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Mouse B cells express CD38, whose ligation by anti-CD38 Ab induces their proliferation and protection from apoptosis. We previously showed that stimulation of mouse splenic B cells with IL-5 together with CS/2, an anti-mouse CD38 mAb, induces production of IgG1 and IgM. Here we examined the role of IL-5 and CS/2 in the expression of germline γ1 transcripts and the generation of reciprocal products forming DNA circles as byproducts of μ-γ1 switch recombination. By itself, CS/2 induced significant expression of germline γ1 transcripts in splenic naïve B cells, whereas IL-5 neither induced nor enhanced germline γ1 expression. Increased cellular content of reciprocal product, which is characteristic of μ-γ1 recombination, was not observed after culturing B cells with CS/2, but increased reciprocal product, along with high levels of IgG1 secretion, was found when B cells were cultured with CS/2 plus IL-5. Although IL-4 did not, by itself, induce μ-γ1 recombination in B cells stimulated with CS/2, in conjunction with CS/2 plus IL-5, IL-4 dramatically enhanced sterile γ1 transcription and IgG1 production. These results demonstrate that CD38 ligation induces only germline γ1 transcription and that IL-5 promotes both μ-γ1 switch recombination and IgG1 secretion in an IL-4-independent manner. The Journal of Immunology, 1999, 162: 2812–2819.
as IgG1 production. These IL-5-evoked effects could not be reproduced using IL-4. Our findings demonstrate that IL-5 has the potential to induce Ig isotype switching from IgM to Syl even in the absence of IL-4 and supports a three-component model of Ig class switching that includes DNA synthesis, transcriptional activation of the germline HC genes, and a component necessary for recombination (4).

Materials and Methods

Animals

Female C57BL/6 mice were purchased from Japan SLC (Hamamatsu, Japan) and were used at 8–10 wk of age. STAT6-deficient (STAT6−/−) mice were generated as described (39) and maintained in the animal facility at the Institute of Medical Science (University of Tokyo) under pathogen-free conditions. All experiments were conducted according to our institution’s guidelines for the care and treatment of experimental animals.

Reagents

RA3-6B2 (rat anti-B220), Bet2 (rat anti-mouse IgM), and 2-G2 (rat anti-mouse FcγR) were obtained from the American Type Culture Collection (ATCC, Manassas, VA). CS2 (rat anti-mouse CD38; Ref. 32) and CS15 (rat anti-mouse IgD) were kindly provided by Dr. K. Miyake (Saga Medical College, Saga, Japan). LB429 (rat anti-mouse CD40; Ref. 40) was kindly provided by Dr. N. Sakaguchi (Kumamoto University, Kumamoto, Japan). Biotinylated 28-1 mAb (rat anti-mouse Syndecan-1) and FITC-labeled rat anti-mouse IgG1 mAb were obtained from Pharmingen (San Diego, CA) and Zymed Laboratories (San Francisco, CA), respectively. R-phycocerythrin-labeled streptavidin (PE-av) was purchased from Life Technologies (Gaithersburg, MD). Mouse IL-4 was purchased from R&D Systems (Minneapolis, MN). Mouse IL-5 was prepared and purified with the mouse FcγR, cells were stained in the presence of DNA and RNA, B cells were cultured with their respective stimuli in six-well plates at a density of 106 cells/ml. Two different combinations were used: 1) IL-5 and CS/2, 2) IL-5 and CS/2 plus B220. Enrichment of sIgD+ B cells

Splenic B cells were isolated from 8-wk-old mice after T cell depletion by treatment with anti-mouse Thy1.2 mAb and guinea pig complement as described previously (42). To purify naive slgD+ B cells, they were stained with FITC-labeled anti-B220 mAb and biotinylated anti-IgD mAb plus PE-av; slgD+ B220+ cells were then isolated by sorting using a FACS Vantage (Becton Dickinson, Mountain View, CA). The purified B cell population contained >99% slgD+ cells, as assessed by fluorescence analysis using the FACS Vantage (Becton Dickinson).

