CD27/CD70 Interaction Augments IgE Secretion by Promoting the Differentiation of Memory B Cells into Plasma Cells

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CD27/CD70 Interaction Augments IgE Secretion by Promoting the Differentiation of Memory B Cells into Plasma Cells

Haruo Nagumo,* Kazunaga Agematsu,1* Koji Shinozaki,* Sho Hokibara,* Susumu Ito,† Masaya Takamoto,‡ Toshio Nikaido,§ Kozo Yasui,* Yoshiro Uehara,¶ and Atsushi Komiyama*

The induction of IgE switching in B cells requires several signals given by cytokines and cell contact-delivered signals. Here, we investigated the role of CD27/CD70 interaction in B cell IgE synthesis. The addition of CD27 ligand (CD70) transfectants to B cell cultures increased the IgE synthesis synergistically in the presence of IL-4 plus anti-CD40 mAb (anti-CD40). The effect of CD70 transfectants was dose dependent and was completely blocked by anti-CD70 mAb. CD27+ B cells had the ability to produce IgE, which was increased by contact with CD70 transfectants, whereas CD27− B cells did not produce IgE. CD27/CD70 interaction enhanced B cell proliferation in the presence of IL-4 or IL-4 plus anti-CD40. The augmentation of B cell proliferation by CD70 transfectants was apparent in CD27+ B cells, but was mild in CD27− B cells. The helper activity for IgE synthesis by the CD27/CD70 interaction did not contribute to the enhancement of germline e transcripts. Flow cytometric and morphological analyses demonstrated that the addition of CD70 transfectants to B cell cultures remarkably promoted differentiation into plasma cells in the presence of IL-4 and CD40 signaling. Finally, CD27 cross-linking resulted in the up-regulation of positive regulatory domain I-binding factor-1. Taken together, our findings indicate that signaling via CD27 on B cells induces IgE synthesis, in cooperation with IL-4 and CD40 signaling, by promoting the generation of plasma cells through up-regulation of positive regulatory domain I-binding factor-1. The Journal of Immunology, 1998, 161: 6496–6502.

The IgE immune response is an important event in vivo because it may lead to the development of allergic diseases such as atopy and asthma (1). The induction of IgE switching in B cells requires at least two signals: a class switching factor, most commonly IL-4, and the engagement of CD40. The combination of IL-4 and anti-CD40 mAb can stimulate IgE production in vitro (2–4), whereas several other regulatory molecules may be involved in the regulation of B cell IgE secretion (5–7). The enhancement of IgE synthesis by CD4+ T cell clones in the presence of IL-4 plus CD40 signaling suggest a different B cell pathway for IgE production (4). Life et al. demonstrated that T cell clones from an X-linked hyper-IgM syndrome patient induced IgE synthesis in vitro despite the expression of nonfunctional CD154, indicating the involvement of T cell surface molecules other than CD154 in the induction of IgE synthesis (8).

CD27, characterized in detail by van Lier et al. (9, 10), is a type I glycoprotein expressed on T and B cells and in B cell malignancies (11). Recently, we have demonstrated that the interaction between CD27, which belongs to the nerve growth factor receptor/TNF receptor family (12), and CD27 ligand (CD70), which belongs to the TNF family (13) and is expressed not only on activated CD4+ CD45RO T cells (14), can enhance Ig production by B cells (15, 16). In addition, it has been demonstrated that the CD27/CD70 interaction is involved in the differentiation of B cells into plasma cells (17, 18). In the present study we clarified the role of CD27/CD70 interaction in B cell IgE synthesis by investigating the effects of CD27 signaling on B cell proliferation, germline e transcripts, differentiation into plasma cells, and the expression of transcription factors involved in B cell differentiation such as positive regulatory domain I-binding factors 1 (PRDI-BF1)2 and B cell-specific activator protein (BSAP).

