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Deletional switch recombination occurs in interleukin-4-induced isotype switching to IgE expression by human B cells

(immunoglobulin/DNA rearrangement)

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ABSTRACT **There is controversy as to whether deletional rearrangement occurs between the IgM and IgE switch regions (S_μ and S_ε, respectively) during switching to the IgE isotype. We have addressed the issue by stimulating normal human B cells, sorted for lack of expression of surface IgE, to produce IgE by infection with Epstein–Barr virus (EBV) in the presence of interleukin 4 (IL-4). Genomic DNA was amplified for S_μ/S_ε switch junction fragments by utilizing the nested-primer polymerase chain reaction. Switch junction fragments were amplified from B cells infected with EBV in the presence of IL-4 but not from B cells infected with EBV alone. The DNA sequence of these "switch fragments" revealed direct joining of S_μ to S_ε in each case. The recombination sites within S_μ were clustered within 900 base pairs at the 5′ end of the switch region, suggesting that there are "hot spots" for recombination within S_μ. The S_ε recombination sites were scattered throughout the S_ε region. These findings indicate that IL-4-induced isotype switching to IgE production in human B cells is accompanied by DNA rearrangements with joining of S_μ to S_ε.**

Diversification of antibody biological function is achieved by the process of heavy chain class switching. Isotype switching occurs by molecular recombination between switch (S) regions (1–3) located upstream of each heavy chain constant (C_H) region gene with intramolecular deletion of the DNA segment between the recombined S regions (4–6). Mitogens and T-cell-derived lymphokines regulate isotype switching. These agents presumably modulate the accessibility of specific S regions to a putative switch recombinase (7–9).

Switching to IgE requires interleukin 4 (IL-4) and a B-cell activation signal provided by T cells (10, 11) or by infection with Epstein–Barr virus (EBV) (12, 13). Recent studies have suggested that, unlike switching to other isotypes, switching to IgE expression may not involve DNA rearrangement (14–16) but would instead occur by alternative splicing of a long mRNA transcript or transsplicing of a productive variable–diversity-joining $(VDJ)_\mu$ transcript to a downstream germ-line transcript. On the other hand, Thyphronitis *et al.* (17) have recently demonstrated by Southern blot analysis the rearrangement of one $C_{H\varepsilon}$ gene and deletion of both $C_{H\mu}$ genes in two EBV-transformed IgE-secreting B-cell clones, suggesting that deletional mechanisms could underlie isotype switching to IgE. However, because these two clones were derived from an unfractionated population of B cells, it was not clear whether they represented cells that were actually induced by IL-4 to undergo isotype switching. More importantly, because of its limited sensitivity, Southern blot analysis may not be useful for the study of populations of B cells in which only a small percentage have undergone switching.

Here we utilized the nested-primer polymerase chain reaction (PCR) to demonstrate that deletional recombination occurs in human B cells, sorted for lack of expression of surface IgE ($sIgE^-$) and stimulated to switch to IgE synthesis by EBV infection in the presence of recombinant IL-4 (rIL-4). Sequence analysis of amplified S_μ/S_ε "switch fragments" revealed direct joining of S_μ to S_ε with a clustering of S_μ recombination sites within the 5′ end of the S region, suggesting possible "hot spots" for recombination within S_μ. Thus, nested-primer PCR provided the sensitivity to analyze switching in polyclonal populations of cells that was not available with Southern blotting techniques.

MATERIALS AND METHODS

B-Cell Preparation/EBV Transformation. B-cell-enriched populations were prepared from peripheral blood mononuclear cells of nonatopic subjects (serum IgE < 70 international units/ml) as previously described (10). $sIgE^-$ B cells were sorted as previously described (13). EBV B-cell transformation in the presence or absence of rIL-4 has been described (18). Growth and passaging of the cells has been previously described (13).

IgE Assay. Cell lines were cultured at 0.5×10^6 cells per ml and supernatants were collected for assay after 3–4 days of growth. IgE was measured by a radioimmunoassay (19) with a limit of sensitivity of 150 pg/ml.

Preparation of Cellular DNA and Southern Blotting. High molecular weight DNA was prepared from 50×10^6 cultured cells by utilizing the A.S.A.P. genomic DNA isolation kit (Boehringer Mannheim). After cleavage with restriction endonucleases, the DNA was fractionated in 0.7% agarose gels and transferred to nylon membranes (Schleicher and Schuell). The $C_{H\varepsilon}$ probe was prepared by nick-translation (ref. 20, pp. 10.6–10.10) of the gel-purified 0.88-kilobase (kb) *Hin*fI fragment encompassing the first two exons of the $C_{H\varepsilon}$ gene and the introns bordering $C_{H\varepsilon 2}$ (described in ref. 13). Blots were hybridized and washed as described in the protocol for Nytran membranes (Schleicher and Schuell).

