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Murine B-cell stimulatory factor 1 (interleukin 4) increases expression of the Fc receptor for IgE on mouse B cells

(lymphokine/class II major histocompatibility complex)

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ABSTRACT **We have studied the activity of mouse B-cell stimulatory factor 1 (interleukin 4, IL-4) on resting splenic B cells and on a B-cell hybridoma. Purified T-cell-derived as well as recombinant IL-4 was shown to increase the expression of the low-affinity Fc receptor for IgE (FcεR) on a majority of B lymphocytes in a 24-hr culture period. Levels of FcεR expression increased 2- to 3-fold on splenic B cells and up to 6-fold on a B-cell hybridoma. The effect was inhibited by an anti-IL-4 monoclonal antibody and by mouse γ-interferon. Other recombinant lymphokines exhibited no effect on either FcεR expression or the induction by IL-4. The presence of IgE during the stimulation with IL-4 resulted in an additional increase in FcεR expression. These data and results showing that IgE prevents FcεR turnover while IL-4 increases the rate of FcεR synthesis suggest that the mechanisms by which IgE and IL-4 increase FcεR expression are likely to be different. The starting population of splenic B cells expressed low levels of FcεR and was relatively uniform in size (small). After >48 hr of culture with IL-4, viable B cells had not undergone DNA synthesis and consisted mainly of larger highly FcεR-positive cells (23%) and medium-sized FcεR-positive cells (60%). A possible role for FcεR in certain B-cell maturation pathways is discussed.**

B lymphocytes undergo a process of activation and differentiation to antibody-secreting cells that is initiated by antigen and T lymphocytes and regulated by several cytokines (1, 2). B-cell stimulatory factor 1 (previously designated B-cell growth factor I), renamed interleukin 4‡ (IL-4), is a T-cell-derived lymphokine that has multiple actions on immature B cells. IL-4 was originally characterized as a costimulator with anti-IgM antibodies that drives B cells into S phase (3). Resting B lymphocytes respond to IL-4 by increased expression of class II major histocompatibility complex (MHC) molecules (4, 5), increased cell volume (6, 7), and preparation for entry into S phase in response to anti-IgM (6, 8). IL-4 also preferentially stimulates lipopolysaccharide-activated B cells to secrete IgG_1 (9–11) and IgE (11) and thus may be involved in class switching. In addition to these activities on B cells, IL-4 has been shown to stimulate the growth of T cells and mast cells (12–15). Recently, a cDNA clone that encodes the multiple IL-4 activities has been isolated from a mouse helper T-cell cDNA library (12, 16).

Receptors with a low affinity for the Fc region of IgE (FcεR) have been identified on macrophages, platelets, eosinophils, B lymphocytes, and T lymphocytes (17, 18). Macrophage and eosinophil FcεR seems to be involved in mediating IgE-dependent cytotoxicity reactions (17, 19). The B-cell FcεR in mice and humans consists of a single glycosylated polypeptide that has an apparent molecular weight of 46,000–49,000 (20, 21). However, its function on the B-cell surface is not clear. One proposal for its role in the immune response has been that the FcεR is involved in regulating IgE synthesis by B cells (22). Paradoxically, a large proportion of B cells from humans and rodents bear FcεR, whereas only a very small percentage of B cells, even in hyper-IgE states, produce IgE (<1%) (18, 23, 24). Recently it has been suggested that FcεR may serve as a stage-specific marker in human B-cell differentiation (25).

With the objective of defining the function of FcεR on B cells and in view of the role of IL-4 in promoting IgE synthesis (11), we examined the response of B cells to IL-4. In this study, we present data showing that purified T-cell-derived, as well as recombinant, mouse IL-4 stimulate a striking increase in the expression of FcεR on normal mouse B cells and on a mouse B-cell hybridoma. Recently similar observations have been made on human B cells with human IL-4 (25, 26).

