Immunoglobulin Recombinase Gene Activity Is Modulated Reciprocally by Interleukin 7 and CD19 in B Cell Progenitors

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Summary

Bone marrow stromal cells promote B cell development involving recombinase gene–directed rearrangement of the immunoglobulin genes. We observed that the stromal cell–derived cytokine interleukin 7 (IL-7) enhances the expression of CD19 molecules on progenitor B-lineage cells in human bone marrow samples and downregulates the expression of terminal deoxynucleotidyl transferase (TdT) and the recombinase-activating genes RAG-1 and RAG-2. Initiation of the TdT downregulation on the first day of treatment, CD19 upregulation during the second day, and RAG-1 and RAG-2 downmodulation during the third day implied a cascade of IL-7 effects. While CD19 ligation by divalent antibodies had no direct effect on TdT or RAG gene expression, CD19 cross-linkage complete blocked the IL-7 downregulation of RAG expression without affecting the earlier TdT response. These results suggest that signals generated through CD19 and the IL-7 receptor could modulate immunoglobulin gene rearrangement and repertoire diversification during the early stages of B cell differentiation.

The commitment of hemopoietic stem cell progeny to the B lineage and the progression of differentiation along this pathway are dependent on environmental cues provided by specialized cells in hemopoietic tissues (1–3). Stromal cells have been shown to mediate the development of B cell precursors through intercellular contact and via the action of secreted factors (4, 5), including the stromal cell–derived cytokine IL-7 (6, 7). The IL-7 receptors (IL-7R),[1] a signal-transducing complex composed of α and γ_c chains (8, 9), are expressed by pro-B and early pre-B cells (10, 11), and IL-7 has been shown to induce proliferation of these B cell progenitors (7, 12, 13). The IL-7R is constitutively associated with the *src* family kinase $p59^{fyn}$, and receptor engagement results in tyrosine phosphorylation of multiple substrates, including phosphatidylinositol phospholipase C in some cell types (14–18). Other soluble factors, such as stem cell factor (SCF), may potentiate the IL-7 proliferative effect (19, 20).

B lymphopoiesis in the mammalian bone marrow is a complex process that involves an orderly rearrangement of Ig V(D)J gene segments, whereby lymphocyte precursors are able to generate their enormous range of antigen binding specificities (21). Recombinase enzymes are postulated to catalyze the Ig gene rearrangement process, which must be regulated to avoid productive V(D)J rearrangements on both chromosomes (22). Although all of the recombinase components have yet to be defined, the expression of the recombinase-activating genes RAG-1 and RAG-2 correlates well with recombinase activity (23), and disruption of either of these genes prevents the rearrangement of Ig and TCR genes in mice (24, 25). The analysis of RAG gene transcription therefore provides a convenient index of V(D)J recombination activity and its regulation during lymphoid development. The Ig heavy chains themselves have been implicated in the allelic exclusion phenomenon (26–29) and cross-linkage of the Ig receptors on immature B cells aborts their development (30–32). The signals for recombinase machinery downregulation in early B-lineage cells, however, have not yet been identified.

One of the earliest markers of B lineage differentiation is CD19, a transmembrane glycoprotein whose expression begins in early B cell progenitors and increases with further differentiation until it is extinguished in plasma cells (33, 34). This 540–amino acid protein, possessing a large ~240 amino acid cytoplasmic domain, has been implicated as a key signal transduction molecule at several stages of B cell

[1] *Abbreviations used in this paper:* FBM, fetal bone marrow; IL-7R, IL-7 receptor; RT, reverse transcriptase; SCF, stem cell factor; sIg, surface Ig; TdT, terminal deoxynucleotidyl transferase.

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development. Antibody cross-linking of CD19 triggers a cascade of signaling events including calcium mobilization, activation of phospholipase C and protein tyrosine and serine kinases, association with PI 3-kinase, and increased NFκB-binding activity (35–40). Signal transduction via CD19 has been examined extensively at the B cell stage, where the net effect of these signaling events depends on the nature and sequence of other transmembrane receptor signals that the B cell receives. For example, ligation of CD19 on B cells inhibits subsequent anti–surface Ig (sIg)–induced proliferation, while coligation of CD19 and sIg decreases the activation threshold necessary for cell proliferation (35, 41–44). Even before sIgM is expressed, CD19 may function as a signal transduction molecule in pro- and pre-B cells, where it has been shown to be physically and functionally associated with *src* family kinases (45). Among the phosphorylated substrates is CD19 itself (40, 46). Intersection of the CD19 pathway with other signaling pathways is suggested by the finding that engagement of sIgM, CD40, or CD72 also induces phosphorylation of CD19 (45, 46).

In this report, we show that treatment of human bone marrow cells with rIL-7 results in a complex series of unanticipated effects: terminal deoxynucleotidyl transferase (TdT) downregulation, CD19 upregulation, and lastly, RAG-1 and RAG-2 downregulation. The downregulatory effect of IL-7 on RAG-1 and RAG-2 expression could be reversed by CD19 antibodies, while CD19 ligation did not counter the negative IL-7 effect on TdT gene activity. These results suggest reciprocal involvement of the CD19 and IL-7R in the fine tuning of early B cell differentiation and repertoire development.