PCR Primers, Amplification of S_μ/S_ε Switch Fragments. Thirty-base-pair (bp) single-stranded DNA oligonucleotide primers for PCR were prepared by utilizing the PCR-Mate DNA synthesizer (Applied Biosystems). PCR amplification of the S_μ/S_ε fragment from the IgE-secreting plasmacytoma U266 (21) was performed with primers S6/S4 (see Fig. 2) in a reaction mixture containing each dNTP at 200 μM, 5 mM KCl, 1.0 mM $MgCl_2$, 10 mM Tris·HCl (pH 8.3), 0.01% gelatin, 1 mM dithiothreitol, 150 ng of each PCR primer, 1 μg of genomic template DNA, 2.5 units of AmpliTaq DNA poly-

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Abbreviations: S, switch; C_H, heavy chain constant; VDJ, variable–diversity-joining; EBV, Epstein–Barr virus; IL-4, interleukin 4; rIL-4, recombinant IL-4; $sIgE^-$, surface IgE-negative.

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merase (Perkin–Elmer), and 0.5 unit of Perfect Match polymerase enhancer (Stratagene) in a 50-μl volume under a 50-μl layer of mineral oil. The reaction mixture was cycled in a Perkin–Elmer/Cetus DNA thermal cycler for 35 cycles with 1 min at 94°C, 1 min at 65°C, and 4.5 min at 72°C. PCR amplification of S_μ/S_ε fragments from the EBV-transformed B cell DNA was similarly performed with primers S6/S4 followed by a second round of PCR utilizing the nested primers S8/S3 (Fig. 2) in a reaction mixture containing the same concentrations of all of the above constituents, except for 1.5 mM $MgCl_2$ and a 5-μl aliquot of the previous PCR reaction mixture that served as DNA template. The reaction mixture was cycled in the thermal cycler under the same conditions as described above.

Purification, Cloning, and Sequencing of PCR-Amplified DNA Fragments. PCR-amplified fragments were fractionated on 5% polyacrylamide gels, electroeluted from cut sections of the gel, and isolated by precipitation with ethanol (ref. 20, pp. 6.28–6.29, 6.39–6.44). Purified fragments were ligated into the *Sma* I site of pUC19 (22). Regions of the cloned fragment were subcloned in the replicative form I DNA for M13mp18 and M13mp19 (Pharmacia LKB), and single-stranded template DNA was isolated according to the protocols of New England BioLabs. All cloning steps utilized the *Escherichia coli* strain JM109 (23), a recombination-deficient strain that protects against bacterial-directed mutation or recombination within cloned fragments. Constructs were sequenced by the dideoxy chain-termination method (24) using Sequenase Version 2.0 (United States Biochemical). Additional 17-bp sequencing primers were prepared with the PCR-Mate DNA synthesizer.

RESULTS

Analysis of Cell Lines. Table 1 lists the cell lines studied. IgE was detected only in culture supernatants of B cells infected with EBV and cultured in the presence of IL-4. The percentage of cells in each cell line with intracytoplasmic IgE never exceeded 5% (13), even in the A5 cell line, which secreted IgE in levels comparable to those secreted by the U266 plasmacytoma line.

Table 1. Cell lines

Line	Sorted*	IL-4†	Time in culture, days	IgE, pg/ml
U266	NA	NA	NA	74,909
A5	−	+	>180	59,797
Semie	−	−	>180	<150
HJS6	+	+	43	2,199
HJS7	+	+	44	5,687
HJS02	+	−	57	<150

NA, not applicable.
*A + denotes that B cells were sorted for lack of expression of surface IgE prior to initiation of culture.
†Cell lines were incubated with (+) or without (−) rIL-4 at the time of EBV infection. rIL-4 was maintained in the media of the + cell lines.

