MOLECULAR AND CELLULAR BIOLOGY, Apr. 1990, p. 1672–1679
0270-7306/90/041672-08$02.00/0

Vol. 10, No. 4

Structure and Expression of Germ Line Immunoglobulin Heavy-Chain ε Transcripts: Interleukin-4 Plus Lipopolysaccharide-Directed Switching to Cε

PAUL ROTHMAN,[1,2] YUNN-YI CHEN,[1] STUART LUTZKER,[1] SUZANNE C. LI,[1,2] VALERIE STEWART,[1] ROBERT COFFMAN,[3] AND FREDERICK W. ALT[1]*

The Howard Hughes Medical Institute and Departments of Biochemistry and Microbiology[1] and Department of Medicine,[2] College of Physicians and Surgeons of Columbia University, New York, New York 10032, and the DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, California 94304[3]

Received 27 September 1989/Accepted 7 December 1989

We have isolated cDNA clones complementary to a truncated immunoglobulin heavy-chain Cε RNA transcript previously found to be induced in B lymphoid cells by treatment with lipopolysaccharide (LPS) combined with interleukin-4 (IL-4). We demonstrate that this transcript initiates from a promoter upstream of the germ line ε class-switch recombination region (Sε region). The major germ line Cε transcript contains a small 5′ exon contributed by sequences upstream of the Sε region spliced to the normal Cε exons. Treatment of splenic B lymphoid cells with LPS plus IL-4 induces the expression of transcripts from the germ line ε transcription unit followed by expression of normal immunoglobulin ε heavy-chain mRNA. Furthermore, we demonstrate that similar treatment of transformed precursor B cell lines induces the expression of germ line ε transcripts followed by class switching to ε expression in these lines. This is the first demonstration of switching to ε in cells of the pre-B stage. The general structure of the germ line ε transcript and transcription unit is similar to that previously characterized for germ line γ2b transcripts. However, expression of these two germ line transcription units in B-lineage cells is inversely regulated by IL-4 (plus LPS) treatment, correlating with the effects of these treatments on switching to these loci.

Immunoglobulin molecules consist of a pair of identical heavy (H) polypeptide chains and a pair of identical light (L) polypeptide chains. H and L immunoglobulin chains have N-terminal variable and C-terminal constant regions of amino acid sequence. Variable regions of H and L chains interact to contribute the antigen-binding domain of the molecule. Variable regions are encoded by separate germ line gene segments that are assembled into complete variable region genes during precursor B lymphocyte differentiation. The immunoglobulin H-chain variable region is encoded by three DNA segments termed the V_H, D, and J_H segments (38). The constant region of the immunoglobulin H chain (the C_H region) determines effector functions of the immunoglobulin molecule. Murine C_H genes are located downstream of the variable-region locus. The eight murine C_H genes are found within a 200-kilobase region and are ordered (from 5′ to 3′) μ, δ, γ3, γ1, γ2b, γ2a, ε, α (31). The type of C_H gene expressed in an antibody molecule determines its class (or isotype); for example, an immunoglobulin molecule containing ε H-chain constant region is termed IgE. The variable-region gene is attached to the immediate downstream constant-region gene via RNA processing mechanisms.

Differentiating B lymphocytes first produce μ chains and, as a result, express an IgM surface receptor. Upon stimulation with polyclonal activators, B cells can be induced to secrete large amounts of immunoglobulin and may change the C_H region of the antibody produced. The latter process, termed H-chain class switching, allows a clonal lineage of B cells to produce an antibody that retains variable-region specificity in association with a different C_H effector function. The common mechanism of class switching in terminally differentiated B lineage cells is through a recombination event that juxtaposes a downstream C_H gene to the expressed V_HDJ_H gene (reviewed in references 13 and 20). This recombination process usually involves areas of repetitive sequences, termed switch (S) regions, that lie upstream of the various C_H genes. In animals, particular antigen-driven responses can lead to production of antibodies with distinct C_H subsets. For example, high levels of IgG2a occur during murine viral infections (10). In contrast, murine parasitic infections lead to high-level IgE production (16). Recent work has indicated that lymphokines secreted by T lymphocytes influence which C_H genes are produced by B cells (reviewed in references 8 and 24), perhaps as a result of a direct influence on the class-switch process (21).

The role of external agents in regulating immunoglobulin isotype production has been studied by using cell culture systems in which B lymphocytes are polyclonally activated with compounds such as bacterial lipopolysaccharide (LPS) along with purified lymphokines. Splenic lymphocytes cultured with LPS produce, in addition to μ, large amounts of γ2b and γ3 (45); when the T cell lymphokine interleukin-4 (IL-4) is added to LPS cultures, levels of γ2b and γ3 decrease, while production of ε and γ1 increases (reviewed in references 24 and 25). These responses occur, at least in large part, due to outgrowth of B-lineage cells that have undergone recombination-deletion class-switch events (26, 40). The propensity of normal and transformed B-lineage cells to switch to certain H-chain isotypes may be correlated with expression of the corresponding germ line C_H gene (reviewed in reference 27). For example, treatment of Abelson murine leukemia virus (A-MuLV)-transformed pre-B cells or normal splenic lymphocytes with LPS induces transcription through the germ line S and Cγ2b region prior to actual recombination to Sγ2b. Furthermore, treatment of normal spleen cells with IL-4 in combination with LPS

* Corresponding author.

inhibits both production of germ line γ2b transcripts and switching to γ2b (21). Germ line γ2b transcripts initiate upstream of the Sγ2b region and terminate at normal sites downstream of Cγ2b (19); thus, these transcripts traverse the region targeted for switch recombination. Such findings suggest that IL-4 and LPS direct class-switching in B cells, possibly by modulating the accessibility of different C_H regions to a common recombination system (21, 33). In the context of this model, transcription of the germ line C_H gene may be either a direct or indirect manifestation of accessibility.

