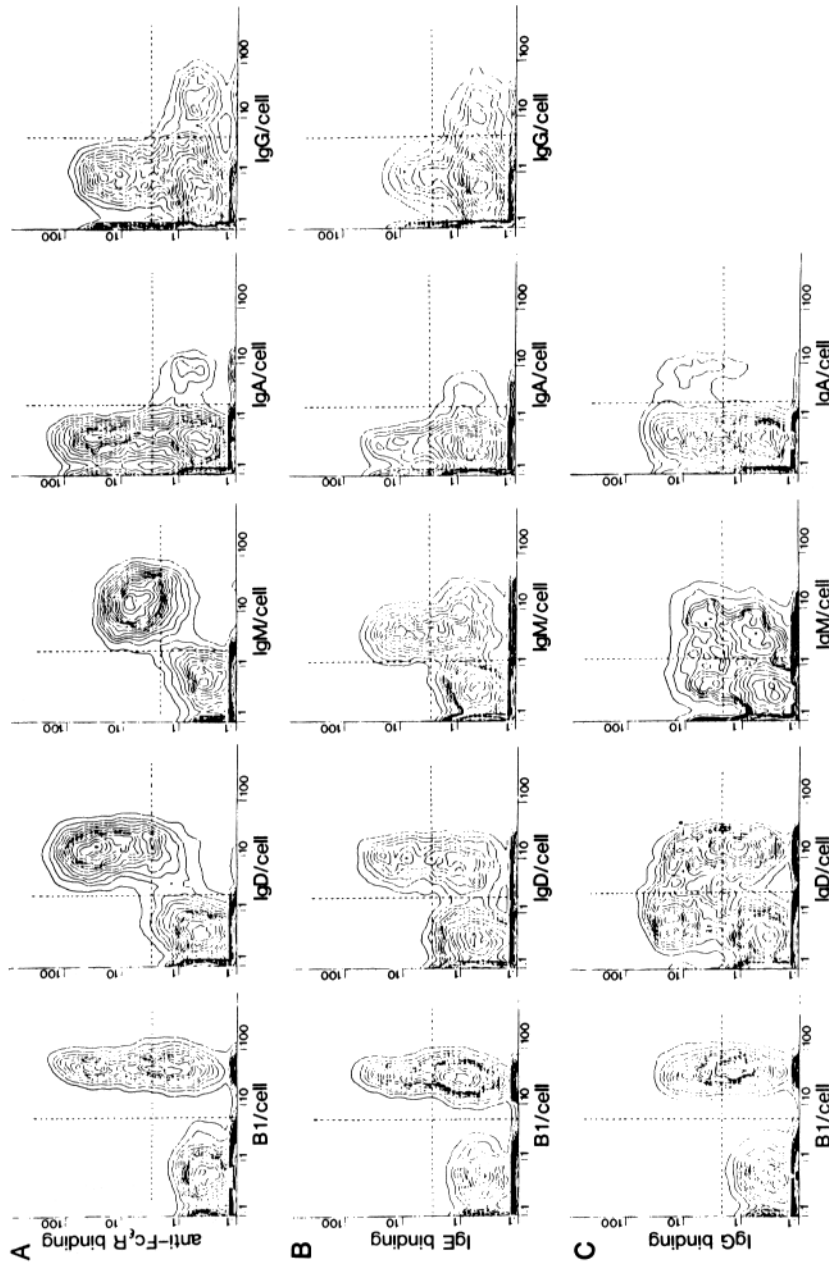


FIGURE 1. Expressions of FcεR and FcγR on tonsillar MNC. Tonsillar MNC were doubly stained with (A) biotinylated anti-FcεR and FITC-anti-B1, FITC-anti-IgM, FITC-anti-IgA, or PC-anti-IgG; (B) biotinylated human IgE and FITC-anti-B1, FITC-anti-IgM, FITC-anti-IgA, or PC-anti-IgG; (C) biotinylated human IgG1 and FITC-anti-B1, FITC-anti-IgD, PC-anti-IgM, FITC-anti-IgA, or PC-anti-IgG. Biotinylated reagents were developed with TR-avidin for double staining with FITC-antibodies, or with FITC-avidin for double staining with PC-antibodies.