Materials and Methods

Abs and reagents

Anti-CD27 mAb (SH5; IgG1), which does not block the ligation of CD27/CD70 (16, 19), was provided by Dr. T. Morimoto (Dana-Farber Cancer Institute, Boston, MA). FITC-conjugated anti-CD20 mAb and PE-conjugated anti-CD20 mAb were purchased from Dako Japan (Tokyo, Japan). FITC-conjugated anti-CD38 mAb (T16; IgG1), anti-CD40 mAb (MAB89; IgG1), anti-CD70 mAb (HNE51; IgG1), and anti-CD62L mAb (TQ1; IgG1) were purchased from Immunotech (Marseille, France). PE-conjugated anti-mouse Ig mAb was obtained from Southern Biotechnology Associates (Birmingham, AL). Conjugation of biotin to anti-CD27 mAb (SH5) was performed by the standard technique, using N-hydroxysuccinimido-biotin (Sigma, St. Louis, MO) in our laboratory. G418 was purchased from Life Technologies (Grand Island, NY), and human IL-4 was obtained from Genzyme (Cambridge, MA). The L cell line expressing murine FcγII receptor (CD32L) was obtained from American Type Culture Collection (Manassas, VA). The CD154-CD8 fusion protein (cell culture supernatant, soluble CD154) was provided by Dr. A. Aruffo (Bristol-Myers/Squibb Pharmaceutical Research Institute, Seattle, WA).

Cell preparation

Adult human PBMCs were isolated from healthy volunteers by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density gradient centrifugation and

2 Abbreviations used in this paper: PRDI-BF1, positive regulatory domain I-binding factor-1; BSAP, B cell-specific activator protein; PL, phycoerythrin; E−, erythrocyte rosette-negative; SAC, Staphylococcus aureus Cowan strain; Blimp-1, B lymphocyte-induced maturation protein-1.
were separated with 5% sheep erythrocytes into erythrocyte rosette-positive and -negative (E- ) populations (20). After depleting monocytes with silica (IBL, Fujikyo, Japan) or by adherence to the plastic surface, E- cells were further purified into B cells by positive selection with anti-CD19 mAb-coated immunomagnetic beads (Dynal, Oslo, Norway), and then anti-CD19 mAb was removed by the use of Detach a Bead (Dynal). Ninety-seven percent of the B cell population was reactive with anti-CD20 mAbs. Proliferation and activation were not observed from the B cells thus obtained. CD20-CD27 or CD20+CD27+ B cells were isolated from the monocyte-depleted E- cells by sorting with a FACStarPlus (Becton Dickenson, Mountain View, CA) under sterile conditions. Both populations obtained were >98% pure.

Preparation of transfectants

The preparation of CD70 transfectants or mock transfectants has been described previously (16). Briefly, total RNA was isolated by the single-step method (21) from the CD70- human B cell line (Daudi cells). After RT-PCR, the amplified cDNA was digested with EcoRI and XhoI and ligated with the mammalian expression vector pCDLSRz296 (22). The resulting plasmid or the vector alone was transfected into the murine pre-B cell line, 300-19 (23) by electroporation. The transfectants were selected by growth on G418 (Life Technologies) at 1 mg/ml.

Flow cytometric analysis

Activated purified B cells were stained with anti-CD38-FITC and anti-CD20-PE. Two-color analysis of B cell surface molecules was performed by FACScan (Becton Dickinson). Ab-coated cells were gated on living cells by cell size and granularity, and dead cells were removed by propidium iodide staining and then enumerated by flow cytometric analysis.

Fixation of transfectant cells

The CD70/300-19 or mock/300-19 transfectants were incubated with 1% paraformaldehyde in PBS for 5 min. After washing with PBS three times, the cells were cultured in RPMI 1640 and 10% FCS for 30 min and then used for the analysis.