Flow cytometric analysis

Expression of surface molecules on freshly isolated or cultured cells was analyzed by flow cytometry. Surface expression of IgG1 was determined on day 5 of culture by flow cytometry. Cells were stained with FITC-labeled anti-mouse IgG1 mAb or FITC-labeled anti-IgD mAb plus PE-av; slgD+ B220+ cells were then isolated by sorting using a FACS Vantage (Becton Dickinson, Mountain View, CA). The purified B cell population contained >99% slgD+ cells, as assessed by fluorescence analysis using the FACS Vantage (Becton Dickinson).

B cells were cultured in RPMI 1640 medium supplemented with 8% heat-inactivated FCS, 2 µM 2-ME (Merck, Rahway, NJ), 100 U/ml penicillin, and 100 µg/ml streptomycin. To determine Ig secretion, naive slgD+ B cells were plated in flat-bottom 96-well plates at a density of 104 cells/well in a final volume of 0.2 ml and cultured for 7 days. Either CS/2 (0.5 µg/ml), IL-5 (100 U/ml), IL-4 (200 ng/ml), or a selected combination of those agents was added at the time the cells were plated. Each culture was set up in triplicate. The concentrations of IgM, IgG1, IgG2a, IgG2b, IgG3, and IgA in culture supernatants were measured by ELISA according to previously described procedures (43). For FACS analysis and preparation of DNA and RNA, B cells were cultured with their respective stimuli in six-well plates at a density of 105 cells/ml.

Semiquantitative analysis of germline γ1 transcripts by RT-PCR

Cells were collected after 2 days of culture, and total RNA was prepared using the acid guanidine isothiocyanate-phenol-chloroform method (44). cDNA synthesis was conducted in 30-µl aliquots of reaction mixture containing 10 µg total RNA, random hexamer (Takara, Kyoto, Japan), 0.01 M DTT, 0.4 mM dNTPs, and 10 U/ml (RT) RNase H− reverse transcriptase (Life Technologies), as described previously (43). For semiquantitation, serial dilutions of the cDNA templates were subjected to PCR amplification using the following primers: germline γ1 transcript (sense, 5′-CAGCTG GTGTCACAATGCA-3′; anti-sense, 5′-ACGTTCGCGTGAGCGTCTC-3′; Ref. 43), germline γ3 transcript (sense, 5′-CAAGTGATGCTACCAA-3′; anti-sense, 5′-GGCTCATAGTCCATT-3′; Ref. 46); β-actin (sense, 5′-ATGATGACGATATCGCT-3′; anti-sense, 5′-ATGAGTATGCTGTTT CAGGGT-3′; Ref. 43). PCR products were separated by electrophoresis on 1.5% agarose gels and visualized by ethidium bromide staining.

PCR analysis of γ1-μ recombinant DNA reciprocal recombination

Total DNA (genomic and circular) was extracted using a QIAamp Blood Kit (Qiagen, Düsseldorf, Germany), and DNA concentrations were measured by spectrophotometry. For amplification of γ1-μ recombinant products, 200 ng of total DNA from cultured or freshly isolated B cells were subjected to PCR amplification in 50-µl volumes containing LA PCR Buffer II (Takara), 2.5 mM MgCl2, 0.4 mM dNTP, 2.5 U LA Taq (Takara), 1 µg mouse-strand-specific 5′-Syl primer(5′-CAGTTCGGGTGAAACACATC CTAC-3′); nucleotides 262–289 in region 5′ of the Syl repeats; MUSHI GAND in combination with an antisense 3′ Sμ primer (5′-AGC TAACTTATGGCTATGGCAA-3′); nucleotides 1347–1319 in region 3′ of the Sμ repeats; MUSIGAND09. Reciprocal γ1-μ products were amplified using 35 cycles of 1 min of melting at 94°C and 8 min of annealing and extension at 69°C.