Materials and Methods

Cells. Human bone marrow cells were obtained from aborted fetuses in accordance with policies established by an institutional review board. Mononuclear cells from fetal bone marrow (FBM) were isolated by centrifugation of cell suspensions over a Ficoll-Hypaque gradient as described previously (47).

Antibodies. mAbs used for cell surface analysis included anti-CALLA, which recognizes CD10; anti-Leu-12, which recognizes the CD19 pan-B cell antigen; anti-Leu-6, which recognizes the more mature B lineage antigen CD20; and anti–HPCA-2, which identifies the early stem cell marker CD34 (Becton Dickinson & Co., Mountainview, CA). Isotype control antibodies were also obtained from Becton Dickinson. Other mAbs included purified anti-CD19 from clone 25C1 (a generous gift from Dr. Stephen Pieper, University of Louisville, Louisville, KY), anti–IL-7R antibodies from clone R34-34 (a generous gift from Dr. Sam Saeland, Schering-Plough, Dardilly, France), anti–human μ chain, SA-DA4-4 (48), anti–human surrogate light chain, SLC2 (49), and anti–human B29, CB3-2 (50). Rabbit anti-TdT antibodies (Supertechs, Bethesda, MD) and goat antibodies to hIgM (Southern Biotechnology Associates, Birmingham, AL) were used for intracellular staining.

Short-term Culture. Mononuclear FBM cells ($1–2 \times 10^6$/ml) were cultured in complete RPMI 1640 media (GIBCO BRL, Bethesda, MD) containing 5% heat-inactivated FCS (HI-FCS; Hyclone Laboratories, Logan, UT), 5×10^{-5} mol/liter 2-ME, essential and nonessential amino acids (Gibco), 2 mmol/liter L-glutamine, MEM vitamins, and 1% penicillin/streptomycin (Gibco). rhIL-7 (100 ng/IL-7/ml; PeproTech, Inc., Rocky Hill, NJ) was added to some cultures.

Immunofluorescence Analysis. For two-color surface antigen analysis, viable cells were incubated with FITC-conjugated CD34 for 15 min on ice, washed with PBS containing 2% HI-FCS and 0.1% azide, and counterstained with PE-conjugated mAbs specific for CD19. For intracellular immunofluorescence analysis, cells previously stained for surface antigens were permeabilized by fixation in 2% formaldehyde for 20 min. Intracellular staining for μ heavy chain and TdT was performed at room temperature in the presence of the mild detergent 0.5% saponin (Sigma Immunochemicals; St. Louis, MO). Isotype control antibodies were used to stain cells to determine background levels. Stained cells were washed and then analyzed by flow cytometry using a FACScan® instrument (Becton Dickinson). Cells were analyzed within the lymphocyte gate that was determined by forward light scatter and side light scatter properties.

Isolation of Pro-B Cells by Immunofluorescence Cell Sorting. From the FBM population of cells stained as described above, the $CD34^+CD19^+$ or $CD34^+CD10^+$ subpopulations of lymphocytes were sorted using immunofluorescence and light scatter characteristics with a FACStar® instrument (Becton Dickinson). Cell purity of >98% was verified by reanalysis of the sorted cells.

Analysis of Gene Expression. $CD34^+CD19^+$ cells ($0.3–2.3 \times 10^5$) were cultured under various conditions with medium alone, IL-7 (100 ng/ml), and/or anti-CD19, anti-μ, anti-SLC, and anti-B29 antibodies. The antibodies were used at a concentration of 10 ng/ml, which was determined by immunofluorescence assays of B lineage cell lines to be a supersaturating level for the relevant cell surface molecules. Cells were then collected, counted, and total RNA was extracted using Tri-Reagent (Molecular Research Center, Inc., Cincinnati, OH), a modification of the acid phenol technique of Chomczynski and Sacchi (51). Equal amounts of RNA were reverse-transcribed with 1 μM oligo(dT), dNTPs (5 mM), 20 U of RNAsin (Promega, Madison, WI), and 5 U of AMV reverse transcriptase (RT) in a total buffer volume of 10 μl. PCR amplification was performed using primers for β-actin (5′ GCG GGA AAT CGT GCG TGA CAT 3′ and 5′ GTG GAC TTG GGA GAG GAC TGG 3′), RAG-1 (5′ TAC CTC CAG AAG TTT ATG AAT 3′ and 5′ ATG ACA GCA GAT GAC CTC CTA 3′), RAG-2 (5′ TTC TTG GCA TAC CAG GAG ACA 3′ and 5′ CTA TTT GCT TCT GCA CTG AAA 3′), TdT (5′ ACA CGA ATG CAG AAA GCA GGA 3′ and 5′ AGG CAA CCT GAG CTT TTC AAA 3′), IgM (5′ AAC GGC AAC AAA GAA AAG AAC 3′ and 5′ TGG TGG CAG CAA GTA GAC ATC 3′), mb-1