IgE antibodies are important in defense against parasitic infections and are also potent stimulators of histamine release in allergies and anaphylaxis (reviewed in reference 3). IL-4 treatment can stimulate production of IgE by B cells both in vitro and in vivo (7, 12). Treatment of normal and/or transformed B-lineage cells with LPS plus IL-4 induces the expression of truncated Cε-hybridizing transcripts (28, 33). If IL-4 induces switching to ε in the context of the mechanism proposed for its inhibitory effects on switching to γ2b, one would predict that the truncated ε transcripts should represent germ line transcripts that traverse the Sε region. A different, but not mutually exclusive, model to explain a correlation between germ line C_H transcription and class switching would involve an active role for the transcript in the process. Such a model might predict structural or sequence homology among different germ line C_H transcripts. To further elucidate the role of mitogens and lymphokines in regulating class switching to ε, we have defined the IL-4-plus LPS-inducible ε transcription unit and analyzed the relationship between germ line ε transcription and switching to ε in both normal B cells and a pre-B cell line.

MATERIALS AND METHODS

Cell culture. The 18-81A20 (A20) A-MuLV-transformed cell line has been described previously (1). Splenocyte isolation and culture conditions were as previously described (28).

Preparation of RNA, cDNA cloning, Southern blotting, and preparation of labeled probe. Total RNA was prepared from cells by the guanidinium thiocyanate extraction procedure (6). Poly(A)$^+$ RNA was prepared by oligo(dT)-cellulose chromatography (2). For cloning purposes, cDNA was synthesized by using a cDNA synthesis kit (Pharmacia, Inc.) and cloned into lambda gt11. Library screening, plaque purification, and the isolation and subcloning of inserts were performed as previously described (2). The ε C_H probe was described previously (28). DNA blotting and preparation of ^{32}P-labeled probes by nick translation was as described previously (2).

Nucleotide sequencing and DNA cloning. The upstream genomic ε fragment was subcloned from a lambda bacteriophage clone, ε-12 (31), provided by Phil Tucker. A 1.1-kilobase *Pst*I fragment that hybridized to an *Eco*RI-PPM1 probe from the 5′ exon of the CE6 cDNA was subcloned into pUC18. Nucleotide sequences were determined by the dideoxy method (30), with protocols and a modified T7 DNA polmeryase (Sequenase) obtained from U.S. Biochemical Corp.

S1 nuclease protection analyses. An *Eco*RI-*Bst*II restriction fragment derived from the CE6 cDNA and a *Pst*I-*Hin*cII restriction fragment derived from the ε-12 genomic phage clone were subcloned into an M13 phage vector, and uniformly labeled single-stranded probes were prepared. Hybridization to RNA, S1 digestion, and analyses on 5% polyacrylamide–7 M urea gels were performed as described previously (17).

Primer extensions. A 24-base-pair (bp) oligonucleotide (5′-CTGAGGTCCTCTGATAAGTGAGTG-3′) complementary to the sequence of the CE6 cDNA that spanned the splice between the 5′ exon and Cε was end labeled with ^{32}P by using T4 polynucleotide kinase (2) and employed as a primer. Hybridization to RNA, extension with reverse transcriptase, and polyacrylamide gel analysis were performed as described by Yokota et al. (44).

Polymerase chain reactions. A 26-bp oligonucleotide (5′-ATTGGATCCTTTACAGGGCTTTAAGG-3′) complementary to a sequence in the C_H1 exon of the Cε gene, with a *Bam*HI restriction site added to the 5′ end, was used as a primer, and cDNA was synthesized by the primer extension method referred to above. The cDNA was amplified with the same Cε oligonucleotide and an 18-bp oligonucleotide corresponding to the sequence 5′-GTACTACCTGCAGTTG AA-3′ of the V_H3660 nucleotide sequence by the polymerase chain reaction (29). Reaction products were Southern blotted and probed with an end-labeled 17-bp oligonucleotide probe that corresponded to the sequence 5′-GGAACCCTCA GCTCTAC-3′, found in C_H1 of the germ line Cε gene; this latter sequence is 5′ to that represented by the oligonucleotide used in the PCR. The PCR product was subcloned into the pUC18 vector, and its nucleotide sequence was determined as indicated above.

RESULTS

Isolation of a cDNA complementary to a germ line ε transcript. We have previously demonstrated that truncated Cε-hybridizing transcripts are produced by the A20 A-MuLV-transformed pre-B cell line after culture with LPS plus IL-4. To define the truncated transcripts, we prepared a cDNA library from the poly(A)$^+$ RNA isolated from A20 cultured in the presence of these agents for 4 days. Seven independent cDNA clones containing inserts that hybridized to a Cε probe were isolated. The longest cDNA insert, CE6, was 1,450 bp long. The nucleotide sequence of this cDNA clone demonstrated that it was composed of a 76-bp novel 5′ sequence attached directly to the C_H1 region of the Cε gene (Fig. 1; only 32 bp of the novel sequence is shown). The novel 5′ sequence had no homology to any known V_H, D, or J_H region. A lambda phage genomic clone that contained the germ line region upstream of the Cε gene was assayed for hybridization to a probe specific for the 5′ sequence of the CE6 cDNA clone; this analysis permitted the isolation of a genomic sequence that was upstream of the Cε gene and shared 100% homology with the 5′ sequence of the CE6 cDNA clone (Fig. 1 and 2). The 5′ 76 bp of the CE6 clone were contained within an 1,130-bp *Pst*I fragment that lies upstream of the Sε region (Fig. 1). The shorter cDNA clones were identical in structure to the CE6 cDNA sequence in that all contained sequences from the same 5′ exon (termed Iε) spliced at identical 3′ splice junctions to the first exon of Cε; the only difference among them was the amount of Iε sequence that they contained (data not shown).