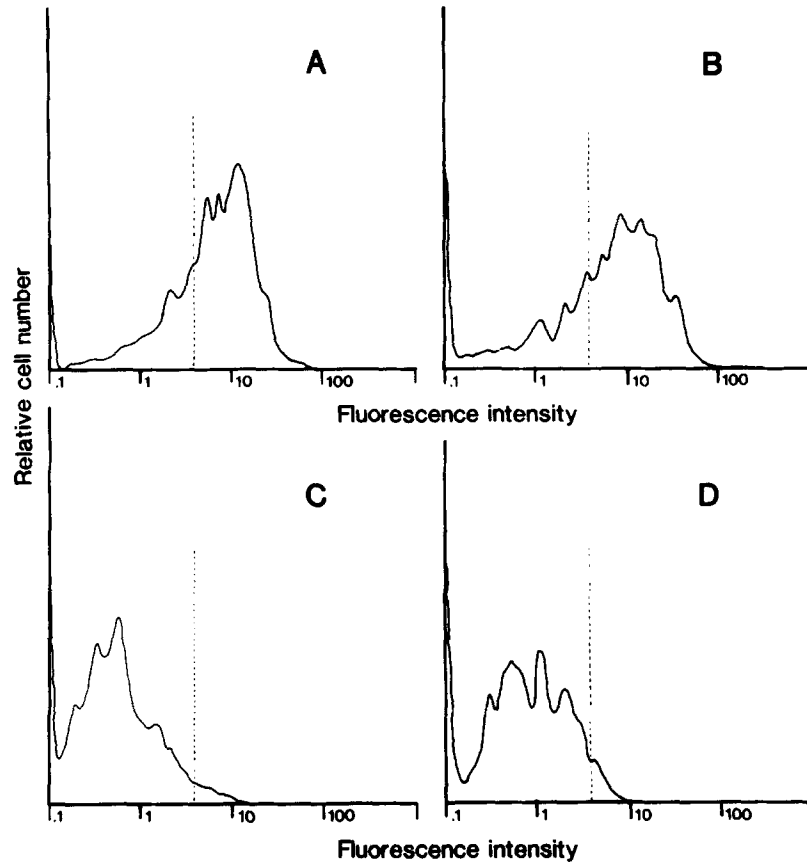


FIGURE 2. Fc ϵ R expression on various isotype-positive B cells from normal PBL. PBL from a normal individual were doubly stained with biotinylated anti-Fc ϵ R and FITC-anti-IgD, PC-anti-IgM, PC-anti-IgG, or FITC-anti-IgA. The fluorescence intensities of Fc ϵ R staining of IgD⁺ (A), IgM⁺ (B), IgG⁺ (C), or IgA⁺ (D) B cells are shown on the histograms. The limit of background fluorescence level is indicated with dotted perpendicular line.

Fc ϵ R even in PBL from patients with hyper-IgE syndrome (Fig. 3, C and D). It is worth noting that the Fc ϵ R expression was not observed in ϵ^+ B cells (Fig. 3 E). All of these results indicate that the Fc ϵ R expression is observed only on mature μ^+ , δ^+ cells, and the expression is lost after the isotype switching from μ^+ , δ^+ B cells to γ^+ , α^+ , or ϵ^+ B cells occurs.

Induction of Fc ϵ R in Bone Marrow and Tonsillar B Cells. As described in Table I, μ^+ , δ^+ B cells in PBL were Fc ϵ R⁺, while B cells in bone marrow were Fc ϵ R⁻, suggesting that only circulating mature B cells are able to express Fc ϵ R. Moreover, experiments were conducted to study whether Fc ϵ R could be induced in bone marrow B cells by certain factors. As shown in Fig. 4, T cell-derived PHA-conditioned medium (PHA-sup) could induce the Fc ϵ R expression on B1⁺ B cells (Fig. 4 A), particularly on δ^+ B cells (Fig. 4 B). The addition of IgE but not IgG could augment the PHA-sup-induced expression of Fc ϵ R. However, IgE alone, without PHA-sup, showed no effect on Fc ϵ R expression.

