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L Ramakrishnan and N Rosenberg
Novel B-Cell Precursors Blocked at the Stage of DJH Recombination

LALITA RAMAKRISHNAN AND NAOMI ROSENBERG*

Immunology Graduate Program and Departments of Pathology and Molecular Biology and Microbiology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, Massachusetts 02111

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Abelson murine leukemia virus-transformed cells have provided the principal model for study of the early events in immunoglobulin gene rearrangements. In this communication, we describe a new type of Abelson virus-transformed pre-B-cell line that is arrested at the DJH stage of the recombination process. These cells differ from other pre-B transformants with respect to two properties associated with the immunoglobulin rearrangement process. First, in contrast to cell lines undergoing VH-to-DJH joining in vitro, none of these cell lines contained detectable levels of RNAs transcribed from their unrearranged VH genes. Second, only some of the cell lines recombined exogenous heptamer-nonamer sequences, indicating that many of them have lost at least a portion of the enzymatic machinery that mediates recombination. The correlation between the absence of unrearranged VH RNAs and the inability to rearrange endogenous immunoglobulin gene segments suggests that VH gene transcription is required both to maintain an active recombination system and for the final step in variable-region formation.

Early B-cell differentiation is characterized by assembly of a functional heavy-chain gene involving two recombinatorial events between three separate elements, VH, D, and JH (reviewed in reference 37). Studies of Abelson murine leukemia virus (Ab-MLV)-transformed and other tumor cells have revealed that the recombination process is ordered, with D-to-JH recombination preceding VH-to-DJH recombination (reviewed in reference 1). The mechanism directing the ordered rearrangement of immunoglobulin genes is not understood. Accessibility of the participating gene segments to the enzymes and factors involved in recombination is likely to play a key role in the process (4, 39). Consistent with this notion, both unrearranged VH segments and rearranged DJH segments are transcribed in early B-cell precursors that are in the process of VH-to-DJH joining and RNAs transcribed from unrearranged VH segments are prominent in tissues rich in early B-cell precursors (2, 40).

Analysis of immunoglobulin structure and expression has identified two types of Ab-MLV-transformed pre-B cells. Cell lines of the first type are most often derived from fetal liver and usually contain only 1 to 10% cytoplasmic μ-positive cells (38). These cells have undergone DJH rearrangements and continue the rearrangement process in vitro as evidenced by the accumulation of VH-to-DJH and D replacement rearrangements in the cells (3, 11, 23, 24). This type of continually rearranging cell line will be referred to here as DJ-R (rearranging). In contrast, nearly all the cell lines derived from adult bone marrow that have been analyzed have completed VH-to-DJH rearrangement at least on one allele (3). While most of these cell lines contain 50 to 100% cytoplasmic μ-positive cells, some of them are μ negative because the imprecision inherent to the rearrangement process has created nonsense or missense mutations (reviewed in reference 1; 3).

Because previous analyses of Ab-MLV-transformed cells derived from adult tissues have centered on μ-positive cells (3), we examined the immunoglobulin gene structure in a panel of μ-negative transformants derived from the bone marrow of normal adult mice. This study revealed a new type of μ-negative Ab-MLV-transformed B-cell precursor that has undergone only DJH joining and does not undergo further rearrangement in vitro at an appreciable frequency. These cell lines are similar to other Ab-MLV-transformed cells with respect to expression of differentiation markers associated with early B-lineage cells (20; reviewed in reference 25). However, only some of these cell lines possess an active recombination system as judged by their ability to recombine exogenous signal sequences. In addition, although all the cell lines contain RNAs transcribed from their DJH units, none of these cell lines contain detectable levels of RNAs transcribed from unrearranged VH genes. This new cell type, arrested at the stage of DJH rearrangement, provides a unique model system to study the regulation of the two recombinatorial events in immunoglobulin heavy-chain gene assembly.