Initiation of germ line ε transcripts. Potential transcription initiation sites for the ε germ line transcripts were initially defined by S1 nuclease protection assays. For these analyses, a 501-bp probe from a *Hin*cII-*Pst*I subclone of a genomic region upstream of the Sε region (Fig. 1) was uniformly labeled with ^{32}P, hybridized to total RNA isolated from the A20 cell line cultured under different conditions, and digested with S1 nuclease and the protected fragments

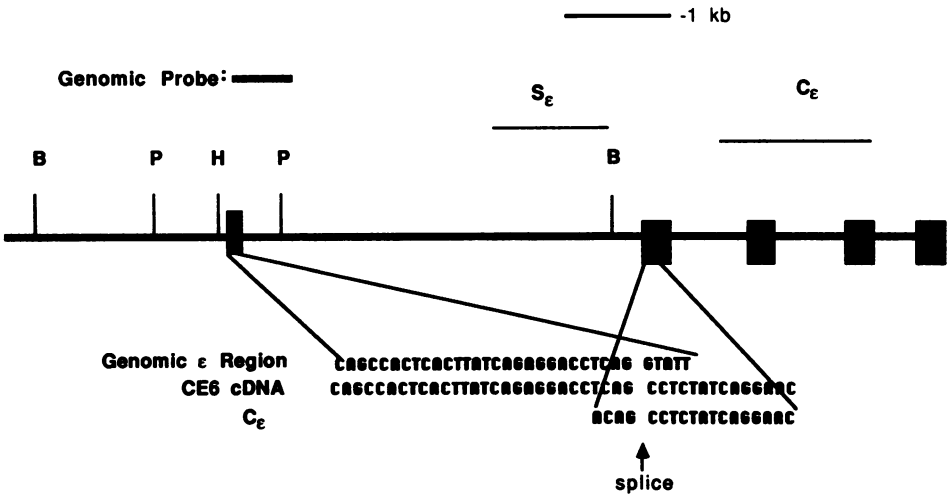

FIG. 1. Characterization of the germ line ε transcript. The upper portion of this figure shows a map of the germ line ε region, with the genomic probe used in the studies shown in Fig. 3 indicated (—). The locations of the Sε and Cε sequences are shown. Abbreviations of restriction endonucleases: B, *Bam*HI: P, *Pst*I: H, *Hin*cII. Below the map, the nucleotide sequence of the CE6 cDNA in the region surrounding the Iε/Cε splice junction is compared with the sequence of the 5′ end of the ε C_H gene and the area of upstream germ line region with sequence homology. The probable splice junction of I to Cε is noted. This junction was chosen on the basis of the presence of consensus splice donor and acceptor sequences.

were analyzed by electrophoresis through acrylamide gels (Fig. 3). This probe was not protected at detectable levels by transcripts produced by A20 cultured in the absence of exogenous agents; however, A20 cells cultured with LPS plus IL-4 produced transcripts that protected a series of fragments of the genomic probe (Fig. 3). In multiple sets of experiments, the two strongest sets of protected fragments were 77 to 87 and 92 to 100 bp in length; these represent the major transcription initiation sites for the germ line ε transcripts (see below). Larger protected fragments (350 to 400 bp) were also detected at various levels of intensity in particular RNA preparations (usually at significantly lower relative levels than those detectable in the experiment shown). The latter fragments are larger than the 220-bp region of the probe that is 5′ to the splice site defined by the seven isolated Cε-hybridizing cDNA clones (see above); therefore, they must have been protected by RNA sequences derived from the genomic region 3′ to the splice donor site of the 5′ Cε exon. Thus, the most likely explanation for their origin is that they represent precursor RNA transcripts present within the total RNA preparations used for these studies. Finally, S1 protection assays, with additional genomic probes representing sequences 5′ to those of the probe described above, demonstrated that there were no detectable transcripts containing sequences derived from the 700-bp genomic region 5′ to the putative germ line ε transcription start sites indicated above (data not shown).

```
CTGCAGCTGAGACAGACACTCACTAGTACCCCAGAAAGCTGCTGAGCCAAAGCCCAGCCCTCA      62
CACCATCTTTACCCTCATCCCTCCCTCAGTGCAGACATAGACCACAGGCCTGGAAGAGACGTT     125
AGCTGTTTCTACACAGCTCCGTGAAACCCAGTCACAACCCAGATGTGCTCTGTCCTTCTGGACT     189
CCTTGCCAGAGTAGCAGGTAGAGGACCTCAAGCTGAAAGATAATCACTTGTGAGTGGGCACCAG     253
GGAAGGCCACTGTCCCTCGCATGCCAGCTCCAAAGCTGATACAGGAACTAGGGTGCCTCTATCA     317
GAGGCCCTGCAATGTCATATCTGGCCACAGGCTGTTCCTCTTTGTGCACCATTAATAACTTACA     381
AAGTGACAGCCACACTCCTGAAGGGCTGCCAAAGGAACAGAAAAAGCAATGGCGAGGGTCTAGT     445
CCTCAGGGCATGACTCCAAAGGGGCAGGCATGGTGACTGCACGCACACACATGCAAGGCTTTAA     509
TACGAGAGCTATGCAAGGAGACCTGGGATCAGACGATGGAGAATAGAGAGCCTTGACCAGAGTG     573
TGCAGGTGTGTCTCCTAGAAAGAGGCCTCACCTGAGACCCCACTGTGCCTTAGTCAACTTCCCAA     638
GAACAGAATCAAAAGGGAACTTCCAAGGCTGCTAAGGCCGGGGGTTCCCACCCCACTTTTAGCTG     703
AGGGCACTGAGGCAGAGCGGCCCCTAGGTACTACCATCTGGGCATGAATTAATGGTTACTAGAGA     768
TTCACAACGCCTGGGAGCCTGCACAGGGGGCAGAAGATGGCTTCGAATAAGAACAGTCTGGCCAG     833
CCACTCACTTATCAGAGGACCTCAGGTATTACAACCCATGGGACCCTGAGCAAAAGGGTTTGCCT     899
AAGGAGAAGGGACAAACAGGTTACAGGGTCCTGGGTGGGGAAGGGGACACCTGGGCTGCCTTCTAA     965
TGTGGACAGTCTCTTGACCACCGAATGTCCTTCAGCTATCACTTCCCTGCACTAAGGCACACAGGT     1031
ATTAGAAACTGCTATAGCTATTCATGAAGACGGGGGACTGTGGATCTCAACCAGAGAGGGCTGAAC     1096
CAAGATAAACTGAATATGTTGTGAGAAACTCAAAACTGCA                               1136
```

FIG. 2. Nucleotide sequence of the genomic region encoding the 5′ exon of the ε germ line transcript. The nucleotide sequence of the 1,130-bp *Pst*I fragment that hybridized to the 5′ exon of the CE6 cDNA was determined. The upstream ε sequences found in the CE6 cDNA are boxed. The predicted regions of major transcriptional initiation as determined from the analyses of Fig. 3 are underlined.