Southern Blot Analysis of IgE-Secreting B-Cell Lines. We performed Southern blot analysis (Fig. 1*A*) on the high-IgE-producing line A5 and on the non-IgE-producing line Semie, which were derived from unsorted B cells from two different donors. A5 had been cultured with rIL-4, while Semie had been grown in the absence of rIL-4. U266 and a λ library clone derived from human placenta that contains the S_ε and $C_{H\varepsilon}$ region in the unrearranged configuration (25) were used as controls. The ε λ clone shows no rearrangement involving S_ε (5.5-kb *Pst* I fragment). U266 DNA had a single rearrangement (3.3-kb *Pst* I fragment) corresponding to the S_μ/S_ε recombination event depicted in Fig. 1*B*. The single rearrangement in U266 indicates either that both chromosomes are identically rearranged or that only one copy of this region is present in the cell line. The second explanation is more likely, since karyotype analysis revealed that U266 is not diploid for chromosome 14 (data not shown). The Southern blot pattern for both the IgE-secreting and non-IgE-secreting cell lines was that of an unrearranged chromosome (5.5-kb *Pst* I fragment). These results indicated that Southern blot analysis for the detection of S_μ/S_ε recombination events in polyclonal populations of B cells is not informative.

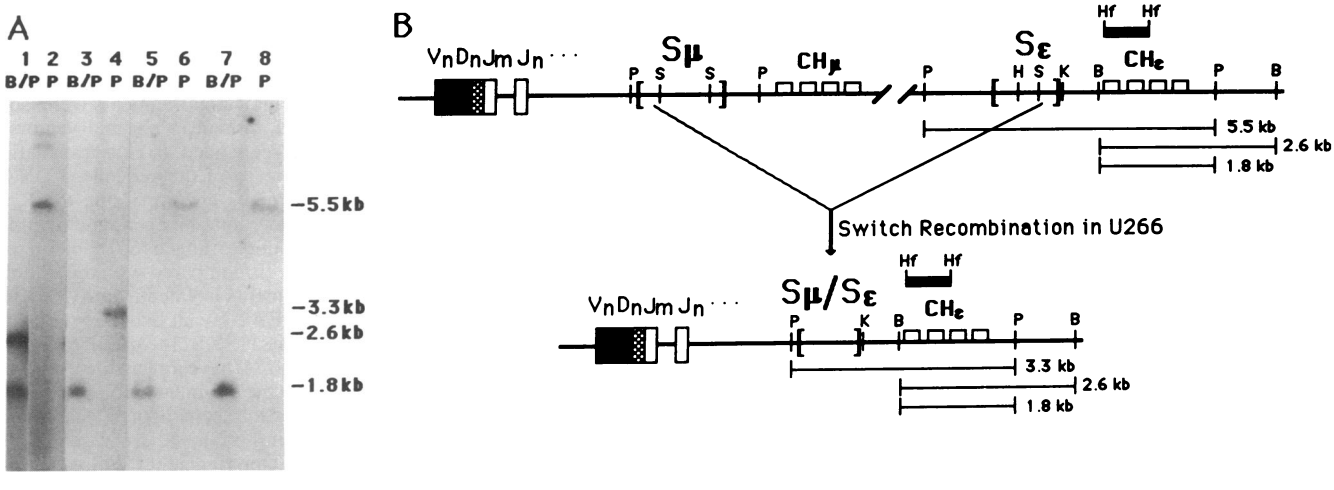

FIG. 1. Arrangement of the S_ε region in U266, IgE-secreting (A5), and non-IgE-secreting (Semie) lymphoblastoid lines. (*A*) Southern blot analysis of cell lines compared with the λ clone 38A (25), isolated from a human placenta genomic library. High molecular weight DNA samples isolated from the ε λ clone (lanes 1 and 2), U266 (lanes 3 and 4), and lymphoblastoid cell lines (A5 in lanes 5 and 6 and Semie in lanes 7 and 8) were digested with *Bam*HI/*Pst* I (B/P) or with *Pst* I (P) alone. Samples on this Southern blot were hybridized with the 0.88-kb *Hin*fI fragment encompassing $C_{H\varepsilon 1}$ and $C_{H\varepsilon 2}$ (shown above $C_{H\varepsilon}$ in *B*). All four DNA samples show the 1.8-kb *Bam*HI–*Pst* I fragment encompassing $C_{H\varepsilon}$ that does not undergo rearrangement (lanes 1, 3, 5, and 7). The λ clone has an unrearranged S_ε region represented by a 5.5-kb *Pst* I fragment (lane 2). The additional bands in lanes 1 and 2 are the result of incomplete digestion with *Pst* I. U266 DNA has only a 3.3-kb *Pst* I fragment, consistent with rearrangement involving the S_ε region (lane 4). Both A5 and Semie cellular DNA have a 5.5-kb *Pst* I fragment, denoting an unrearranged S_ε region (lanes 6 and 8). No other bands were detected in the lymphoblastoid cell lines, even with overexposure of the autoradiogram. (*B*) Diagrammatic summary of the rearrangement event that occurred in U266. Brackets represent the probable extent of the switch regions. B, *Bam*HI; H, *Hin*dIII; Hf, *Hin*fI; K, *Kpn* I; P, *Pst* I; S, *Sac* I.

