

Interleukin-10 Induces Immunoglobulin G Isotype Switch Recombination in Human CD40-Activated Naive B Lymphocytes

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Summary

Upon activation, B lymphocytes can change the isotype of the antibody they express by immunoglobulin (Ig) isotype switch recombination. In previous studies on the regulation of human IgG expression, we demonstrated that interleukin 10 (IL-10) could stimulate IgG1 and IgG3 secretion by human CD40-activated naive (sIgD⁺) tonsillar B cells. To assess whether IL-10 actually promotes the DNA recombination underlying switching to these isotypes, we examined the effect of IL-10 on the generation of reciprocal products that form DNA circles as by-products of switch recombination. The content of reciprocal products characteristic of μ - γ recombination was elevated after culture of CD40-activated tonsillar sIgD⁺ B cells with either IL-4 or IL-10, although high levels of IgG secretion were observed only with IL-10. Unlike IL-4, IL-10 did not induce reciprocal products of μ - ϵ and γ - ϵ switch recombination. These results demonstrate that IL-10 promotes both switching to γ and IgG secretion.

IgM is the first Ig secreted by a naive B lymphocyte in response to antigen stimulation. IgM is encoded by a functional variable gene (VDJ) and the constant region C μ gene. The heavy chain variable domain (V_H) confers antigen specificity, whereas the constant region (C_H) confers effector functions to the Ig molecule. As B lymphocytes differentiate, they can substitute IgG, IgE, or IgA for the initially secreted IgM by a process known as isotype switching (1, 2); this permits B cells to express antibodies with unaltered antigen specificity but with different effector functions. The isotype switch is mediated by a DNA recombination that moves the VDJ gene from its initial position upstream of the C μ gene, deleting the DNA between the recombination breakpoints. These breakpoints occur within (or near) internally repetitive sequences, known as switch (S)¹ regions, that lie upstream of each C_H gene (except δ ; 2–4). The recombination generates a hybrid switch region composed of the 5' part of S μ joined to the 3' part of the switch region associated with the new C_H region. The deleted DNA can recircularize to form "switch circles" or reciprocal recombination products that

contain the 3' part of S μ joined to the 5' part of the S region of the new isotype (5–8).

Isotype switch recombination is a highly regulated process, controlled by soluble cytokines and by T cell membrane interaction with the CD40 molecule on the B cell surface (9, 10). In humans, switching to IgE and IgG is stimulated by the cytokine IL-4, whereas IgA expression is promoted by TGF- β 1 (11–15). However, cytokine regulation of IgG subtype expression is less well understood. We previously showed that IL-10 induces sIgD⁺ naive CD40-activated B cells to secrete IgG1 and IgG3 (16). However, because IL-10 is known to promote differentiation of B cells and to enhance Ig synthesis by CD40-activated isotype-committed cells (17, 18), we could not formally conclude from these experiments that IL-10 was directly promoting switch recombination to C γ genes. In the present work, we have examined this question by assessing the effect of IL-10 on reciprocal circles produced by cultured B cells undergoing μ - γ switch recombination. Switch circles are expected to exist only transiently in the B cell population as they would not contain replication origins and centromeres. Proliferation makes the copy number of circular DNA decrease relative to the number of cells. Therefore, these circles represent a reliable marker of in vitro switch recombination. Using this method, we compared the capacity of IL-10 and IL-4 to induce isotype switching and/or differentiation into IgG-secreting B lymphocytes.

¹Abbreviations used in this paper: CD40L, human ligand for CD40; LCD40L, CD40L-transfected L cell; S, switch; sCD40L, soluble human CD40L.