To confirm that the protected bands observed in the S1 analyses represented true transcription initiation sites, poly(A)$^+$ RNA from the IL-4- plus LPS-treated A20 cells was reverse transcribed from an oligonucleotide primer that spanned the splice junction between Cε and the 5′ exon of the CE6 cDNA. In multiple experiments, the primer-extended products consisted of heterogeneous sets of reverse transcripts, with the longest species ranging from approximately 70 to 100 bp; on the basis of their size, the 5′ termini of the primer extension products roughly corresponded to the 5′ borders of the 70- to 100-bp sets of S1 nuclease-protected genomic fragments described above (data not shown). Thus, based on a combined analysis of multiple cDNA clones, S1 nuclease analyses, and primer extension analyses, the transcription of the major steady-state species of germ line ε transcripts initiates from a series of sites spread over an approximately 30-bp region upstream of the Sε region (underlined in Fig. 2) and proceeds through the Sε and Cε loci. From this primary (unprocessed) transcript, a 60- to 100-bp exon, termed Iε, is spliced to Cε exons to encode a processed transcript approximately 300 bp smaller than mature IgE mRNA. This predicted size corresponds to the size of the truncated Cε-hybridizing transcript previously

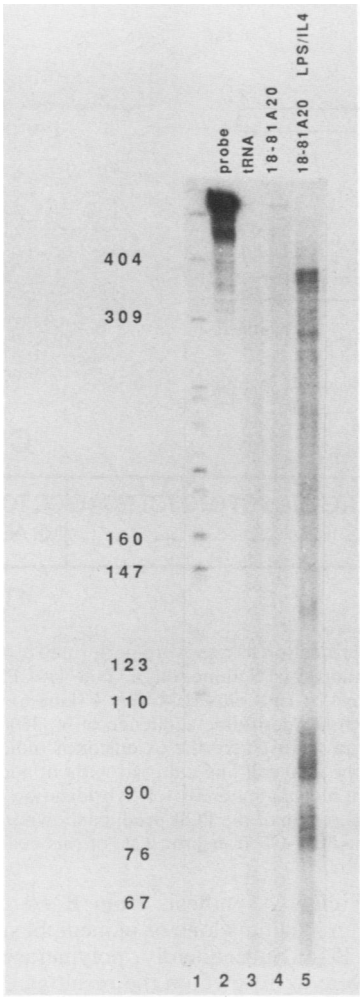

FIG. 3. Transcriptional initiation sites of germ line ε RNA. An S1 nuclease protection analysis was performed by using a uniformly labeled genomic probe from the *Hin*cII-*Pst*I genomic fragment (Fig. 1) and total RNA from the A20 cell line cultured under various conditions. Lanes: 1, markers (pBR322/MspI); 2, probe alone; 3, tRNA; 4, A20 RNA; 5, RNA from A20 cells grown with LPS plus IL-4 for 4 days.

identified by Northern (RNA) blotting (28, 34). These studies do not eliminate the possibility of lower-abundance transcripts that originate downstream of the Iε exon; however such transcription units, if they exist, do not contribute significantly to the steady-state levels of germ line ε RNA sequence.

The nucleotide sequence of the Iε exon defined by these studies, along with the surrounding germ line sequence, is shown in Fig. 2. The Iε exon (shown in the box) has a stop codon in the Cε reading frame 44 bp from the splice junction with Cε. No significant homology was found between the Iε exon and that previously defined for germ line γ2b transcripts (20). With respect to the potential promoter region upstream of the germ line ε transcription initiation site, there were no striking homologies to the regions surrounding other IL-4-inducible genes such as major histocompatibility complex class II genes (5, 11).

Expression of germ line transcripts and ε switching in splenic lymphocytes. To elucidate potential relationships between germ line transcription and switching to Sε, we

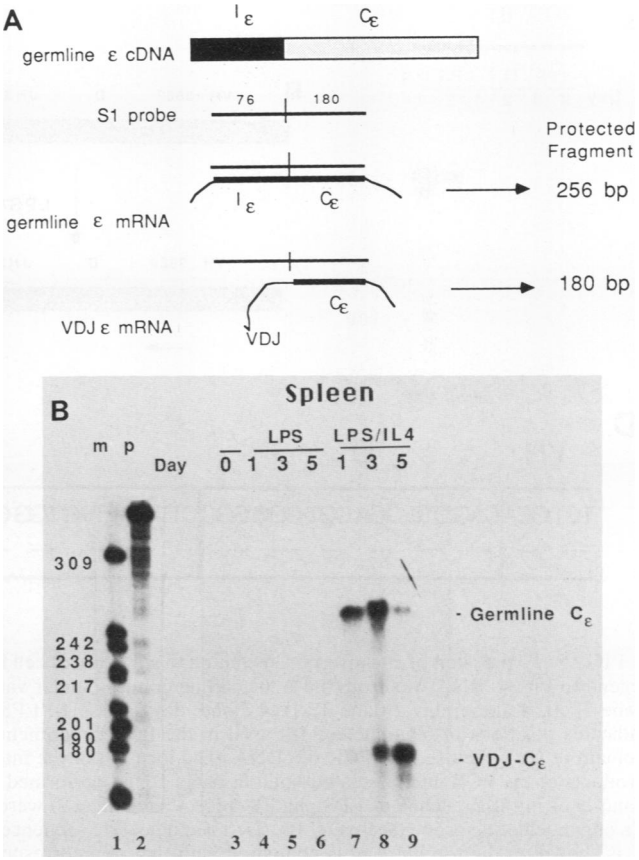

FIG. 4. Expression of ε transcripts in mouse splenocytes. (A) Diagram depicting the sizes of S1 nuclease-protected fragments of 256-bp uniformly labeled probe derived from the 5′ end of the CE6 cDNA following hybridization to germ line εRNA or εmRNA. (B) S1 protection analyses as outlined in panel A with RNA from splenocytes cultured under various conditions as indicated below. Lanes: 1, markers (pBR322/MspI); 2, probe alone; 3, day 0 cells; 4 to 6, cells cultured with LPS alone for 1, 3, and 5 days, respectively; 7 to 9, cells cultured with LPS plus IL-4 for 1, 3, and 5 days, respectively.

