Brief Definitive Report

Molecular Analysis of the Induction of Immunoglobulin E Synthesis in Human B Cells by Interleukin 4 and Engagement of CD40 Antigen

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Summary

The molecular events leading to immunoglobulin E (IgE) synthesis in human sIgE$^-$ B cells stimulated with interleukin 4 (IL-4) and anti-CD40 monoclonal antibody (mAb) 626.1 were analyzed. Anti-CD40 mAb increased the levels of IL-4–induced germline Cε transcripts and induced the production of mature Cε mRNA. These effects were dependent on the presence of IL-4. Nested primer PCR revealed deletional switch recombination occurring only in B cells stimulated with both IL-4 and anti-CD40 mAb. DNA sequence analysis of switch fragments showed direct Sμ/Sε joining, without the deletions or duplications within Sμ often found in B cells stimulated with IL-4 and Epstein-Barr virus. Analysis of the switch junction map sites showed "hot spots" for recombination within Sμ, but not within Sε. These findings indicate that IL-4 provides a signal to B cells to induce germline Cε transcription and concurrent CD40 engagement induces Sμ/Sε deletional switch recombination, production of mature Cε mRNA, and IgE synthesis.

Engagement of the B cell antigen CD40 by mAb has been shown to provide the second signal required for induction of human IgE synthesis by IL-4 (1, 2). The CD40/IL-4 system is T cell independent, and therefore amenable to further molecular analysis. We show herein that engagement of CD40 significantly enhanced the level of IL-4-induced ε germline transcripts (GLT)[1] and resulted in the production of mature Cε transcripts. A recently established nested primer PCR approach (3) showed that deletional switch recombination underlies IgE class switching in the CD40/IL-4 system.

Materials and Methods

B Cell Preparations. Highly purified human peripheral blood B cells from nonatopic subjects were isolated and cultured as previously described (1). sIgE$^-$ B cells were obtained by cell sorting (3). Resting B cells were collected at the 50–60% interface after Percoll gradient centrifugation (4).

Northern Blot Analysis. Northern blot analysis was performed as previously described using a ^{32}P-labeled 0.88 kb HinfI fragment which spans the first two exons of Cε (5), and a human cDNA β-actin probe provided by Dr. C. Terhorst (Dana-Farber Cancer Institute, Boston, MA). Densitometric analysis was performed with an Ultrascan XL Densitometer (LKB Instruments, Inc., Bromma, Sweden).

PCR Primers/Amplification, Cloning, and Sequencing of Sμ/Sε Switch Fragments. Nested PCR runs for switch (S)μ/Sε switch fragments were performed on high molecular weight DNA isolated from B cells as described (3). Four PCR primers were used (see Fig. 4): S6 (5'-CTGCAGACTCAGAAGGGAGGGGATGCTCCG-3'); S4 (3'-ACTGATCCAAGACAGGAGTGTGGCGGATGT-5'); S7 (5'-GAGGGTGGTAATGATTGGTAATGCTTTGGA-3'); S9 (3'-GAACCCTGAGGTCCGGTCCCCGCTTCCCGG-5'). The first round of PCR was performed using primers S6/S4. The second round of PCR was performed on a 5-μl aliquot of the first round PCR mixture with primers S7/S9. PCR amplification and purification, cloning, and sequencing of the amplified fragments were carried out as described (3).

Results and Discussion

Role of CD40 Engagement in Cε Transcription. Highly purified B cells were incubated with anti-CD40 mAb 626.1 (5 μg/ml) (4), rIL-4 (100 U/ml), or both for 5 and 10 d, and their RNA was analyzed by Northern blot using the 0.88 kb HinfI probe which hybridizes to both 1.8 kb ε GLT and 2.0 kb mature Cε transcripts (5). A representative experiment is shown in Fig. 1. As previously shown (5), 1.8 kb ε GLT, but not mature 2.0 kb Cε mRNA were detected in B cells stimulated with IL-4 for 5 or 10 d. Induction of ε GLT did not require in vivo preactivation of B cells because IL-4 induced ε GLT in small resting B cells prepared by Percoll gradient centrifugation (data not shown). Anti-CD40

[1] *Abbreviations used in this paper:* GLT, germline transcripts; S region, switch region.