As described earlier, circulating δ^- B cells, which are mainly α^+ or γ^+ , do not

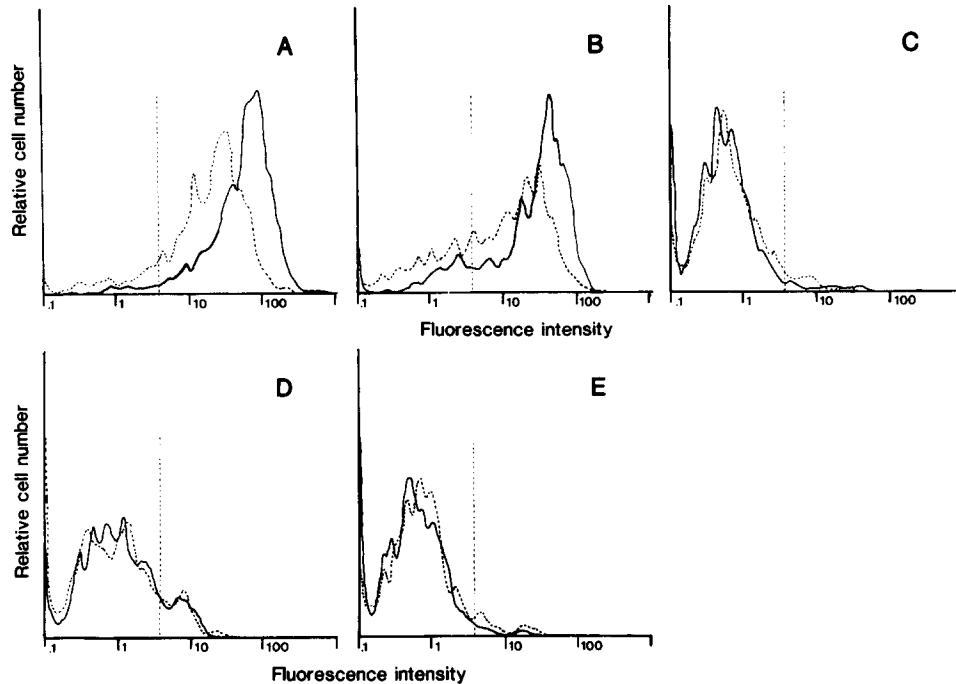


FIGURE 3. Fc ϵ R expression on various isotype-positive cells from PBL of patients with hyper-IgE syndrome. PBL from two hyper-IgE patients were doubly stained with biotinylated anti-Fc ϵ R and FITC-anti-IgD, PC-anti-IgM, PC-anti-IgG, FITC-anti-IgA, or PC-anti-Fc ϵ R and biotinylated anti-IgE. Biotinylated antibodies were developed with TR-avidin or FITC-avidin. The fluorescence intensities of Fc ϵ R staining of IgD $^+$ (A), IgM $^+$ (B), IgG $^+$ (C), IgA $^+$ (D), or IgE $^+$ (E) B cells are shown on the histograms. Solid and dotted lines represent the histograms of PBL from each of two patients.

express Fc ϵ R. To study whether Fc ϵ R is inducible in those cells, δ^- B cells were sorted from tonsillar B cells and incubated with PHA-sup and IgE. As shown in Fig. 5 A, stimulation did not induce expression of Fc ϵ R on circulating δ^- B cells, indicating that the loss of the Fc ϵ R expression was irreversible in the cells that had already completed isotype switching. A small fraction of δ^+ B cells in tonsil did not express Fc ϵ R. However, as shown in Fig. 5 B, PHA-sup plus IgE could induce the Fc ϵ R expression in these cells, suggesting that they may be in an immature stage of differentiation. Therefore, these results indicate that there are two different varieties of Fc ϵ R $^-$ B cells: immature B cells, and B cells that had already switched to other isotypes.