PCR products were transferred onto nylon membranes (GeneScreen; NEN, Boston, MA) using standard procedures and then hybridized with 32P-labeled DNA fragments. As an Syl probe, we used the 1.1-kb BamHI-Bg/II fragment (nucleotides 537–1674, MUSHIGAND) in the 5′ region of the Sμ repeats from pyl/EH100.0 (47); the Sμ probe was a 99-bp PCR-amplified fragment (nucleotides 1210–1308, MUSIGAND09) starting directly downstream of the Sμ region. Blots were analyzed with a Fujix BAS1000 Bioimaging Analyzer (Fuji Photo Film, Tokyo, Japan).

Cloning and Sequencing

PCR products were cloned using the TA cloning method according to the manufacturer’s instructions (Invitrogen, San Diego, CA). Sequencing was performed using the ALF Express DNA Sequencer System (ALF Express Auto Cycle Sequencing Kit; Pharmacia Biotech, Uppsala, Sweden). We used the universal M13-40 primer, M13 reverse primer, or appropriate specific primers synthesized based on available sequences as follows: MUSIGAND 09 (1461 bp, Sμa) and MUSIGHAND (8966 bp, Syl).

Results

Stimulation of slgD+ B cells with CS/2 and IL-5 induces production of IgG1

We previously reported that CD38 ligation by CS/2 in mouse B cells induces proliferation and tyrosine phosphorylation of Btk and enhances surface expression of IL-5Rα (35). CS/2 and IL-5 act synergistically to elicit remarkable levels of B-cell proliferation, blimp-1 gene expression, and IgG1 and IgM secretion (35, 36). Because of the dual effects of IL-5 on B cell proliferation and differentiation, IgG1 secretion induced by CS/2 plus IL-5 could result from either isotype switch recombination or expansion of a pool of IgG1-committed B cells driven to differentiate toward plasma cells. To investigate in more detail the role of CS/2 and IL-5 in IgG1 secretion, we purified slgD+ B cells as naive B cells from spleen. The purity of this population was >99%, and it contained less than 0.1% slgG1+ B cells. The cells thus obtained were cultured for 7 days with either CS/2, IL-5, or CS/2 plus IL-5. After the culture period, the quantities of Abs secreted into the culture supernatants were estimated by ELISA. Stimulation of slgD+ B cells with either CS/2 or IL-5 alone did not induce Ig production (Table I). Consistent with earlier results, slgD+ B cells stimulated with CS/2 plus IL-5 produced IgM and IgG1 and, to a lesser extent, IgG2b and IgG3.

The above result suggests that IgG1 production elicited by culturing slgD+ B cells in the presence of CS/2 plus IL-5 does not reflect simply the expansion and differentiation of a preexisting pool of slgG1+ B cells. To confirm this conclusion further, we
examined the capacity of IL-5 and IL-4 to induce surface IgG1 expression in splenic B cells with or without CS/2 stimulation. It was observed that neither CS/2 alone nor CS/2 plus IL-4 induced significant increases in the number of B cells expressing surface IgG1 (Fig. 1). On the other hand, stimulation with CS/2 plus IL-5 elicited significant increases (4.1%; p < 0.01) in the numbers of slgG1+ B cells. Thus, we conclude that IL-5-induced IgG1 production does not result from the expansion of a pool of IgG1-committed B cells driven to differentiate toward IgG1-producing plasma cells.

Since IL-4 is known to be an IgG1-switching factor, it is possible that, in our system, endogenously produced IL-4 might also be involved in CS/2- and IL-5-dependent IgG1 production. B cells from STAT6-deficient mice do not respond to IL-4 (39). To evaluate a possible involvement of IL-4 in our culture system, therefore, we cultured splenic B cells from STAT6-deficient mice and their wild-type littermates with CS/2 plus IL-5 for 7 days; as a control, the cells were also stimulated with anti-CD40 mAb (LB429) plus IL-4. As can be seen in Table II, IgG1 production was induced in B cells from STAT6−/− mice by both LB429 plus IL-4 and CS/2 plus IL-5. B cells from STAT6−/− mice responded similarly to CS/2 plus IL-5 but were insensitive to LB429 plus IL-4, clearly indicating that IgG1 production induced by CS/2 plus IL-5 was IL-4 independent. We also found that naive B cells from IL-4-deficient mice also produced IgG1 in response to CS/2 plus IL-5 (data not shown).

