Brief Definitive Report

MITOGEN PLUS INTERLEUKIN 4 INDUCTION OF Cε TRANSCRIPTS IN B LYMPHOID CELLS

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The Ig H chain locus contains multiple C region genes that encode different effector functions; the order of these genes from 5′ to 3′ is μ, δ, γ3, γ1, γ2b, γ2a, ε, and α (1). Recombination events between sets of repetitive elements located upstream of each C region gene (switch regions) can replace the μ gene with a downstream C region gene (termed HC class or isotype switching), and thereby allow an antibody of given specificity (variable region) to change constant region effector function (for review see reference 2).

Certain lymphokines modulate production of specific Ig HC isotypes by activated B cells (for review see references 3, 4). For example, activation of B cell cultures by addition of bacterial LPS increases production of γ3 and γ2b as a result of class-switch recombinations to these loci, and concomitant addition of IL-4 abrogates this response (5, 6). Expression of germline γ2b transcripts precedes switching to this locus in activated B cells; this expression is induced by LPS and abrogated by simultaneous treatment with IL-4 (7). The correlation between the effects of these agents on production of germline transcripts and class switches has been interpreted in the context of a model that suggests such agents direct switching to specific isotypes by modulating recombinational "accessibility" of specific switch regions (7–9).

Simultaneous treatment of B cells with LPS and IL-4 elicits *increased* production of γ1 and ε (10, 11). A prediction of the above model is that this treatment may induce specific switches to these loci by altering their accessibility. As a test of this hypothesis, we have examined the effects of LPS and IL-4 on Cε and Cγ1 gene expression in B lineage cells.

Materials and Methods

Cell Culture. The 18–81A20 (A20) Abelson murine leukemia virus (A-MuLV)-transformed cell line is a subclone of the 18–81 line. The A20 line produces μ H chains (12). WCPR8 was derived by preculturing BALB/c mouse bone marrow with IL-4 (150 U/ml) for 12 h before infection with A-MuLV. Viral infections, propagation of cells, and LPS or IL-4 treat-

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ments were as described (7, 13). Spleen cells were isolated from 3–4-wk-old female BALB/c mice, depleted of T cells by treatment with an mAb to Thy-1.2 (30-H12) (a gift of N. Braunstein, Columbia University) and complement, and were cultured as described previously (11). LPS was used at 10 μg/ml. rIL-4 (purified by affinity chromatography from extracts of *Escherichia coli*) expressing an IL-4 cDNA; >96% pure) was used at a 150 U/ml final concentration. Anti-IL-4 mAb 11B11 was used at 5 μg/ml final concentration.

Nucleic Acids. Preparation of RNA, Northern blotting, and labeling of probes with ^{32}P were performed as previously described (9). The Cε probe was a germline 4.8-kb Bam HI fragment that contained the ε constant region (derived from plasmid PE, which was generously provided by Phil Tucker, University of Texas Southwestern Medical Center, Dallas, TX).

Results

IL-4 Induction of ε Transcripts in Pre-B Cell Lines. The A20 cell line expresses a germline γ2b transcript and switches to γ2b when treated with LPS (7); others have reported Cε transcripts in a B cell lymphoma that switches spontaneously to ε (8). To assay effects of LPS and IL-4 on expression of the A20 ε gene, this line was cultured with one or a combination of these agents and assayed for Cε-hybridizing RNA. When cultured without added agents (not shown), LPS alone (Fig. 1 *A*), or IL-4 alone (Fig. 1 *B*), no ε transcripts were detected. However, when cells were cultured with LPS plus IL-4, a 1.9-kb Cε-hybridizing transcript was produced (Fig. 1, *A* and *B*). This transcript was detected after 24 h, reached maximum level at day 4, and gradually disappeared (Fig. 1, *A* and *B*). The 1.9-kb transcript is smaller than ε mRNA (e.g., 2.2-kb VDJCε mRNA; reference 14) and did not hybridize to a V_H probe specific for the single V_HDJ_H rearrangement in A20; in addition, the line had no detectable level of rearrangements at the Cε region before or after treatment (not shown). These data indicate that the 1.9-kb Cε-hybridizing transcript derives from the germline ε locus rather than from the V_HDJ_H rearrangement. The transient nature of the induction of Cε transcripts in A20 could have several explanations, including a potential growth disadvantage of cells that produce the transcript or are responsive to IL-4. No switching to Cε was detected in A20 after treatment; however, we would not have detected switching at low level by the analyses used.