MATERIALS AND METHODS

Cell lines. Transformed pre-B-cell clones were derived from normal adult bone marrow (1881, 192-11, 298-13, 298-18, 298-26, 300-3, 300-9, 300-10, 300-16, 300-19, 300-25, 300-26, 300-30, 300-31, 300-35, C-9, C-11, 480-35, 480-36, 480-39, and 481-29), from bone marrow of adult mice treated with 250 mg of cyclophosphamide per kg of body weight (14-4, 2-1-1, 2-10-2, 2-3-1, 3-1-1, 14-1, 7-8, 2-6-2, 21-7, 2-11-2, 21-10, 21-6, 7-4, and 2-3-2), or from fetal liver (22D6, 22D10, 38B9, 40E1, 40E3, and 41B1) as previously described (26). All the cell lines classified in the Results section as DJ-R contain a single, unique Ab-MLV integration except for 2-1-1, which contains two molar integration sites (3; data not shown). All DJ-F cells contain an intact JH-Cμ intron in the vicinity of the EcoRI site Y' of JH4 (data not shown). Established cell lines were maintained in RPMI 1640 medium supplemented to contain 10% fetal calf serum and 5 x 10^{-5} M 2-mercaptoethanol. Some cell lines were subcloned by plating the cells at a density of 0.3 cells per well in flat-bottomed 96-well plates (Becton Dickinson Labware, Oxford, Calif.). After 10 days, subclones were transferred first to 24-well plates and then to 60-mm plates and maintained in the same fashion as other transformants (27).

Immunofluorescence analysis. Expression of cytoplasmic μ protein was determined by staining with a fluorescein is-
thiocyanate-conjugated goat anti-mouse immunoglobulin M (IgM) antibody (Organon Teknika, Malvern, Pa.) (38). In most cases, the synthesis of IgM was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of [125I]methionine-labeled immunoprecipitates (28). Expression of I55(B220) and the BPI determinant was monitored by surface immunofluorescence (33) with appropriate antibodies. The mouse monoclonal IgG2a antibody BPI (9) gift of M. D. Cooper, University of Alabama at Birmingham, the rat monoclonal anti-I55 IgM antibody RA3.3A1/6.1 (7), and the biotinylated monoclonal rat anti-Thyl.2 antibody 30-H12 (16) were used in conjunction with goat anti-mouse IgG2a, goat anti-rat IgM (Southern Biotechnology Associates), and fluorescein isothiocyanate-avidin (E-Y Laboratories, Inc.), respectively.

DNA and RNA analysis. DNA and RNA were obtained by the method of Chirgwin and co-workers (6). Briefly, cells were lysed in a 4 M guanidinium lysis buffer, and the cell lysates were centrifuged through a 5.7 M CsCl gradient. DNA was obtained by collecting the viscous material just above the CsCl cushion and was recovered by precipitation in 2 volumes of 95% ethanol. The DNA was washed with 70% ethanol and suspended in 10 mM Tris–1 mM EDTA (pH 8.0). Total cellular RNA was obtained from the same CsCl gradient by suspending the pellet at the bottom of the tube following centrifugation (6). DNA from subclones was prepared by adding lysis buffer (10 mM EDTA, 10 mM Tris [pH 7.4], 25 mM EDTA, 1% sodium dodecyl sulfate) to approximately 5 × 10⁷ cells and incubating the mixture with 50 μg of proteinase K per ml for 1 h (W. Frankel, Ph.D. thesis, Albert Einstein College of Medicine, Bronx, N.Y.). The lysates were extracted twice with phenol-chloroform-isooctyl alcohol (25:24:1) and twice with ether and precipitated in 0.5 M ammonium acetate and 2.5 volumes of 95% ethanol at room temperature. The DNA was then washed twice in 70% ethanol, and the total yield was used in a single restriction digestion without quantitation. The DNA was digested with EcoRI (New England Biolabs, Inc., Beverly, Mass.), fractionated through 0.8% agarose, and transferred to GeneScreen Plus membranes (Dupont, NEN Research Products, Boston, Mass.). The membranes were hybridized as recommended by the supplier to JH and 5′ D probes (3) (gifts of F. Alt, Columbia University) that had been labeled with [α-32P]dCTP (3,000 Ci/mmole; Dupont, NEN Research Products) by the random oligonucleotide priming method (10). The RNAs were fractionated through formaldehyde-agarose gels and transferred either to nitrocellulose (Schleicher & Schuell, Inc., Keene, N.H.) or to GeneScreen Plus. The membranes were hybridized as recommended by the suppliers to a J558 VH probe (5) (gift of P. Brodeur, Tufts University School of Medicine), and to oligonucleotide probes directed against J12, J13, and J14, or the lambda 5 gene (30). The J558 probe was labeled by the random priming method (10), while the oligonucleotides were labeled with polynucleotide kinase and [γ-32P]ATP (7,000 Ci/mmol; Dupont, NEN Research Products) (36).