assayed for germ line ε transcripts and normal ε mRNA expression in mouse splenic B cells. For this analysis, a portion of the CE6 cDNA that contained the 76-bp Iε-derived sequence and 180 bp of the Cε exon was uniformly labeled with ^{32}P and used as a probe in S1 nuclease protection assays. Hybridization of this probe to the full-length germ line transcript should yield a protected band of 256 bp, whereas hybridization to a VDJ-Cε mRNA transcript should protect only the Cε portion of the probe and yield a band of 180 bp (Fig. 4A). Spleen cells cultured with LPS alone produced no detectable ε transcripts, either germ line or mRNA (Fig. 4B, lanes 4 to 6). However, exposure of spleen cells to LPS plus IL-4 induced the production of a significant level of germ line ε transcripts within the first 24 h of culture (Fig. 4B, lane 7); this level of germ line ε transcripts was maintained through day 3 of culture and declined significantly by day 5 (Fig. 4B, lanes 7 to 9). Transcripts corresponding in size to mature ε mRNA were barely detectable at day 1 of the cultures with LPS plus IL-4, but their level increased dramatically by day 3 and continued to increase by day 5 (Fig. 4B, lanes 7 to 9). Therefore, induction of germ line ε transcripts precedes the accumulation of ε mRNA in LPS- plus IL-4-treated splenic lymphocytes.

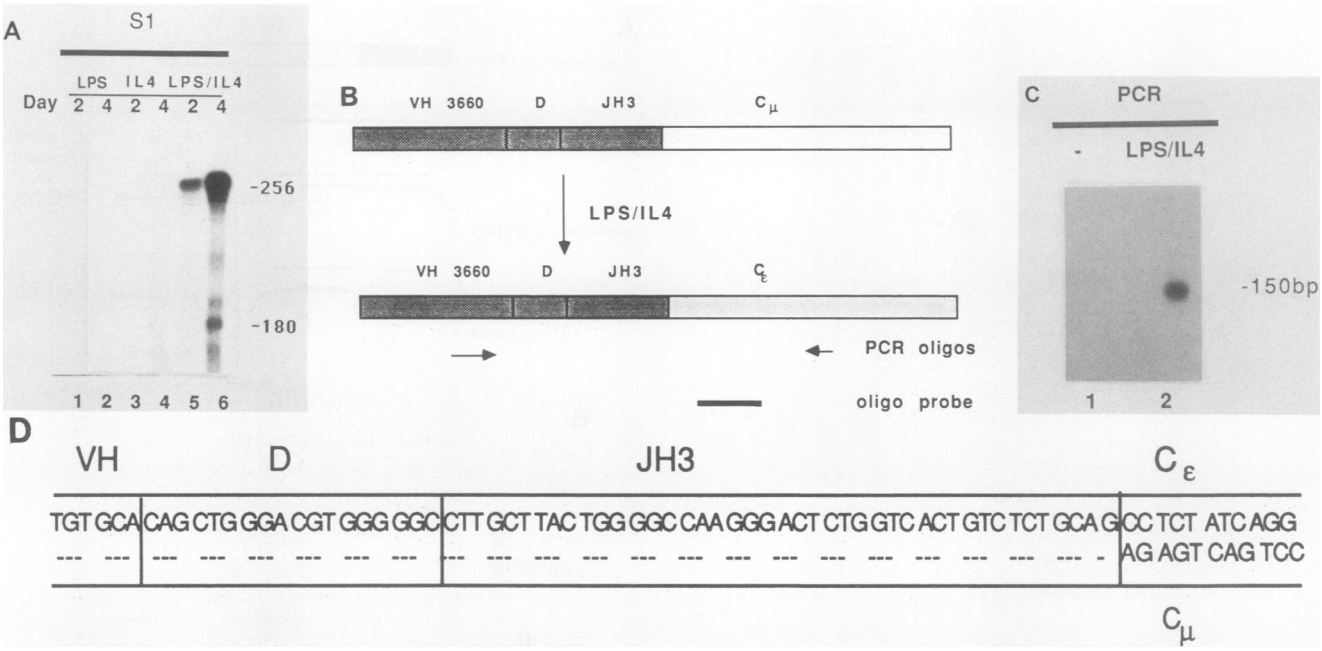

FIG. 5. Expression of ε transcripts switching to ε in a pre-B cell line. (A) S1 nuclease protection assays were performed as outlined in the legend to Fig. 4. RNA was from the A20 cell line cultured under various conditions as follows: LPS alone, day 2 (lane 1); LPS alone, day 4 (lane 2); IL-4 alone, day 2 (lane 3); IL-4 alone, day 4 (lane 4); LPS plus IL-4, day 2 (lane 5); LPS plus IL-4, day 4 (lane 6). (B) This map indicates positions of oligonucleotides used in the PCR experiment. Probes derived from the germ line sequence of V_H3660 and the C_H1 domain of Cε were used for PCR of cDNA. The location of the internal oligonucleotide probe used for the extension is indicated. (C) The products of the PCR analysis as outlined in panel B and performed on poly(A) RNA of the A20 cell line cultured without additional agents (lane 1) or in the presence of LPS plus IL-4 for 4 days (lane 2) were assayed by Southern blotting methods for hybridization to the internal Cε oligonucleotide probe (see panel B). (D) The nucleotide sequence of the VDJ junctional region of the PCR product shown in panel C, lane 2, is shown on the top line and is compared with that of a corresponding sequence of the VDJ-Cμ transcript (21) of this cell line.