(5′ GCT CCC CTA GAG GCA GCG ATT AAG GGC TCA 3′ and 5′ AGG GTA ACC TCA CTC TTC TCC AGG CCA GGC 3′), B29 (5′ GAA TCT CTC GCC ACC CTC ACC 3′ and 5′ CCT TGC TGT CAT CCT TGT CCA 3′), κ (5′ TGG CTG CAC CAT CTG TCT TCA 3′ and 5′ TTG AAG CTC TTT GTG ACG GGC 3′), or γ (5′ TCT GTT CCC ACC CTC CTC TGA 3′ and 5′ TGG TGG TCT CCA CTC CCG CCT 3′). cDNA samples were amplified for 30 cycles for RAG-1 and RAG-2 at an annealing temperature of 55°C or for 30 cycles for β-actin and the others at an annealing temperature of 60°C. For each cDNA preparation, a control synthesis reaction was performed without RT to ensure that there was no contaminating genomic DNA. PCR conditions were optimized so that 30 cycles was in the exponential phase of the amplification. The samples were electrophoresed on a 1% agarose gel and blotted onto Nytran membranes (Schleicher & Schuell, Inc., Keene, NH). The membranes were prehybridized (1 M NaCl, 0.2 M Tris, pH 7.5, 0.1% SDS, and 200 μg/ml salmon sperm DNA), then hybridized using internal probes for β-actin (5′ CCG GCC CCT CCA TCG TCC AC 3′), RAG-1 (5′ CCA TTA GGC ATA GAG GAC 3′), RAG-2 (5′ GAG TCT TCA AAG GGA GTG G 3′), TdT (5′ AAC AGA GGA TGA AGA GCA 3′), IgM (5′ CTA CAA GGT GAC CAG CAC ACT 3′), mb-1 (5′ TGT CCC ACT CTT CTT CCC TCT 3′), B29 (5′ CGTG TTT GAG GAC AAT GGC ATC 3′), κ (5′ CAT CTT CCC GCC ATC TGA TGA 3′), or γ (5′ GCC ACA CTG GTG TGT CTC ATA 3′) that had been end labeled with ^{32}P. The blots were washed in 1× SSC + 0.1% SDS, scanned with a phosphorimager (Molecular Dynamics Inc., Sunnyvale, CA) to quantify signal intensity, and exposed to x-ray film. To obtain a semiquantitative estimate of gene expression levels, signal intensity of the B lineage genes was normalized to the β-actin signal.

Cell Viability. Cellular viability was estimated over the culture interval by trypan blue exclusion and by propidium iodide uptake. Cells were incubated with 1.0 μg/ml PI in PBS + 2% HI-FCS for 5 min on ice, and propidium iodide uptake was analyzed by automated flow cytometry using a FACScan® instrument. The two methods of cell viability analysis yielded comparable results.

Results

The Effect of IL-7 on FBM Mononuclear Cells. Human mononuclear FBM cells cultured in the presence or absence of IL-7 (100 ng/ml) were collected after 3 d, counted, and analyzed for the B lineage markers CD34, CD19, CD10, CD20, CD24, CD45, and sIgM. The IL-7 treatment resulted in an increase in the cell surface expression of the CD19 pan-B cell marker, as noted previously by Wolf and co-workers (52). The pro-B cell population appeared to be the most responsive to IL-7, as indicated by a three- to fourfold increase in the mean fluorescence intensity of CD19 on the $CD34^+$ subpopulation (Fig. 1 *A*). IL-7 also induced a twofold increase in the relative frequency of $CD20^+$ cells (mean of 56.2 ± 6.4% SE versus 28.5 ± 6.4% control values; Student's *t* test, $P < 0.005$) in keeping with the results of Saeland and co-workers (12). The frequency of $CD34^+$ lymphocytes, which also expressed CD20, increased from 3 to 6% after culture with IL-7. The IL-7 treatment had no effect on the expression of other cell surface markers of B lineage cells, including IgM, CD10, CD24, and CD45 (data not shown).