Assay for recombination activity. The plasmid pJH201 (12) (gift of M. Gellert and M. Lieber, National Institutes of Health) was introduced into 2 × 10⁷ cells as described previously (12). Following transfection, the cells were plated in 0.5 ml of growth medium in 24-well plates. A small-scale plasmid preparation protocol (19) was used to recover the plasmid DNA from the cells 48 h after transfection. The RNAs were digested or mock digested with DpnI, and 1 μl of the mixture was used to transform 20 μl of competent Escherichia coli DH5α cells (Bethesda Research Laborato-

ries, Inc., Gaithersburg, Md.). The cells were then plated onto agar plates containing either 100 μg of ampicillin per ml or 100 μg of ampicillin and 11 μg of chloramphenicol per ml, and the number of colonies growing on each type of plate was counted 18 to 20 h later. The ratio of colonies in the two types of plates was used to calculate the frequency of recombination. Most cell lines were transfected two to three times, and because similar recombination frequencies were obtained from each preparation, the data from individual experiments were combined.

In the initial experiments, the proportion of ampicillin-resistant colonies obtained from DpnI and mock-digested DNAs was used to measure the frequency of replicated molecules in each cell line (17). Subsequent experiments used only undigested DNA, and the number of colonies obtained was adjusted to reflect the frequency of recombination among replicated molecules. In several cases, including those involving cells classified as DJ-F, representative chloramphenicol-resistant colonies were analyzed to examine the authenticity of the recombination event. For this analysis, DNA from chloramphenicol-resistant colonies was digested with HgiAI and fractionated through a 1.2% agarose gel to detect unique fragments diagnostic of heptamer-nonamer-mediated recombination (12). In all cases, a pattern consistent with heptamer-nonamer-mediated recombination was observed.

RESULTS

Several μ-negative Ab-MLV transformants have a unique immunoglobulin gene structure. The immunoglobulin heavy-chain gene structure in a panel of 11 clonally derived, μ-negative, Ab-MLV-transformed lymphoid cells derived from normal adult mouse bone marrow was examined by Southern blotting. First, EcoRI-digested DNAs were analyzed with a JH₁ region probe to identify rearranged variable-region fragments. Then the DNAs were reprobed with a mixture of 5′-flanking D-region probes to assess the presence and linkage of the D₁-H segments to V₇H segments. Immunoglobulin heavy-chain genes that have completed variable-region assembly have deleted all D-related fragments and thus do not hybridize to this probe mixture (3, 27). In contrast, heavy-chain genes that have only completed D-to-J₇H joining contain rearranged D-related fragments that co-migrate with the rearranged J₇H fragments. Thus, this assay, which has been validated by cloning and analysis of 27 heavy-chain gene segments (3), can be used to assess the nature of immunoglobulin heavy-chain gene rearrangements. Analysis of the panel of μ-negative cells revealed that, like virtually all Ab-MLV transformants analyzed to date (reviewed in reference 1), all the cell lines contained rearranged J₇H fragments on both alleles (data not shown). In addition, 4 of the 11 cell lines had at least one rearrangement that was not associated with a rearranged D-related gene segment, indicating the presence of a V₇H-D₁-H joint at that allele. A representative cell line in which both J₇H fragments are rearranged and all D-related fragments are deleted is shown in Fig. 1B. In contrast to this pattern, 7 of the 11 μ-negative cell lines analyzed had undergone only DJ₁ rearrangements on both alleles. Representatives of this type of cell line are shown in Fig. 1G through K. In this respect, these cell lines are similar to the predominant type of cell line isolated from fetal liver (3) or cyclophosphamide-treated bone marrow (L. Ramakrishnan and N. Rosenberg, manuscript in preparation). However, in contrast to these previously described cells, the DJ₁ cells in the bone marrow panel lacked multiple
submolar JH fragments such as those evident in lanes 1 of Fig. 1C through F. As shown by others (3, 23, 24), such fragments represent new DJH or VHDJH recombinations in subpopulations of clonal DJ-R cell lines, and their absence in the cell lines analyzed in Fig. 1G through K suggests that these cells may have ceased rearrangement at the stage of DJ recombination. We have termed this type of cell line DJ-F (fixed).