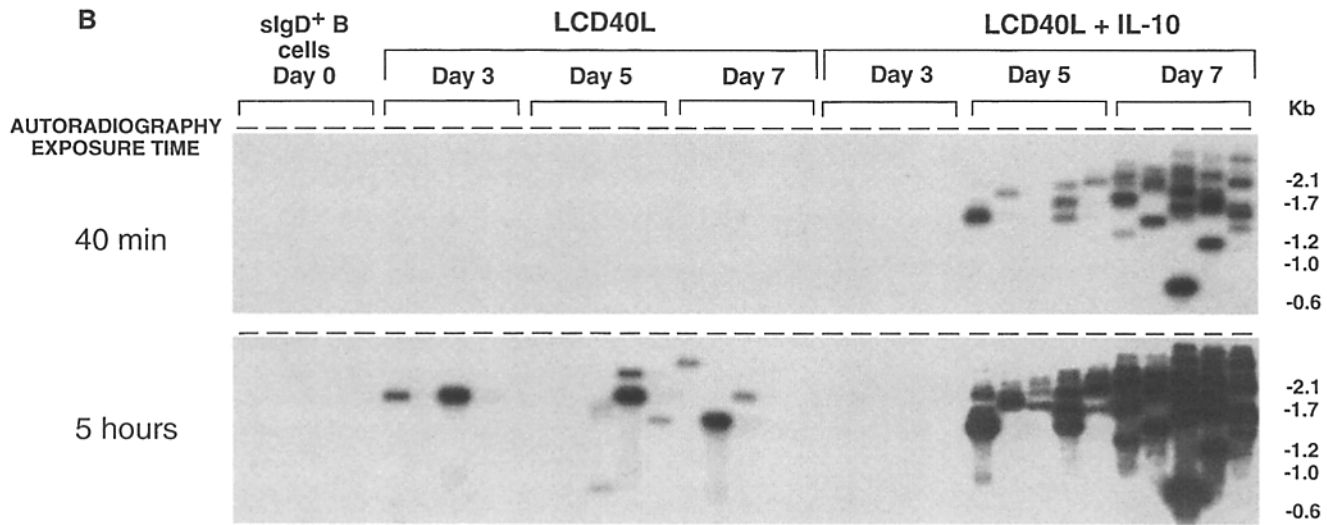
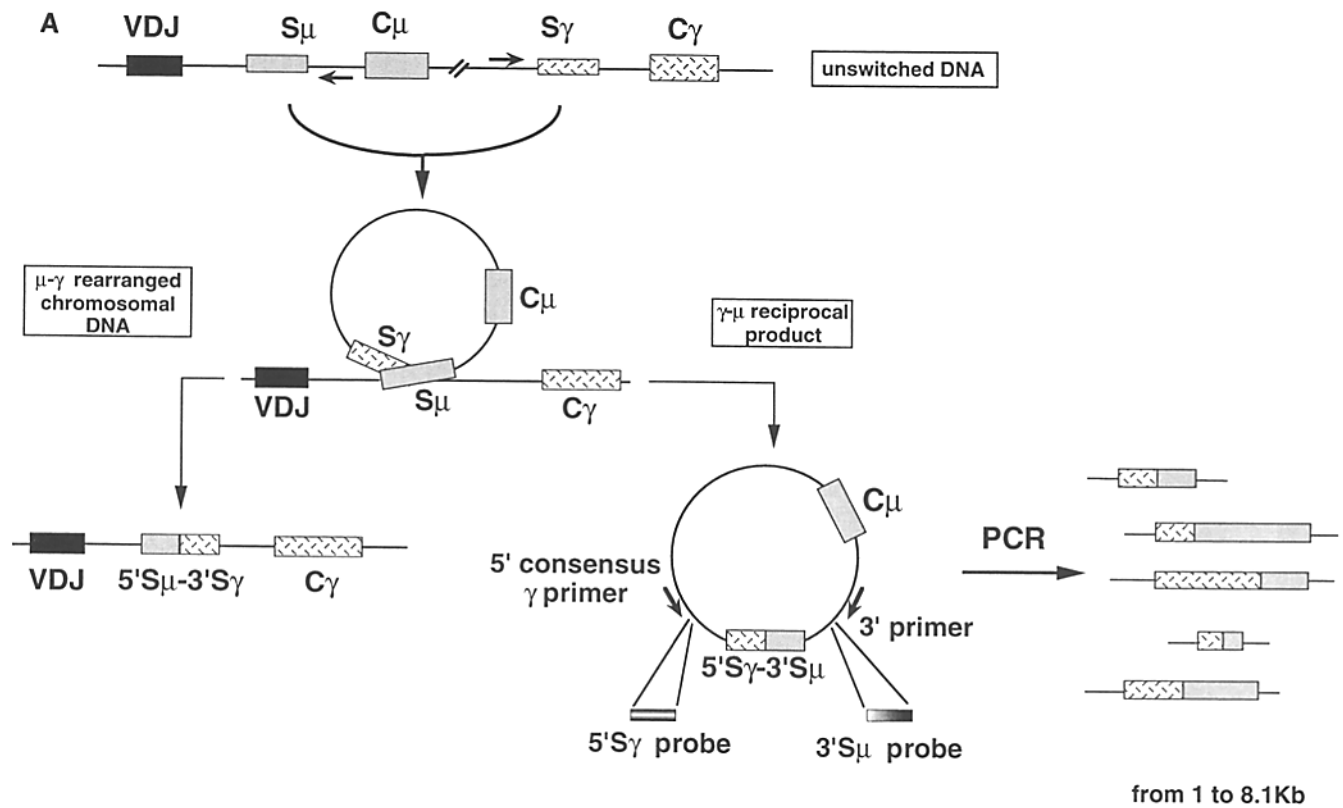
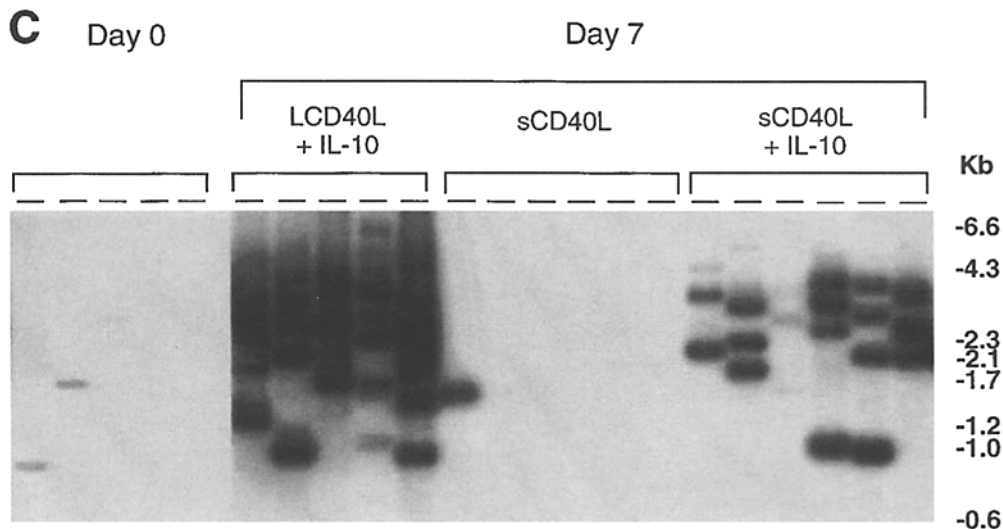


Figure 1. PCR analysis of $S\gamma$ - $S\mu$ switch circles. (A) Schematic $S\gamma$ - $S\mu$ switch circle formation and localization of PCR primers and probes. (B) 100 ng of total DNA from either freshly isolated $sIgD^+$ B cells or $sIgD^+$ cells cultured 3, 5, or 7 d on CD40L-transfected L cells (*LCD40L*) in the absence or presence of IL-10, was amplified with 5' $S\gamma$ and 3' $S\mu$ primers using Taq polymerase and hybridized with the 5' $S\gamma$ probe as depicted in A. (C) $sIgD^+$ B cells were cultured on CD40L-transfected L cells (*LCD40L*) or with soluble CD40L (*sCD40L*) in the absence or presence of IL-10 as described in Materials and Methods. 500 ng of total DNA from cells cultured 7 d under different conditions was amplified with 5' $S\gamma$ and 3' $S\mu$ primers using rTth DNA polymerase, XL, and hybridized with the 5' $S\gamma$ probe as depicted in A. Autoradiographs were exposed for 4 h.