Induced switching to ε in a pre-B cell line. It is not known when the competence to switch to ε occurs during B cell ontogeny. We have previously demonstrated that certain μ H-chain-producing A-MuLV-transformed cell lines constitutively produce germ line γ2b transcripts and correspondingly switch spontaneously to γ2b production as a result of S-region-mediated class-switch recombination (1, 19, 43); treatment of these lines with LPS augments germ line γ2b expression and increases the frequency of switching in these pre-B lines (21). Pre-B cells or cell lines have never been observed to make germ line ε transcripts or to switch to ε expression. To elucidate whether such cells are capable of undergoing switching to ε upon appropriate stimulation, we assayed the populations of the A20 cell line treated with either LPS alone or LPS plus IL-4 for production of germ line ε transcripts and mature ε mRNA. A20 cells cultured with LPS alone (Fig. 5A, lanes 1 and 2) or IL-4 alone (lanes 3 and 4) produced no detectable transcripts of any type. However, when this line was cultured in the presence of both LPS and IL-4, expression of germ line ε transcripts was readily detectable by day 2 of culture and further increased by day 4 of culture (lanes 5 and 6). Significantly, after 4 days of culture in the presence of LPS plus IL-4, transcripts corresponding in structure (protected size) to those of mature ε mRNA were detectable, suggesting that cells in the A20 population may have actually switched to ε production (lane 6). We have obtained similar results from analyses of several other independently derived A-MuLV-transformed pre-B cell lines (data not shown).

To confirm that cells in the A20 population had actually switched from μ to ε mRNA production, an oligonucleotide complementary to a sequence in the Cε C_H1 domain was used to prime cDNA synthesis from RNA of A20 cells cultured either in medium alone or in medium supplemented with LPS and IL-4. Subsequently, polymerase chain reactions (PCRs) were performed on the resulting cDNA preparations, with an oligonucleotide sequence derived from the Cε1 domain and an oligonucleotide specific for the productive V_H region expressed in the μ mRNA of this cell line as primers (Fig. 5B). The PCR products were then assayed for the presence of RNA corresponding in structure to ε mRNA by Southern blotting procedures for hybridization to a diagnostic oligonucleotide probe corresponding to a Cε sequence 5′ to that used in the probe used for the PCRs (Fig. 5B). Under these conditions, only RNA from the LPS-plus-IL-4-cultured A20 cells produced a PCR product that hybridized to the diagnostic Cε probe (Fig. 5C shows typical results; the experiment was performed several times). Comparison of the nucleotide sequence of this product with that of the μ mRNA produced by the parent A20 cells clearly demonstrated that the PCR product derived from an mRNA encoding the productively rearranged V_HDJ_H gene of this cell line correctly spliced to the C_H1 of Cε (Fig. 5D).

DISCUSSION

LPS- plus IL-4-directed switching to ε in splenic lymphocytes is preceded by transcription of the germ line ε gene. LPS treatment of normal spleen cells induces the appearance of cells that have switched to γ2b (and γ3) production, whereas treatment of these normal cells with IL-4 plus LPS abrogates the appearance of γ2b-producing cells, but now stimulates the appearance of cells that produce ε (and γ1) (7, 14, 39). We previously have demonstrated that the effects of these

agents on class switching to γ2b directly correlates with their effects on production of germ line γ2b transcripts. Thus, culture of splenic B cells with LPS induces the expression of germ line γ2b transcripts followed by the accumulation of cells that have switched to γ2b, while simultaneous addition of IL-4 to the LPS-cultured cells abrogates the induction of germ line γ2b transcripts and switching to γ2b (21). In striking contrast, we demonstrate that treatment of splenic lymphocytes with LPS alone has absolutely no effect on the expression of the germ line ε transcription unit (and switching to ε), whereas treatment with IL-4 plus LPS dramatically induces germ line ε transcripts in these cells followed by switching to ε. There is substantial evidence that switch region rearrangement is a major pathway in the production of IgE in such systems (15, 23, 32, 37). Therefore, our findings provide strong support for the notion that expression of germ line transcripts by B lineage cell lines is directly related to factors involved with directing the class-switch process. Our findings also are consistent with models which predict that class switch recombination is regulated by exogenous agents via modulation of the accessibility of S regions to a common recombination system (28, 33, 34, 43).

Role of germ line transcription in switching. The precise role of germ line transcription with respect to directed class switching remains to be elucidated. Similar to the previously defined germ line μ and γ2b transcripts (18, 19, 22), the major germ line ε transcripts initiate 5′ of the switch region. Thus, the IL-4-induced ε transcripts derive from a transcription unit that proceeds through the Sε region prior to class switching to ε This finding is consistent with the possibility that transcription through a targeted region is involved directly in promoting accessibility. In this regard, several recent studies have shown that increased transcription of certain regions of DNA in yeast can be correlated with increased rates of recombination (36). Likewise, transcription of germ line immunoglobulin or T-cell receptor variable-region loci has been shown to correlate with their ability to be rearranged by a common VDJ recombinase system (4, 41, 42). With respect to the latter system, a direct role for transcriptional control elements in regulating VDJ recombination has been implied from experiments which demonstrate that a DNA fragment containing the immunoglobulin H-chain transcriptional enhancer element targets associated transgenic T-cell receptor variable-region gene segments for rearrangement by the VDJ recombinase (P. Ferrier, B. Krippl, T. K. Blackwell, A. J. Furley, H. Suh, A. Winoto, W. Cook, L. Hood, F. Constantini, and F. W. Alt, EMBO J., in press).

Transcription may not be directly responsible for targeting germ line C_H (and other) genes for rearrangement; several other, not necessarily mutually exclusive, possibilities may be considered. For example, transcription may be a by-product of other events (perhaps binding of specific factors) involved in promoting recombinational accessibility. Other possible explanations for the correlation between switching and expression of germ line transcripts include a potential role in the process for the germ line transcripts themselves. The structures of the germ line C_H gene transcripts we have elucidated, including γ2b (19), γ3 (P. Rothman et al., manuscript submitted, and ε (this study), are all very similar to each other and to that of germ line μ transcripts (18). Each consists of a 5′ exon (I exon) derived from germ line sequences upstream of the corresponding S region that is spliced correctly to the C_H1 of the immediate downstream C_H gene. The 5′ exon of the germ line ε transcript initiates from several sites within a small region 5′ to the ε switch region. This pattern of heterogeneous initiation sites was also found for both the germ line γ2b and μ transcripts. The similarities in the structures of the spliced germ line transcripts may indicate that they perform some analogous function, although they contain no significant sequence homologies. The upstream germ line exons of all of these transcripts have stop codons in the open reading frame of the C_H region to which they are spliced; therefore, they do not appear capable of encoding large proteins. It has been suggested that germ line RNA transcripts themselves may promote specific recombination events through the stabilization of triplex structures that can be formed within S-region DNA (9). Another conceivable role for the germ line transcripts would be their use in putative *trans* RNA splicing mechanisms for H-chain class switching (20). Transplicing, which has been observed in trypanosomes (35), would allow a cell to utilize the same V region and different C_H regions without deletional recombination and would provide a convenient mechanism by which some B cells could express a downstream immunoglobulin isotype without S-region recombination while targeting the S region of that isotype for a future S-region recombination.