D replacements and VH-to-DJH rearrangements do not occur frequently in DJ-F cells. The presence of cytoplasmic μ-positive cells in a DJ-R population is a sensitive indicator of ongoing formation of productive heavy-chain variable-region genes, and a typical DJ-R cell population contains 1 to 10% μ-positive cells (3, 37; unpublished data). Analysis of cytoplasmic μ production in all seven of the DJ-F cells revealed that fewer than 1 in 2,000 cells in each population synthesized μ, suggesting that, consistent with the Southern analysis, productive VHDJH rearrangements were not being formed in culture. However, the DJ-F cells could have been undergoing a very high frequency of either nonproductive VHDJH rearrangements or D replacement rearrangements (23, 24). Neither of these types of rearrangements would give rise to cytoplasmic μ-positive cells, and the presence of a large number of different rearrangements might not be detected in the Southern blotting assay.

To determine whether the DJ-F cell lines were actually undergoing rearrangements at a high frequency, we prepared subclones of two representative DJ-F and DJ-R cell lines. DNA from randomly chosen subclones was examined for changes in immunoglobulin gene structure. As expected, subclones with new JH rearrangements were detected in both DJ-R populations (Fig. 2D). In the representative

FIG. 1. Immunoglobulin gene structure in pre-B-cell lines. Southern analysis of 10 µg of EcoRI-digested genomic DNA was performed first with a JH probe (lanes 1) and subsequently with a mixture of the 5′-D-flanking probes 5′DSP2 and 5′DFLI6 (lanes 2) (3). Liver DNA (A); DNA from 300-16, a representative VDJ cell line (B); DNA from 2-1-1, 14-4, 2-10-2, and 481-29, representative DJ-R cell lines (C through K, respectively); and DNA from 300-3, 300-30, 300-31, 300-25, and 300-10, representative DJ-F cell lines (G through K, respectively) are shown.

FIG. 2. Immunoglobulin gene structure in subclones of DJ-R and DJ-F cell lines. Southern analysis of genomic DNA from subclones was done with a JH probe. DNA was obtained by a small-scale preparation technique (Frankel, Ph.D. thesis), digested with EcoRI, and used for the analysis without quantitation. Analysis of representative subclones from the DJ-R cell line 481-29 (A) and two DJ-F cell lines, 300-31 and 300-35 (B and C respectively), is shown. L, Liver DNA; P, DNA from parental cell line.
sample shown (Fig. 2A), new JH fragments were detected in some clones (lanes 5, 7, 8, 11, 15, and 16), while others had lost a parental JH fragment without acquiring a new one (lanes 3, 6, 10, 13, and 14). In contrast, only one subclone (Fig. 2B, lane 10) from both of the DJ-F cell lines examined (Fig. 2B and C) had undergone a change in JH gene structure. In this case, only one of the new fragments was associated with a newly rearranged D-related fragment, suggesting that the second was a VH-DJH recombination. The possibility that this rearrangement reflected a deletion affecting the EcoRI site in JH-Cα was not examined because all the subclones were analyzed by small-scale DNA preparations. However, assuming that this rearrangement represented a VH-to-DJH joining event, the frequency of rearrangement in the DJ-F cells examined is at least 15 times lower than in the DJ-R cells (Fig. 2D).

Consistent with the results obtained in the Southern blotting, immunofluorescence analyses revealed that 7 of the 10 subclones examined from one of the DJ-R cells expressed μ (Fig. 2D). Indeed, those subclones in which rearrangement appeared to be most active based on the results of the Southern analysis contained 1 to 10% μ-positive cells. This frequency is consistent with the idea that many new rearrangements are occurring, a fraction of which lead to immunoglobulin production. In contrast, none of the subclones from either of the DJ-F cell lines expressed μ protein (Fig. 2D).

DJ-F cells resemble B-lineage precursors. Previously characterized Ab-MLV-transformed lymphoid cells share a number of differentiation markers with normal pre-B cells (reviewed in reference 25). However, DJH rearrangements have been observed in some Ab-MLV-induced thymic tumors (8) and in other cells of the T and myeloid lineages (13, 14), raising the possibility that the DJ-F cells are not related to cells of the B lineage. To address this issue, we examined expression of the B-lineage-specific form of Iṣ5 (7) and the Thy1 (16) and BPI determinants (9) by immunofluorescent staining. As expected for cells related to early B-cell precursors, all the DJ-F cells expressed Iṣ5 (B220) and BPI and none of them expressed Thy1 (Table 1). This pattern was indistinguishable from that observed with DJ-R and VDJ-transformed B cells.