Materials and Methods

Reagents. Purified human IL-4 (10^7 U/mg) and IL-10 (2×10^7 U/mg) were obtained from Schering-Plough Research Institute (Kenilworth, NJ) and were used at 50 U/ml and 200 ng/ml, respectively. FITC-conjugated anti-IgM, -IgD, and control mAbs were from Dako (Glostrup, Denmark) and anti-IgG from

Kallestad (Austin, TX). FITC-conjugated rat anti-mCD8 α mAb was obtained from Boehringer Mannheim (Mannheim, Germany). The rat anti-mouse CD8 α mAb (clone 53.6.72) was a kind gift from Dr. A. Zlotnik (DNAX, Palo Alto, CA) and was used as culture supernatant.



Isolation of sIgD⁺ B cell Population. Tonsillar B cells were purified as described earlier (12). Briefly, mononuclear cells separated by standard Ficoll-Hypaque gradient were first rosetted with sheep red blood cells. Nonrosetting cells were further incubated with anti-CD2, -CD3, and -CD14 mAbs (Becton Dickinson & Co., Mountain View, CA) and submitted to negative selection performed with magnetic beads coated with anti-mouse IgG (Dyna-beads; Dynal, Oslo, Norway). Naive B lymphocytes were then labeled with biotinylated goat anti-IgD antibody (Sigma Chemical Co., St. Louis, MO) and streptavidin microbeads (Miltenyi Biotec GmbH Bergisch, Gladbach, Germany) and were separated using a preparative magnetic sorter (MACS[®], Miltenyi Biotec) as described earlier (12). IgD was expressed on >99% of the sIgD⁺ B cell subpopulation, as assessed by fluorescence analysis using a FACScan[®] (Becton Dickinson & Co.).

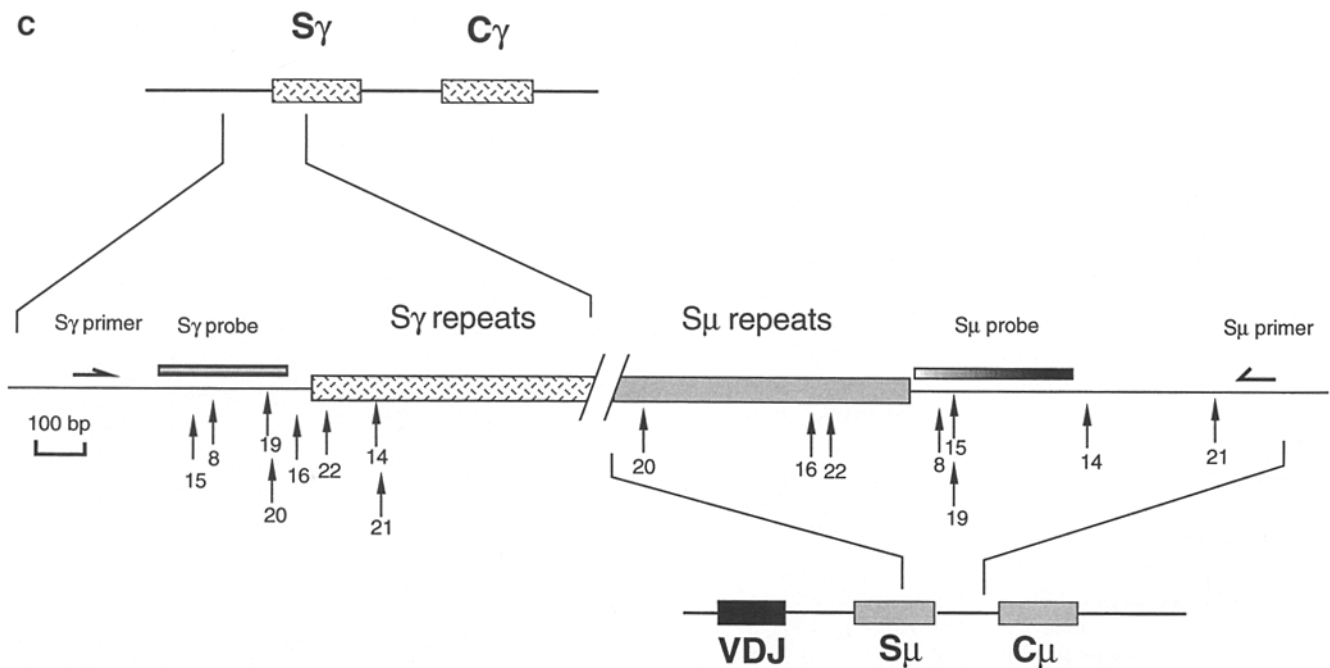
B Cell Cultures. All cultures were performed in Iscove's medium, enriched with 50 µg/ml human transferrin, 5 µg/ml bovine insulin, 0.5% BSA, 5 × 10⁻⁵ M 2-β mercaptoethanol (all from Sigma Chemical Co.), and 5% heat-inactivated fetal bovine serum (GIBCO BRL, Gaithersburg, MD). Naive sIgD⁺ B cells were cultured in flat-bottomed 24-well plates at a density of 10⁵ cells/well, in a final volume of 1 ml. When indicated, either IL-10 (200 ng/ml) or IL-4 (50 U/ml) was added at the onset of the cultures. Cultures of naive sIgD⁺ B cells were performed either on irradiated (7,000 rad) murine Ltk⁻ L cells (10⁴ cells/well) stably transfected with the human ligand for CD40 (CD40L), or with soluble CD40L (sCD40L) composed of the extracellular domain of human CD40L fused to the extracellular domain of murine CD8α. CD40L transfections and sCD40L construction were previously described (19). sCD40L was used as Cos cell supernatant (12% vol/vol), and in order to cross-link this molecule, a rat anti-murine CD8α antibody was added (0.2% vol/vol of culture supernatant). Specific binding of sCD40L was assessed: the same percentages of CD40-positive B cells were detected with either FITC-conjugated anti-CD40 mAb (mAb89 produced in our own laboratory; 20) or sCD40L revealed by FITC-conjugated rat anti-murine CD8α. Cells were harvested at the indicated times and surface expression was determined at day 7 by fluorescence staining. Secretion of IgM, IgG, and IgE at day 14 was determined by standard ELISA techniques as previously described (21, 22).