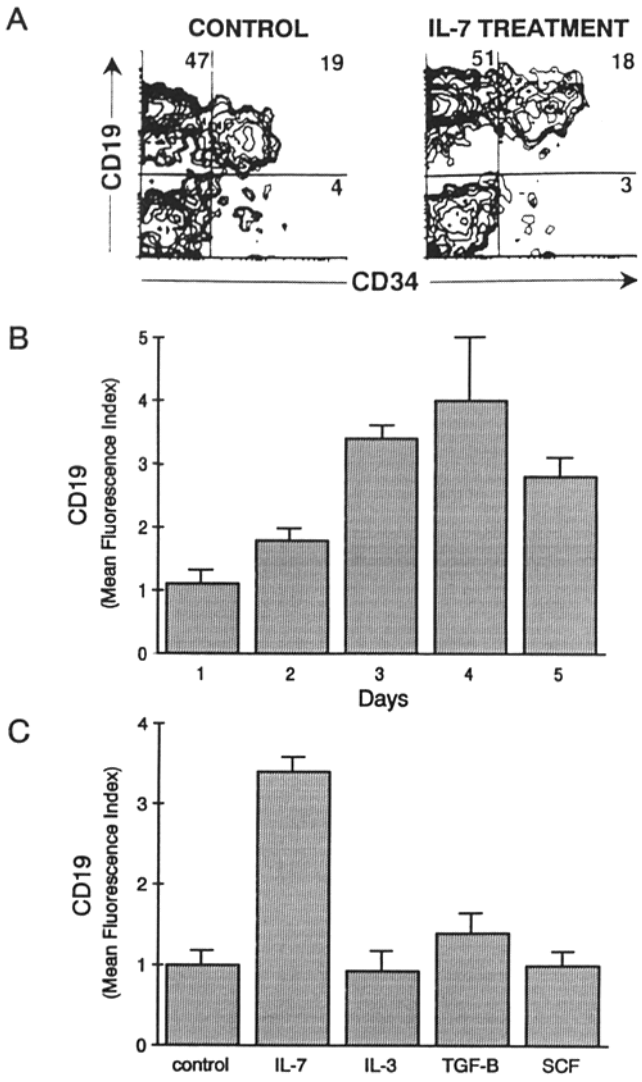

Figure 1. (*A*) Effect of IL-7 treatment on B lineage cells in FBM. Analysis of mononuclear FBM cells expressing both CD34 and CD19 after culturing in medium alone or 100 ng/ml IL-7 for 3 d. Data is represented by the percentage of expressing cells within the lymphocyte gate. (*B*) Time course of IL-7 treatment on CD19 expression of FBM cells. CD19 expression by mononuclear FBM cells treated with 100 ng/ml IL-7 for 5 d at daily intervals was analyzed. CD19 expression was measured by the mean fluorescence index with the medium control mean fluorescence value equal to 1 and the IL-7 value being relative to that value. (*C*) Effect of other growth factors on CD19 expression in FBM. Analysis of CD19 expression by mononuclear FBM cells treated with medium alone, 100 ng/ml IL-7, 100 U/ml IL-3, 20 ng/ml TGF-β, 10 ng/ml SCF for 3 d in three experiments. CD19 expression was measured by mean fluorescence index as described above. A significant increase in CD19 expression was induced by IL-7 ($P < 0.005$), whereas the other cytokines had no demonstrable effect ($P > 0.10$).

A time course analysis of the effect of IL-7 on CD19 expression revealed that the IL-7–induced increase in cell surface expression of CD19 was initiated between 1 and 2 d, and the maximal effect occurred by day 3 (Fig. 1 *B*). Other growth factors, including the SCF, IL-3, and TGF-β, had no effect on CD19 expression (Fig. 1 *C*). These results suggest that the specific effects of IL-7 on early B lineage cells in humans include a pronounced enhancement of CD19 expression, which is a normal feature of their differentiation.

Effect of IL-7 Treatment on Intracellular Proteins. The enzyme TdT is expressed in the nucleus of the progenitors of B and T cells (53–55), where it catalyzes the random incorporation of nonencoded nucleotides (N residues) during the rearrangement of Ig and TCR (56–58). When we used intracellular immunofluorescence to evaluate TdT expression by FBM cells, IL-7 treatment was found to decrease TdT levels by ~35% in the pro-B subpopulation of cells (Fig. 2).

Because the intracellular expression of μ heavy chains can be used to characterize the pre-B cell stage (59), we also examined the IL-7 effect on μ chain expression. The outcome of these experiments revealed that the frequency of bone marrow lymphocytes expressing μ heavy chains in their cytoplasm was unaffected by IL-7 treatment (control: 47 ± 6% versus IL-7: 45 ± 9%), thus reinforcing the idea that IL-7 acts primarily on pro-B cells.

IL-7 Effects on Gene Expression by Pro-B Cells. To explore further their response to IL-7, the $CD34^+CD19^+$ population of pro-B cells was isolated and the cells were cultured for 3 d with and without rIL-7. IL-7 did not enhance the growth of the pro-B population; 95 ± 4% of the initial input was recovered after 3 d in control cultures versus 96 ± 3% for the IL-7–treated cultures. In addition, cell viability was not significantly altered by IL-7 treatment during the 3-d culture interval; 93 ± 4% of the IL-7–treated cells were viable after 3 d versus 92 ± 3% of the nontreated cells. Since only limited numbers of $CD34^+CD19^+$ cells were available ($0.3–2.3 \times 10^5$ in a typical experiment), the effect of IL-7 on pro-B cell gene expression was measured by RT-PCR. In keeping with the protein analysis, TdT message levels were downregulated by IL-7 (Fig. 3 *A*). The IL-7–induced TdT^+ inhibition was striking but never complete. A mean of 65% inhibition (± 17% SE; $P < 0.005$) of the TdT transcript levels (normalized to β-actin) was observed in 10 experiments. Of the other genes examined, the most remarkable effect of IL-7 was a decrease in the levels of RAG-1 and RAG-2 mRNA to ~25% and 48% of the respective control values (Fig. 3, *A* and *B*). In view of this RAG-1 and RAG-2 downmodulation, the progression of heavy chain gene rearrangement to generate μ^+ pre-B cells would not be anticipated, and the lack of an