MATERIALS AND METHODS

Cell Lines and Mice. 01.2A3 hybridoma cells were grown as described (27). BALB/cByJ mice were purchased from The Jackson Laboratory and used when 6–8 weeks old.

Antibodies. Monoclonal anti-I-A^d, MK-D6, was purchased from Becton Dickinson. A rat monoclonal antibody to mouse IL-4, 11B11 (6, 28), was a gift from W. Paul (National Institutes of Health). B220, a B-cell-specific glycoprotein (29), was detected by the monoclonal antibody, RA3-6B2-1, which was a gift from R. Coffman (DNAX). Rat IgE was purified as described (30) from ascites of LOU/MN rats bearing the IR162 immunocytoma (31). The purified MK-D6 and IR162 antibodies were biotinylated as described (32). Monoclonal antibodies 30H12, anti-thy1.2 (33), and GK-1.5, anti-L3T4 (34), were grown as described (33, 34) and used as culture medium for cytotoxicity. Affinity-purified, fluorescein isothiocyanate (FITC)-conjugated $F(ab')_2$ fragment of rat anti-mouse IgG (anti-heavy and anti-light chain) was purchased from Pel-Freez. FITC-conjugated avidin was purchased from Tago, Burlingame, CA.

Lymphokines. Purified human interleukin 1α (IL-1α) was purchased from Genzyme, Norwalk, CT, and used at 1 unit/ml. Recombinant mouse interleukin 2 (IL-2) produced in *Escherichia coli* was obtained from Schering and used at 400 units/ml. Culture medium of S15 72F-D11 cells (35) was used as a source of recombinant mouse interleukin 3 (IL-3) at 12 units/ml. Recombinant mouse granulocyte/macrophage colony-stimulating factor (GM-CSF) was expressed in COS monkey cells and used as culture medium (36). Purified

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Abbreviations: IL-4, interleukin 4 (formerly called B-cell stimulatory factor 1); FACS, fluorescence-activated cell sorter; FcεR, Fc receptor for IgE; sIg, surface immunoglobulin; IFN-γ, interferon-γ; FITC, fluorescein isothiocyanate; MHC, major histocompatibility complex; GM-CSF, granulocyte/macrophage colony-stimulating factor.

‡The name interleukin 4 was recommended recently for this lymphokine by an ad hoc subcommittee of the International Union of Immunological Societies/World Health Organization.

recombinant mouse γ-interferon (IFN-γ) was obtained from Schering and had a specific activity of 2.4×10^6 units/mg analyzed by cytopathic effect assay. Mouse IL-4 purified from the medium of a concanavalin A-stimulated T-cell line was a generous gift from C. Smith (DNAX). Recombinant mouse IL-4 expressed in COS cells (12) was partially purified by Schering. The specific activity in the T-cell growth assay (12, 37, 38) was 6.5×10^7 units/mg. One unit of activity per ml has been defined as the concentration that produces 50% of maximal response.

Preparation of B Cells. T-cell-depleted spleen cells were prepared from BALB/cByJ mice essentially as described (3). Briefly, the spleen cells were passed twice over columns of Sephadex G-10 (Pharmacia) and treated with rat monoclonal antibodies to thy1.2 (30H12) and L3T4 (GK-1.5) and complement (Cedarlane Low-Tox-M rabbit complement, Accurate Chemicals, Westbury, NY). Live cells were separated on Ficoll (Histopaque 1119; Sigma) and seeded at 5×10^5 cells per ml in B-cell culture medium [RPMI 1640 medium supplemented with 10% fetal bovine serum (Hyclone, defined)/10 mM Hepes, pH 7.3/2 mM L-glutamine/0.1 mM nonessential amino acids/1 mM sodium pyruvate/50 μM 2-mercaptoethanol/penicillin (50 units/ml)/streptomycin (50 μg/ml)/gentamicin sulfate (50 μg/ml)]. B cells isolated by this procedure were >97% surface immunoglobulin (sIg)-positive when stained with FITC rat anti-mouse immunoglobulin and analyzed in the fluorescence-activated cell sorter (FACS). 01.2A3 hybridoma cells were seeded at 5×10^4 cells per ml in growth medium (27) for 24-hr incubations. For longer incubations (up to 72 hr), cells were plated at 5×10^3 cells per ml.