In addition to differences in expression of surface markers, hematopoietic cells can be distinguished based on the expression of particular lineage-specific genes. One such differentiation marker is expression of lambda 5. This gene is related to the light-chain locus (30) and appears to be expressed exclusively in early cells of the B lineage (31). To determine whether DJ-F cells expressed lambda 5, total cellular RNA from a panel of cell lines was analyzed by Northern blotting. All the DJ-F cells expressed the 1.2-kilobase lambda 5 RNA (Fig. 3, lanes 4 to 11) at levels comparable to those of the DJ-R and VDJ cell lines (Fig. 3, lanes 1 to 3 and 12 to 15, respectively). As expected, the mature B-cell lines A105 (J. Runnels and N. Rosenberg, unpublished data) and WEHI-231 (39) and the hybridoma TIB-97 (21) did not express this RNA (Fig. 3, lanes 15 to 17). Consistent with their relationship to cells of the B lineage, Southern analysis revealed that none of the cell lines had rearranged their T-cell-receptor β or γ1 and γ2 genes (Table 1; data not shown). These analyses coupled with the analysis of surface antigen expression demonstrate that the DJ-F cells are related to cells of the B-lymphocyte lineage.

DJ-F cells lack unrearranged VH RNAs. Transcription of unrearranged VH genes is prominent in late gestation fetal liver and has been observed in all DJ-R-transformed cells and in many transformants that have completed VH-DJH rearrangement (41). DJ-R cells also express RNAs containing DJH and Cα sequences that initiate 5′ of the rearranged D element (2, 22). Other studies have suggested that transcriptional activity is associated not only with variable-region formation (4) but also with heavy-chain class switching (18, 35, 42). Thus, in several instances, transcription of a region and recombination in that area are at least temporarily associated.

To determine whether the levels of RNAs from the variable region in the DJ-F cells were similar to those in DJ-R cells, we analyzed total cellular RNAs from the DJ-F cells and a panel of other B-lineage cells for the presence of unrearranged VH RNAs and RNAs transcribed from the JH region. All the DJ-R cell lines examined expressed RNAs of the appropriate size for unrearranged VH genes of the J558 family (Fig. 4A, lanes 1, 8, and 9). An RNA of appropriate size for full-length γ2b mRNA was detected in MPC-11 (lane 10), a myeloma which expresses a member of this VH family (5), and consistent with published data (41), the mature B-cell lymphoma WEHI-231 (39) did not express unrearranged VH RNAs. In contrast to the DJ-R cell lines, none of the DJ-F cell lines expressed these genes at a detectable level (Fig. 4A, lanes 2 to 7 and 12). However, analysis of the same filter with a mixture of oligonucleotides specific for JH-coding sequences revealed that all the DJ-F cells expressed RNAs from the DJH region (Fig. 4B, lanes 2 to 7 and 12) and that the levels were at least as great as those seen in the DJ-R samples (lanes 1, 8, and 9). These RNAs are of the appropriate size for the truncated μ RNAs that have been observed in DJ-R cell lines (2, 22). These data suggest that recombination in the DJ-F cells is limited by either the

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### Table 1. Characteristics of DJ-F cell linesa