Ig assay by ELISA

Highly purified peripheral blood B cells were cultured with 50 ng/ml of IL-4 and 1 μg/ml of anti-CD40 in the presence of various concentrations of fixed CD70/300-19 or mock/300-19 with or without 1 μg/ml of anti-CD70 mAb for 12–14 days at 37°C in a humidified atmosphere with 5% CO2. The final cell density was 5.10 × 10^3/ml in a volume of 200 μl/well. The cultured supernatants were harvested, and the supernatants and the standard human IgE (Chemicon, Temecula, CA) were added to 96-well flat ELISA plates (Nunc, Roskilde, Denmark). The plates were coated with human IgE mAbs (CIA-E-7.12 and CIA-E-4.15, provided by Dr. A. Saxon, Division of Clinical Immunology/Allergy, University of California School of Medicine, Los Angeles, CA). After 2 h of incubation at room temperature, color detection was performed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (Sigma). Calibration was performed with PBS at standard zero levels. The limit of sensitivity for IgE was 5 ng/ml.

B cell proliferation assay

Highly purified unseparated B cells and CD20-CD27+ and CD20+CD27+ B cells were cultured with fixed mock/300-19 or CD70/300-19 (20% of the B cells added) in the presence of 50 ng/ml IL-4 or IL-4 plus 1 μg/ml anti-CD40 at a final cell density of 2.5–5 × 10^5/ml in a volume of 200 μl/well in 96-well round-bottom plates (Nunc) for 3 days at 37°C in a humidified atmosphere with 5% CO2. Then the cultures were pulsed with 0.5 μCi of [3H]thymidine. After 18 h of incubation, the cells were harvested with an automatic cell harvester (Packard, Meriden, CT), and [3H]thymidine incorporation was measured on a liquid scintillation analyzer (Packard).

RT-PCR of germline C, BSAP, and PRDI-BF1

Highly purified unseparated B cells and CD20-CD27+ or CD20+CD27+ B cells (1 × 10^6 cells) were stimulated with 50 ng/ml IL-4 or IL-4 plus 1 μg/ml anti-CD40 in the presence of mock transfectants or CD70 transfectants for 16 h or 4 days. Total RNA was extracted by the acid- guanidine thiocyanate-phenol-chloroform method using a RNAzol rapid RNA purification kit (Biotex, Houston, TX). First-strand cDNA copies were synthesized by using murine Moloney leukemia virus reverse transcriptase (Life Technologies, Grand Island, NY) with oligo(dT) (Life Technologies) as a primer in a total volume of 20 μl, and then PCR was performed. The following oligonucleotide primers were used for PCR: for Ce exon, sense primer, 5'-CATGCGGTCCAGCACCAGAAC-3'; antisense primer, 5'- CCACTGCACAGCTGATGGAG-3'; for BSAP, 5'-CAACCAAC CAGTCCCAAGCTC-3' and 5'-TCACAAATGGGTTAGACCTGCG-3'; and for PRDI-BF1, 5'-AGCTGACATGTAATCTCA-3' and 5'-CT TGGGTATGACGCGTTA-3'. Two microliters of cDNAs were amplified in PCR using each primer and Taq DNA polymerase (Life Technologies). The amplified products were analyzed on a 1.2% agarose gel containing ethidium bromide and visualized by UV light illumination. The amplified product was determined by PAGE. To ensure that the amounts of germline Ce cDNA in the samples were the same, the amounts of the β-actin cDNA were evaluated as an internal mRNA control.

Results

Augmentation of B cell IgE production by CD27/CD70 interaction

Since triggering via CD27 by CD70 transfectants, which greatly express CD70 on the surface (Fig. 1A), induces IgA, IgM, and IgG synthesis in B cell activation systems with SAC plus IL-2 (15) and IL-10 (25), we investigated whether CD27/CD70 interaction participates in B cell IgE synthesis. To clarify the direct effect of CD27 signaling on IgE synthesis, we highly purified B cells from adult peripheral blood. As reported previously (26), anti-CD40 or IL-4 alone did not induce IgE synthesis by B cells, whereas in the presence of anti-CD40 plus IL-4, B cells produced substantial amounts of IgE. Most significantly, CD70 transfectants greatly augmented IgE production in the presence of IL-4 plus anti-CD40, which was about fourfold compared with that after the addition of mock transfectants (Fig. 1B). The augmentation of IgE synthesis by CD70 transfectants was dose dependent, and the enhancement of IgE production by CD70 transfectants was completely blocked by the initial addition of anti-CD70 mAb (Fig. 1B), but not by the control mAb (data not shown). On the other hand, the addition of IL-4 or anti-CD40 to CD70 transfectants did not induce detectable levels of IgE secretion (Fig. 1B). When we stimulated B cells by cross-linking with anti-CD40 plus CD32 transfectants and by the addition of soluble CD154 in the presence of IL-4, IgE production was 32 ± 7 and 21 ± 5 ng/ml, respectively. These data demonstrate that triggering via CD27 by its ligand, CD70, synergistically induces B cell IgE synthesis in the presence of IL-4 plus anti-CD40, indicating that the CD27/CD70 interaction is involved in B cell IgE synthesis by direct cell to cell contact.