**Induction of germline γ1 transcripts in B cells by CD38 ligation**

Ig isotype switching in B cells is preceded by cell proliferation and transcription of the corresponding unrearranged or constant region gene of the germline heavy chain (7, 8). To clarify the role of signaling from CD38 and IL-5R in the preferential induction of IgG1, we examined the expression of germline γ1 transcripts following stimulation with CS/2 alone, CS/2 plus IL-5, IL-4, and CS/2 plus IL-4 in 3-day-old cultures. The results revealed that, before culture, slgD+ B cells did not exhibit significant expression of germline γ1 gene transcripts (Fig. 2). B cells cultured in the presence of CS/2 exhibited significant induction of germline γ1 and γ3 transcripts (Fig. 2A) but no induction of the γ2a, α, or ε transcripts was observed (data not shown). IL-5 neither induced detectable levels of germline γ1 transcripts (data not shown) nor enhanced the expression of transcripts induced by CS/2 on day 2 (Fig. 2A) and on day 3 (data not shown). In contrast, IL-4 significantly induced germline γ1 transcription and enhanced the expression of germline γ1 transcripts in CS/2-stimulated B cells (Fig. 2, A and B). The capacity of IL-5 to induce expression of IgG1 but not germline γ1 transcription in CS/2-activated B cells suggests that IL-5 plays an important role in mediating γ1 switch rearrangement of the HC gene.

**IL-5 induces γ1-μ switch recombination in CD38-activated slgD+ B cells**

The looping-out and deletion model of switch recombination predicts that, during the course of joining the 5′ segment of Sμ to the 3′ segment of Sγ1, intervening DNA between switch regions is excised as a circle (Fig. 3A; Refs. 20–23). At least some of the deleted DNA fragments circularize to form reciprocal Sγ1-Sμ junctions that are potential targets for PCR amplification. In the absence of replication origins, these circles would not be replicated during cell division. Thus, after expansion of switched cells in culture, each switch recombination event should be represented by multiple copies of the chromosomal Sμ-Sγ1 junction, but by only a single reciprocal circle. The content of reciprocal Sγ1-Sμ junctions, therefore, should reflect the frequency of switch recombination events regardless of subsequent proliferation.

Initially, we devised a method to detect γ1-μ circular DNA by PCR. We set up a 5′ primer in the 5′ region of the Sγ1 repeats and a 3′ primer in the 3′ region of Sμ repeats. Total DNA from cells was obtained after 3 days of culture in the presence of LB429 plus IL-4; the DNA was amplified by PCR and subjected to hybridization with the 5′ Sγ1 probe and thereafter with the 3′ Sμ probe.
digested PCR fragments, we detected a single 1.4-kb band with the products. A and amplified (9) cultured for 3 and 5 days in the presence of CS/2, CS/2 plus IL-4, 9 and Bgl II generation of S m region of the S gion and a Hinc II site (position 1674, MUSIGHANB) in the 5 S yl repeat. PCR products digested by Hinc II or HindIII were hybridized to our 5 S yl probe and then to the 3 S m probe. With the HincII-digested PCR fragments, we detected a single 1.4-kb band with the S yl probe (Fig. 3C), whereas, with the HindIII-digested PCR fragments, the 3 S m probe detected a single band of about 140 bp. These findings show that all of the PCR products hybridized with our 5 S yl and 3 S m probes and had the expected 5 S yl–3 S m structure.

