

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 C ϵ transcripts and induced the production of mature C ϵ 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 S μ /S ϵ joining, without the deletions or duplications within S μ 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 S μ , but not within S ϵ . These findings indicate that IL-4 provides a signal to B cells to induce germline C ϵ transcription and concurrent CD40 engagement induces S μ /S ϵ deletional switch recombination, production of mature C ϵ 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 C ϵ 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 ³²P-labeled 0.88 kb HinfI fragment which spans the first two exons of C ϵ (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).

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

PCR Primers/Amplification, Cloning, and Sequencing of S μ /S ϵ Switch Fragments. Nested PCR runs for switch (S) μ /S ϵ 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'-ACTGATCCAAGACAGGAGTGTGGCCGATGT-5'); S7 (5'-GAGGGTGGTAATGATGGTAATGCTTTGGA-3'); S9 (3'-GAACCCTGAGGTCCGGTCCCCGCTTCCCCG-5'). The first round of PCR was performed using primers S6/S4. The second round of PCR was performed on a 5- μ l 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 C ϵ Transcription. Highly purified B cells were incubated with anti-CD40 mAb 626.1 (5 μ g/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 C ϵ 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 C ϵ 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

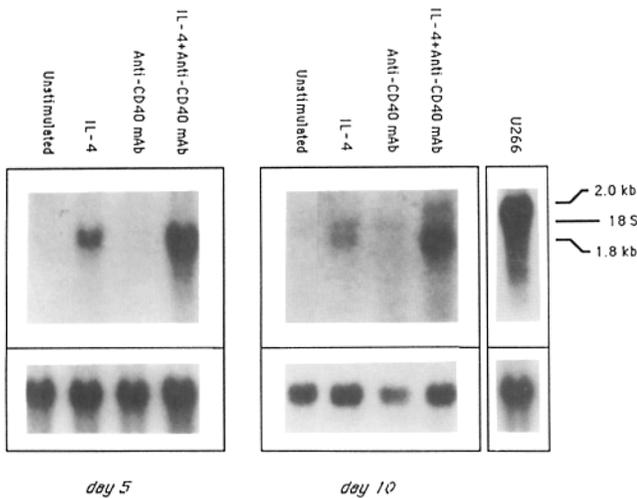


Figure 1. Induction of germline and mature C ϵ transcripts in normal human B cells stimulated with rIL-4 and anti-CD40 mAb. Total RNA (10 μ g) from normal peripheral blood B cells stimulated with rIL-4 and/or anti-CD40 mAb for 5 and 10 d and from U266 cells was subjected to electrophoresis on a 1% formaldehyde-agarose gel, transferred to nitrocel-

lulose by itself did not induce either GLT or mature C ϵ 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 S μ (6–8), whereas the CD40-derived signal may function as an activator of DNA recombination.

lulose and hybridized to ³²P-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.