Production of IgE by CD27+ B cells, but not by CD27- B cells, in the presence of IL-4 plus anti-CD40

Having demonstrated that peripheral blood B cells can be separated by CD27 expression, and that CD27+ B cells, but not CD27- B cells, produce large amounts of IgA, IgM, and IgG (27), we conducted additional experiments to determine whether B cell subpopulations separated by CD27 have different functions with regard to the IgE synthesis. We purified CD20+CD27+ B cells and CD20+CD27- B cells by flow cytometry; the purities of the two...
addition of CD70 transfectants in the presence of IL-4 or IL-4 plus anti-CD40. IL-4 alone induced low levels of proliferation of untransfected B cells, but was very mild in CD27+ B cells, whereas mock transfectants or CD70 transfectants with or without anti-CD70 mAb at a final cell density of 1 × 10^5/well for 12–14 days. IgE content was measured by ELISA. The data were representative of three independent experiments. Results are expressed as the mean ± SD of triplicate determinations.

Table I. B cell proliferation by CD70 transfectants in the presence of IL-4 or IL-4 plus anti-CD40 mAb

<table>
<thead>
<tr>
<th></th>
<th>Expt. 1</th>
<th>Expt. 2</th>
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<tbody>
<tr>
<td>Medium</td>
<td>239 ± 52b</td>
<td>322 ± 73</td>
</tr>
<tr>
<td>IL-4c</td>
<td>356 ± 80</td>
<td>723 ± 175</td>
</tr>
<tr>
<td>+ mock-trans.</td>
<td>387 ± 78</td>
<td>572 ± 101</td>
</tr>
<tr>
<td>+ CD70-trans.</td>
<td>1227 ± 315</td>
<td>1389 ± 216</td>
</tr>
<tr>
<td>+ CD70-trans. + HNE51c</td>
<td>365 ± 81</td>
<td>654 ± 155</td>
</tr>
<tr>
<td>+ CD70-trans. + TQ1c</td>
<td>1305 ± 322</td>
<td>1443 ± 319</td>
</tr>
<tr>
<td>IL-4c + anti-CD40c</td>
<td>1161 ± 216</td>
<td>2949 ± 465</td>
</tr>
<tr>
<td>+ mock-trans.</td>
<td>1396 ± 329</td>
<td>2820 ± 498</td>
</tr>
<tr>
<td>+ CD70-trans.</td>
<td>2193 ± 476</td>
<td>8174 ± 1279</td>
</tr>
<tr>
<td>+ CD70-trans. + HNE51c</td>
<td>1185 ± 237</td>
<td>3066 ± 793</td>
</tr>
<tr>
<td>+ CD70-trans. + TQ1c</td>
<td>2218 ± 531</td>
<td>7792 ± 1378</td>
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</table>

* a Proliferation assay was performed as described in Materials and Methods using 5 × 10^4/well of highly purified peripheral blood B cells.
  b Results are expressed as the mean ± SD of triplicates.
  c The final concentration of IL-4 and anti-CD40 mAb was 50 ng/ml and 1 μg/ml, respectively.
  d The transfectants were present as 20% of the B cell numbers added.
  e Both Abs were added at a concentration of 1 μg/ml at the initiation of cell culture.
results suggest that the ligation of CD27 on B cells by CD70 transfectants enhances B cell proliferation in the presence of IL-4 or IL-4 plus anti-CD40.