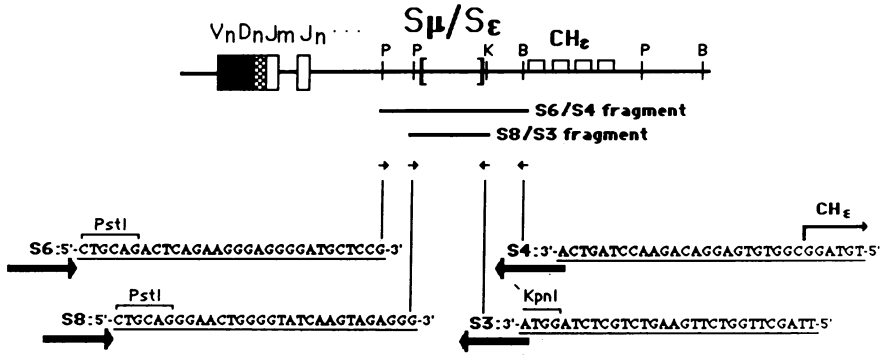

FIG. 2. PCR amplification of S_μ/S_ε junction fragments. A representative fragment containing an S_μ/S_ε junction is shown. The PCR primers used for amplification of the region are pictured below the map. For nested primer PCR, the first round uses primers S6/S4 while the second (nested) round of PCR uses primers S8/S3. B, *Bam*HI; K, *Kpn* I; P, *Pst* I.

PCR Amplification and Sequencing of the Switch Fragment from U266. Genomic DNA from the U266 cell line was subjected to one round of PCR amplification utilizing the primers S6/S4 or S8/S3 (Fig. 2). Amplified fragments were subcloned and DNA sequencing showed that a complex rearrangement had occurred between S_μ and S_ε regions. A 243-bp internal deletion occurred within S_μ, followed by an additional 186 bp of S_μ sequence (Fig. 3*B*). At the termination of the S_μ sequence, there was a 34-bp insertion segment, not homologous to either S_μ or S_ε (Fig. 4). Part of this insertion segment contained pentameric repeats typical of S region sequences (26, 27). However, comparison of this insertion segment with S_μ, S_ε, and $S_{\gamma 4}$ sequences (28) failed to reveal an unambiguous origin for this insert. The 34-bp insertion segment was ligated to an S_ε sequence that continued downstream without interruption into the IgE heavy chain gene coding region, $C_{H\varepsilon}$ (Fig. 3*B*).

The sequence of the fragment amplified by PCR from U266 DNA was identical to the sequence of a fragment isolated from a U266 genomic library that encompassed the S_μ/S_ε junction (unpublished data). Thus, PCR-amplified switch fragments accurately reflect the switch rearrangement in genomic DNA.

Nested-Primer PCR Amplification of Switch Fragments from IgE-Secreting B-Cell Lines. Because <5% of B cells in our cell lines contained cytoplasmic IgE, we tested the sensitivity of the PCR amplification method used above. Various dilutions of U266 DNA were mixed with fibroblast genomic DNA such that the final concentrations of U266 DNA in the mixtures were 20%, 2%, 0.2%, 0.02%, and 0.002%. The final dilution represented the DNA equivalent of two to three U266 cells in a 1-μg sample of fibroblast DNA. When PCR was performed utilizing the primers S6/S4 (Fig. 2), no PCR-amplified fragments were identified by gel elec-