FACS Analysis. Determination of FcεR by FACS analysis was essentially as described (27). Each sample was assayed in duplicate and contained 2×10^5 cells in 0.1 ml of FACS buffer (Hanks' balanced salt solution without phenol red supplemented with 10 mM Hepes, pH 7.3/3% fetal bovine serum). Incubation with biotinylated rat IgE (10 μg/ml) was for 1 hr at 0°C. After washing, FITC avidin (50 μg/ml) was added for 30 min at 0°C.

For determination of class II MHC expression, cells were incubated with biotinylated MK-D6 antibody (6 μg/ml) for 1 hr at 0°C followed by FITC avidin as described above. sIg was detected by incubating cells with FITC rat anti-mouse immunoglobulin at a concentration of 60 μg/ml.

Samples were analyzed with a FACS IV (Becton Dickinson) equipped with a software program (LYSIS) developed by Raymond LeFevre (Becton Dickinson). A number representing the relative integrated fluorescence (total relative cell surface molecules) was calculated by multiplying the mean fluorescence by the total number of cells in a defined window and subtracting the appropriate background (FITC avidin without biotinylated antibody). The integrated fluorescence of untreated cells was assigned the relative level of 1.0. The *P* values for relative levels were calculated and used to determine the significance of the differences from 1.0.

RESULTS

IL-4 Increases FcεR Levels on B Cells. The mouse B-cell hybridoma 01.2A3 has been shown to bear up to 6×10^4 FcεR per cell (27). Culturing these cells for 24 hr with rat IgE (10 μg/ml) caused a further increase in cell-surface FcεR (Fig. 1; ref. 27). IgE has been shown to increase expression of FcεR on these B cells by preventing turnover and promoting accumulation of newly synthesized FcεR on the cell surface (39). To examine the effect of IL-4 on B-cell FcεR expression, 01.2A3 hybridoma cells were cultured for 24 hr with either purified IL-4 from the supernatant medium of a concanavalin A-stimulated mouse T-cell line or with highly purified recombinant (mammalian expressed) IL-4. FcεR

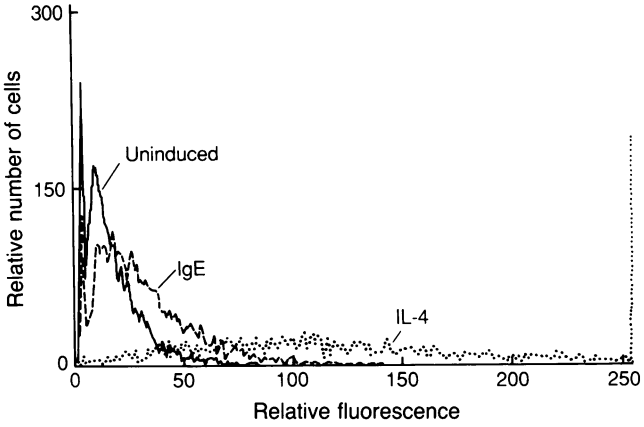

FIG. 1. Increase in FcεR expression on the 01.2A3 B-cell hybridoma in response to IgE and IL-4. Cells were cultured for 24 hr in medium (—), rat IgE at 10 μg/ml (---), or recombinant mouse IL-4 at 56 units/ml (···). After acid stripping, cells were stained with biotinylated IgE and FITC avidin and analyzed in the FACS in a linear mode.

levels, as measured by binding of biotinylated IgE and FACS analysis, increased 6-fold when compared with uninduced or IgE-incubated cells (Fig. 1). Similar effects of IL-4 were found on normal B cells purified from spleens of BALB/c mice. IL-4 treatment of splenic B cells for 18 hr resulted in a 2- to 3-fold increase in FcεR levels (see below, Table 1).