Lymphokine(s) Responsible for Fc ϵ R Expression. To examine the nature of lymphokine(s) responsible for the induction of Fc ϵ R, bone marrow MNC were cultured with recombinant or highly purified lymphokines in the presence of IgE. As shown in Fig. 6, none of the recombinant or purified lymphokines, including IL-1 (Fig. 6 B), IL-2 (C), IFN- β (D), IFN- γ (E), and BCDF (F) and crude B-BCGF (Fig. 6 G) induced the expression of Fc ϵ R. A mixture of these lymphokines (Fig. 6 H) also failed to induce the Fc ϵ R expression. On the other hand, PHA-sup (Fig. 6 I) could induce the Fc ϵ R expression in more than half of the bone marrow B cells. As shown (Fig. 6 J), IFN- γ almost completely abrogated

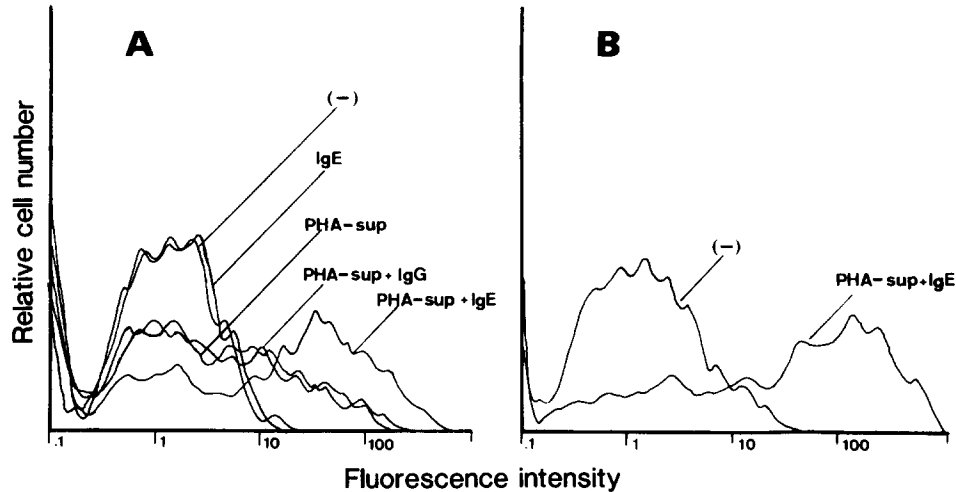


FIGURE 4. Induction of FcεR on bone marrow B cells. Bone marrow MNC from an immunologically normal adult donor were incubated without (-) or with IgE (10 μg/ml), PHA-sup (10% vol/vol), PHA-sup plus IgG (10 μg/ml), or PHA-sup plus IgE, for 3 d. After culture, cells were doubly stained with biotinylated anti-FcεR and FITC-anti-B1 or FITC-anti-IgD, and developed with TR-avidin. Cells were analyzed by FACS. The intensities of FcεR staining on B1⁺ B cells (A) and IgD⁺ B cells (B) were shown in the histogram.

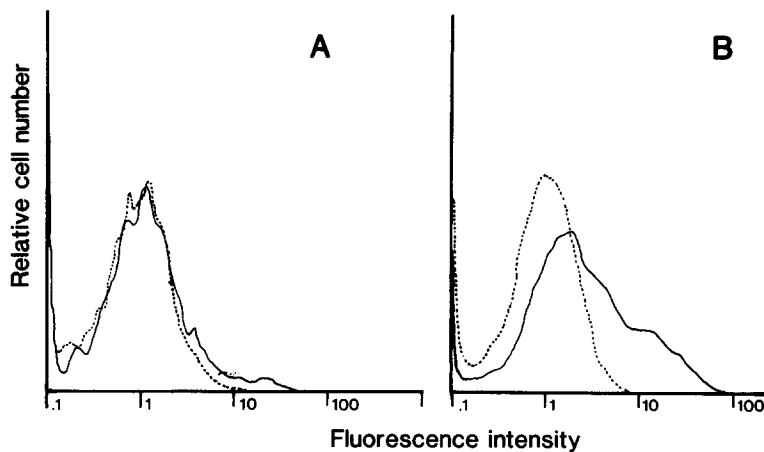


FIGURE 5. FcεR can be induced on δ⁺ cells but not δ⁻ cells in tonsils. Tonsillar B cells were stained with anti-FcεR and anti-IgD and δ⁻, FcεR⁻ (A) and δ⁺, FcεR⁻ (B) cells were sorted by FACS. Sorted cells were incubated with (solid line) or without (dashed line) PHA-sup (10% vol/vol) and IgE (10 μg/ml) for 3 d, and stained with anti-FcεR and anti-B1. The intensities of FcεR staining of B1⁺ B cells were shown in the histograms.

the induction of the FcεR expression by PHA-sup. Since it is known (29) that IFN-γ inhibits the BSF-1 activity in the activation of murine B cells, it may be possible that BSF-1-like activity is involved in the induction of FcεR expression, although the human counterpart of murine BSF-1 has not yet been characterized.