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<th>DJ-F</th>
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<td>10/10</td>
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<tr>
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<td>11/12</td>
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<td>1/12</td>
<td>0/3</td>
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<td>4/4</td>
<td>4/4</td>
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<tr>
<td>TCry rearranged</td>
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<td>J558 VH RNAs</td>
<td>0/7</td>
<td>12/12</td>
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a DJ-F transformants had <0.01% μ-positive cells, most DJ-R transformants had 1 to 10% μ-positive cells, and VDJ RNAs were either 20 to 100% or <0.01% μ-positive cells. In B220, 100% of the cells in all the populations expressed the determinant. Populations containing >10% BPI-reactive cells were considered positive. For Thy1, 10% of the cells in the positive cell line reacted with the antibody. The DJ-F cell lines were 300-3, 300-10, 300-25, 300-26, 300-30, 300-31, and 300-35, except that TCR receptor gene structure was not examined in 300-26. The DJ-R cell lines examined for cytoplasmic μ expression were 38B9, 22D6, 41B1, 40E3, 2-10-2, 2-12-2, 2-10, 21-10, 192-11, and 481-29; B220 and Thy1 expression were examined in 22D10, 40E1, 38B9, 22D6, 41B1, 40E3, 2-10-2, 2-11-2, 2-3-2, 2-1-1, 21-10, and 192-11; BPI expression was examined in the same group and also in 2-6-2 and 21-7; lambda 5 expression was examined in 14-4, 300-19, 481-29, and 298-13; TCRβ RNAs were examined in 481-29, 2-1-1, and 192-11; TCRγ RNAs were examined in 192-11; J558 RNAs were 192-11, 481-29, 2-10-2, 2-11-2, 2-1-1, 2-3-2, 21-10, 192-11, 21-10, 14-4, 21-6, 300-19, and 298-13. The VDJ RNAs and DJ-R cell lines examined for cytoplasmic μ were 2-3-1, 14-1, 3-1-1, 300-19, 7-4, 1881Y, C-11, C-9, 298-26, 480-36, 480-39; lambda 5 was examined in 2-3-1, 1-7, 14-1, 3-1-1, Thy1 was examined in 298-18, 1881Y, and 480-36; B220 was examined in 2-3-1, 298-18, 300-16, 7-4, 1881, 480-36, 480-39; BPI was examined in the same panel plus 7-8, 3-1-1, 298-26, 300-9 and 480-35; J558 RNAs were examined in 7-8, 14-1, 7-4, C-11, C-9, and 298-26; TCR RNAs were examined in 298-18, 300-16, and 298-26.  

b Number of cell lines with characteristic/number of cell lines tested.  

c TCR, T-cell receptor.
decreased transcriptional accessibility of the $V_H$ gene segments or the low level of these products. As judged by the presence of RNAs that hybridize with $J_H$-region probes, the DJ$_H$ regions of these cells appear to be accessible for transcription and presumably recombination.

**Some DJ-F cells recombine an exogenous recombination substrate.** The lack of transcriptional activity at the $V_H$ locus in DJ-F cells suggested that the configuration of these genes was limiting their recombination potential. However, an inactive recombination system could also be limiting rearrangement. To distinguish between these possibilities, we assessed the ability of the DJ-F cells to rearrange a substrate containing heptamer-nonamer recognition sequences with an extra-chromosomal substrate in a transient transfection assay (12). This assay employs the pH201 plasmid, which carries the genes for ampicillin and chloramphenicol resistance, the $E. coli$ and polyomavirus origins of replication, and heptamer-nonamer recognition elements. Recombination of the heptamer-nonamer sequences activates expression of the chloramphenicol resistance gene, which is monitored following transformation of $E. coli$. Although levels of activity in transformed pre-B-cell lines vary from 0.5 to 30% and do not correlate precisely with differentiation as judged by immunoglobulin gene structure or synthesis (17), all cell lines of this type and early T cells contain the activities required to rearrange this plasmid, while other hematopoietic and non-hematopoietic cells do not (17).

Transfection of VDJ and DJ-R cells with pH201 revealed that these cells contained recombination activities similar to those reported for such cell lines (Table 2) (17). Six of seven DJ-F cell lines were analyzed in the recombination assay; the seventh cell line, 300-3, did not take up sufficient DNA to be tested. Among those studied, at least two categories were found (Table 2). Two of the cell lines did not have detectable activity, and the frequencies obtained for two others, 300-25 and 300-35, were in the range of those found in some cell lines of myeloid origin that have been considered to be greatly reduced levels by others (17). Thus, the block in rearrangement in these four cell lines may reflect the loss of an active recombination system. However, two of the cell

FIG. 3. Expression of lambda 5 RNA. Northern (RNA) analysis of 20 μg of total RNA was performed with an oligonucleotide probe homologous to lambda 5 coding sequences (30). RNAs from the DJ-R cell lines 300-19 (22), 481-29, and 298-13 (22) (lanes 1 to 3, respectively); from the DJ-F cell lines 300-25, 300-35, 300-3, 300-30, 300-26, and 300-10, and 300-31 (lanes 4 to 10, respectively); and from the VDJ cells 3-1-1, 14-1, 2-3-1, and 7-8 (lanes 11 to 14, respectively) were analyzed. RNAs from mature cells of the B lineage (A105, WEHI-231, and TIB-97) were analyzed in lanes 15 to 17, respectively.