Using this primer pair and the S yl probe, we analyzed the generation of S yl-S m reciprocal products by naive slgD+ B cells cultured for 3 and 5 days in the presence of CS/2, CS/2 plus IL-4, CS/2 plus IL-5, or CS/2, IL-5 and IL-4. Total DNA (200 ng) was amplified by PCR and hybridized with the 5 S yl or 3 S m probe. Three independent amplifications were performed on identical aliquots of DNA template to improve detection of rare events and assess reproducibility. As shown in the upper panel of Fig. 4, few if any reciprocal S yl-S m junctions were amplified in unstimulated (day 0) slgD+ B cells, and only small numbers of amplified products were detected from cells cultured with CS/2 and CS/2 plus IL-4. On the other hand, the quantity of γ1-μ switch circles (ranging from 2 to 10 kb) was substantially increased in cells cultured in the presence of CS/2 plus IL-5, indicating that IL-5 induces μ to γ1 switch recombination in B cells stimulated with CS/2.

When the blots were stripped, reprobed with the 3 S m probe (see Materials and Methods), and autoradiographed again, the resultant images were virtually identical to those previously obtained using the 5 S yl probe (Fig. 4; compare upper and lower panels), indicating that virtually all of the amplified products contain both the 5 S yl and 3 S m segments. Three independent amplifications using aliquots of the same DNA preparations showed multiple bands with different sizes in each replicate sample, which is consistent with amplification of multiple single-copy templates with S m and S yl recombination breakpoints in different positions. The variation in intensity among bands may be explained by the variable amplification efficiency of different template sequences or by comigration of similar sized bands.

To verify the identity of the PCR products, we cloned the amplified DNA segments prepared from B cells cultured for 6 days with CS/2 plus IL-5 and performed a sequence analysis on 12 randomly selected 5 S yl-positive clones. The clones all contained the 5 S yl and 3 S m sequences in a 5’-3’ orientation (Table III). Among them, we could determine the nucleotide sequence of γ1-μ switch junction in six clones, because the junction was too far from the ends of the cloned segments. These sequence analyses confirmed the interpretation that the amplified bands in Figs. 3C and 4 represent the exerted 5’ S yl-3’ S m structure.

Comparison of IL-5 and IL-4 in promoting switch recombination

Given that IL-5 induces switch recombination to IgG1 in CS/2-stimulated slgD+ B cells, we wished to compare its effects with those of IL-4, which is known to promote switching to IgG1 but has also been reported to induce IgE expression (10, 11). We first compared the number of γ1-μ switch circles in B cells stimulated with CS/2, IL-5, and IL-4 with those stimulated with CS/2 plus IL-5. The number of γ1-μ switch circles was approximately doubled when slgD+ B cells were stimulated with a combination of CS/2, IL-5, and IL-4 (Fig. 4). We described earlier how IL-5 increased the percentage of slgG1+ B cells among CS/2-activated B cells (4%: Fig. 1). Addition of IL-4 together with CS/2 plus IL-5 increased the percentage of slgG1+ cells up to 11% (data not shown). The proportion of Ab-forming cells (AFCs) in the B cell cultures was examined by staining with anti-Syndecan-1 mAb, and IgG1 in the culture supernatants was quantified after culture periods of various duration. The numbers of AFC (Syndecan-1+ cells) and mean fluorescence intensity of Syndecan-1 increased upon stimulation with CS/2 plus IL-5, and this response was augmented by addition of IL-4 (Fig. 5A). Moreover, in the presence of the combination of CS/2, IL-5, and IL-4, production of IgM and IgG1 was increased at every observed time point during the culture period (Fig. 5B). These results suggest that IL-4 increases the frequency of HC switch recombination from γ1-μ and that IL-4 enhances differentiation of B cells stimulated with CS/2 plus IL-5 into IgG1-secreting cells.

We next examined the ability of IL-5 and IL-4 to promote HC switch recombination from IgM to IgE in CS/2-stimulated B cells. Stimulation of B cells with neither CS/2 plus IL-4 nor a mixture of CS/2, IL-4, and IL-5 induced detectable levels of IgE production (<0.3 ng/ml), although both stimulations induced germline e transcripts (data not shown). Then we devised a method to detect e-μ circular DNA by PCR and analyzed the generation of Se-S m reciprocal products in B cells stimulated with CS/2 plus IL-4, a mixture of
CS/2, IL-4, and IL-5, or with LB429 plus IL-4. IL-4 induced \( \mu-e \) switch recombination in LB429-stimulated B cells, while it was incapable of inducing \( \mu-e \) DNA recombination in B cells stimulated with CS/2 (data not shown). Furthermore, IL-5 did not induce \( \mu-e \) switch recombination in B cells stimulated with CS/2 plus IL-4 (data not shown). These results suggest that IL-5 preferentially induces \( \mu-Y1 \) switch recombination and IgG1 production.