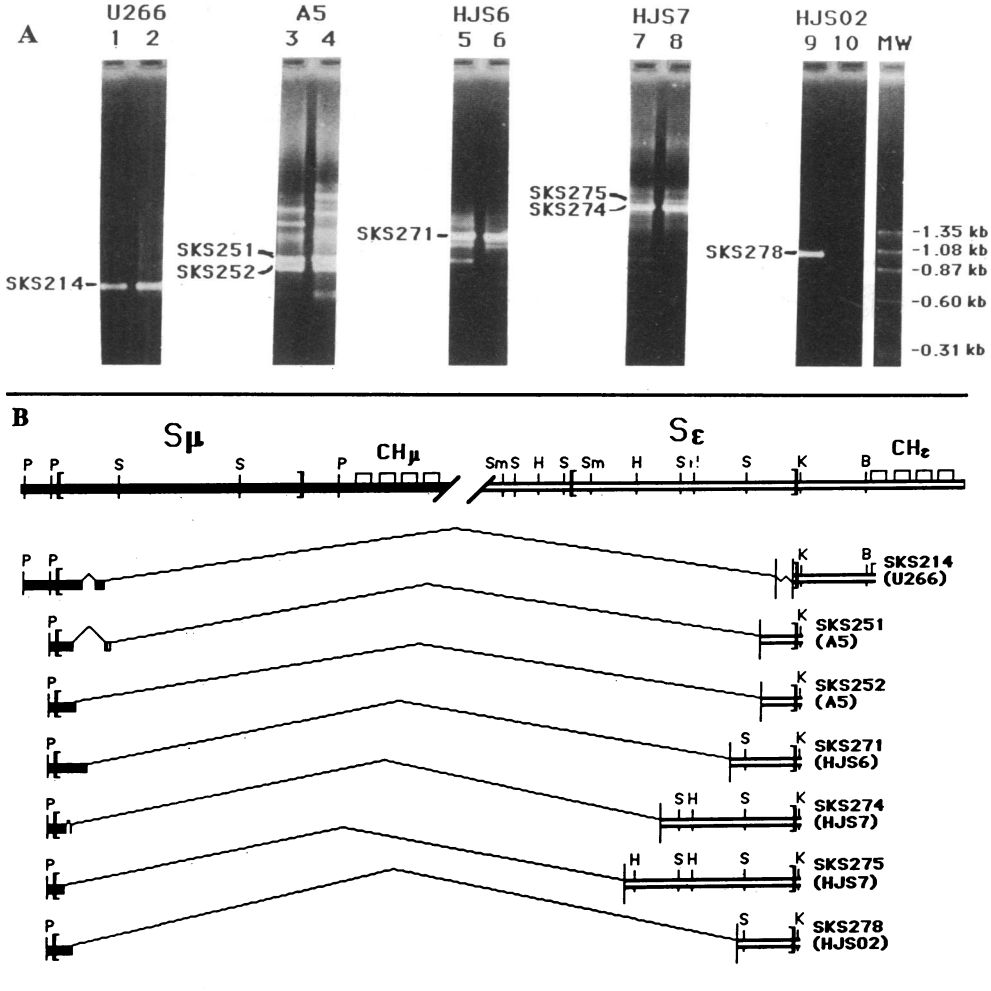

FIG. 3. Nested primer PCR products of U266, IgE-secreting (A5, HJS6, and HJS7), and non-IgE-secreting (HJS02) lymphoblastoid lines. (*A*) Agarose gel electrophoresis of PCR products. Each pair of samples represents two independently run nested-primer PCR products from the same genomic DNA preparation. The indicated fragments were isolated, cloned in plasmid vectors, and sequenced. (*B*) Diagrammatic summary of the sequenced DNA fragments encompassing the S_μ/S_ε junction. The genomic map of the unrearranged S_μ and S_ε regions is compared with the switch fragments isolated from U266 and the EBV B-cell lines. Three fragments (SKS214, SKS251, and SKS274) have internal deletions of S_μ. One fragment (SKS214) has an insertion segment of unclear origin joining S_μ to S_ε (represented as W). One fragment (SKS251) has a 26-bp duplication of a portion of S_μ at the 3′ end of the S_μ sequence. One example of an unreproducibly amplified fragment from the non-IgE-secreting cell line (HJS02) is shown in lane 9; this fragment is not present in the replicate sample shown in lane 10. Brackets represent the probable extent of the switch regions. B, *Bam*HI; H, *Hin*dIII; K, *Kpn* I; P, *Pst* I; S, *Sac* I; Sm, *Sma* I.