Preparation of Genomic DNA. Total DNA containing both genomic and circles was extracted by standard procedures (23).

Briefly, cells were lysed in 10 mM Tris, pH 8, 10 mM EDTA, and 1% SDS. Proteinase K was added to a final concentration of 0.1 mg/ml, and the samples were incubated for 3–6 h at 37°C. DNA was then extracted with phenol and chloroform, followed by ethanol precipitation, after which the samples were resuspended in 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA. DNA samples were then digested overnight at 37°C with RNase (RNAce it cocktail; Promega, Madison, WI) in order to degrade RNA, and in combination with EcoRI to reduce the viscosity of the DNA, thus facilitating reproducible pipetting. Digestion with EcoRI does not alter the amplification of S-S junctions as described below since none of the human switch regions (S_μ, S_γ, or S_ε) contain internal EcoRI sites (24). DNA concentrations were measured by spectrophotometry, and aliquots were used for the amplification of reciprocal products.

PCR Amplification of DNA Switch Circle Fragments. For γ-μ amplifications, 100 or 500 ng, as indicated, of total DNA from unstimulated or cultured sIgD⁺ B cells, or either 1 or 10 copies of the artificial construct plasmids (data not shown) was amplified in 25-µl PCR reactions. These control plasmids were constructs containing ~2-kb 5' S_γ segments from S_γ1 (HindIII-PstI), S_γ2 (HindIII-HaeIII), S_γ3 (HindIII-PstI), or S_γ4 (HindIII-Bsu36I), ligated to a 3' 0.9-kb SstI-EcoRI fragment containing the 3' portion of S_μ. The amplifications contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1 mM MgCl₂, 200 nM of each of the four deoxynucleotide triphosphate (dNTPs), 5% DMSO, 0.625 U of Amplitaq Polymerase (Perkin-Elmer Cetus, Branchburg, NJ), and 100-nM concentrations of sense-strand 5' S_γ primer (5' AAGAGTCCAGG-GAGGCCAGAAAGGCCAG 3', nucleotides 1193-1222 in the consensus 5' S_γ sequence, a segment 100% conserved among all the human S_γ regions; reference 25, and see Fig. 2 A), in combination with an antisense 3' S_μ primer (5' TGAGTGCCCTCACTACTTGCCTCCCG 3', nucleotides 4373-4345 in the region 3' of the S_μ repeats; reference 26, and see Fig. 2). Reciprocal γ-μ products were amplified using 35 cycles of 1-min melting at 94°C, 2-min annealing at 72°C, and 6-min extensions at 72°C.

For ε-μ, ε-γ, and γ-μ amplifications, when indicated, amplifications were performed using 500 ng of DNA in 25-µl volumes containing 1 U of XL rTth DNA polymerase (Perkin-Elmer Cetus) with 1× buffer supplied by the manufacturer, 1 mM magnesium acetate, and 100-nM concentrations of a sense 5' S_ε primer (5' GCTGATCTTGCAAGTCCGAGCTGGGCGACTG 3',



nucleotides 1041-1072 in the S ϵ sequence; reference 26, and see Fig. 4) used in combination with either the antisense 3' S μ primer described above in the protocol for γ - μ reciprocal product amplification or an antisense 3' S γ primer (5' CCTGCCTC-CCAGTGCCTGCATTACTTCTG 3', nucleotides 747-776 in the consensus 3' S γ sequence; reference 25, and see Fig. 2 C). Amplifications were carried out using 35 cycles of 30-s melting at 94°C, 2-min annealing at 72°C, and 6-min extensions at 72°C.

Southern Transfer and Hybridization. PCR products were transferred onto nylon membrane (Hybond-N; Amersham International, Amersham, Bucks, UK) by standard procedures (23) and hybridized with a specific internal probe. For 5' S γ and 3' S μ flanking regions, the probes were generated by PCR. The S μ probe spans a segment localized between nucleotides 3711 and 4041 in the 3' region of S μ repeats (26, and see Fig. 2). The S γ probe spans a segment localized between nucleotides 1280 and 1546 of the S γ 1 flanking region (25, and see Fig. 2, A and B). The 3' S γ probe is a 339-bp PstI fragment 3' to S γ 4 repeats (26). Hybridization was performed overnight at 65°C, in 2 \times SSPE (1 \times SSPE is 20 mM phosphate buffer, pH 7.4, 300 mM NaCl, and 2 mM EDTA), 1% SDS with ³²P-labeled probes. Blots were then washed for 30 min in 2 \times SSPE, 1% SDS at room temperature; 30 min in 2 \times SSPE, 1% SDS at 65°C, and 20-30 min in 0.1 \times SSPE, 1% SDS at 65°C. Blots were then autoradiographed.

Cloning and Sequencing. PCR products were cloned by the TA cloning method as provided by the manufacturer (Invitrogen, San Diego, CA). Sequencing was performed using both the Taq-DyeDeoxy™ termination and Dye-primer™ Cycle sequencing kits and was analyzed on an automatic DNA sequencer (model 373A; both from Applied Biosystems, Inc., Roissy, France).

Results

IL-10 Induces IgG Switch Recombination in CD40-activated sIgD⁺ B Cells.

Rationale. Because of the dual effects of IL-10 on B cell proliferation and differentiation, IgG secretion induced by this

cytokine could be the result of either isotype switch recombination or the expansion of a pool of IgG-committed B cells driven to differentiate towards plasma cells (27, 28). Therefore, to determine whether IL-10 can promote switch recombination to IgG, we devised a PCR strategy to detect the circular DNA formed as a by-product of the recombination. In the course of joining the 5' part of S μ to the 3' part of S γ , the intervening DNA is looped out and deleted (Fig. 1 A). At least some of the deleted DNA fragments circularize to form reciprocal S γ -S μ junctions that are potential targets for PCR amplification. In the absence of replication origins, each circle 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 S μ -S γ junction, but only a single reciprocal circle. Therefore, the content of reciprocal S γ -S μ junctions should reflect the frequency of switch recombination events independent of subsequent proliferation.