**CD27 signaling in B cells does not enhance germline e transcripts**

Since B cell IgE production was strongly enhanced by the addition of CD70 transfectants in the presence of IL-4 plus anti-CD40, we investigated the expression of germline e transcripts, which is an early molecular marker of the isotype switch process, to examine possible early switch events being induced by CD70 transfectants. Germline e transcripts from highly purified B cells were not obtained by the addition of medium, CD70 transfectants, or anti-CD40 alone (data not shown). Representative data by quantitative RT-PCR showed that germline e transcripts were induced by IL-4. In our experiments, the transcript expression by B cells was not enhanced by the addition of anti-CD40 to IL-4 (Fig. 3). We were unable to find differences in the expression of germline e by B cells in CD70 transfectant-treated vs untreated B cells in the presence of IL-4 or IL-4 plus anti-CD40 (Fig. 3). The addition of CD70 transfectants also had no enhancing effect on transcription in the presence of IL-4 plus anti-CD40 plus CD32 transfectants (data not shown). This effect did not change with various culture times from 16–48 h (data not shown). Although CD27+ B cells did not produce IgE (Fig. 2), their germline e transcripts were obtained in the presence of IL-4 plus anti-CD40 (Fig. 4). Thus, it appears that the increased IgE secretion from peripheral blood B cells by CD27/CD70 interaction does not occur in the process of germline e transcripts.

**Induction of plasma cells by CD27/CD70 interaction with IL-4 plus anti-CD40**

Recently, we demonstrated that triggering via CD27 by CD70 on purified peripheral blood B cells yielded an increase in the number of plasma cells in the presence of IL-10 (17). The finding prompted us to ascertain whether CD27/CD70 interaction induced differentiation of B cells into plasma cells secreting IgE in the presence of IL-4 plus anti-CD40. Accordingly, we studied the effect of CD27 signaling on peripheral blood B cell differentiation stimulated with IL-4 plus anti-CD40 by flow cytometric and morphological analyses. Human peripheral blood B cells expressed high levels of CD20 and CD40 and a low level of CD38, and plasma cells expressed CD38 strongly. Most interestingly, B cells stimulated with CD70 transfectants in the presence of IL-4 plus anti-CD40 for 12–14 days differentiated into plasma cells. In contrast, IL-4 plus anti-CD40 without CD27 signaling induced a slight increase in the number of plasma cells. The increase in the differentiation from B cells to plasma cells disappeared by the blockage between CD27 and CD70 interaction with anti-CD70 mAb (Fig. 5A). Morphological analysis showed that CD20− CD38high cells, which were increased by CD27/CD70 interaction in the presence of IL-4 plus anti-CD40, were induced by the addition of CD27/CD70 transfectants to IL-4 or IL-4 plus anti-CD40 for 16 h. After extraction of total RNA, quantitative RT-PCR was performed as described in Materials and Methods. Each template contained the same amount of cDNA from RNA extracted from 10^6 highly purified B cells and one of fivefold dilutions of germline Ce transcript competitor from 3.2 × 10^−4 to 2.6 × 10^−3 attomoles. The amounts of the germline Ce mRNA were quantitated by the amounts of the competitive template that most nearly equaled the amplification of the cellular cDNA (arrows). T, target cDNA products; C, competitive products. The competitive PCR assay of the β-actin in the same samples is shown, confirming that the amounts of cDNA used for the assay were the same. The fractions above represent the dilution series of the competitive Ce DNA and the competitive β-actin DNA (1 corresponds to 3.2 × 10^−4 attomoles). The data shown are representative of five separate experiments.