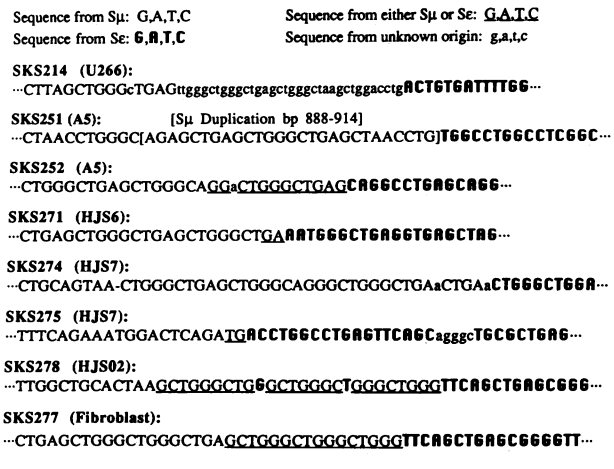

FIG. 4. Sequence of the S_μ/S_ε switch junction in the cloned fragments. The DNA sequence data encompassing the S_μ/S_ε switch junction of the fragments in Fig. 3*B* are shown. The key at the top indicates origins of the nucleotides. Nucleotides that could originate from either S_μ or S_ε generally encompass pentameric repeat sequences that are common to both switch regions. The presence of sequences common to both S_μ and S_ε as in fragments SKS277 and SKS278 is indicative of *in vitro* artifactual switching. The common sequence in the SKS252 fragment is interrupted by a nucleotide not present in either switch region, thus suggesting that this fragment is not artifactual. The origin of the insertion segment in SKS214 is not known. The 26-bp duplicated segment in SKS251 is identical to bp 888–914 and is shown between brackets within the sequence. The hyphen in the SKS274 sequence represents a 1-bp deletion. The sequence of unknown origin in SKS275 within the S_ε region may not be a true insertion/deletion but may represent a polymorphism.

trophoresis, even when 20% of the template DNA was from U266. We hypothesized that this failure resulted from competition for the primers by unrearranged DNA. To dilute out this competition, we performed a second round of nested-primer PCR amplification utilizing primers S8/S3 (Fig. 2) on an aliquot from each first round of PCR. The expected 618-bp U266 switch fragment was amplified from all the samples (data not shown). These results demonstrated that nested primer PCR could amplify an S_μ/S_ε switch fragment when it was present at only two or three copies in a sample of unrearranged DNA.

Genomic DNA samples from three IgE-secreting cell lines (A5, HJS6, HJS7) and one cell line that did not produce IgE (HJS02) were amplified by the nested-primer PCR technique. As expected, no amplified fragments were detected in the DNA from the cell lines after the first round of PCR (data not shown). However, after the nested-primer round of PCR, multiple fragments were reproducibly detected in replicate samples from all three IgE-secreting cell lines (Fig. 3*A*). In contrast, PCR amplification of DNA from non-IgE-secreting cell lines revealed either no PCR fragments or, only occasionally (<30% of all PCR runs), gave rise to PCR fragments that were never reproducibly observed in duplicate runs. An example of one such fragment is seen in lane 9 of Fig. 3*A*; this fragment was not seen in the duplicate PCR run shown in lane 10. Similarly, PCR fragments were occasionally amplified from fibroblast DNA (data not shown). Sequence data revealed that these fragments represented artifactual PCR-generated "*in vitro* switching" (see below).

Sequence Analysis of Switch Fragments from IgE-Secreting B-Cell Lines. Five switch fragments (SKS251, SKS252, SKS271, SKS274, and SKS275) reproducibly amplified from DNA of the IgE-secreting cell lines were purified, cloned, and sequenced. Fig. 3*B* compares these cloned fragments to the genomic S_μ and S_ε maps. The sequences of these fragments around the S_μ/S_ε junction are shown in Fig. 4. In each case there was direct joining of S_μ to S_ε. Two of the five fragments (SKS251 and SKS274) had internal deletions within S_μ of 551 bp and 10 bp, respectively (Fig. 3*B*). However, these deletions had different 5′ and 3′ ends than the deletion seen in U266.

Analysis of the sites of switch recombination and internal deletion within S_μ demonstrated a clustering of these sites within 900 bp at the 5′ end of the S region. In contrast, the S_ε recombination sites were scattered throughout the S_ε region. These data suggest that there may be hot spots for recombination within the S_μ region.