Discussion

We studied the molecular mechanisms of IgG1 production by sIgD \(^+\) B cells stimulated with CS/2 and IL-5 and made four intriguing observations. 1) Stimulation of naive (sIgD \(^+\)) B cells with IL-5 and CS/2 induces production of IgG1 and, to a lesser extent, IgG3 and IgG2b; among the various cytokines tested, only IL-5 had this synergistic effect on IgG1 production with CS/2. 2) By itself, CD38 ligation with CS/2 induced significant expression of sterile \( Y1 \) transcripts, whereas IL-5 was incapable of inducing the sterile \( Y1 \) transcripts. 3) In CS/2-stimulated sIgD \(^+\) B cells, IL-5 enhanced the cellular content of reciprocal products forming DNA circles as byproducts of \( \mu-Y1 \) recombination; the amplified products of the DNA circles all contained 5’-Sy1 and 3’-Sm sequences in a 5’-3’ orientation. 4) Stimulation with IL-4 in combination with CS/2 plus IL-5 further enhanced expression of sterile \( Y1 \) transcripts and IgG1 production. These results demonstrate that IL-5...
promotes \( \mu \)-\( \gamma_1 \) switch recombination and terminal differentiation of \( \text{sIgG}^+ \) B cells into IgG1-producing cells in an IL-4-independent manner. Nevertheless, IL-4 enhanced IgG1 production by \( \text{sIgG}^+ \) B cells stimulated with CS/2 plus IL-5 by increasing induction of \( \gamma_1 \) sterile transcripts and terminal differentiation.

DNA synthesis, expression of sterile transcripts of a particular Ig isotype, and switch recombination are all known to be required for the Ig isotype switch. At the molecular level, the predominant mode of Ig isotype switching consists of a recombination event that includes looping out and deletion of all HC genes, with the exception of the one that is to be expressed (19–23). In \( \text{sIgM}^+ \) B cells, the switch recombination event occurs between the \( \text{S} \) and the \( \text{S} \) regions of the downstream HC gene. In the mouse, LPS induces IgG3 and IgG2b class switching, even in the absence of cytokines. Addition of T cell cytokines (e.g., IL-4) to LPS cultures suppresses switching to IgG3, while switch recombination to IgG1 is induced. This system has been used extensively to study isotype switching in vitro: in LPS-stimulated B cells, IL-4 stimulates expression of IgE and IgG1; IFN-\( \gamma \) stimulates expression of IgG2a; and TGF-\( \beta \) promotes expression of IgA and IgG2b (16). Stimulation of mouse B cells with CD40 plus IL-4 induces IgG1 and IgE (48).

Three methodologies have been utilized to assess Ig isotype switching: 1) semiquantitative RT-PCR for comparative assessment of germline HC RNA expression; 2) digestion circularization-PCR (DC-PCR) for quantifying specific switch rearrangement events at the level of the DNA (49); and 3) flow cytometric analysis of the B cell populations expressing various Ig isotypes as a consequence of in vitro activation. In the present study, we amplified deleted circular DNA fragments containing reciprocal Syl-S\( \mu \) junctions to detect switch recombination, since the cellular content of reciprocal Syl-S\( \mu \) junctions should reflect the frequency of switch recombination events regardless of subsequent proliferation.