Initial experiments were performed with T-cell-derived purified IL-4. Recombinant IL-4 exhibited similar activities on both splenic B cells and 01.2A3 cells (Table 1). Because

Table 1. FcεR induction is specific to IL-4 and is inhibited by IFN-γ

Lymphokine	Relative level of FcεR	
	Spleen	01.2A3
None	1.00	1.00
IgE	1.75	1.55
rIL-4	2.56	6.21
nIL-4	3.18	5.15
IgE + rIL-4	4.42	8.14
11B11	1.15	0.85
IgE + 11B11	1.70	1.51
rIL-4 + 11B11	0.93	0.88
rIL-4 + 11B11 mock	2.46	5.84
IgE + rIL-4 + 11B11	1.38	2.22
Hu IL-1α	1.20	1.08
Mu IL-2	1.22	0.78
Mu IL-3	1.06	1.16
Mu GM-CSF	1.06	ND
Mu IFN-γ (1 unit/ml)	1.01	ND
Mu IFN-γ (30 units/ml)	0.78	ND
Mu IFN-γ (100 units/ml)	0.69	1.14
Mu IFN-γ (1 unit/ml) + IL-4	1.49	5.27
Mu IFN-γ (30 units/ml) + IL-4	1.17	ND
Mu IFN-γ (100 units/ml) + IL-4	1.10	1.21

Splenic B cells were cultured for 18 hr and 01.2A3 hybridoma cells were cultured for 24 hr with or without the addition of lymphokines. T-cell-derived IL-4 (nIL-4) was used at 25 units/ml for spleen cells and at 128 units/ml for 01.2A3 cells. Recombinant IL-4 (rIL-4) was used at 12 units/ml for spleen cells and at 56 units/ml for 01.2A3 cells. rIL-4 was used with the IFN-γ for spleen cells, and nIL-4 was added with IFN-γ for 01.2A3 cells. FcεR levels were analyzed in the FACS. Based on *P* value calculations, numbers > 1.3 were considered significantly different from 1.0. Hu, human; Mu, murine. ND, not determined.

recombinant IL-4 was more readily available in quantity, it was used for further studies.

The FcεR-inducing activity of each IL-4 preparation was titrated on both 01.2A3 cells and splenic B cells. With increasing IL-4 concentrations, the increase in FcεR expression reached a plateau. The concentration of IL-4 that produced 50% of the plateau FcεR increase on splenic B cells corresponded to 1.1 units of T-cell growth factor activity per ml. The induction of class II MHC molecules by IL-4 followed a titration curve that was identical to the increase in FcεR expression (data not shown). Thus, the activities and titrations in these assays were comparable. When compared with 01.2A3 cells, splenic B cells showed a maximal FcεR increase with a 2 to 4 times lower IL-4 concentration. This was most likely a result of the rapid growth rate and metabolic activity of 01.2A3 cells.

The Increase in FcεR on B Cells Is Specific to IL-4 and IgE. Splenic B cells or 01.2A3 cells were treated with both IgE and IL-4 or with different lymphokines to determine the specificity of the response to IL-4 (Table 1). Incubation of splenic B cells with optimal concentrations of IL-4 and IgE together resulted in at least an additive increase in the level of FcεR (Table 1; see also Fig. 3*A*). Similar results were found with 01.2A3 cells.

Addition of anti-IL-4 monoclonal antibody prevented the IL-4-stimulated increase in FcεR in both IL-4 and IL-4 plus IgE-treated splenic B cells and 01.2A3 cells (Table 1). Incomplete inhibition was observed in the presence of IgE, suggesting that the IgE effect was maintained after antibody treatment.