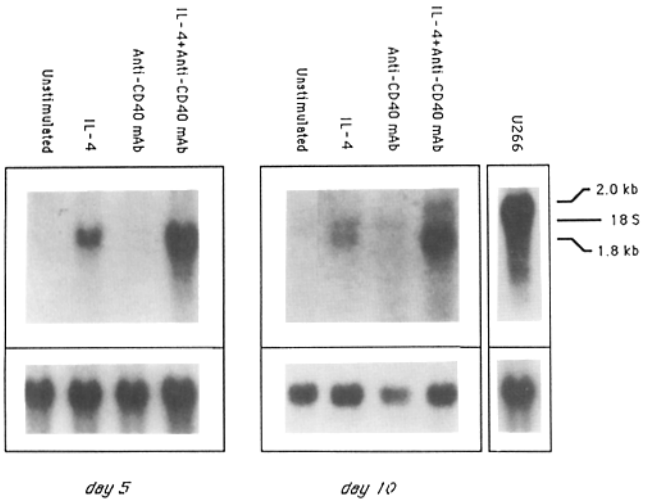

Figure 1. Induction of germline and mature Cε transcripts in normal human B cells stimulated with rIL-4 and anti-CD40 mAb. Total RNA (10 μg) from normal peripheral blood B cells stimulated with rIL-4 and/or anti-CD40 mAb for 5 and 10 d and from U266 cells was subjected to electrophoresis on a 1% formaldehyde-agarose gel, transferred to nitrocellulose and hybridized to ^{32}P-labeled 0.88 kb HinfI (*top*) or actin (*bottom*) probes. Top panels were exposed for 3 d, with the exception of the U266 lane (4 h). Bottom panels were exposed for 1 d. Similar results were obtained in two additional experiments.

mAb by itself did not induce either GLT or mature Cε transcription, but synergized with IL-4 to enhance ε GLT accumulation. Densitometric analysis showed a sevenfold increase in ε GLT RNA in comparison with actin RNA following addition of anti-CD40 mAb, at both time points. A 2.0 kb band that comigrated with the major band in RNA from the IgE-secreting plasmacytoma U266 and corresponded to mature ε mRNA (5) was detected only in B cells stimulated with both IL-4 and anti-CD40 mAb for 10 d. These results indicate that CD40 engagement in the presence of IL-4–induced isotype switching and the production of mature ε mRNA. These findings are compatible with the hypothesis that induction of GLT may result in increased accessibility of the downstream S region for deletional switch recombination with Sμ (6–8), whereas the CD40-derived signal may function as an activator of DNA recombination.