CD40L-transfected L Cells and IL-10 Induce IgG Switch Recombination. To allow amplification of S γ -S μ reciprocal junctions from any of the four human γ subclasses, we chose a consensus primer from 5' of the S γ repeat region, a region highly conserved across the four human IgG subclasses (25). The antisense primer was based on the sequence 3' of the S μ repeats. Using this primer pair, we analyzed the generation of S γ -S μ reciprocal junctions by naive sIgD⁺ B cells at different times (days 3, 5, and 7) after initiation of culture with CD40L-transfected L cells (LCD40L) in the presence or absence of IL-10. Four to six independent amplifications were performed in identical aliquots of DNA template to improve detection of rare events and assess reproducibility. Depending on the tonsils, no reciprocal S γ -S μ junctions or very few, were amplified in unstimulated (day 0) sIgD⁺ B cells (Fig. 1, B and C). The rare amplified products may represent recently switched cells. A few amplified

products were detected from cells cultured with LCD40L, but the number of these products was greatly augmented by the addition of IL-10 to the medium, especially after 7 d of culture (Fig. 1 B). Five independent amplifications from aliquots of the same DNA preparations showed multiple bands with different sizes (0.6–3 kb) in each replicate sample. This result is consistent with amplification of multiple single-copy templates with different positions of their S μ and S γ recombination breakpoints, thus generating different-sized PCR products. The different band patterns generated from replicate DNA samples presumably result from sampling different sets of nonreplicated switch circles in each aliquot of DNA template amplified. The variation in intensity between bands may be explained by the variable amplification efficiency of different template sequences or by comigration of similar sized bands. Replicated template molecules or repeated recombination at “hotspots” seem unlikely explanations for the intense bands because either of these models would predict the appearance of identical intense bands in different replicate lanes; this is not observed.

CD40L and IL-10 Are Sufficient to Induce IgG Switch Recombination. To verify that the costimulation by LCD40L was due to their expression of CD40L rather than nonspecific effects of the fibroblast cell line, sIgD⁺ B cells were cultured with or without IL-10 in the presence of soluble human CD40L (sCD40L). Lower but significant levels of IgG (1.2 μ g/ml) were produced by sIgD⁺ B cells in the presence of IL-10 when sCD40L was used instead of LCD40L (2.4 μ g/ml). S γ -S μ reciprocal junctions were analyzed at day 7 under these culture conditions. As shown in Fig. 1 C, significant numbers of γ - μ switch circles (ranging from 1 to 6 kb) could be detected in the presence of both IL-10 and sCD40L. It is noteworthy that the number of switch events was lower than in the same cells stimulated by the LCD40L in the presence of IL-10. These data demonstrate that CD40L and IL-10 induce sIgD⁺ naive B cells to switch towards IgG.

PCR Products are Bonafide 5' S γ -3' S μ Reciprocal Switch Junctions. To verify that PCR products contained the expected 5' S γ -3' S μ structure, Southern blots of amplified products from day 5 of a LCD40L plus IL-10 culture were hybridized to our 5' S γ probe and autoradiographed. The blots were then stripped and reprobated with the 3' S μ probe (see Materials and Methods) and reautoradiographed. As shown in Fig. 2 A, the near identity of the two autoradiograms indicated that almost all the amplified products contained both 5' S γ and 3' S μ segments. The occasional band missing with one or the other probe may reflect particular junctions in which the recombination has deleted most of one probe sequence (see below). The identity of the PCR products was confirmed by cloning amplified DNA segments and by sequence analysis of eight randomly picked clones (designated CIR). As shown in Fig. 2 B, the clones all contained 5' S γ sequence joined directly to 3' S μ sequence in a 5'-3' orientation. The recombination breakpoints for each clone could be deduced by alignment of the CIR sequence with the germline S γ and S μ region sequences, and are

Table 1. Homology of CIR S γ Segments to Germline S γ Sequences

Clone	PCR size	S γ length segment	Percent homology			
			S γ 3	S γ 1	S γ 2	S γ 4
CIR8	820	206	95.7	96.6	96.6	82.5
CIR14	850	545	91.6	97.1	92.7	85.9
CIR15	750	172	96.6	97.1	97.1	84
CIR16	1300	374	93.7	98.4	94.4	87
CIR19	900	318	94.4	98.4	94.1	85.8
CIR20	1500	326	97.6	95.4	96.4	87
CIR21	650	568	90	89.4	93	85.3
CIR22	1300	437	96.4	88.9	94.5	88

schematically summarized in Fig. 2 C. In most cases, recombination occurred outside the tandem repeats of the S γ regions (CIR clones 8, 15, 16, 19, and 20) and the S μ regions (CIR clones 8, 14, 15, 19, and 21). Several clones deleted most or all of the DNA representing one or the other flanking probes, supporting the explanation suggested above for the discordant hybridizing bands in the two panels of Fig. 2 A. Recombination breakpoints outside the switch region repeats are frequently observed (29, 30). The known bias for amplification of smaller templates (31) may have increased the frequency of such clones in our sample above their actual frequency in the switched genomic DNA templates. Two of the clones shared an almost identical S μ breakpoint (CIR clones 15 and 19). Several sequences contained deviations from the corresponding germline sequences including point mutations, insertions, and deletions; these could represent polymorphisms, PCR and/or sequencing errors, or mutations introduced by the switch recombination event (32). Because of these mutations, and the short lengths of S γ sequences in the clones, as well as the high sequence conservation of the four human γ subtypes 5' of S γ (25), it was impossible to assign all the sequences unequivocally to specific γ subtypes. The best sequence alignments, as summarized in Table 1 and showing the percentage of homology of the S γ segments to the four S γ germline subtypes, suggested that γ 1, γ 2, and γ 3 genes contributed to these eight clones. These sequence analyses validate the interpretation that the amplified bands in Fig. 1 B represent the expected 5' S γ -3' S μ structure.