Treatment of cells with human IL-1α or the recombinant mouse lymphokines IL-2, IL-3, GM-CSF, or IFN-γ produced no significant increase in FcεR expression (Table 1). IFN-γ at concentrations >50 units/ml sometimes caused a decrease in the level of FcεR expression on splenic B cells (Table 1). IL-1α, IL-2, IL-3, or GM-CSF had no additional effect on B-cell FcεR expression when each was added with IL-4 (data not shown). Recombinant IFN-γ at 100 units/ml eliminated the IL-4-stimulated increase in FcεR (Table 1). The inhibition was still observable on splenic B cells at 1 unit of IFN-γ per ml. These results are consistent with the inhibitory effect of IFN-γ on other well-characterized activities of IL-4 on B cells (40). Cycloheximide treatment of cells also prevented the IL-4-dependent increase in FcεR (data not shown). Thus protein synthesis is required for the increased FcεR expression.

Time Course of IL-4 Treatment. The level of FcεR was examined at various times after treatment of B cells with optimal concentrations of either IgE or IL-4. FcεR expression on 01.2A3 cells reached a plateau after a 24-hr incubation with either IgE or IL-4 and remained high when the IL-4 concentration was saturating (Fig. 2*A*). Treatment of 01.2A3 cells with IL-4 and IgE together resulted in a higher plateau in FcεR expression after 24 hr (data not shown).

The maximum FcεR expression on splenic B cells occurred after an 18- to 24-hr incubation period with IL-4 or IgE. However, FcεR levels on the total B-cell population declined after >30 hr incubation with IL-4 (Fig. 2*B*). This observation was reproducible and occurred when the IL-4 concentration was increased 5-fold or an equivalent amount of IL-4 was added after 48 hr. The decline was prevented by including IgE in the culture (Fig. 2*B*).

Effect of IL-4 and IgE on Levels of Class II MHC and sIg Expression. Splenic B cells were incubated with IL-4, IgE, or both, and expression of FcεR, class II MHC, and sIg was measured. IgE or IL-4 treatment increased the expression of sIg by ≈50% (Fig. 3*C*). The increase in FcεR levels was significantly greater than that of sIg and was augmented with both IL-4 and IgE (Fig. 3*A*). In confirmation of the work of others (4, 5), IL-4 produced an increase (3.1-fold) in expression of class II MHC molecules (Fig. 3*B*). IgE alone or in conjunction with IL-4 had no effect.

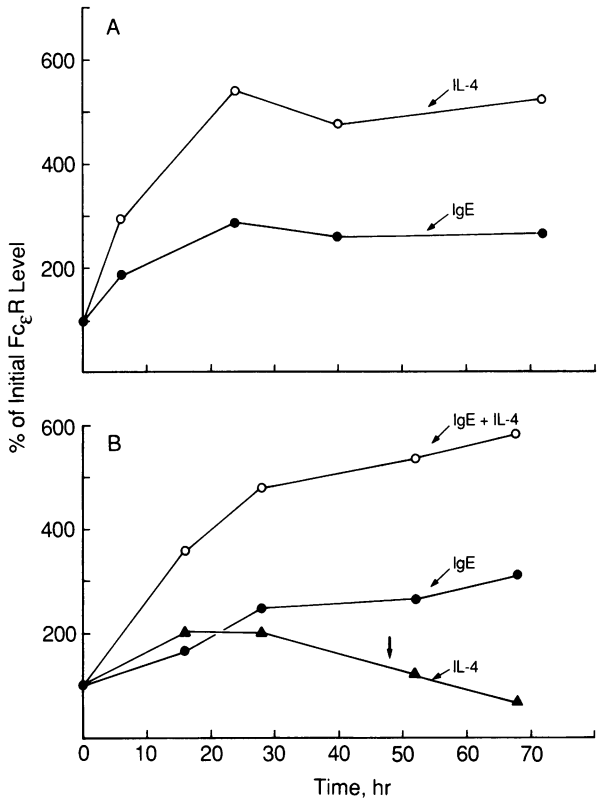

FIG. 2. Time course of changes in FcεR expression on 01.2A3 and splenic B cells. (*A*) IL-4 at 56 units/ml (○) or IgE at 10 μg/ml (●) was added at various times after initiation of the culture. (*B*) IL-4 at 56 units/ml (▲), IgE at 10 μg/ml (●), or IgE plus IL-4 (○) was added. At 48 hr (arrow), additional IL-4 was added to the IL-4 plus IgE and IL-4 cultures to bring the final concentration to 112 units/ml. Identical results were obtained when IL-4 or IgE was added at the initiation of the culture, and cells were analyzed for FcεR at various times.