**Table II. CD27+ and CD27− B cell proliferation by CD70 transfectants in the presence of IL-4 or IL-4 plus anti-CD40 mAb**

<table>
<thead>
<tr>
<th>[3H] Thymidine Incorporation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>CD27+ B cells</th>
<th>CD27− B cells</th>
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<tbody>
<tr>
<td>IL-4&lt;sup&gt;b&lt;/sup&gt; + mock-trans.&lt;sup&gt;c&lt;/sup&gt;</td>
<td>537 ± 144&lt;sup&gt;d&lt;/sup&gt;</td>
<td>781 ± 180&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-4 + CD70-trans.&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1511 ± 325</td>
<td>1023 ± 293</td>
</tr>
<tr>
<td>IL-4 + anti-CD40&lt;sup&gt;b&lt;/sup&gt; + mock-trans.</td>
<td>1101 ± 291</td>
<td>889 ± 283</td>
</tr>
<tr>
<td>IL-4 + anti-CD40 + CD70-trans.</td>
<td>2054 ± 446</td>
<td>1240 ± 305</td>
</tr>
</tbody>
</table>

<sup>a</sup> Proliferation assay was performed as described in Materials and Methods using 3 × 10^3/well of CD27+ or CD27− B cells.

<sup>b</sup> The final concentration of IL-4 and anti-CD40 mAb was 50 ng/ml and 1 μg/ml, respectively.

<sup>c</sup> The transfectants were present as 20% of the B cell numbers added.

<sup>d</sup> Results are expressed as the mean ± SD of triplicates.

**FIGURE 3.** CD27 signaling in B cells does not enhance germline e transcript expression. The highly purified B cells were cultured with IL-4 or IL-4 plus anti-CD40 for 16 h. After extraction of total RNA, quantitative RT-PCR was performed as described in Materials and Methods. Each template contained the same amount of cDNA from RNA extracted from 10^6 highly purified B cells and one of fivefold dilutions of germline Ce transcript competitor from 3.2 × 10^−4 to 2.6 × 10^−3 attomoles. The data shown are representative of five separate experiments.

**FIGURE 4.** Germline e transcript expression by CD27+ B cells and CD27− B cells. CD27+ and CD27− B cells were stimulated with IL-4 plus anti-CD40 for 16 h. After extraction of total RNA, quantitative RT-PCR was performed as described in Materials and Methods. Each template contained the same amount of cDNA from RNA extracted from 10^6 highly purified B cells and one of fivefold dilutions of germline Ce transcript competitor from 3.2 × 10^−4 to 2.6 × 10^−3 attomoles. The data shown are representative of three separate experiments.
anti-CD40, were typical plasma cells with a basophilic cytoplasm with a pale Golgi zone and an eccentric nucleus. In contrast, CD20<sup>1</sup>CD38<sup>low</sup> cells revealed the morphology of B cells (Fig. 5B). These results clearly demonstrated that, in agreement with the IgE production measurement, CD27/CD70 interaction promotes plasma cell differentiation in the presence of IL-4 plus anti-CD40.

**Effect of CD27/CD70 interaction on the expression of PRDI-BF1 and BSAP**

As a further assessment of the role of CD27 signaling on B cell differentiation into plasma cells, we finally investigated the effect of CD27/CD70 interaction on the mRNA expression of PRDI-BF1/Blimp-1, which has been shown to be involved in multiple steps of B cell differentiation leading to plasma cell development (28) and the transcription factor BSAP. As shown in Fig. 6, PRDI-BF1 mRNA expression was up-regulated by the addition of CD70 transfectants compared with that with mock transfectants in the presence of IL-4 plus anti-CD40 after both 16 h and 4 days of culture. Up-regulation of BSAP mRNA expression was observed, although it was not significant, in CD70 transfectant-treated B cells in the presence of IL-4 plus anti-CD40. The enhancement of PRDI-BF1 and BSAP mRNA levels by CD70 transfectants was completely blocked by the initial addition of anti-CD70 blocking mAb (data not shown). The murine pre-B cell lines, mock/300-19 and CD70/300-19, did not express mRNA for human PRDI-BF1, BSAP, or b<sub>2</sub> m (Fig. 6, lanes 1 and 2). These studies indicate that while CD27/CD70 interaction does not reduce the expression of BSAP, it promotes the generation of plasma cells at least in part by up-regulating PRDI-BF1 levels.