Interaction with lymphokines may alter a precommitment to switch to particular C_H genes. A-MuLV transformed pre-B cell lines often spontaneously undergo recombination-deletion class switching to the γ2b gene; treatment of these cells with LPS can increase the spontaneous rate of switching to γ2b and also induce switching to γ3 (21; Rothman et al., submitted). We now demonstrate that A-MuLV-transformed pre-B cells can be induced to switch to ε expression when cultured with IL-4 in addition to LPS. This finding demonstrates for the first time that transformed cells of the pre-B lymphocyte stage can functionally respond to IL-4 and suggests the possibility that normal pre-B cells will respond similarly. In addition, because the pre-B lines tested do not put immunoglobulin on their surface, it is highly unlikely that IL-4 selects cells that have switched to ε on the basis of surface IgE expression. Therefore, our findings support prior evidence (reviewed in reference 8) that IL-4 can actually direct class-switch recombination and also provides the first evidence that a precommitment to switch to a particular isotype in a clonal pre-B cell line can be altered by treatment with exogenous agents.

Our findings suggest the possibility that differentiating B lineage cells inherit a precommitment to switch to γ2b and γ3 (20); a manifestation of this commitment is the LPS induction of γ2b and γ3 transcripts in these cells. Interaction of B lineage cells with lymphokines such as IL-4 may change this commitment, as manifested by the observation that treatment of splenic B cells with LPS combined with IL-4, in direct correspondence to the effect on switching patterns, abrogates germ line γ2b expression and induces the accumulation of germline ε transcripts. Our current findings also suggest that this change in commitment to switch can occur even in pre-B cells. In contrast to the A20 pre-B line, the I.29 B-cell lymphoma line can switch to ε when induced with LPS alone (32). The difference between the requirements for ε switching in these two cell lines may be due to their stage in B-cell ontogeny or, more probably, may be related to an event prior to transformation of the I.29 line that might have included interaction with T cells and/or their products. The molecular basis of the precommitment of a cell to switch to certain isotypes has not been defined. The further characterization of the regions and events involved in controlling the expression of germ line C_H transcripts should allow more precise elucidation of the mechanisms by which exogenous

agents direct class-switch recombination and help define the molecular elements involved in the commitment of B lineage cells to differentiate into various types of effector cells.

ACKNOWLEDGMENTS

This work was supported by the Howard Hughes Medical Institute and by Public Health Service grants AI200047 and CA40427 to F.W.A. and by Physician Scientist Award DK01336 to P.R. from the National Institutes of Health. S.C.L. is the recipient of an Arthritis Foundation grant, and S.L. was supported by training grant GM-07367 from the National Institutes of Health.

Our thanks to Noboyuki Harada and Rob Kastelein for help in preparing recombinant IL-4.