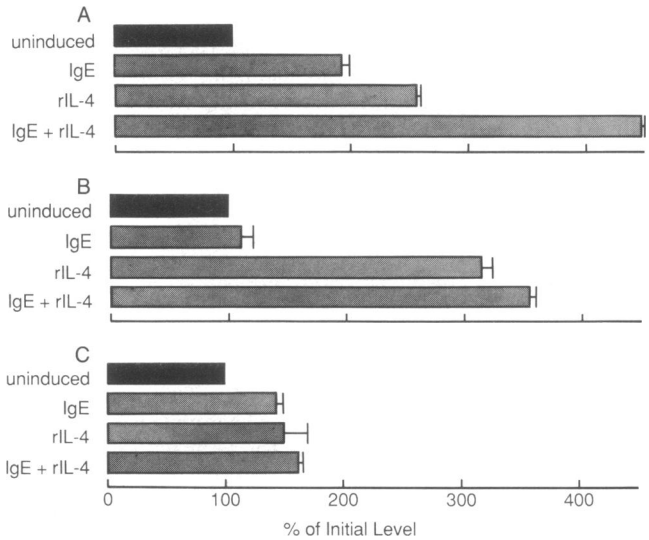

FIG. 3. Analysis of the levels of FcεR, class II MHC molecules, and sIg on splenic B cells after treatment with IL-4 and IgE. Splenic B cells were incubated for 18 hr in medium (uninduced), IgE at 10 μg/ml, recombinant IL-4 (rIL-4) at 12 units/ml, or both IgE and rIL-4. Levels of surface FcεR (*A*), class II MHC molecules (*B*), and sIg (*C*) were analyzed in the FACS.

IL-4 Changes B-Cell Size Distribution and FcεR Density. One of the effects of IL-4 on splenic B cells is to increase their susceptibility for entry into S phase without causing cell divisions (8) and to produce an increase in cell volume (6, 7). To correlate these responding B cells with the populations that were expressing FcεR, forward light scatter and FcεR levels were measured during a time course of IL-4 treatment. The initial population of splenic B cells shown in Fig. 4 (unstimulated) consisted primarily of minimally scattering (small) cells. Approximately 50% of the freshly prepared cells expressed low levels of FcεR as measured with either monomeric IgE (Fig. 4) or with a monoclonal antibody to FcεR (ref. 41; data not shown).

Incubation of the cells for periods of up to 67 hr with IgE alone did not change the light-scatter distribution of the small cells but resulted in an increase in FcεR levels to generate more FcεR-positive ($Fc\varepsilon R^+$) cells in region B from population D cells (data not shown). In contrast, IL-4 or IL-4 and IgE-treated B cells showed a progressive increase in the more highly light scattering (larger) cells in regions B and C (data for IL-4 plus IgE shown in Fig. 4). Staining of these stimulated cells with anti-B220 confirmed that the cells were B cells (2.9% of the cells were B220 negative at 67 hr). In parallel experiments, [^{3}H]thymidine uptake of IgE or IL-4-treated cells was found to be no different from unstimulated cells. Thus, the larger $Fc\varepsilon R^+$ B cells in regions B and C must have been generated without cell division from small cells in the original population.

A significant effect of IL-4 during the *in vitro* culture was to increase the cell viability from 25–30% (uninduced, 67 hr) to 60–70% (Table 2). The changes in distribution of cells in the regions indicate that in the absence of IL-4 both $Fc\varepsilon R^+$ and FcεR negative ($Fc\varepsilon R^-$) cells were dying in the culture (Table 2). Only a minority of cells (≈9% in region D) remained $Fc\varepsilon R^-$ and small after 67 hr treatment with IL-4 (Table 2). Either the majority of the $Fc\varepsilon R^-$ cells have become $Fc\varepsilon R^+$ or IL-4 preferentially maintained the viability of the $Fc\varepsilon R^+$ population.