**Discussion**

B cell IgE production is regulated by cytokines such as IL-4 (29–31) and IL-13 (5, 6) and by direct contact between B and Th cells (2, 29, 32, 33), in which CD40/CD154 interaction is important (3). Gascan et al. reported that the addition of both anti-CD40 and the CD4<sup>+</sup> T cell clones to purified B cells in the presence of IL-4 had synergistic effects on IgE synthesis, suggesting that different B cell activation pathways can result in IgE switching (4). Life et al. (8) also suggested the involvement of T cell surface molecules, other than CD154, in the induction of IgE synthesis, because T cell clones with nonfunctional CD154 molecules obtained from X-linked hyper-IgM syndrome patients induced IgE synthesis. As previously reported, peripheral blood B cells can be separated by CD27 expression, and CD27<sup>+</sup> B cells produce IgA, IgM, and IgG, which are augmented with CD70 transfectants by promoting their differentiation into Ab-secreting cells in various B cell stimulation conditions.
systems (17, 18, 27). These findings prompted us to ascertain whether CD27/CD70 interaction participates in B cell IgE synthesis.

It is known that IL-4 has a potent function in the production of IgE by B cells (34, 35). However, IL-4 alone induces B cell germline \( e \) transcripts but not IgE production. By the addition of CD40 signaling to IL-4, the mature \( e \) transcripts can be induced, and IgE synthesis finally occurs (4, 3, 26). In our experiments, CD70 transfectants significantly augmented IgE production by highly purified B cells cultured with IL-4 plus anti-CD40. Since the blockage of CD27/CD70 interaction by mAb completely blocks the augmentation of B cell IgE production by CD70 transfectants (Fig. 1), CD27 signaling evidently plays an important role in IgE synthesis. When we used highly purified CD27\(^{+} \) B cells, their IgE production was increased more than that of unseparated B cells in the presence of IL-4 plus anti-CD40, whereas CD27\(^{-} \) B cells did not entirely produce IgE in the presence of IL-4 plus anti-CD40 (Fig. 2). These findings support the view that CD27\(^{+} \) B cells are primed memory B cells, and CD27\(^{+} \) B cells are unprimed naive B cells as we previously demonstrated in the synthesis of IgA, IgM, and IgG (27).

Several reports indicated that CD70 transfectants induced B cell proliferation in the presence of SAC plus IL-2 (15, 16, 18). In our experiment, CD70 transfectants augmented B cell proliferation to about 3 times that found in the presence of IL-4 (Table I). In disagreement with our data, Jumper et al. reported that stimulation via CD27 had no effect on B cells in the presence of IL-2 or IL-4, suggesting that the role of CD70 in regulating B cell growth and differentiation is questionable (36). The reasons for the discrepancy in B cell proliferation via CD27 between the reported data (36) and our present results are not clear, but several possibilities could be postulated. One possibility is that the degree of the CD70 expression of the transfectants may be different; CD70 expression of our transfectants is prominent compared with that of the reported cells (36). Alternatively, the concentrations of cytokines used in the studies are probably different between the two studies.

The levels of B cell proliferation in our system using IL-4 plus anti-CD40 were very low compared with the proliferation effects previously reported (36). As cross-linking of anti-CD40 by CD32 transfectants or soluble CD154 in the presence of IL-4 greatly promoted B cell proliferation, the effects of our stimulation systems with IL-4 plus anti-CD40 alone appear to be suboptimal. Enhancement of B cell proliferation by CD70 transfectants were remarkable in the presence of IL-4 plus anti-CD40, but were not prominent in the presence of IL-4 plus anti-CD40 plus CD32 transfectants or IL-4 plus soluble CD154. Thus, our present suboptimal activation system is suitable for observing the enhancing effects of CD27/CD70 interaction on B cell proliferation. On the other hand, IgE synthesis was not as significantly increased in the presence of IL-4 plus anti-CD40 plus CD32 transfectants or IL-4 plus soluble CD154 compared with that in the presence of IL-4 plus anti-CD40. The enhancement of IgE synthesis via CD27 signaling was most significant in the presence of IL-4 plus anti-CD40.