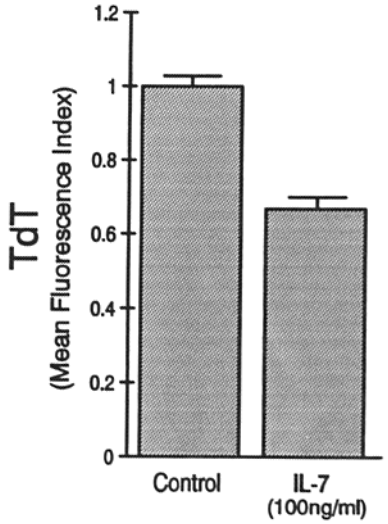

Figure 2. TdT downregulation by IL-7 treatment. Sorted $CD34^+$-$CD19^+$ FBM cells were placed into culture for 3 d in the presence and absence of 100 ng/ml IL-7. The cells were then permeabilized by formaldehyde fixation, stained with anti-TdT antibody, and analyzed by FACS® for intracellular TdT expression. The data were derived from three separate experiments.

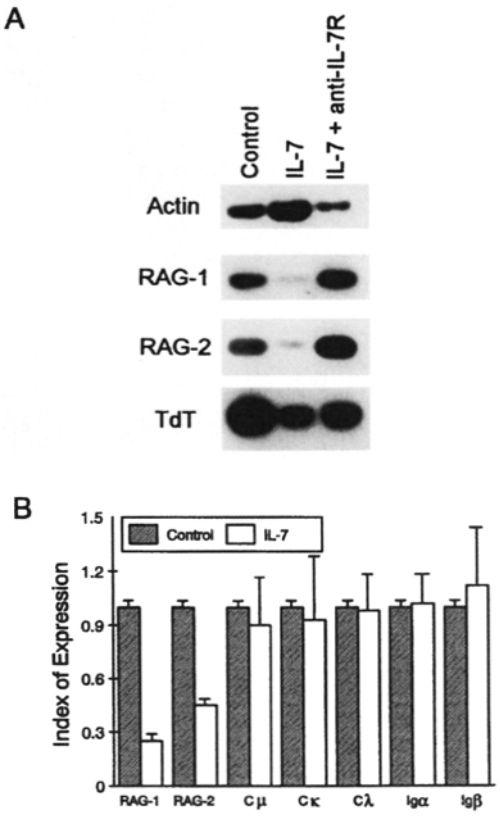

Figure 3. (*A*) IL-7 downregulates RAG-1/2 and TdT gene expression in $CD34^+CD19^+$ FBM cells, and anti–IL-7R antibody negates these effects. $CD34^+CD19^+$ cells were placed into culture for 3 d with medium alone, 100 ng/ml IL-7, or IL-7 + anti–IL-7R. (*B*) IL-7 treatment does not affect expression of IgM, mb-1, B29, κ, and λ genes. Sorted $CD34^+CD19^+$ FBM cells were placed into culture with medium alone or 100 ng/ml IL-7 for 3 d. The index of expression was derived from the measurement of band intensity normalized to the β-actin band intensity. The data shown were derived from four identical experiments in which pro-B cells were isolated from different FBM samples. Only the RAG-1 and RAG-2 values were significantly affected ($P < 0.005$, Student's *t* test).

IL-7 effect on cytoplasmic μ chain expression was confirmed by immunofluorescence analysis after permeabilization of the isolated pro-B cells. The addition of an antagonistic anti–IL-7R antibody (60) to the IL-7–treated cultures blocked the downregulation of RAG and TdT mRNA levels, thus confirming the involvement of the IL-7R in mediating the IL-7 effects (Fig. 3 *A*). IL-7 treatment did not alter transcript levels for μ heavy chains, mb-1 (Igα), B29 (Igβ), and κ or λ light chains (Fig. 3 *B*), suggesting that the effects of this cytokine on pro-B cells are relatively specific.

As an initial approach to determining the mechanism(s) of the IL-7 effects, the kinetics of the suppression of RAG-1, RAG-2, and TdT gene expression were examined. $CD34^+CD19^+$ pro-B cells were cultured as before and harvested daily for analysis. The downregulation of TdT gene expression occurred during the 1st d (Fig. 4). Additional studies revealed that the TdT downregulation occurred within the first 6 h of IL-7 treatment (data not shown). In contrast, the downregulation of RAG-1 (Fig. 4) and RAG-2 gene expression (not shown) did not occur until after the second day of IL-7 treatment, thus implying that a cascade of effects is initiated via the IL-7R on the pro-B cells.