The S_μ/S_ε sequence of the PCR fragment amplified from the DNA of the non-IgE-secreting cell line HJS02 (lane 9, Fig. 3*A*) is shown as SKS278 in Fig. 4. Unlike switch fragments amplified from the IgE-secreting cell lines, SKS278 contained at the junction between S_μ and S_ε a 9-bp sequence that is common to S_μ and S_ε regions and, therefore, could have derived from either S region. Allowing for two mismatches, the sequence in SKS278 common to both S regions would be 26 bp. Switch fragment SKS278 likely represents an *in vitro* artifact that arose because single-stranded PCR products bound to each other due to complementarity at their 3′ ends. Elongation of the annealed product resulted in amplification of the fragment leading to artifactual *in vitro* switching. The sequence of the switch junction of an artifactual PCR fragment amplified from fibroblast genomic DNA (SKS277) similarly contains a segment at the S_μ/S_ε junction (16 bp) that is common to the two S regions. We have confirmed the presence of the switch fragments in the IgE-secreting cell lines and the artifactuality of fragments amplified from HJS02 and fibroblast DNA by using another pair of primers (S7 and S9) for the nested-primer round of PCR. Switch fragments of the expected sizes were amplified with these primers from DNA of the IgE-secreting cell lines. In contrast, none of the artifactual switch fragments were amplified from either HJS02 or fibroblast DNA (data not shown).

One of the amplified fragments (SKS252) from the IgE-secreting cell lines had a segment at the S_μ/S_ε junction that could have derived from either S_μ or S_ε. However, this fragment could not have arisen by *in vitro* switching because within the 12-bp sequence common to S_μ and S_ε is a nucleotide insertion (adenine) not present in either S_μ or S_ε. Moreover, this fragment was reproducibly amplified from A5 genomic DNA, and this PCR product from two different isolates of A5 DNA had identical sequences. These data indicate that this fragment represents a true switch recombination event.

DISCUSSION

To date, direct analysis of switch recombination has been restricted to clonal populations of cells because Southern blot analysis, as illustrated in our study, is not applicable to the study of B-cell populations in which only a small percentage of cells have undergone isotype switching. In the present study, we have used the nested-primer PCR technique to demonstrate deletional S_μ to S_ε switch recombination in polyclonal normal human B cells induced to switch in culture to IgE isotype expression by stimulation with EBV and IL-4. Deletional switch recombination must have occurred *de novo*, because B cells were sorted for lack of expression of surface IgE prior to culture.

Nested-primer PCR can amplify a switch fragment even when it is present as only a single copy among more than 250,000 unrearranged DNA segments. Though this procedure occasionally amplifies PCR fragments from DNA of non-IgE-secreting cell line controls and from fibroblast DNA, this amplification is not reproducible and the overlapping sequence at the switch junction indicates that these fragments are generated via an artifactual *in vitro* switching event.

Three of the switch fragments (SKS214, SKS251, and SKS274) had internal deletions within S_μ. One switch fragment had a 26-bp duplication of a portion of S_μ sequence juxtaposed to the junction with S_ε. Several point mutations or 1-bp insertions or deletions were also observed in the vicinity of switch junctions. These findings are consistent with the models in which error-prone DNA synthesis occurs during switch recombination (29–31).

The data presented here do not exclude the possibility that S_μ/S_ε recombination occurred on chromosomes that have not undergone productive VDJ rearrangement. Nonproductive chromosomal rearrangements have been described in hybridomas and normal murine B cells (32–34). The fact that we observed S_μ/S_ε rearrangements only in IgE-secreting lymphoblastoid cells, and not in the non-IgE-secreting controls, strongly suggests that S_μ/S_ε switch fragments have the necessary upstream VDJ regions for productive IgE expression. The recent observation that an EBV-transformed B-cell clone has deleted $C_{H\mu}$ on both chromosomes indicates that S_μ/S_ε switching occurs on chromosomes that have productively rearranged their VDJ regions (17).

Our observations of S_μ/S_ε deletional switch recombination are in contrast to the results of MacKenzie and Dosch (15), who failed to detect S_μ/S_ε recombination by Southern blot analysis of IgE-secreting human B-cell clones. The discrepancy may be due to different modalities used to induce IgE synthesis. MacKenzie and Dosch cultured unsorted B cells in the presence of EBV and irradiated T-T hybridoma filler cells in the absence of exogenous IL-4. Nonetheless, our results cannot exclude nondeletional mechanisms of IgE isotype switching.

The approach we have used to study the mechanism of isotype switching to IgE by nested-primer PCR amplification of S_μ/S_ε junctions is applicable to the study of switch recombination to other isotypes and could prove useful in the study of potential isotype switch defects in patients with antibody deficiency syndromes.