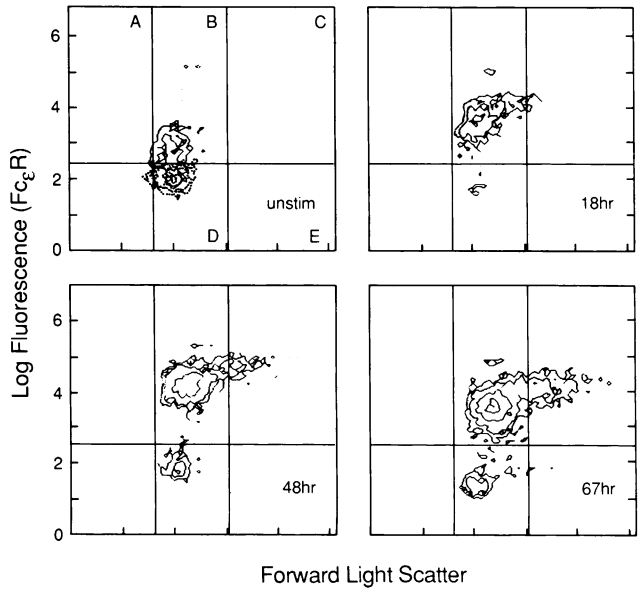

FIG. 4. Time course of IgE plus IL-4-promoted increase in FcεR expression on splenic B cells. B cells were cultured for 67 hr at 5×10^5 cells per ml. IgE at 10 μg/ml and recombinant IL-4 at 56 units/ml were added at the indicated times after initiation of the culture. FcεR levels were analyzed in the FACS in a logarithmic mode. Dead cells that stained with propidium iodide appeared in region A and are not shown. Cell doublets and aggregates were off scale and were not collected. Background fluorescence from cells stained with FITC avidin is shown in the "unstim" panel to define the $Fc\varepsilon R^-$ cells (···).

Table 2. Changes in FcεR level on splenic B cells treated with IL-4 and IgE

Treatment		% cells in population				
		A Dead	B S/M^+	C L^+	D S/M^-	E L^-
Uninduced	0 hr	3.9	51.3	1.9	44.5	0.9
	18 hr	41.7	33.2	2.0	24.9	0.3
	67 hr	74.6	16.8	2.4	7.0	0.5
IL-4	0 hr	3.9	51.3	1.9	44.5	0.9
	18 hr	22.2	50.8	7.0	21.2	0.4
	67 hr	33.6	41.8	15.5	9.4	1.9
	67 hr*	30.3	42.0	17.1	10.3	2.6
IgE + IL-4	0 hr	3.9	51.3	1.9	44.5	0.9
	18 hr	28.7	49.8	6.2	16.1	0.4
	67 hr	37.1	37.8	16.9	8.5	0.9
	67 hr*	38.1	38.7	16.7	6.5	1.2

Recombinant IL-4 at 56 units/ml or IL-4 and IgE was added at the initiation of the B-cell culture. Cells were stained for FcεR at the indicated times. The five populations of cells are shown in Fig. 4 (designated A–E). S/M, small to medium cells; L, large cells; +, $Fc\varepsilon R^+$; −, $Fc\varepsilon R^-$.
*Additional IL-4 was added to the culture at 48 hr.

After culture with IL-4, the more highly scattering cells in region B exhibited a significant increase in FcεR level, and the large cells in region C exhibited the highest FcεR levels (Fig. 4). Thus, there seemed to be a correlation between increased cell size and expression of high levels of FcεR. The effect of IgE treatment in conjunction with IL-4 was to maintain the high levels of FcεR on cells in regions B and C (see Fig. 2*B*) while not significantly affecting the proportion of $Fc\varepsilon R^-$ cells.