Most A-MuLV-transformed pre-B lines have very low levels of IL-4-R (15). A-MuLV transformants were derived by preincubating bone marrow cells with IL-4 before viral infection in hopes of obtaining lines more responsive to IL-4. Treatment of one such line (WCPR8) with LPS alone or LPS plus IL-4 yielded results similar to those obtained with A20, except that both a 1.9- and 2.2-kb (the latter corresponding in size to ε mRNA) were seen at day 5; induction of the 2.2-kb ε transcript was preceded (at day 3) by the induction of the 1.9-kb Cε-hybridizing transcript (Fig. 1 *C*). Subclones of WCPR8 have been assayed and give a similar response (data not shown).

LPS plus IL-4 Induction of ε Transcripts in Normal Spleen Cell. T cell–depleted splenocytes cultured with LPS produce no detectable Cε-hybridizing transcripts (Fig. 2). In contrast, when cultured with LPS plus IL-4 these cells express the 1.9-kb Cε-hybridizing transcript; expression of this transcript is detectable by day 2 and increases by day 4 (Fig. 2). No transcripts corresponding in size to ε mRNA are detectable at day 2; however, day 4 cells contain a substantial amount of 2.2-kb ε mRNA sequences (Fig. 2). Accumulation of ε mRNA at day 4 correlates with appearance of ε protein–producing cells in LPS plus IL-4 cultures (11). Addition of 11B11, an mAb against IL-4 (16), to the LPS plus IL-4 cultures, reduces accumulation of the

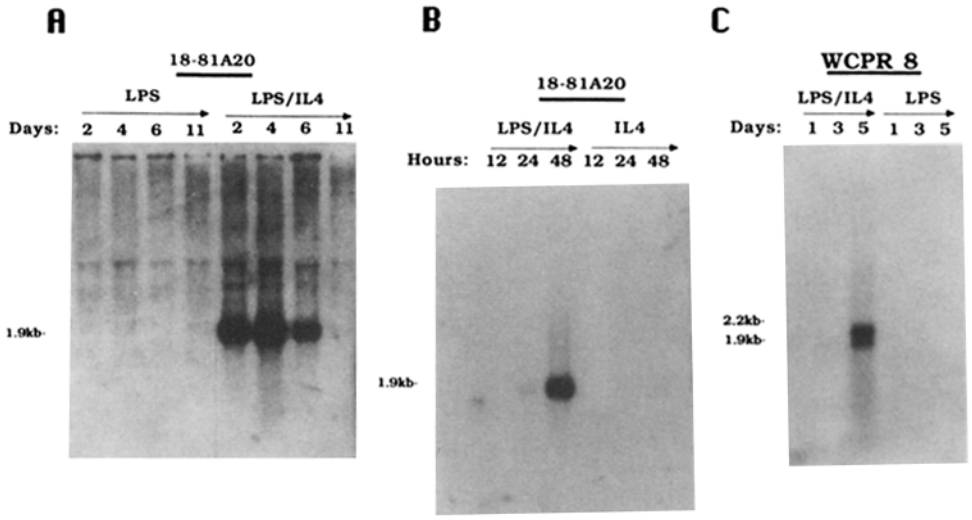

FIGURE 1. IL-4 induction of ε transcripts in A-MuLV transformants. The cell lines were cultured and total RNA was assayed by Northern blotting for Cε hybridizing transcripts as indicated in Materials and Methods. (*A*) The A20 cell line was cultured with 10 μg/ml LPS, with or without IL-4(150 U/ml). (*B*) The A20 line was cultured with IL-4, with or without LPS (as above). (*C*) WCPR8 was cultured with LPS with or without IL-4 (as above).