Anti-CD19 Counteracts the IL-7–induced RAG-1 and RAG-2 Downregulation, but not the Effect on TdT Gene Expression. Because the downregulatory effect of IL-7 on RAG-1 and RAG-2 gene expression followed the increase in cell surface expression of CD19, we examined the effect of anti-CD19 antibodies on the outcome of IL-7 treatment. Ligation of the CD19 molecules on the pro-B cells by treatment with anti-CD19 antibodies completely abrogated the downregulatory effects of IL-7 on RAG-1 and RAG-2 gene expression, but had no effect on the IL-7–mediated downregulation of TdT expression (Fig. 5). We also used the CD10 antibody instead of anti-CD19 to isolate $CD34^+$ pro-B cells to ensure that the CD19 antibody staining was not responsible for the downregulatory effect. The results in these experiments were the same, regardless of whether the CD19 or CD10 antibodies were used with the CD34 antibody for the isolation of pro-B cells. In contrast to the anti-CD19 effect, incubation of the pro-B cell population with antibodies to μ heavy chains, surrogate light chains, and the Igβ chains, which are essential components of both pre-B and B cell receptors, had no effect on expression of the recombinatory genes, RAG-1, RAG-2, or TdT either in the presence or absence of IL-7 (Fig. 6 and data not shown).

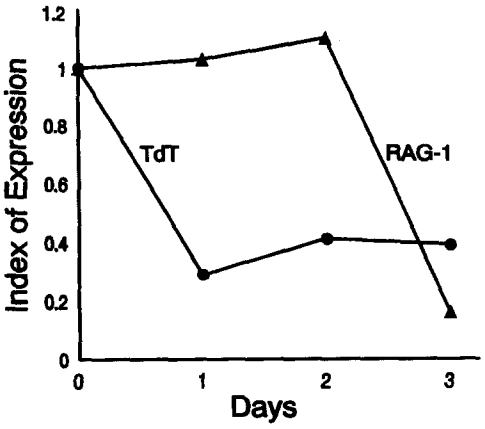

Figure 4. Time course analysis of the IL-7 effects on RAG-1 and TdT gene expression by pro-B cells. Sorted $CD34^+CD19^+$ FBM cells were placed into culture with medium alone or 100 ng/ml IL-7. Cells were collected daily for 3 d. PCR products measured by phosphorimage scanning and the index of expression was calculated as described in Fig. 3.

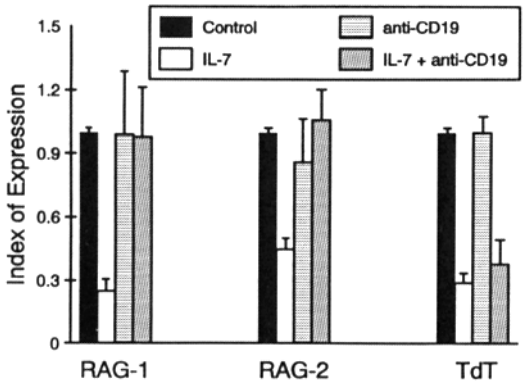

Figure 5. The effect of CD19 ligation on RAG-1/2 and TdT gene expression. Sorted $CD34^+CD10^+$ FBM cells were placed into culture with medium alone, 100 ng/ml IL-7, 10 ng/ml anti-CD19, or IL-7 and anti-CD19. The data obtained by phosphorimage scanning were normalized to β-actin expression as described in Fig. 3. The data from five experiments indicate that the downregulation of RAG-1, RAG-2, and TdT gene expression by IL-7 was statistically significant for each of these genes (Student's *t* test, $P < 0.005$). The values for these genes found when anti-CD19 was added together with IL-7 were also significantly different from those found with IL-7 alone ($P < 0.005$).

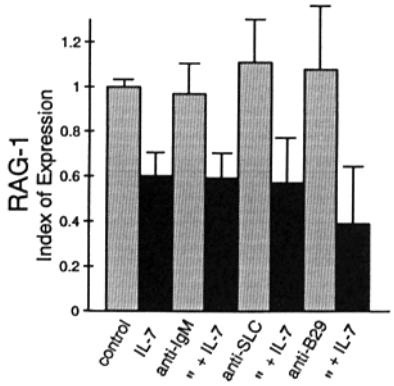

Figure 6. RAG-1 expression in IL-7–treated pro-B cells is unaffected by treatment with anti-BCR antibodies. $CD34^+CD19^+$ cells were placed into culture with medium alone, 10 ng/ml anti-IgM, 10 ng/ml anti-SLC, or 10 ng/ml anti-B29 in the presence and absence of IL-7. The data obtained from three experiments were derived by phosphorimage scanning. The data showed that these antibodies had no demonstrable effect ($P > 0.10$) on RAG-1 values.

Discussion

This study provides evidence for a novel regulatory potential of two key circuits in the interaction between stro-

mal cells and the progenitors of B cells in human bone marrow, namely (*a*) the stromal cell–derived cytokine IL-7 and its complementary IL-7R on pro- and pre-B cells; and (*b*) the signal-transducing CD19 molecule on B lineage cells and its postulated ligand on stromal cells. The results indicate that while the first of these circuits (IL-7→IL-7R) may enhance the second by upregulating CD19 expression, the two types of interactions exert antagonistic effects on expression of genes intimately involved in the assembly of Ig variable region genes.