By itself, CS/2 stimulated naive B cells to synthesize DNA. Despite the capacity of CS/2 to induce germline \( \gamma_1 \) transcription in cultured naive B cells (Fig. 2), however, no corresponding increases in the percentage of \( \text{sIgG}^+ \) cells or \( \text{S} \mu \)-S\( \gamma_1 \) rearrangement events were observed. Surprisingly, further addition of IL-5 strongly induced \( \text{S} \mu \)-S\( \gamma_1 \) rearrangement, as well as the appearance of \( \text{sIgG}^+ \) cells (Figs. 1 and 4); moreover IL-5 elicited this effect without altering the levels of germline \( \gamma_1 \) transcripts on day 2 (Fig. 2) and on day 3 (data not shown). In addition, in the absence of CS/2-mediated targeting of the HC\( \gamma_1 \) gene, IL-5 failed to induce switching to IgG1. IL-5 responsiveness appears to be influenced by CS/2, such that more cells are responsive to undergoing proliferation and switch recombination, because CS/2 stimulation enhances the IL-5R expression on B cells (35). These results demonstrate that, while the signals mediated by CD38 ligation are essential, they are not sufficient to evoke substantial \( \text{S} \mu \)-S\( \gamma_1 \) rearrangement. Furthermore, results support the notion that IL-5 induces \( \text{S} \mu \)-S\( \gamma_1 \) switch recombination. It still remains unclear whether IL-5 induces Ig isotype switching directly or indirectly. Our work confirms and extends analogous work by Mandler et al. (50) and Purkerson and Isakson (51), which demonstrate IL-5 activity on Ig isotype switch recombination. The system used by Mandler et al. (50) consisted of dextran-conjugated anti-IgD Ab (anti-\( \delta \)-dex)-activated B cells that were stimulated with IL-4 and IL-5. In that case, although anti-\( \delta \)-dex Abs stimulated resting B cells to synthesize DNA and IL-4 induced germline \( \gamma_1 \) transcription in anti-\( \delta \)-dex-activated B cells, no corresponding increase in the percentage of \( \text{sIgG}^+ \) cells or \( \text{S} \mu \)-S\( \gamma_1 \) rearrangement events

![FIGURE 5. Costimulatory effect of IL-4 on induction of AFC and Ig production in B cells activated with CS/2 plus IL-5. A, Splenic B cells were cultured for 5 days in the presence of CS/2, CS/2 plus IL-4, CS/2 plus IL-5, or the combination of CS/2, IL-4, and IL-5. The cells were stained with FITC-labeled B220 mAb and biotinylated anti-Syndecan-1 mAb plus PE-av. The percentages of Syndecan-1\(^{+} \) B220\(^{+} \) cells in each sample are given in the upper right corner of the respective panels. B, Splenic B cells were cultured for various periods of time in the presence of CS/2 (filled squares), CS/2 plus IL-4 (open squares), CS/2 plus IL-5 (filled circles), or the combination of CS/2, IL-4, and IL-5 (open circles). IgM and IgG1 levels in culture supernatants were determined by ELISA.](http://www.jimmunol.org/)

### Table III. Cloned amplified DNA\(^{a}\)

<table>
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<th>Clone</th>
<th>PCR Size (kb)</th>
<th>Switch Recombination</th>
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<td>SC2</td>
<td>2.8</td>
<td>( \gamma_1/\mu )</td>
<td>No</td>
</tr>
<tr>
<td>SC3</td>
<td>5.0</td>
<td>( \gamma_1/\mu )</td>
<td>Yes</td>
</tr>
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</tr>
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<td>SC28</td>
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\(^{a}\)The amplified DNA segments were cloned by the TA cloning methods and subjected to hybridization with the 5'.Syl probe. Twelve 5'.Syl-positive clones were randomly selected, and the structures of the amplified DNA were analyzed by DNA sequencing.
was observed. Parkerson and Isakson used Separose coupled anti-IgM-activated B cell blasts that were stimulated with LPS plus IL-4. In their system, IL-5 was required for production of IgG1 and IgE, but germline γ1 and e RNA expression was not (51). A difference between our experimental protocols and those described above is that IL-4 was not required in our system but was required in theirs. Consequently, because use of IL-4 could be avoided, we were able to more directly examine the role of IL-5 in Ig class switching.