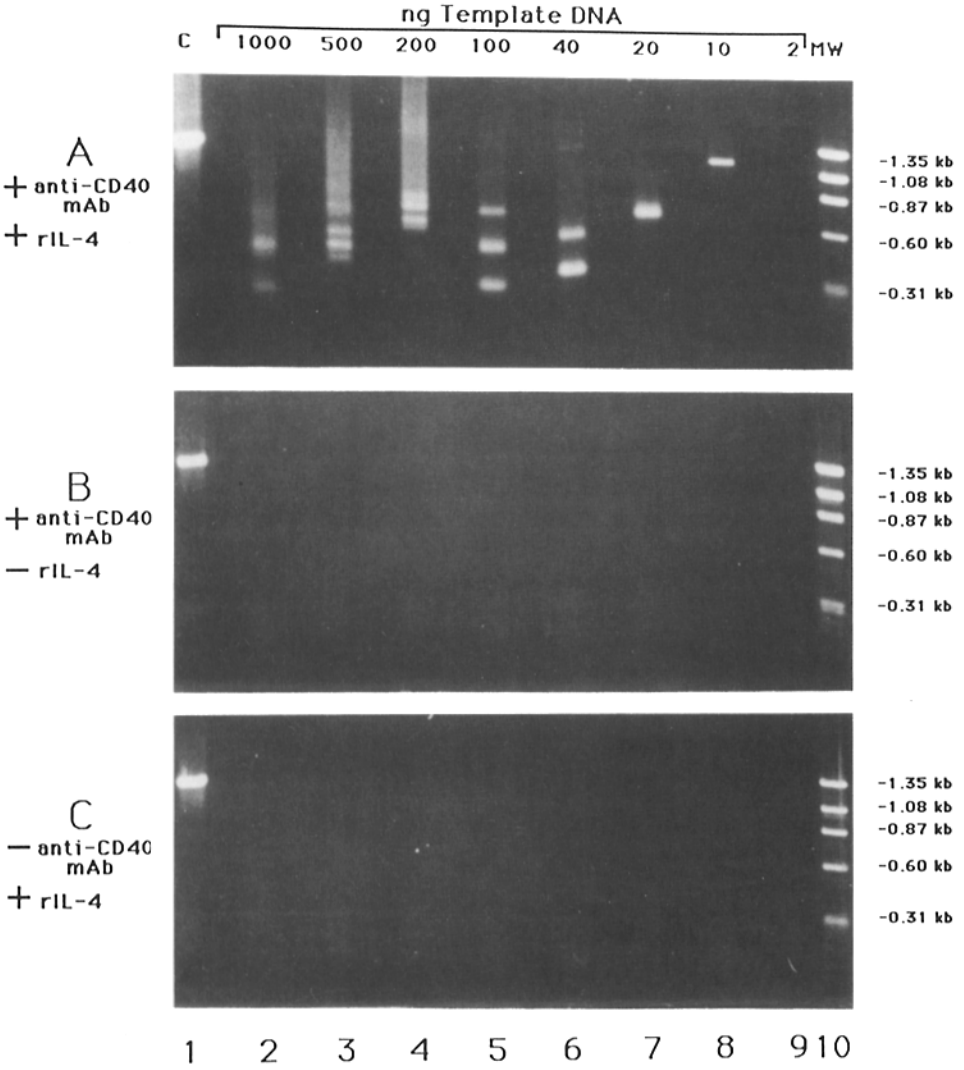

Figure 2. Nested primer PCR amplification of switch fragments. Serially diluted aliquots of total cellular DNA from B cells stimulated with anti-CD40 mAb and IL-4 (*A*), anti-CD40 mAb (*B*), or IL-4 (*C*) were amplified by nested primer PCR. The amounts of DNA used in the first round of PCR are noted above the gel in *A*. The second or nested round of PCR utilized 10% of the original PCR reaction mixture as DNA template. Final PCR products were subjected to agarose gel electrophoresis. PCR-amplifiable DNA was initially present in all three samples, as evident by the control (*C*) band (lane *1*), for which primers specific for the human IL-1β promoter (−1323/+72) were used.

Sequence from Sμ: G,A,T,C
Sequence from Sε: G,A,T,C
Sequence from either Sμ or Sε: G,A,T,C

SKS281: ···TGGGCTGAGCTGGGCAGGGCTGTCTACGCTGGGCTGAACTGAGCA···

SKS282: ···TGAGTTGAACTGGGTTGAGCTGAACTGGGTTGAGCTGGGGAGGACT···

SKS283: ···TGGGCTGAGTTGAACTGGGTTGATGAACCTAGCTGGGCTGGGCTAAA···

SKS285: ···TTTCAGAAATGGACTCAGATGGGGGAGGACTAGGCTGGGTGAGTGAC···

SKS286: ···TTGAACTGGGTTGAGCTGAGCTGATTGAGTTGGCTGGACTGAAATAA···

SKS287: ···GAGACAAAAGATGGAAGCCAGCCTAGGTTGGGCTGGGTTGGGCTAAA···

SKS288: ···GAGCTGAACTGGGCTGAGTTGAATGGAGCTGGGCTGAGCTGGCCTGG···

Figure 3. Sequence of the Sμ/Sε switch junction. The key indicates nucleotides that originate from Sμ (*capital letters*), Sε (*bold letters*), or from either S region (*underlined letters*). The adenine noted by an asterisk in SKS286 is a cytosine in the published Sε sequence, and could represent a DNA polymorphism, a PCR-generated error, or a point mutation generated during the switch event.

PCR Amplification of Sμ/Sε Switch Fragments from Cultured B Cells. Nested primer PCR was carried out to amplify Sμ/Sε switch fragments. As shown in Fig. 2 *A*, distinct fragments were amplified when ≥10 ng of template DNA from sIgE⁻ B cells stimulated with both IL-4 and anti-CD40 mAb for 10 d were used in the first round of PCR, whereas fragments were rarely amplified from less than 10 ng of DNA. No fragments were amplified from DNA of B cells cultured with either anti-CD40 mAb (Fig. 2 *B*) or IL-4 (Fig. 2 *C*), or medium (data not shown). Although it is possible that some switch fragments represent recombination events which have occurred on a chromosome carrying a nonproductive VDJ rearrangement (9–11), the detection of such fragments only in DNA from IgE-secreting B cell cultures suggests that at least some of the Sμ/Sε fragments represent recombination events on the productive chromosome.

We have previously shown that nested primer PCR amplifies switch fragments from as little as 10 pg (one diploid genome) of DNA from the IgE plasmacytoma U266 (3). If all the B cells containing cytoplasmic IgE in the CD40/IL-4 system (≈10% of the total B cells) had undergone deletional switch recombination, switch fragments should have been detected in 100 pg DNA samples. In our experiments (Fig. 2), 100-fold more DNA (10 ng) was required to amplify switch fragments. These data suggest that either only a fraction of the switch fragments could be amplified, possibly because PCR is biased against amplification of fragments longer than 1.6–2 kb, and/or that non-deletional recombination mechanisms for IgE switching (12–14) were also operative.