B cell IgE synthesis is preceded by transcription of the germline \( e \) gene, and the relationship between this expression and subsequent switching to Ce has been reported (37, 38). CD20\(^{+} \) CD27\(^{+} \) and CD20\(^{+} \) CD27\(^{-} \) B cells from healthy adults did not differentiate in their ability to produce \( e \) transcripts by exogenous IL-4 (Fig. 4). Therefore, IL-4-induced \( e \) transcripts occur similarly in CD20\(^{+} \) CD27\(^{-} \) naive B cell and CD20\(^{+} \) CD27\(^{+} \) B cell populations. Jumper et al. (36) demonstrated that the addition of CD70 transfectants into EBV-transformed B cells did not augment the germline \( e \) expression. Also, triggering via CD27 by CD70 in the present study exerted no enhancing effect on the expression of the germline \( e \) in the presence of IL-4 or IL-4 plus anti-CD40 (Fig. 3). These findings suggested that CD27/CD70 interaction does not participate in the IL-4-induced germline \( e \) expression. However, one possibility remains that CD27 signaling may induce IgE class switching following the increase in germline \( e \) transcripts with IL-4 or IL-4 plus CD40 signaling. To clarify this point, we performed quantitative RT-PCR using mature \( e \) primers. Unfortunately, as we could not detect mature \( e \) bands using several primer pairs in the presence of IL-4 plus anti-CD40 plus CD32 transfectants, we failed to estimate the effects of CD27/CD70 interaction in the IgE class switching.

As reported previously, the CD27 signal resulted in the terminal differentiation of peripheral blood memory B cells into plasma cells in B cell activation systems by IL-10 or IL-10 plus IL-2 (17) and SAC or SAC plus IL-2 (18). In our experiments, B cells also differentiated into plasma cells by triggering via CD27 in the presence of IL-4 plus anti-CD40 (Fig. 5), but not in the presence of IL-4 alone (data not shown). Since CD154 is expressed on T cells soon after the activation, and CD70 is expressed on T cells later after the activation (17), CD40 triggering probably participates in the early steps of the IgE switch recombination and then CD27/CD70 interaction may promote the differentiation of class-switched B cells into IgE-producing plasma cells (35, 39, 40). CD40/CD154 interaction also induces the expansion of memory B cells expressing CD27 on the surface. CD27 may be important in the induction of plasma cells when the expansion of CD27-positive B cells has already occurred (17, 18). Thus, as we demonstrated here and previously, CD27 is crucial in controlling the differentiation of all isotype-committed memory B cells into plasma cells.

PRDI-BF1/Blimp-1, a recently described zinc finger transcription factor, is involved in various steps of B cell terminal differentiation (28), indicating that its expression participates in the generation of plasma cells. Since CD27 cross-linking on B cells enhanced the mRNA expression of PRDI-BF1/Blimp-1, even after the overnight activation, the induction of plasma cells, which was demonstrated by FACS, is probably due to the effect of the expression of PRDI-BF1/Blimp-1 induced by CD27/CD70 interaction. We also investigated the transcription factor BSAP/Pax-5, which is involved in the regulation of B cell proliferation, because it is expressed in all B cell developmental stages except for the plasma cell stage (41). BSAP mRNA expression was unexpectedly up-regulated by triggering via CD27 on B cells. A large majority of the cultured cells in our system was composed of B cells, and plasma cells constituted a small population, even after 8 days of culture. Thus, the remaining B cells may express BSAP. At least, CD27/CD70 interaction on B cells did not directly reduce BSAP expression.

In summary, CD70 transfectants significantly augmented IgE production from highly purified B cells, especially CD27\(^{-} \) B cells cultured with IL-4 plus anti-CD40, by enhancing B cell proliferation and differentiation into plasma cells without any increase in the expression of germline \( e \). Our findings presented in this paper should be tested in the murine system including CD27\(^{-}/- \) mutant mice (42), although the effects of CD27/CD70 interaction on B cell Ig synthesis in the murine and human systems are somewhat different (J. Borst, unpublished observations). Experiments to examine these aspects are underway.

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References

AUGMENTATION OF IgE SYNTHESIS BY CD27/CD70