1.9-kb transcript to very low levels and correspondingly abrogates accumulation of ε mRNA at day 4 (Fig. 2).

LPS plus IL-4 Induction of Germline Cγ1-Region Transcripts. Northern blotting analyses of RNA from LPS plus IL-4-treated splenocytes or A20 cells with a Cγ1 probe revealed (at prominant levels) only transcripts corresponding in size to γ1 mRNA (not shown). However, LPS plus IL-4 treatment of these cells also resulted in accumulation of a very heterogeneous set of transcripts that hybridized to a germline γ1 switch region probe (not shown). The heterogeneous size of these transcripts precluded a simple analysis of their structure; however, their hybridization pattern clearly indicated germline origin (confirmed by molecular cloning of one species).

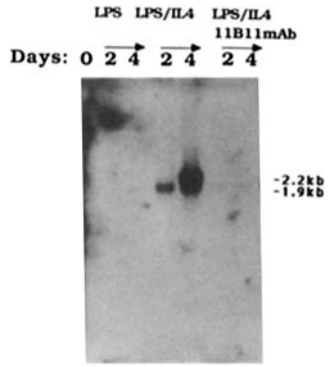

FIGURE 2. Expression of ε transcripts by IL-4- and LPS-treated splenocytes. BALB/c mouse spleen cells were depleted of T cells and cultured in the presence of LPS (10 mg/ml) with or without IL-4 (150 U/ml). Total RNA was assayed by Northern blotting for Cε transcripts.

Discussion

We previously demonstrated that treatment of pre-B lines or normal spleen cells with LPS leads to induction of germline γ2b transcripts followed by class-switch recombinations leading to accumulation of γ2b mRNA sequences (7). Concomitant treatment of splenocytes with IL-4 plus LPS abrogates LPS induction of germline γ2b transcripts and subsequent class-switch events (7). More recently, we found that these agents have similar effects on the γ3 gene (Rothman, R., S. Lutzker, R. Coffman, and F. W. Alt, manuscript in preparation). Based on these findings, we proposed that switching to γ2b and γ3 is induced by LPS treatment and that this induction is inhibited by IL-4 in the context of a recombinase accessibility mechanism. In the context of this model, one prediction is that *positive* influences of IL-4 on class-switch events at the γ1 and ε loci should correlate with expression of corresponding germline constant region genes. Our present study demonstrates that treatment of the A20 line with IL-4 and LPS together induces production of Cε-hybridizing transcripts that by all tested criteria appear germline. Furthermore, expression of these transcripts precedes switching to Cε in normal splenocytes. We have found similar evidence for LPS plus IL-4 induction of transcripts from the germline Cγ1 region. Thus, our current findings on *positive* effects of IL-4 in the context of class-switching complement results of previous studies on *negative* effects of this lymphokine on that process. Together, these studies support the notion that the activity of this lymphokine on B lineage cells can alter the class-switch program effected by mitogen activation in the context of a recombinase accessibility mechanism. Whether accumulation of germline transcripts from the various C region loci is simply a manifestation of accessibility, or the transcripts play a more active role in directing switching is not known. Also, it is conceivable that differential expression of germline transcripts in B lineage cells after treatment with various agents could also permit downstream isotype expression (before switching at the DNA level) through the recently defined trans-RNA splicing process (17).

Summary

To elucidate the mechanism of IL-4-induced enhancement of IgE and IgG1 production, murine splenic B cells and A-MuLV-transformed cells were cultured with LPS and IL-4 and assayed for ε and γ1 transcripts. Concomitant treatment with IL-4 and LPS induced expression of Cε transcripts in both normal and transformed cells. Expression of these truncated Cε transcripts preceded accumulation of normal ε mRNA in treated cells. Consistent data were obtained with respect to γ1 RNA expression. These results suggest that IL-4 can direct class switching in the context of a mechanism associated with differential expression of germline constant region genes.

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