FIG. 4. Expression of unrearranged $V_H$ RNAs in DJ-F cell lines. Northern analysis of 20 μg of total RNA was performed with a J558 $V_{H\gamma}$ probe (5) (A). RNAs in lanes 1, 8, and 9 are from the DJ-R cell lines 481-29, 14-4, and 3-3-1, respectively; lanes 2 to 7 and lane 12 contain RNAs from the DJ-F cell lines 300-3, 300-30, 300-25, 300-35, 300-31, 300-10, and 300-26, respectively. Lane 10 contains RNA from the $\gamma_2b$-producing myeloma cell line MPC 11 that expresses a J558 $V_{H\gamma}$ gene, and lane 11 contains RNA from the B-cell lymphoma WEHI-231 that does not express a J558 family $V_{H\gamma}$ gene. The J558 $V_{H\gamma}$ probe was washed off, and the filter was reprobed with a mixture of oligonucleotide probes directed against the $J_{H\gamma}^\alpha$, $J_{H\gamma}^\beta$, and $J_{H\gamma}^\mu$ coding regions (B). This mixture will not detect RNAs in which $J_{H\gamma}$ sequences are used.
lines, 300-10 and 300-30, retained levels of activity similar to those found in most transformed pre-B cells, suggesting that recombination of the endogenous genes in these cells is not limited by an inactive recombination system.

DISCUSSION

Ab-MLV-transformed lymphoid cells have provided one of the principal model systems for analysis of early B-cell differentiation (reviewed in reference 1). The DJ-F cells described here define a new type of transformant that is isolated at a high frequency from normal adult mouse bone marrow. The tissue-specific distribution of these transformants and those of the DJ-R and VDJ type (3; Ramakrishnan and Rosenberg, in preparation) is consistent with the notion that the phenotype of the transformant reflects the predominant types of target cell present in the tissue. This observation and the fact that VDJ and DJ-R transformants appear to be accurate representations of early B-cell precursors (reviewed in reference 1) suggests that normal lymphoid cells of DJ-F type exist as well. Presumably, these cells would arise during the course of normal differentiation and be dead-end by-products of the pathway eliminated through either senescence or some active mechanism.

Although the DJ-F cells studied here are transformed, it is unlikely that the transformation process itself arrests differentiation in these cells. Although the direct effect of v-abl expression on rearrangement awaits analysis of normal cells or cells transformed by temperature-sensitive mutants of Ab-MLV, DJ-R transformants display all the characteristics of typical Ab-MLV-transformed cells and continue rearrangement in vitro (3, 23, 28, 38). The DJ-R cells, already known to be capable of generating VDJ cells in vitro (23, 24), may give rise to DJ-F cells as well. Preliminary analysis of one typical DJ-R cell line has revealed that a clone lacking submol JH fragments does not have detectable levels of unarranged VH gene RNAs, while a second clone which has undergone V-to-DJ rearrangement retains expression of those RNAs (unpublished data).

DJ-F cells fail to undergo both VH gene appendage and secondary D gene replacements at a significant frequency. Secondary D rearrangements, observed in DJ-R cells at approximately the same frequency as VH rearrangements (24), involve replacement of the existing DJ units with new units formed by recombination of a 5' D with 3' JH segment. All the DJ-F cells retain large numbers of VH genes (data not shown) and multiple D segments of their JH rearrangements and many of them retain at least one 3' JH segment, indicating that the elements needed to participate in both types of recombinations are present. Because D replacements often involve use of the 5'-most D segment (24), the fact that most DJ-F cells retain some D segments suggests that rearrangement in the DJ-F cells ceased after the initial recombination. Thus, our data suggest that both VH-to-DJH recombinations and secondary DJH recombinations require the same set of signals.

The failure of DJ-F cells to undergo further rearrangement suggests that they lack some of the cellular factors required for the process. It is likely that the cell lines which recombine an introduced extrachromosomal recombination substrate retain the subset of these signals that are required for recombination of heptamer-nonamer units but lack those specifically required for rearrangement of endogenous gene elements. One of these signals may be reflected by the decreased expression of unarranged VH RNAs. The absence of these RNAs in DJ-F cells coupled with their prominence in other transformants and the tissues where B-cell generation is ongoing (41) suggests that transcription of these regions is functionally important in variable-region assembly. Further experiments are needed to determine whether the presence of these RNAs merely reflects a chromatin configuration that is compatible with recombination (reviewed in reference 1) or whether they or the putative peptide products specified by them play an active role in recombination.