We and others have reported that, in contrast to humans, CD38 is expressed in both primary follicular B cells and follicular mantle B cells in the spleens of immunized mice but is down-regulated in GC B cells (28, 29). Stimulation of mouse GC B cells with CS/2 and IL-5 did not induce B cell proliferation or IgG1 production, whereas stimulation of non-GC B cells with CS/2 and IL-5 induced both cell proliferation and IgM and IgG1 production (36), suggesting that isotype switching occurs in B cells outside of the GC under certain conditions. If that is the case, CD38 ligation by a natural ligand for CD38 and by cytokines including IL-5 may play a key role in isotype switch recombination.

IL-5 was originally defined as a cytokine by ourselves and others based on its ability to promote B cell (particularly B-1 cell) growth and by its role in stimulating maturation of B cells into AFC in an Ig-isotype-independent manner (37, 52–54). In addition, we showed that IL-5 up-regulates IgM, IgG1, and IgA, at least in part, by preferential processing of RNA for the secretory form of HC over that of the membrane form (55). The data presented here, however, confirm an additional role for IL-5 in promoting switch recombination of HC genes. Confirmation of a more general capacity of IL-5 to stimulate switch recombination will await further studies, in which DNA circle formation will be used as an index of switch recombination to analyze HC gene rearrangement for other isotypes. As described, stimulation of B cells with a mixture of CS/2, IL-4, and IL-5 did not induce either μ-ε switch recombination or IgE production, although germline e transcripts were detected (data not shown). In contrast, stimulation with anti-CD40 mAb plus IL-4 could induce μ-ε switch circles and IgE production. These results indicate that IL-5 does not induce μ-ε switch recombination in B cells activated with CS/2 plus IL-4, although stimulation by CS/2 plus IL-4 induces sterile e transcripts. There are at least three possibilities that could account for this result. First, insufficient levels of sterile e transcripts were induced by CS/2 plus IL-4 to elicit switch recombination by IL-5. Second, sterile e transcripts induced by CS/2 plus IL-4 were not sufficient, by themselves, to elicit IL-5-dependent switch recombination; rather CS/2-induced sterile HC gene expression might also be required. Third, IL-5 might preferentially switch μ to γ1 and not μ to e. Further analysis will be required to address these issues.

Although the molecular mediators responsible for effecting switch recombination events are unknown, potential candidates include a variety of regulatory proteins that participate in DNA replication, repair, and/or recombination and whose expression can be regulated. One such potential mediator of isotype switch recombination is the Ku protein complex (56). This heterodimer composed of Ku70 and Ku80 subunits binds in a sequence-independent manner to double-strand breaks, nicks, gaps, and hairpins in DNA and has been implicated in mediating repair of DNA breaks, as well as V(D)J recombination (57, 58). Once bound to DNA, the Ku protein complex binds and activates the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs). A defective DNA-PKcs gene underlies the SCID mutation (59), which exhibits impaired switch recombination (60). B cells that are deficient in Ku70 or Ku80 are unable to undergo Ig HC class switching (61, 62).

These results indicate that DNA-PKcs is a key mediator of switch rearrangement. In anti-6-dex-activated B cells, IL-5 has been shown to elicit double-strand breaks in the Sy3 region of DNA (63) and to variably enhance Ku expression (64). If the level of expression of the functional Ku complex in B cells can be regulated by IL-5 during activation of CS/2-stimulated B cells, this would represent a potential pathway for regulating Sµ-Sγ1 recombination by IL-5 independent of alterations in germine HC transcription.

In conclusion, we have demonstrated that IL-5 induces Sµ-Sγ1 recombination in CS/2-activated murine B cells independent of IL-4. CS/2 induces germine y1 transcripts, an effect widely regarded as necessary for targeting the HCγ1 gene for recombination. Elucidation of a more general capacity of IL-5 to stimulate switch recombination and the precise mechanism by which IL-5 mediates Ig switch recombination await further study.

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