Comparison between IL-10 and IL-4 in Promoting Switch Recombination

Given that IL-10 induces switch recombination to IgG in human B cells, we wished to compare its effects to those of IL-4, which is known to induce IgE expression, but has also been reported to promote switching to IgG (13–15). We first examined the capacity of IL-10 and IL-4 to induce

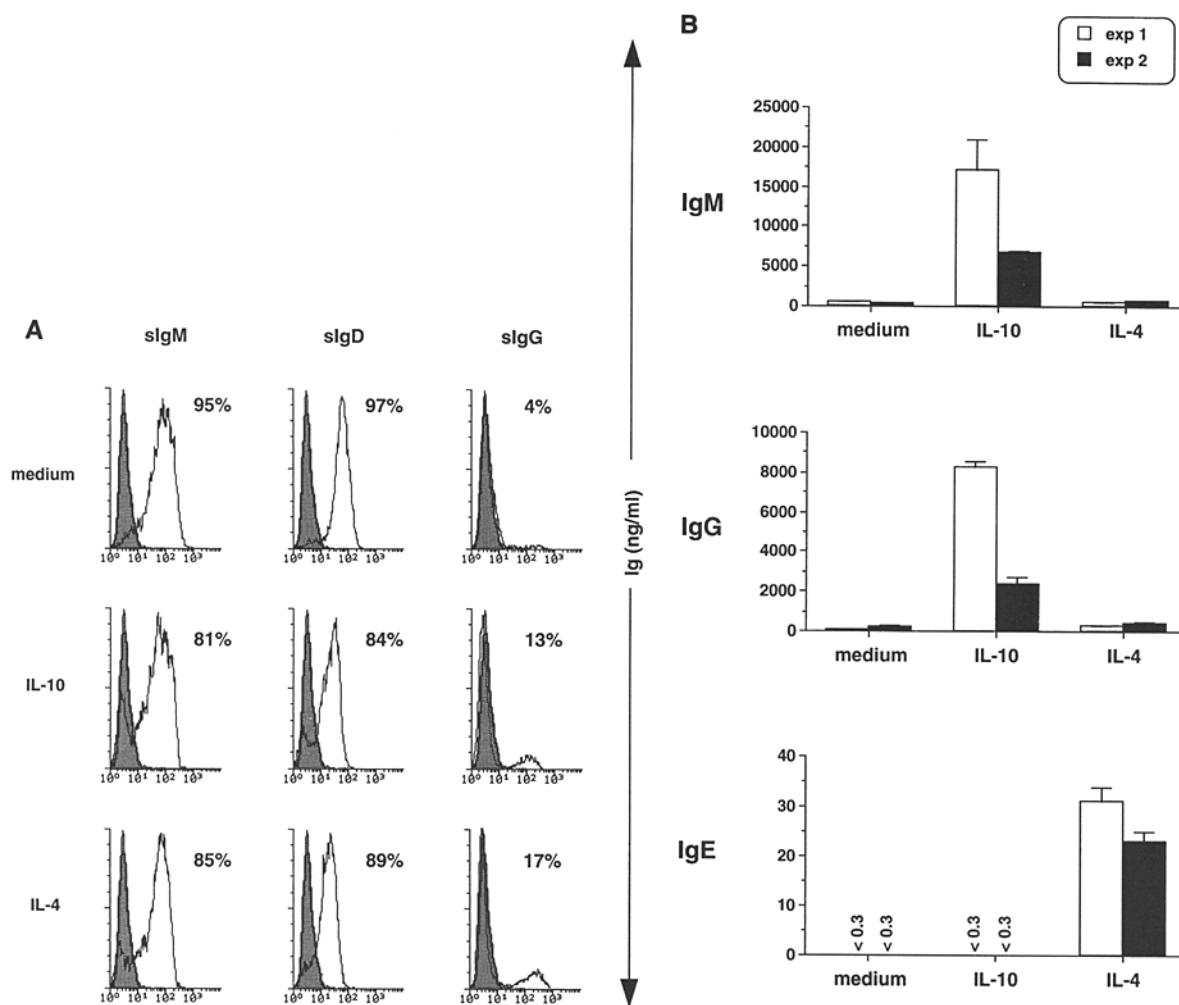


Figure 3. Both IL-4 and IL-10 induce sIgG expression whereas only IL-10 induces IgG secretion by CD40-activated naive sIgD⁺ B cells. 10⁵ MACS[®]-purified sIgD⁺ B cells were cultured with 10⁴ irradiated human LCD40L with or without either 200 ng/ml IL-10 or 50 U/ml IL-4. (A) Surface expression analysis of sIgM, sIgD, and sIgG was performed at day 7. (B) Supernatants were harvested after 19 (*exp 1*) or 14 d (*exp 2*) and IgM, IgG, and IgE levels were determined by ELISA. The limits of sensitivity for IgE ELISA are 0.3 ng/ml.

surface Ig expression and/or differentiation into Ig-secreting cells. As can be seen in Fig. 3 A, IL-4 and IL-10 induced roughly equal percentages of B lymphocytes (~15%) to express surface IgG upon activation through CD40 antigen. As expected, CD40 triggering alone was not sufficient to lead to Ig synthesis by sIgD⁺ B cells (33) whereas addition of IL-4 but not IL-10 induced IgE (between 20 and 30 ng/ml, depending on the experiment). It is noteworthy that, both IL-4 and IL-10 induced similar sIgG expression, but only IL-10 could promote the differentiation of sIgG⁺ B cells into B lymphocytes secreting high levels of IgG (ranging from 2,300 to 8,200 ng/ml), and IgM (ranging from 7,000 to 17,300 ng/ml).