IL-7 serves as an essential growth factor for B lineage precursors in mice via its interaction with the signal-transducing IL-7R complex (IL-7R$\alpha\gamma_c$) that is expressed on pro- and pre-B cells (11). Treatment of mice with either anti–IL-7 or anti–IL-7Rα antibodies blocks the early development of B lineage cells in the bone marrow (11, 61), and a similar developmental block is seen in gene-targeted mice whose IL-7γ_c gene is disrupted (62). By contrast, in humans, an essential growth-promoting role for the IL-7/IL-7R interaction is not well established for B lineage precursors. Only a modest IL-7 proliferative effect could be demonstrated for human bone marrow precursors by Saeland and coworkers (12) and in the present studies. Our results confirm an approximately twofold increase in the frequency of cells that express CD20, but fail to show growth of the pro-B cell population. The existence of other growth-promoting factors for human B cell precursors is implied by the development of normal numbers of B cells in boys who have X-linked severe combined immunodeficiency because of a function loss mutation in their IL-7 γ_c gene (63). These observations indicate the existence of significant differences in the biological roles for IL-7 and IL-7R, in B lineage cells of mice and humans.

IL-7 treatment has been reported to sustain expression of the RAG-1 and RAG-2 genes and to enhance V(D)Jβ rearrangement by murine pre-T cells (64). Those studies used an intact thymic organ culture system, where it was impossible to determine whether the IL-7 effects were direct or indirect. Other murine studies have shown that pre-B cell lines established from Eμ-bcl2 transgenic mice proliferate in the presence of stromal cells and IL-7, but the removal of IL-7 results in transient upregulation of RAG-1/2 gene expression followed by a decrease in expression with the progression of differentiation into IgM-bearing B cells (65). This suggests that IL-7 may have a biphasic effect on RAG-1/2 expression. These results in mice raise interesting questions about potential differences between IL-7 effects on early T and B lineage cells.

The present data suggest that IL-7 promotes the differentiation of human B progenitors. In keeping with the observations of Wolf and co-workers (52), IL-7 treatment enhanced the level of CD19 expression by the $CD34^+$ population of pro-B cells. A similar effect was noted for the $CD34^-$ population of pre-B cells, although the natural level of CD19 expression is already higher by this differentiation stage. More compelling evidence for a differentiation promoting effect of the IL-7/IL-7R interaction was observed in our analysis of B-lineage–specific gene activity. IL-7 treatment resulted in diminished expression of the recombinase genes RAG-1 and RAG-2, as well as a rapid downregulation of TdT gene transcripts. Since the analysis of RAG-1 and RAG-2 gene expression provides an indirect measure of V(D)J rearrangement, and TdT activity is essential for N sequence diversification of CDR3 V(D)J joins, this suggests that IL-7 could play an important role in regulating repertoire development during B lymphopoiesis. This interpretation is reinforced by the specificity of the IL-7 effect in that the expression of genes encoding the Ig receptor components IgH, IgL, Igα, and Igβ was totally unaffected by IL-7 treatment. Moreover, the downregulatory effects of the IL-7 treatment on the TdT, RAG-1, and RAG-2 genes were abrogated by an anti–IL-7R antibody with proven antagonist characteristics (60).

The temporal relationship of these IL-7 effects suggests a cascade phenomenon. While downmodulation of TdT gene expression occurred within the first 6 h of IL-7 treatment, downregulation of RAG-1 and RAG-2 gene expression was not observed until the 3rd day of treatment. The upregulatory effect on CD19 expression began during the 2nd d of IL-7 treatment, and the maximal effect was not seen until the 3rd d of treatment. This temporal association between an increase in CD19 expression and the decrease in RAG gene expression raised the possibility that a signal generated via the CD19 molecule itself might be responsible for silencing RAG expression. Wolf and coworkers (52), who previously noted the upregulatory effect on IL-7 on CD19 expression, demonstrated an increase in homotypic interaction between the CD19 molecules on precursor cells. A CD19 ligand could also be expressed by stromal cells, although we are unaware of published data supporting this hypothesis. On testing the effect of CD19 ligation, we made the surprising observation that CD19 cross-linkage by antibodies completely negates the downregulatory effect of IL-7 on RAG-1/2 expression, while having no demonstrable effect on its own. This remarkable antagonistic effect of CD19 ligation appears relatively specific in that the IL-7 downmodulation of TdT expression, which occurs earlier, was completely unaffected.

A large body of evidence indicates that heavy chain expression, perhaps together with surrogate light chains, results in a negative signal for VDJ_H rearrangement (21, 29, 66, 67), but our results suggest that the stromal cell/precursor cell interactions involving cytokine and other non-Ig cell surface receptors on the B lineage precursors could also influence Ig assembly by modulating the activity of the recombinase genes. To test the alternative possibility that IL-7 treatment might induce expression of an Ig or Ig-like receptor, which in turn could transduce a negative signal for recombinase gene activity perhaps via CD19, we examined the effects of a variety of antireceptor antibodies. None of these antibodies against pre-B or B cell receptor components, which included anti-μHC, anti-ψLC, and anti-Igβ, had a demonstrable effect on recombinase gene activity of pro-B cells cultured either in the presence or absence of

IL-7. These results suggest that the observed IL-7–induced downregulation of recombinase gene activity is not mediated indirectly via Ig or Ig-like receptors.