We also examined the time course of FcεR induction by IgE, IL-4, or both with a biotinylated monoclonal antibody to FcεR (41) to measure FcεR levels. The data correlated closely with measurements using biotinylated IgE. The increase in FcεR expression and the percentages of cells distributed in the regions of Fig. 4 were very similar (data not shown).

DISCUSSION

Increase in FcεR Level on B Cells Is Specific to IL-4 and IgE. IL-4 treatment of both splenic B cells and a B-cell hybridoma was shown to significantly increase (3- to 6-fold) FcεR levels on the cell surface. This effect was specific to IL-4 in that it was observed with both recombinant and T-cell-derived IL-4 but not with other lymphokines and was prevented by a monoclonal antibody to IL-4. IFN-γ, which inhibits other activities of IL-4 on B cells (40), was also shown to inhibit the IL-4-induced increase in FcεR. Comparable results obtained with a monoclonal antibody to FcεR (41) support the notion that the increased binding of IgE to IL-4-treated cells is actually a result of increased FcεR expression and that the induced FcεR is biochemically the same as the endogenous FcεR. In confirmation of our results, it has recently been found that recombinant human IL-4 (42) promoted a dramatic increase in FcεR expression on human B cells (25, 26). Since IL-4 has activities on other cell types (13–15, 43), it will be interesting to determine the effect of IL-4 on FcεR expression on macrophages, eosinophils, and T cells.

Mechanism of Increase in FcεR Expression. Lee *et al.* (39) have proposed that IgE increases FcεR levels on B cells by binding to FcεR, preventing its turnover, and allowing newly synthesized FcεR to accumulate at the same rate on the cell surface. We found that treatment with both IgE and IL-4 seemed to be at least additive in increasing FcεR expression, but that IgE had no additional effect on expression of class II molecules or on the size of the B cells. These results suggest

that the mechanism by which IL-4 increases FcεR expression may be different from the mechanism by which IgE increases FcεR levels. Since IL-4 has been shown to induce mRNA for class II MHC molecules (4, 5, 44), one possibility is that IL-4 directly induces an increase in FcεR by increasing transcription of FcεR mRNA. This is supported by experiments showing that IL-4 increased the rate of FcεR synthesis in a mouse B-cell hybridoma (D.H.C., unpublished data) and increased the levels of FcεR mRNA in human B cells (45).

Significance. Our results support the proposal that FcεR may serve as a marker of a particular stage of B-cell differentiation (24, 25). The other cell-surface protein that has been observed to increase in expression in response to IL-4 treatment, class II MHC, plays an important role in antigen presentation and lymphocyte activation (46, 47). We observed that after extended IL-4 treatment, B cells increased in size but the level of FcεR expression had returned to that of unstimulated cells and was not increased by additional IL-4. This suggests that the cells are progressing through some programmed differentiation in which transiently increased FcεR expression might play some role.

Interestingly, IL-4 also stimulates IgE production by preactivated B cells *in vitro* (11). It is possible that the maintenance of high FcεR levels by IgE might make a significant contribution in certain *in vivo* situations to the subsequent responsiveness or differentiation of the B cell. However, a role for FcεR in regulating IgE responses remains to be elucidated.

The majority of IL-4-stimulated splenic B cells became more highly light scattering (larger) and expressed high levels of FcεR. There remained a nonresponding FcεR$^-$ population of small B cells (≈10%; Fig. 4 and Table 2) that may possibly consist of B cells at a different stage of maturation. We are currently characterizing this cell population with respect to cell-surface phenotype and responsiveness to IL-4.

The correlation of FcεR expression with response to IL-4 suggests that newly induced FcεR on IL-4 activated B cells may not only be a marker of more differentiated B cells but may perform some function that is necessary for the progression of the differentiation or activation process in the majority of B cells. It remains to be determined whether other polypeptides on the B-cell surface change their levels of expression in response to IL-4.

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