To explore the nature of switch recombination promoted by IL-4 and IL-10, we compared the ability of these cytokines to induce switch circles, characteristic of μ to γ , μ to ϵ , and γ to ϵ DNA recombination. Schematic representations of either direct or sequential recombination towards IgE and PCR strategies are depicted in Fig. 4 A. Both

IL-10 and IL-4 were capable of inducing comparable levels of μ to γ recombination (Fig. 4 B, top). On the other hand, direct μ to ϵ switch recombination was exclusively induced by IL-4 (Fig. 4 B, center). In addition, IL-4 but not IL-10, (Fig. 4 B, bottom) induced the generation of ϵ - γ switch circles. Taken together, these data demonstrate that IL-10, like IL-4, induces IgG switch recombination, but only IL-10 leads to differentiation into IgG-secreting cells.

Discussion

The present study demonstrates that IL-10 is an IgG switch factor, promoting switch recombination to C γ genes in CD40-activated human tonsillar sIgD⁺ B cells. It also shows that whereas both IL-10 and IL-4 induce μ to γ DNA recombination and sIgG expression, IL-10 exclusively leads to high levels of IgG secretion. As expected, only IL-4 promoted switch recombination to the C ϵ gene followed by IgE secretion (8, 25, 31, 34).

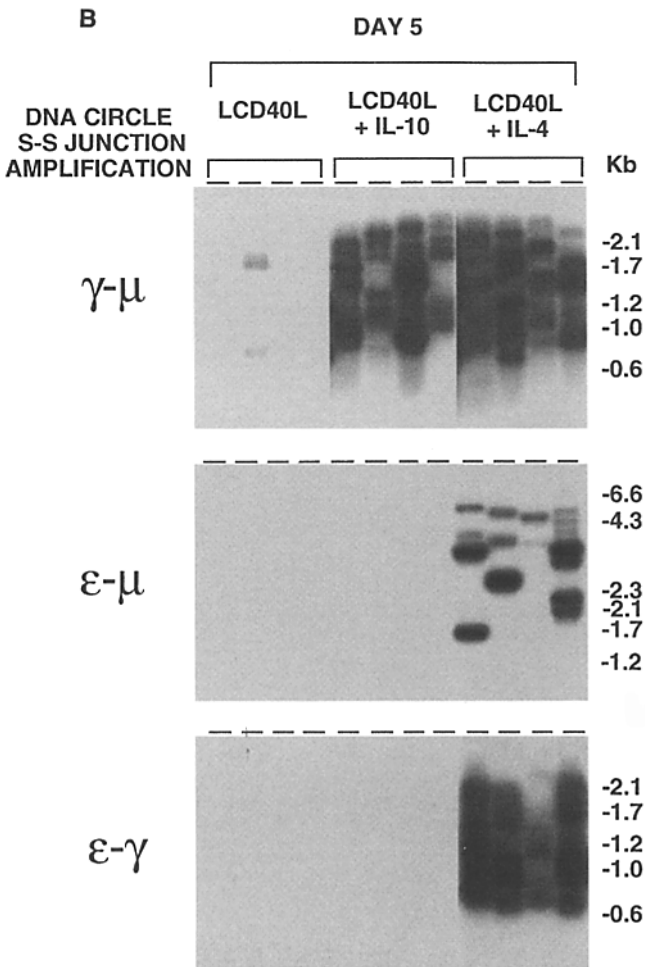
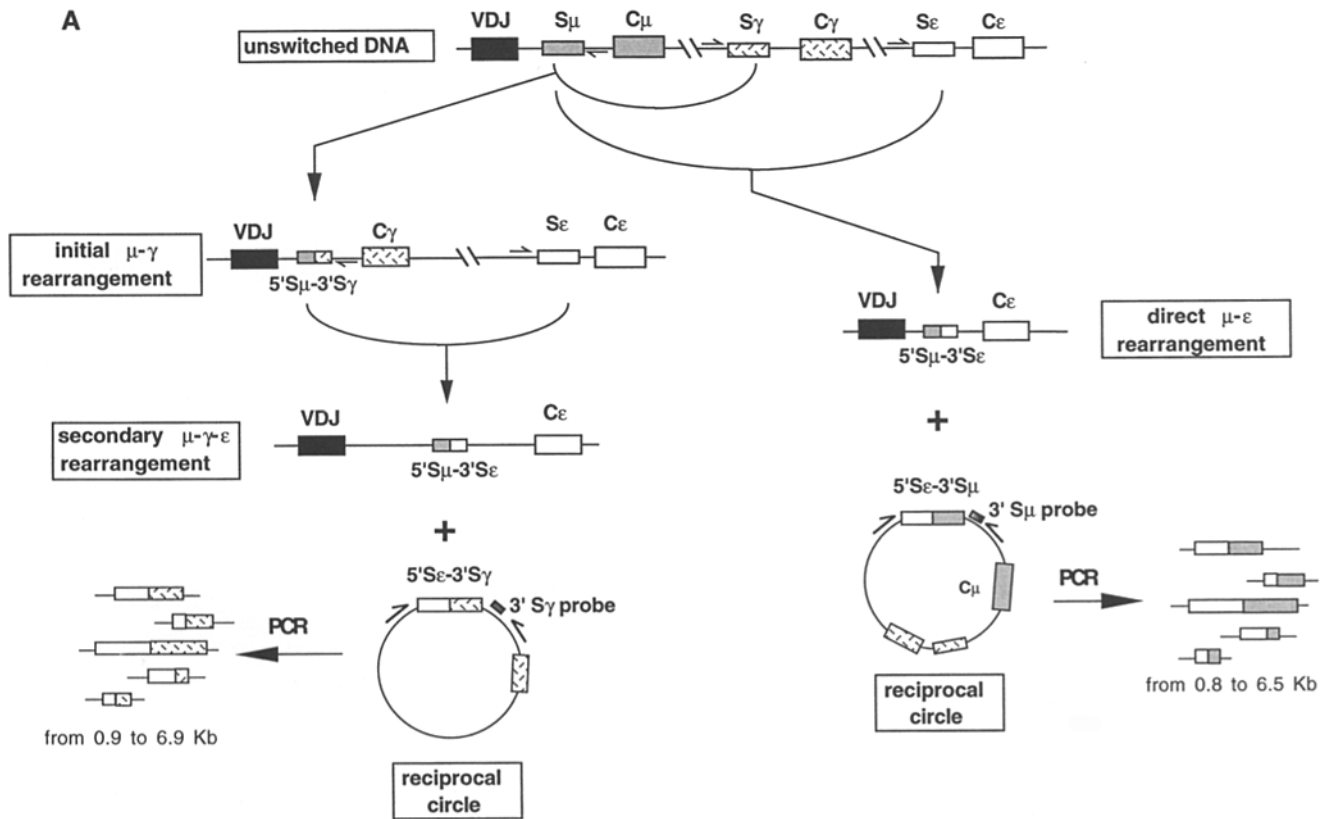