The in vitro demonstration of antagonistic effects of IL-7R and CD19 ligation on Ig gene recombinase activity raises the question of physiological relevance. Our results can be considered within the context of a stepwise differentiation model based on observations indicating that B cell precursors are initially dependent on stromal cell contact, then on stromal cells plus IL-7, and finally IL-7 alone as the precursor cells progressively differentiate to become immature surface Ig-expressing B cells (3, 68). The idea of an intersection between the CD19- and IL-7R-signaling pathways is favored by the observation of the phosphorylation of an unidentified 85–95-kD protein after IL-7R engagement (14, 15); moreover, IL-7 may also induce phosphorylation of CD19 in precursor B cells (45). This type of receptor "cross-talk" is not unique to the IL-7R; aggregation of sIg, CD40, and CD72 also phosphorylates CD19 (45, 46). Previous observations suggest that pro-B cells may interact with stromal cells, possibly via CD19 binding with a stromal cell ligand, to enhance IL-7 production (3, 5, 69), and our data indicate that IL-7 could then interact with IL-7R on pro-B cells to initiate downregulation of TdT expression and the upregulation of CD19 expression. The RAG-1 and RAG-2 genes would continue to be expressed in the presence of IL-7 for as long as the CD19 ligation persists. As the precursor cells respond with an increase in CD19 expression and undergo further maturation to the stromal cell contact–independent stage, however, they may be physically displaced from stromal cell contact as a consequence of population expansion. In the absence of CD19 ligation, IL-7/IL-7R–mediated signals would then initiate the process of RAG gene downregulation. The phosphorylation of a critical threonine residue in RAG-2 by cell division control kinases has been shown to result in a rapid degradation of the protein (70), indicating that the regulation of recombinase activity may be fine tuned further by posttranslational events. Later, when the pre-B cells and newly formed B cells begin to express their receptors, ψ, κ, or λLC/μHC/Ig$\alpha\beta$, these may interact with their ligands to terminate recombinase gene activity (32).

Our data suggest that CD19 and IL-7 may serve as important coregulators of early B lymphocyte differentiation during which pro-B cells progress through the next developmental stage of pre-B cells. One prediction of these results is that loss of CD19 function would interfere with early B cell development unless compensatory signaling pathways exist. CD19 deficiency has not been reported yet, but transgenic mice that overexpress hCD19 display a significant block in B lymphocyte development at the pre-B cell stage, the severity of which correlates with the level of hCD19 expression in different transgenic lines (71). This suggests the human protein may behave as a dominant negative mutant in the mouse. The phenotype of these mice could reflect premature downregulation of RAG-1 and RAG-2 gene activity in pre-B cells in which the IL-7R–negative signal is not counteracted by CD19 ligation. Under these circumstances, light chain gene rearrangement and B cell development would be impaired.

The downregulatory effect of IL-7 on TdT expression by pro-B cells is an unprecedented observation that deserves special comment. This effect occurred within the first 6 h of IL-7 treatment of the pro-B cells and, in further contrast to the RAG1/2 downregulation, could not be reversed by CD19 ligation. Although unnecessary for V(D)J recombination, TdT is responsible for the insertion of nonencoded nucleotides in the V(D)J splice sites, and these N sequences make a major contribution to antibody diversity (21, 56). While N sequences are characteristically found in VDJ_H splices of all heavy chain genes expressed by B cells generated beyond the embryonic phase (72, 73), they are not found in most light chain VJ joins (74, 75). This implies the cessation of TdT activity before most of the light chain genes are rearranged. TdT downregulation via the IL-7 $\rightarrow$ IL-7R interaction suggests a mechanism for the initiation of TdT gene silencing before the recombinase genes RAG-1 and RAG-2, which are needed for completion of the LC gene rearrangement process, and B cell formation.

The complex signal transduction pathways which must exist to mediate the antagonistic effects of IL-7 and CD19 ligation on Ig gene recombinase represent fascinating issues for study. One clue to this puzzle may be provided by the analysis of pro-B cells from patients with X-linked agammaglobulinemia. Function-loss mutations in the cytoplasmic tyrosine kinase, Bruton's tyrosine kinase, result in faulty pro-B/pre-B cell differentiation and pan-hypogammaglobulinemia due to the resultant B-cell deficiency (76, 77). Abnormal carryover of TdT expression into the pre-B cell phase has been observed in XLA patients (78, 79) and, when the effect of IL-7 was examined, we noted that IL-7 did not induce TdT downregulation in XLA pro-B cells, but it still induced RAG-1 and RAG-2 downmodulation. Remarkably, the ligation of CD19 molecules on XLA pro-B cells did not negate the negative IL-7 effect on recombinase gene activity (Billips, L.G., N. Nishimoto, C.A., Nuñez, P.D. Burrows, M.D. Cooper, and H. Kubagawa, manuscript in preparation). These results implicate Bruton's tyrosine kinase as an essential participant in the IL-7R and CD19 signal transduction pathways that modulate recombinase gene activity.

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