Sequence Analysis of Sμ/Sε Switch Fragments. Seven switch fragments amplified from DNA isolated from sIgE⁻ B cells stimulated with IL-4 and anti-CD40 mAb for 10 d were purified, cloned, and sequenced. The sequences of these fragments at the Sμ/Sε junction are shown in Fig. 3. All switch fragments in the CD40/IL-4 system had direct joining of Sμ to Sε without duplication, deletion, insertion, or extensive point mutation at the switch junction. By contrast, in the fragments generated from the plasmacytoma U266, and from

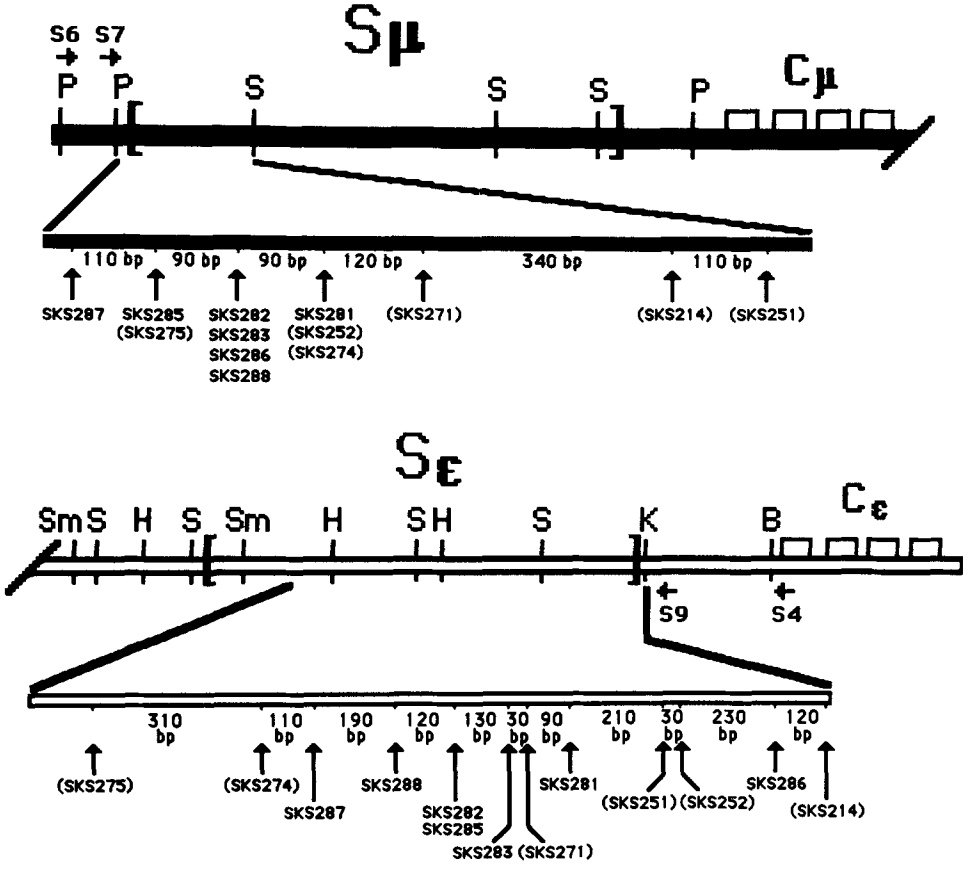

Figure 4. Switch recombination sites for the sequenced Sμ/Sε switch fragments. The positions of the PCR primers and of the recombination sites for the sequenced switch fragments are shown. Sites of recombination for switch fragments isolated from IgE-secreting EBV-B cells and from the IgE myeloma U266 (3) are shown in parentheses. Approximate map distances between recombination sites are noted (rounded off to the nearest 10 bp). B, BamHI; H, HindIII; K, KpnI; P, PstI; S, SacI; Sm, SmaI.

B cells stimulated with IL-4 and EBV, internal deletions or duplications within Sμ and insertions between Sμ and Sε were often found (3). Because the CD40/IL-4 system involves neither immortalization nor viral infection, and thus appears to be more physiologic, direct Sμ/Sε joining with deletion of intervening DNA may underlie IgE switching in vivo.

Fig. 4 shows the map sites of Sμ/Sε recombination in the CD40/IL-4 system, as well as in five switch fragments isolated from IgE-secreting EBV B cell lines and from the IgE-secreting plasmacytoma, U266 (3). The recombination sites in Sμ were clustered within 900 bp at the 5'-end of the Sμ region. There were three sites in Sμ in which more than one recombination event had occurred within a stretch of ten nucleotides. In contrast, the sites for recombination were scattered throughout the smaller Sε region. These data suggest that the specificity for Sμ/Sε switch recombination may lie within hot spots in the Sμ region, although DNA sequence comparison has not yielded a consensus sequence for the putative hot spots.

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