Figure 4. PCR analysis of $S\epsilon$ - $S\gamma$ and $S\epsilon$ - $S\mu$ switch circles resulting from sequential and direct switch, respectively. (A) Schematic $S\epsilon$ - $S\gamma$ and $S\epsilon$ - $S\mu$ switch circle formation and localization of PCR primers and probes. (B) 500 ng of total DNA from $slgD^+$ B cells cultured 5 d on CD40L-transfected L cells (LCD40L), in the absence or presence of either IL-10 or IL-4, was subjected to PCR. (Top) DNA was amplified with 5' $S\gamma$ and 3' $S\mu$ primers using Taq polymerase and hybridized with the 5' $S\gamma$ probe as depicted in Fig. 2 A. (Center) DNA was amplified with 5' $S\epsilon$ and 3' $S\mu$ primers using rTth DNA polymerase, XL, and hybridized with the 3' $S\mu$ probe as depicted in A. (Bottom) DNA was amplified with 5' $S\epsilon$ and 3' $S\gamma$ primers using rTth DNA polymerase, XL, and hybridized with the 3' $S\gamma$ probe as depicted in A. Autoradiography exposure time for the different panels is 4 h.

We had reported before that IL-10 induces IgG3 and IgG1 secretion by CD40-activated sIgD⁺ naive B cells (16). The presently used technique involved amplifying the reciprocal hybrid S γ -S μ junctions that form as by-products of μ - γ switch recombination. Other investigators have shown that these reciprocal products are found in fractions of circular DNA. In our experiments, purification of circular DNA was unnecessary because the strategy used allowed selective amplification of the reciprocal products from total cellular DNA preparations. Our results demonstrate that γ - μ reciprocal recombination products could be detected when naive sIgD⁺ B cells were cultured in the presence of IL-10 and triggered through their CD40 molecule. Sequence analysis of circular DNA fragments from CD40-activated naive B cells cultured with IL-10 revealed that switch recombination sites are scattered along S μ and S γ segments. Two of the sequences displayed nearly identical S μ breakpoints, whereas the other six are distinct, which is consistent with a stochastic distribution of recombination sites (2). Even though we could not quantitatively assess the relative recombination frequency between γ subclasses, assignment of S γ -S μ junctions indicates that γ 1, γ 2, and γ 3 are involved. This is consistent with our previous data at the protein level which showed IL-10 induction of IgG1 and IgG3 (16).

When we compared the capacity of IL-4 and IL-10 to induce specific Ig switch and differentiation, we observed similar percentages of sIgG⁺ B cells. Furthermore, whereas both IL-4 and IL-10 promoted switch recombination to C γ genes, only IL-10 induced high levels of IgG1 and IgG3 secretion. As this manuscript was being completed, Fujieda et al. (30), using a similar strategy to that described here, reported that IL-4 could promote switching to IgG1, IgG3, and IgG4 as judged by increases in the S γ -S μ reciprocal products. The differences between IL-4 and IL-10 could reflect a differential transcription activation at the switch region locus. Indeed, switch regions have been reported to

play a critical role in the upregulation of IgH gene expression in vivo, most likely at the transcriptional level (35). In conclusion, in humans, IL-4 and IL-13 are responsible for the induction of isotype switching towards IgE and IgG (13-15, 36), TGF- β for IgA (11), and IL-10 for IgG1 and IgG3 (16). By analogy to IL-4, which induces switching to ϵ in addition to γ , IL-10 might induce switch recombination to a downstream isotype such as C α . Indeed, previous experiments have shown IgA production, albeit in quantities lower than those of IgG by CD40-activated naive B cells in response to IL-10. It remains to be demonstrated whether IL-10 promotes switch recombination to C α genes.

Isotype switching is a highly CD40-dependent event as demonstrated by the hyper-IgM syndrome in which, as a result of a mutated CD40L, no secondary isotypes are produced (37-40). The fact that neither IL-10 nor IL-4 alone was capable of inducing γ - μ and ϵ - μ switch circles, respectively (data not shown), is consistent with the requirement of CD40 signaling. However, the low viability of B cells at the end of the culture (10-30%), a consequence of the lack of CD40 survival signaling, prevented us from concluding whether IL-10 or IL-4 alone are capable of inducing DNA recombination. Upon CD40 triggering alone, few γ - μ switch circles, and low surface IgG expression after 12 d (data not shown) were observed, in the absence of IgG secretion. Although endogenous production of IL-10 by CD40-activated B cells (41), could presumably be responsible for the small amount of switching to γ , addition of neutralizing IL-10 receptor antibody at the onset of the culture did not reduce the low level of γ - μ reciprocal circles (data not shown). It is noteworthy that CD40 triggering could be achieved either by means of LCD40L or cross-linked soluble CD40 ligand.

In conclusion, the present work provides molecular evidence that IL-10 is an isotype switch factor for IgG, and further extends the notion that both CD40 activation and cytokines are required for isotype switching.

We thank Dr. J. Chiller for support, Dr. S. Saeland for careful reviewing of the manuscript, Ms. Catherine Peronne for DNA sequencing, and Mrs. Sandrine Bonnet-Arnaud for editorial work.

F. Malisan is a recipient of a grant from the Fondation Mérieux.

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Received for publication 21 September 1995 and in revised form 30 November 1995.

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