Failure of FcεR Expression on Lymphocytes from Patients with Certain Immunodeficiencies. PBL from patients with AT or CVI were stained with anti-FcεR and anti-B1 or anti-IgM and anti-IgD and analyzed by FACS. Three cases of CVI

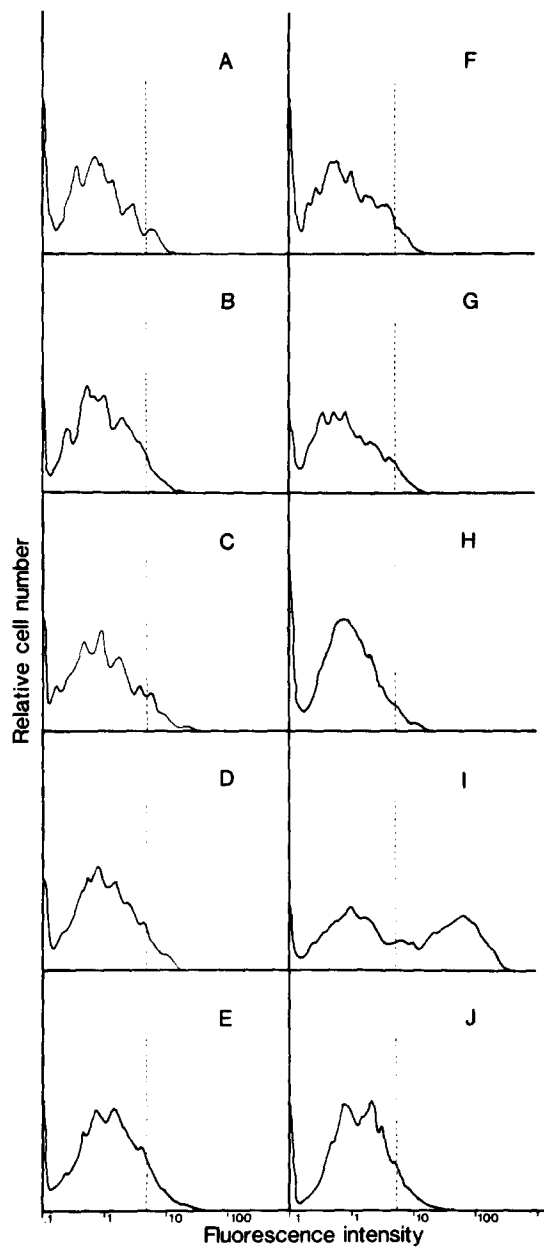


FIGURE 6. Lymphokine(s) responsible for the induction of Fc ϵ R. Bone marrow MNC were incubated in the presence of IgE (10 μ g/ml), without (A), or with rIL-1 (150 U/ml) (B); rIL-2 (150 U/ml) (C); rIFN- β (10⁴ U/ml) (D); rIFN- γ (10⁴ U/ml) (E); purified BCDF (50 U/ml) (F); crude B-BCGF (10% vol/vol) (G); a mixture of IL-1, IL-2, IFN- β , IFN- γ , BCDF, and B-BCGF (H); PHA-sup (10% vol/vol) (I); and PHA-sup plus IFN- γ (10⁴ U/ml) (J). After 3 d incubation, cells were stained with anti-Fc ϵ R and anti-B1 and analyzed by FACS. The intensities of Fc ϵ R staining of B1⁺ B cells are shown in the histograms.