LITERATURE CITED

1. **Alt, F. W., N. Rosenberg, R. Casanova, E. Thomas, and D. Baltimore.** 1982. Immunoglobulin heavy chain expression and class-switching in a murine leukemia cell line. Nature (London) **296**:325–331.
2. **Alt, F., N. Rosenberg, V. Enea, E. Siden, and D. Baltimore.** 1982. Multiple immunoglobulin heavy-chain gene transcripts in Abelson murine leukemia virus-transformed lymphoid cell lines. Mol. Cell. Biol. **2**:386–400.
3. **Bach, M. K.** 1982. Mediators of anaphylaxis and hypersensitivity. Annu. Rev. Microbiol. **36**:371–391.
4. **Blackwell, T. K., M. Moore, G. Yancopoulos, H. Suh, S. Lutzker, E. Selsing, and F. Alt.** 1986. Recombination between immunoglobulin variable region segments is enhanced by transcription. Nature (London) **324**:585–589.
5. **Boothby, M., E. Gravallese, H. C. Liou, and L. Glimcher.** 1988. A DNA binding protein regulated by IL-4 and by differentiation in B cells. Science **242**:1559–1562.
6. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**:5294–5299.
7. **Coffman, R., J. Ohara, M. Bond, J. Carty, A. Zlotnik, and W. Paul.** 1986. B cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide-activated B cells. J. Immunol. **136**:4538–4541.
8. **Coffman, R., H. F. J. Savelkoul, and D. A. Lebman.** 1989. Cytokine regulation of immunoglobulin isotype switching and expression. Semin. Immunol. **1**:55–64.
9. **Collier, D., J. Griffin, and R. Wells.** 1989. Non-B right handed DNA confirmations of homopurine-homopyrimidine sequences in the murine immunoglobulin Cα switch region. J. Biol. Chem. **263**:7397–7405.
10. **Coutelier, J.-P., J. Van der Logt, F. Heessen, G. Warnier, and J. Van Snick.** 1987. IgG2a restriction of murine antibodies elicited by viral infections. J. Exp. Med. **165**:64–69.
11. **Didier, D. K., J. Schiffenbauer, S. L. Woulfe, M. Zacgeis, and B. D. Schwartz.** 1988. Characterization of the cDNA encoding a protein binding to the major histocompatibility complex class II Y boz. Proc. Natl. Acad. Sci. USA **85**:7322–7326.
12. **Finkelman, F., I. Katona, J. Urban, C. Snapper, J. Ohara, and W. Paul.** 1986. Suppression of in vivo polyclonal IgE response by monoclonal antibody to the lymphokine BSF-1. Proc. Natl. Acad. Sci. USA **83**:9675–9678.
13. **Honjo, T., A. Shimizu, and Y. Yaoita.** 1989. Constant-region genes of the immunoglobulin heavy chain and the molecular mechanism of class switching, p. 123–151. *In* F. Alt, T. Honjo, and T. Rabbits (ed.), Immunoglobulin genes. Academic Press, Inc., New York.
14. **Isakson, P., E. Pure, E. Vitteta, and P. Kramer.** 1982. T cell-derived B cell differentiation factor(s). Effect on the isotype switch of murine B cells. J. Exp. Med. **155**:734–746.
15. **Ishida, N., S. Ueda, H. Hayashida, T. Miyata, and T. Honjo.** 1982. The nucleotide sequence of the mouse immunoglobulin epsilon gene: comparison with human epsilon gene sequence. EMBO J. **1**:1117–1123.
16. **Jarrett, E., and H. Miller.** 1982. Production and activities of IgE in helminth infection. Prog. Allergy **31**:178–233.
17. **Kohl, N., E. Legouy, R. DePinho, P. Nisen, R. Smith, C. Gee, and F. Alt.** 1986. Human n-myc is closely related in organization and nucleotide sequence to c-myc. Nature (London) **319**:73–77.
18. **Lennon, G., and R. Perry.** 1985. Cμ-containing transcripts initiate heterogenously within the IgH enhancer region and contain a novel 5′-nontranslatable exon. Nature (London) **318**:475–478.
19. **Lutzker, S., and F. Alt.** 1988. Structure and expression of germ line immunoglobulin γ2b transcripts. Mol. Cell. Biol. **8**:1849–1852.
20. **Lutzker, S., and F. Alt.** 1988. Immunoglobulin heavy chain class switching, p. 691–714. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
21. **Lutzker, S., P. Rothman, R. Pollock, R. Coffman, and F. Alt.** 1988. Mitogen- and IL-4-regulated expression of germline Ig γ2b transcripts: evidence for directed heavy chain class switching. Cell **53**:177–184.
22. **Nelson, K. J., J. Haimovich, and R. Perry.** 1983. Characterization of productive and sterile transcripts from the immunoglobulin heavy-chain locus: processing of μm and μs mRNA. Mol. Cell. Biol. **3**:1317–1332.
23. **Nishida, Y., T. Miki, H. Hisajima, and T. Honjo.** 1982. Cloning of human immunoglobulin ε chain genes: evidence for multiple Cε genes. Proc. Natl. Acad. Sci. USA **79**:3833–3837.
24. **Paul, W. E.** 1989. Pleiotropy and redundancy: T cell-derived lymphokines in the immune response. Cell **57**:521–524.
25. **Paul, W. E., and J. Ohara.** 1987. B-cell stimulatory factor-1/interleukin 4. Annu. Rev. Immunol. **5**:429–459.
26. **Radbruch, A., and F. Sablitzky.** 1983. Deletion of Cμ gene in mouse B lymphocytes upon stimulation with LPS. EMBO J. **2**:1929–1935.
27. **Rothman, P., S. C. Li, and F. Alt.** 1989. The molecular events in heavy chain class-switching. Semin. Immunol. **1**:65–77.
28. **Rothman, P., S. Lutzker, W. Cook, R. Coffman, and F. Alt.** 1988. Mitogen plus interleukin 4 induction of Cε transcripts in B lymphoid cells. J. Exp. Med. **168**:2385–2389.
29. **Saiki, R. K., D. H. Gelfand, S. Soffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239**:487–491.
30. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
31. **Shimizu, A., N. Takahashi, Y. Yaoita, and T. Honjo.** 1982. Organization of the constant region gene family of the mouse immunoglobulin heavy chain. Cell **29**:499–506.
32. **Stavnezer, J., S. Sirlin, and J. Abbott.** 1985. Induction of immunoglobulin isotype switching in cultured I.29 B lymphoma cells. J. Exp. Med. **161**:577–601.
33. **Stavnezer, J., G. Radcliffe, Y.-C. Lin, J. Nietupski, L. Berggren, R. Sitia, and E. Severinson.** 1988. Ig heavy chain switching may be directed by prior induction of transcripts from constant region genes. Proc. Natl. Acad. Sci. USA **85**:7704–7708.
34. **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 J. **5**:95–102.
35. **Sutton, R., and J. Boothroyd.** 1986. Evidence for trans-splicing in trypanosomes. Cell **47**:527–535.
36. **Thomas, B. J., and R. Rothstein.** 1989. Elevated recombination rates in transcriptionally active DNA. Cell **56**:619–630.
37. **Tian, S., and C. Faust.** 1987. Rearrangement of rat immunoglobulin E heavy chain and c-*myc* in the B cell immunocytoma IR162. Mol. Cell. Biol. **7**:2614–2619.
38. **Tonegawa, S.** 1983. Somatic generation of antibody diversity. Nature (London) **302**:575–581.
39. **Vitetta, E., J. Ohara, C. Myers, J. Layton, P. Krammer, and W. Paul.** 1985. Serological, biochemical, and functional identity of B cell stimulatory factor-1 and B cell differentiation factor for IgG1. J. Exp. Med. **162**:1726–1731.
40. **Winter, E., U. Krawinkel, and A. Radbruch.** 1987. Directed Ig class switch recombination in activated murine B cells. EMBO J. **6**:1663–1671.

41. **Yancopoulos, G. D., and F. W. Alt.** 1985. Developmentally controlled and tissue-specific expression of unrearranged VH gene segments. Cell **40**:271–281.
42. **Yancopoulos, G., T. K. Blackwell, H. Suh, L. Hood, and F. Alt.** 1986. Introduced T cell receptor gene segments recombine in pre-B cells: evidence that B and T cells use a common recombinase. Cell **44**:251–259.
43. **Yancopoulos, G., R. DePinho, K. Zimmerman, S. Lutzker, N. Rosenberg, and F. Alt.** 1986. Secondary rearrangement events in pre-B cells: V_HDJ_H replacement by a LINE-1 sequence and directed class switching. EMBO J. **5**:3259–3266.
44. **Yokota, A., H. Kikutani, T. Tanaka, R. Sato, E. L. Barsumian, M. Suemura, and T. Kishimoto.** 1989. Two species of human Fcε receptor II (FcεRII/CD23): tissue-specific and IL-4 specific regulation of gene expression. Cell **55**:621–628.
45. **Yuan, D., and E. Vitteta.** 1983. Structural studies of cell surface and secreted IgG in LPS-stimulated murine B cells. Mol. Immunol. **20**:367–375.