The signals required for transcription of unarranged VH genes have not been defined. If at least some of these genes are expressed from the VH promoter that is used after rearrangement (41), the tissue-specific factors important for transcription of fully rearranged immunoglobulin genes (15, 34) may mediate expression of the unarranged genes. These factors appear to be present in DJ-F cells because cells transfected with a fully rearranged μ gene express it at high levels (unpublished data). Alternatively, because lipopolysaccharide treatment decreases the level of unarranged VH RNAs (41) but stimulates the levels of octamer-binding factor (34), it is possible that expression of unarranged VH RNAs is mediated by different factors. In either case, DJ-F cells may lack a distinct signal that allows, either directly or indirectly, the transcription of unarranged VH genes. Such a factor might be involved in transcription or be important in altering the chromatin structure of the VH region in preparation for recombination.

Some DJ-F cells fail to recombine the extrachromosomal recombination substrate at detectable levels or do so at levels lower than those reported for early B- and T-cell lines (17). Because these cells have completed the first step in rearrangement, it is likely that the entire recombination system was once active but has been turned off prematurely. The absence of the basic components necessary to mediate heptamer-nonamer recombination probably plays a major role in arresting rearrangement in these cells. The nature of these components has yet to be defined, but the observation that a single DNA segment can confer recombination activity to fibroblast cells (32) suggests a central role for a single factor.

Whether the absence of detectable VH RNAs is related to the loss of the recombination system cannot be determined from these experiments. However, the fact that some early B-cell lines which have completed both heavy- and light-chain gene assembly retain the ability to recombine the.

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**TABLE 2. Recombination activity in DJ-F cell lines**

<table>
<thead>
<tr>
<th>Gene structure</th>
<th>Cell line</th>
<th>No. of Amp' colonies</th>
<th>No. of Amp'-Cam' colonies</th>
<th>R × 100^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>VDJ</td>
<td>1881Y</td>
<td>5,493</td>
<td>251</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>298-26</td>
<td>832</td>
<td>28</td>
<td>3.7</td>
</tr>
<tr>
<td>DJ-R</td>
<td>2-11-2</td>
<td>254</td>
<td>8</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>2-10-2</td>
<td>1,178</td>
<td>13</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>2-1-1</td>
<td>436</td>
<td>23</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>22D6</td>
<td>181</td>
<td>6</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>300-19</td>
<td>1,941</td>
<td>77</td>
<td>4.0</td>
</tr>
<tr>
<td>DJ-F</td>
<td>300-31</td>
<td>3,956</td>
<td>0</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td></td>
<td>300-26</td>
<td>1,656</td>
<td>0</td>
<td>&lt;0.060</td>
</tr>
<tr>
<td></td>
<td>300-35</td>
<td>4,474</td>
<td>6</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>300-25</td>
<td>2,695</td>
<td>4</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>300-30</td>
<td>991</td>
<td>28</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>300-10</td>
<td>1,084</td>
<td>166</td>
<td>15.3</td>
</tr>
</tbody>
</table>

* Amp', Ampicillin resistant; Cam', chloramphenicol resistant.

R × 100 = (No. of Amp'-Cam' colonies/Amp' colonies) × 100.
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substrate (17) but do not express unrearranged V\textsubscript{H} RNAs (41) suggests that the recombination machinery is lost gradually. This hypothesis could be extended to suggest that the DJ-F cells which continue to rearrange the recombination substrate but lack detectable V\textsubscript{H} RNAs are still in the process of turning off the recombination system, while those which lack both activities have completed the process. Consistent with this idea, both a DJ-F and VDJ subclone derived from a DJ-R clone rearrange the exogenous substrate, but only the parental DJ-R clone and the VDJ subclone express unrearranged V\textsubscript{H} RNAs (unpublished data).

Irrespective of the relationship between unrearranged V\textsubscript{H} gene transcription and recombination activity, the DJ-F cells represent a unique model system in which this and other features controlling immunoglobulin gene assembly can be explored.

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