TABLE II
*Failure of FcεR Expression on Lymphocytes from
 Immunodeficiency Patients*

Antigen	Percentage of positively staining cells from:				
	CVI-1	CVI-2	CVI-3	AT-1	AT-2
B1	20	2.5	4	30	5
IgD	17	1.8	2	27	2
IgM	19	2.3	3.6	28	4.5
FcεR	0.5	0.1	0.2	0.8	0.05
FcεR, B1*	2.5	4	5	2.4	1.0

PBL from patients with CVI or AT were stained with antibodies against B1, IgD, IgM, or FcεR and analyzed by FACS. Percentages of positive cells were shown.

* Cells were stained with FITC-anti-B1 and biotinylated anti-FcεR, and analyzed by FACS. The percentages of FcεR⁺ cells among B1⁺ cells were assayed by two-color analysis with FACS as described in Materials and Methods.

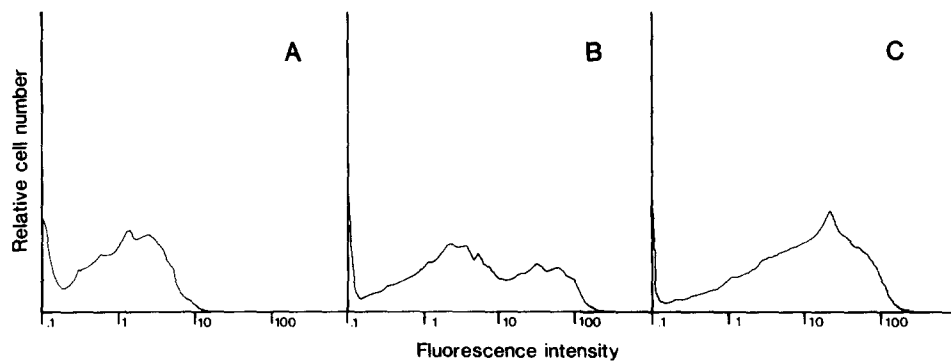


FIGURE 7. FcεR is inducible on FcεR⁻ PBL from a patient with CVI. PBL from a patient with CVI were incubated without (A) or with (B) PHA-sup (10% vol/vol) or PHA-sup and IgE (10 μg/ml) (C) for 3 d. After incubation, cells were stained with anti-FcεR and anti-B1 and analyzed by FACS. The intensities of FcεR staining of B1⁺ B cells are shown in the histograms.

and two cases of AT were studied, and the results are summarized in Table II. In all these cases, most of the B cells expressed both IgM and IgD, although some patients had few B cells. It was interesting to note that >90% of B1⁺ B cells in all these patients did not express FcεR. The results suggest that B cells from these patients are similar in their differentiation stage to μ⁺, δ⁺ B cells found in the bone marrow rather than in PBL. The results furthermore suggest a possible arrest in the maturation of these B cells. To study whether FcεR could be induced by external signals, PBL from a patient with CVI (CVI-1) were cultured with PHA-sup and IgE for 3 d, and the FcεR expression on B1⁺ B cells was examined. As shown in Fig. 7 B, PHA-sup could induce FcεR expression in a certain fraction of those B cells, and the addition of IgE could augment the expression (Fig. 7 C), suggesting that those B cells are in a similar differentiation stage as that of bone marrow B cells.

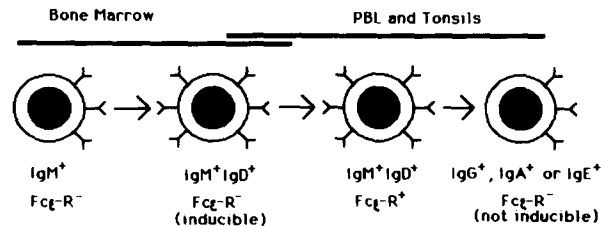


FIGURE 8. A scheme for the correlation of the FcεR expression and human B cell differentiation.

Discussion

The present study shows that FcεR is a B cell-specific surface molecule, the expression of which is strictly correlated with the differentiation stage of B cells: (a) bone marrow B cells with surface IgM and IgD do not express FcεR, although its expression could be induced; (b) circulating B cells with surface IgM and IgD express FcεR; but (c) circulating B cells with no surface IgD, which have already undergone isotype switching into IgG-, IgA-, or IgE-bearing cells, do not express and can not be induced to express FcεR. Therefore, according to these observations, the differentiation of B cells can be divided into three stages as described in Fig. 8: (a) μ^+, δ^+ FcεR⁻ bone marrow B cells, (b) μ^+, δ^+ FcεR⁺ circulating B cells, and (c) μ^-, δ^- FcεR⁻ with γ^+, α^+ , or ϵ^+ B cells.