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1. Davis, M. M., Calame, K., Early, P. W., Livant, D. L., Joho, R., Weissman, I. L. & Hood, L. (1980) *Nature (London)* **283,** 733–739.
2. Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. (1980) *Nature (London)* **286,** 676–683.
3. Kataoka, T., Kawakami, T., Takahashi, N. & Honjo, T. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 919–923.
4. Matsuoka, M., Yoshida, K., Maeda, T., Usuda, S. & Sakano, H. (1990) *Cell* **62,** 135–142.
5. Schwedler, U., Jäck, H.-M. & Wabl, M. (1990) *Nature (London)* **345,** 452–456.
6. Iwamoto, T., Shimuzu, A., Hohjo, T. & Yamagishi, H. (1990) *Cell* **62,** 143–149.
7. Yancopoulos, G. D., DePinho, R. A., Zimmerman, K. A., Lutzker, S. G., Rosenberg, N. & Alt, F. W. (1986) *EMBO J.* **5,** 3259–3266.
8. Stavnezer-Nordgren, J. & Sirlin, S. (1986) *EMBO J.* **5,** 95–102.
9. Lutzker, S., Rothman, P., Pollock, R., Coffman, R. & Alt, F. W. (1988) *Cell* **53,** 177–184.
10. Vercelli, D., Jabara, H. H., Arai, K. & Geha, R. S. (1989) *J. Exp. Med.* **169,** 1295–1307.
11. Vercelli, D., Jabara, H. H., Lauener, R. P. & Geha, R. S. (1990) *J. Immunol.* **144,** 570–573.
12. Thyphronitis, G., Tsokos, G. C., June, C. H., Levine, A. D. & Finkelman, F. D. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5580–5584.
13. Jabara, H. H., Schneider, L. C., Shapira, S. K., Alfieri, C., Moody, C. T., Kieff, E., Geha, R. S. & Vercelli, D. (1990) *J. Immunol.* **145,** 3468–3473.
14. Snapper, C. M., Finkelman, F. D., Stefany, D., Conrad, D. H. & Paul, W. E. (1988) *J. Immunol.* **141,** 489–498.
15. MacKenzie, T. & Dosch, H.-M. (1989) *J. Exp. Med.* **169,** 407–430.
16. Chan, M. A., Benedict, S. H., Dosch, H.-H., Huy, M. F. & Stein, L. D. (1990) *J. Immunol.* **144,** 3563–3568.
17. Thyphronitis, G., Max, E. E. & Finkelman, F. D. (1991) *J. Immunol.* **146,** 1496–1502.
18. Umetsu, D. T., Yunis, E. J., Matsui, Y., Jabara, H. H. & Geha, R. S. (1985) *J. Immunol.* **15,** 356–361.
19. Umetsu, D. T., Leung, D. Y. M., Siraganian, R., Jabara, H. H. & Geha, R. S. (1985) *J. Exp. Med.* **162,** 202–214.
20. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Cold Spring Harbor, NY) 2nd Ed.
21. Seno, M., Kurokawa, Y., Ono, H., Onda, H., Sasada, R., Igarashi, K., Kikuchi, M., Sugino, Y., Nishida, Y. & Honjo, T. (1983) *Nucleic Acids Res.* **11,** 719–726.
22. Messing, J. (1983) *Methods Enzymol.* **101,** 20–78.
23. Yanisch-Perron, C., Vieira, J. & Messing, J. (1985) *Gene* **33,** 103–119.
24. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
25. Max, E. E., Battey, J., Ney, R., Kirsch, I. R. & Leder, P. (1982) *Cell* **29,** 691–699.
26. Nikaido, T., Nakai, S. & Honjo, T. (1981) *Nature (London)* **292,** 845–848.
27. Kataoka, T., Miyata, T. & Honjo, T. (1981) *Cell* **23,** 357–368.
28. Mills, F. C., Brooker, J. S. & Camerini-Otero, R. D. (1990) *Nucleic Acids Res.* **18,** 7305–7316.
29. Kenter, A. L. & Watson, J. V. (1987) *J. Immunol. Methods* **97,** 111–117.
30. Dunnick, W., Wilson, M. & Stavnezer, J. (1989) *Mol. Cell. Biol.* **9,** 1850–1856.
31. Dunnick, W. & Stavnezer, J. (1990) *Mol. Cell. Biol.* **10,** 397–400.
32. Radbruch, A., Müller, W. & Rajewsky, K. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3954–3957.
33. Hummel, M., Berry, J. K. & Dunnick, W. (1987) *J. Immunol.* **138,** 3539–3548.
34. Kepron, M. R., Chen, Y.-W., Uhr, J. W. & Vitetta, E. S. (1989) *J. Immunol.* **143,** 334–339.