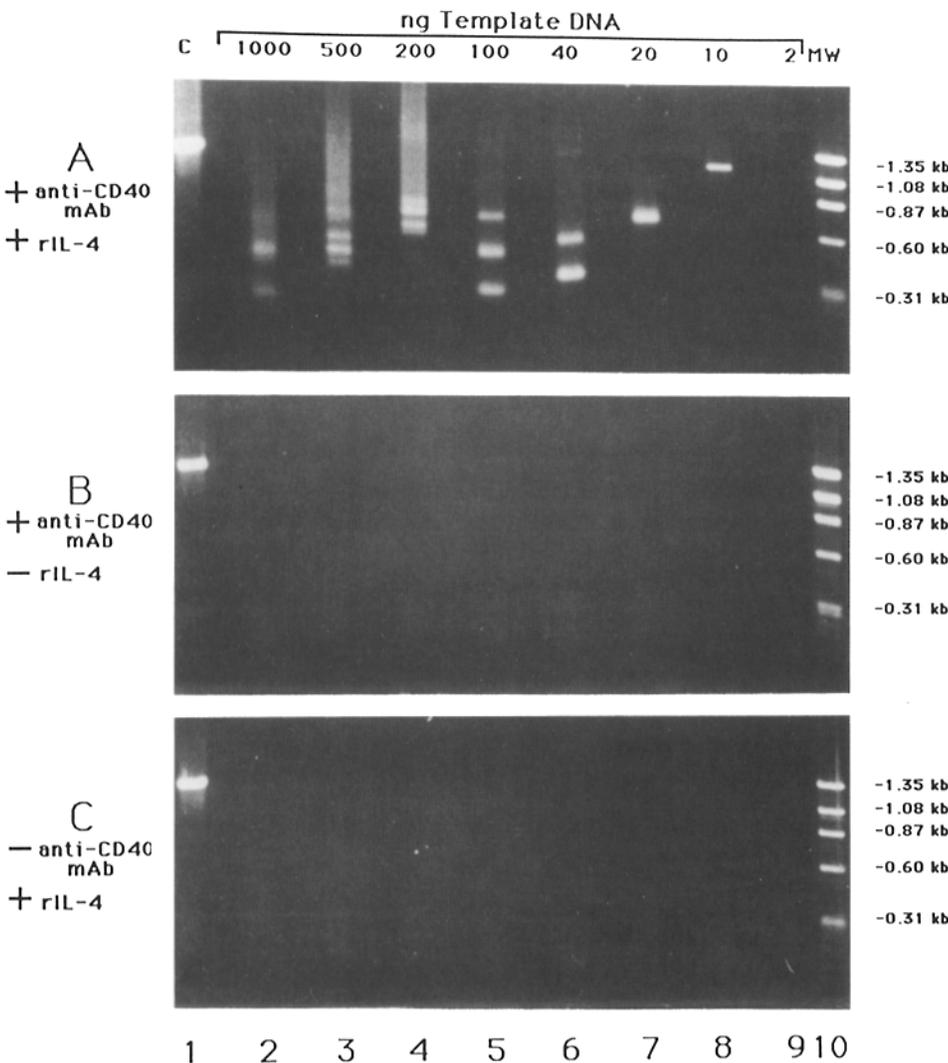


Figure 2. Nested primer PCR amplification of switch fragments. Serially diluted aliquots of total cellular DNA from B cells stimulated with anti-CD40 mAb and IL-4 (A), anti-CD40 mAb (B), or IL-4 (C) were amplified by nested primer PCR. The amounts of DNA used in the first round of PCR are noted above 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-1 β promoter (–1323/+72) were used.

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

Fig. 4 shows the map sites of $S\mu/S\epsilon$ 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 $S\mu$ were clustered within 900 bp at the 5'-end of the $S\mu$ region. There were three sites in $S\mu$ 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 $S\epsilon$ region. These data suggest that the specificity for $S\mu/S\epsilon$ switch recombination may lie within hot spots in the $S\mu$ region, although DNA sequence comparison has not yielded a consensus sequence for the putative hot spots.

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References

1. Jabara, H.H., S.M. Fu, R.S. Geha, and D. Vercelli. 1990. CD40 and IgE: Synergism between anti-CD40 monoclonal antibody and interleukin 4 in the induction of IgE synthesis by highly purified human B cells. *J. Exp. Med.* 172:1861.
2. Zhang, K., E.A. Clark, and A. Saxon. 1991. CD40 stimulation provides an IFN- γ -independent and IL-4-dependent differentiation signal directly to human B cells for IgE production. *J. Immunol.* 146:1836.
3. Shapira, S.K., H.H. Jabara, C.F. Thienes, D.J. Ahern, D. Vercelli, H.J. Gould, and R.S. Geha. 1991. Deletional switch recombination occurs in IL-4 induced isotype switching to IgE expression by human B cells. *Proc. Natl. Acad. Sci. USA.* 88:7528.
4. Gruber, M.F., J.M. Bjorndahl, S. Nakamura, and S.M. Fu. 1989. Anti-CD45 inhibition of human B cell proliferation depends on the nature of activation signals and the state of B cell activation. *J. Immunol.* 142:4144.
5. Jabara, H.H., L.C. Schneider, S.K. Shapira, C. Alfieri, C.T. Moody, E. Kieff, R.S. Geha, and D. Vercelli. 1990. Induction of germ-line and mature $C\epsilon$ transcripts in human B cells stimulated with rIL-4 and EBV. *J. Immunol.* 145:3468.
6. Yancopoulos, G.D., R.A. DePinho, K.A. Zimmerman, S.G. Lutzker, N. Rosenberg, and F.W. Alt. 1986. Secondary genomic rearrangement events in pre-B cells: V_HDJ_H replacement by a LINE-1 sequence and directed class switching. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:3259.
7. Stavnezer-Nordgren, J., and S. Sirlin. 1986. Specificity of immunoglobulin heavy chain switch correlates with activity of germline heavy chain genes prior to switching. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:95.
8. Lutzker, S., P. Rothman, R. Pollock, R. Coffman, and F.W. Alt. 1988. Mitogen- and IL-4-regulated expression of germ-line Ig $\gamma 2b$ transcripts: evidence for directed heavy chain class switching. *Cell.* 53:177.
9. Radbruch, A., W. Müller, and K. Rajewsky. 1986. Class switch recombination is IgG1 specific on active and inactive IgH loci of IgG1-secreting B cell blasts. *Proc. Natl. Acad. Sci. USA.* 83:3954.
10. Hummel, M., J.K. Berry, and W. Dunnick. 1987. Switch region content of hybridomas: the two spleen cell *Igh* loci tend to rearrange to the same isotype. *J. Immunol.* 138:3539.
11. Kepron, M.R., Y.-W. Chen, J.W. Uhr, and E.S. Vitetta. 1989. IL-4 induces the specific rearrangement of $\gamma 1$ genes on the expressed and unexpressed chromosomes of lipopolysaccharide-activated normal murine B cells. *J. Immunol.* 143:334.
12. Snapper, C.M., F.D. Finkelman, D. Stefany, D.H. Conrad, and W.E. Paul. 1988. IL-4 induces co-expression of intrinsic membrane IgG1 and IgE by murine B cells stimulated with lipopolysaccharide. *J. Immunol.* 141:489.
13. MacKenzie, T., and H.-M. Dosch. 1989. Clonal and molecular characteristics of the human IgE-committed B cell subset. *J. Exp. Med.* 169:407.
14. Shimizu, A., M.C. Nussenzweig, H. Han, M. Sanchez, and T. Honjo. 1991. *Trans*-splicing as a possible molecular mechanism for the multiple isotype expression of the immunoglobulin gene. *J. Exp. Med.* 173:1385.