These results show that the majority of circulating $\mu^+\delta^+$ B cells express FcεR, and this is in marked contrast with several previous studies (11–14) in which it was reported that only a few percent of FcεR⁺ cells were detected in PBL by the rosette method with IgE-conjugated ORBC. The discrepancy may be due to the difference in sensitivity between the assay systems used. It is important to note that the FcεR found on the surface of lymphocytes are of relatively low affinity compared with the homologous receptors on mast cells and basophils (30), and therefore the use of highly specific mAbs has helped in monitoring the expression of FcεR. In the present study, the expression of FcεR was restricted to B1⁺ cells even after induction. This is in marked contrast with previously reported studies (13, 26). Therefore, the specificity of the mAbs, used should be confirmed. The specificity of the anti-FcεR antibodies has been extensively studied in our previous report (15). The mAb 8–30 was shown to recognize FcεR⁺ B cell lines such as RPMI-8866 and CESS, but not a FcεR⁻ B cell line, Daudi. The antibody inhibited IgE binding to 8866 cells. The mAb 3–5 had the same staining pattern with 8–30, but did not inhibit the IgE binding. However, two-color FACS analysis showed a correlated expression of the molecules recognized by these mAbs, on B cells or B cell lines. Furthermore, both mAbs (8–30 and 3–5) precipitated surface molecules with M_r 45,000 and 25,000, and preabsorption of the cell lysates with mAb 8–30 removed the molecules reactive with mAb 3–5. Furthermore, we have recently (Owaki et al. manuscript submitted for publication) established mouse L cell transformants that selectively express the human IgE receptor recognized by its ability to bind human IgE and the mAbs used in this study. The receptor on the L cell transformants has been detected both by IgE-ORBC rosetting and FACS analysis. The mAbs did not react with granulocytes fraction of PBL (data not shown). All of these results indicated that these two

mAbs (8-30 and 3-5) recognized different epitopes of the same FcεR on B lymphocytes. The results obtained either with 8-30 or 3-5 were identical with regards to the expression of FcεR on various B cells. The results obtained with biotinylated IgE were also identical with those observed with the mAbs. In the staining with biotinylated IgE, >300 μg/ml IgE could not saturate the receptors. Therefore, the present results indicate that the lymphocyte FcεR with low affinity is a B cell-specific differentiation marker. The discrepancy between our and several other results (11-14, 26) cannot be explained at the present time.

A number of B cell-specific mAbs have been reported. However, surface molecules recognized with most of these antibodies are expressed on B-lineage cells in a wide range of differentiation stages, including immature bone marrow and mature B cells. None of the mAbs so far reported could differentiate bone marrow B cells and circulating B cells, as demonstrated using anti-FcεR and presented in this study. Some of the earlier reported antibodies, however, could react only with activated B cells (9, 10), B blasts (31, 32) or plasma cells (5). In previous studies (10, 33), we prepared a mAb (anti-Ba) that could recognize activated B cells but not resting B cells. Anti-FcεR could react with both Ba⁻ and Ba⁺ cells in PBL and tonsils (data not shown), and activation of resting B cells with anti-IgM or phorbol ester did not augment or abrogate the FcεR expression (data not shown). The results show that the expression of FcεR is not correlated with the activation stage of mature B cells, but rather is related to the differentiation stage of B-lineage cells.

The expression of FcεR was inducible in bone marrow B cells with PHA-sup. However, recombinant IL-2, IL-1, IFN-γ, IFN-β, and purified BCDF did not show any effect on the expression of FcεR. PHA-sup used herein probably contains BSF-1-like activity, since it showed a strong synergy with anti-μ for the proliferation of Ba⁻ resting B cells (33). Therefore, a possible candidate responsible for the FcεR induction may be BSF-1 (34, 35). Furthermore, the observation that IFN-γ could completely inhibit the FcεR expression induced by PHA-sup strongly suggests that the human counterpart of BSF-1 may be responsible for FcεR induction, since IFN-γ is known to inhibit the effect of BSF-1 in the activation of murine resting B cells (29).

The induction of FcεR in bone marrow B cells was augmented by the addition of IgE but not IgG. This may explain the augmented expression of FcεR on B cells of patients with hyper-IgE syndrome. A recent study (36) with murine B cells showed that BSF-1 could augment the production of IgE. Therefore, it might be possible that the overproduction of BSF-1 could be responsible for the augmented expression of FcεR as well as for the high serum concentration of IgE in hyper-IgE syndrome. In contrast, B cells from patients with CVI or AT did not express any FcεR, suggesting possible maturational arrest of B cells. PHA-sup could induce the FcεR expression in these defective B cells, strongly suggesting that B cells in these patients are at a similar differentiation stage as μ⁺, δ⁺ B cells found in the bone marrow of normal individuals. Two alternative possibilities should be considered with regards to their defect: (a) the defect is intrinsic in B cells, and the latter may not be sensitive enough to signals delivered by helper T cells, or (b) the defect may be in the regulatory function of T cells, and the appropriate signals required for the acquisition of FcεR may not be

available or could not be delivered. The second possibility seems to be unlikely, since PHA-sup from T cells of patients could induce FcεR expression on normal bone marrow B cells (data not shown).

One of the most interesting findings in this study was the absence of FcεR expression on γ^+ , α^+ , or ϵ^+ B cells. It could be said that the expression of FcεR is restricted to μ^+ , δ^+ circulating mature B cells, and that its disappearance is correlated with the isotype switching of the B cells. The observations that FcεR is expressed only on μ^+ , δ^+ cells could explain why PBL include more FcεR⁺ cells than found in tonsils. The loss of FcεR on γ^+ , α^+ , or ϵ^+ cells is irreversible even in the presence of PHA-sup and IgE. However, the loss of the expression is not due to the deletion of the gene for FcεR, since RPMI-8866 cells or CESS cells, which express IgG on their surface, also express FcεR. Therefore, the situation may be similar to the loss of δ expression in the final maturation stage of B cells that are committed to IgM secretion.

The FcεR expression is not correlated with the FcγR expression. In fact, FcγR⁺ B cells were observed in the μ^- , δ^- population, particularly in all α^+ cells as well as μ^+ , δ^+ cell population. The result strongly suggests that FcεR may have different immunological functions from FcγR. It is interesting to note that ϵ -bearing cells do not have FcεR even in hyper-IgE syndrome. The function of FcεR on mature B cells is yet to be defined.

Summary

The expression of FcεR on human lymphocytes was studied with the anti-FcεR mAbs. FcεR was expressed on most μ^+ , δ^+ circulating B cells, whereas T cells did not express FcεR even in patients with hyper-IgE syndrome. B cells with γ , α , or ϵ phenotype did not express FcεR, moreover its expression could not be induced, suggesting that the FcεR expression was correlated with isotype switching. μ^+ , δ^+ B cells in bone marrow did not express FcεR, but PHA-sup (supernatant from PHA-stimulated cell cultures) could induce its expression, and the addition of IgE augmented this induction. Recombinant IL-2, IL-1, IFN- γ or - β , or purified B cell differentiation factor (BSF-2 B cell-stimulatory factor 2) could not induce FcεR expression in bone marrow B cells. IFN- γ inhibited the FcεR expression induced by PHA-sup, suggesting that the human counterpart of BSF-1 may be responsible for FcεR expression in bone marrow B cells. B cells from patients with common variable immunodeficiency and ataxia teleangiectasia did not express FcεR, but PHA-sup could induce its expression, indicating that circulating B cells of these patients are at a differentiation stage similar to B cells in bone marrow. The study showed that FcεR is a B cell-specific differentiation marker, the expression of which is restricted to a defined stage of B cell differentiation.

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Note added in proof. Recent experiments with the newly cloned BSF-1 (IL-4) (*Proc. Natl. Acad. Sci. USA.* 83:5894. 1986) and kindly provided by DNAX Corp., revealed that this lymphokine could induce the expression of FcεR on human bone marrow B cells, confirming our observations made earlier in this study.

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