Expression of Human Recombination Activating Genes (RAG1 and RAG2) in Neoplastic Lymphoid Cells: Correlation With Cell Differentiation and Antigen Receptor Expression

By Jean Christophe Bories, Jean Michel Cayuela, Pascale Loiseau, and Francois Sigaux

Regulation of V-(D)-J recombinations that occur in antigen receptor encoding genes remains poorly understood. Recently, two genes, RAG1 and RAG2, that are able to activate rearrangement of synthetic recombination substrates were cloned in mouse and a human gene homologous to RAG1 was described. To define the differentiation stages corresponding to RAG1 and RAG2 RNA expression, we have studied a large number of B- and T-lymphoid neoplasias. First, we show that a human gene homologous to the murine RAG2 is transcribed in humans. Moreover, using a polymerase chain reaction approach, we have shown that RAG are expressed not only in T-cell receptor (TCR)-negative T-cell acute lymphoblastic leukemias (T-ALLs), but also in some cases in which a significant percentage of cells expressed surface TCR. Absence of RAG expression was shown in certain T-ALLs at variable stages of thymic differentiation. Data obtained in B-lineage ALLs show that RAG RNAs are expressed in almost all slg" B-lineage ALLs but are not transcribed in the slg" B-cell proliferations tested, including Burkitt's ALLs, follicular center cell lymphomas, and chronic leukemias. These findings are consistent with the involvement of RAG in the control of in vivo V-(D)-J recombinations. These findings are also of interest in the delineation of potential regulatory factors acting on RAG transcription and in the understanding of the mechanisms of specific chromosomal abnormalities occuring in immature lymphoid cells.

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T HE SPECIFICITY of antigen recognition by lymphoid cells is mediated by surface-expressed antigen receptors whose diversity results mainly from clone-specific somatic rearrangements.1,2 These rearrangements occur during the early phases of lymphoid differentiation and involve multiple and dispersed V, J, and, in some cases, D segments that encode the variable portion of antigen receptors. Rearrangements are mediated by conserved recombination signal sequences (RSS) consisting of a palindromic heptamer and an A-T-rich nonamer separated by a spacer of either 12 or 23 bp. RSS flank each rearranging segment. Use of engineered recombination substrates has shown that RSS alone are necessary and sufficient for targeting recombination irrespective of the coding sequences associated with RSS. The details of V-(D)-J recombination remain poorly understood but may include specific steps (binding to RSS, cleavage near RSS) and non-specific steps (deletions/additions of a random number of nucleotides, repair and ligation).3 Products of genes allowing binding to RSS10 and stabilization of an intermediate complex that holds the RSS in proximity are probably involved, as are certain enzymes (nuclease,7 terminal deoxynucleotidyl transferase,12 and ligase). Most mechanisms are probably common to B and T cells because engineered T-cell receptor (TCR) recombination substrates rearrange when transfected in Abelson-transformed murine pre-B cells.11 Additional and not fully characterized mechanisms ensure that full rearrangements of Ig and TCR genes only occur in B and T cells, respectively.

A fundamental breakthrough in the understanding of antigen receptor assembly was obtained by the recent cloning of two lymphoid-specific genes, RAG1 and RAG2, that activate V-(D)-J recombination.12,14 Murine RAG2 is located 8 kb downstream to RAG1.14 RAG1 and RAG2 appear to be coordinately transcribed in most cells. However, recently, selective expression of RAG1 and RAG2 has been described in murine central nervous system15 and chicken B cells undergoing Ig gene conversion,16 respectively. The nucleotide sequences of these two genes are not homologous. Most of the structural sequences and 3' untranslated regions from RAG1 and RAG2 are encoded by a single exon, suggesting that these genes, conserved through evolution, have evolved from viral or fungal genes. It has been shown that RAG differ from the murine scid gene that leads, when mutated, to a specific defect in V-J joining. When cotransfected into murine fibroblasts, RAG synergistically activate the rearrangement of a synthetic recombination substrate but not of endogenous antigen receptor genes. Because RAG are expressed in murine lymphoid cells at stages when the recombinase activity is high, it has been speculated that these genes are of fundamental importance for the V-(D)-J recombinations occurring in vivo.13,14 Very little is known as yet concerning RAG expression in human cells and human RAG2 has not been definitively identified.

We analyze here the distribution of RAG messenger RNA (mRNA) in human leukemic cells frozen at various stages of differentiation. Using a polymerase chain reaction (PCR)-based strategy, RAG expression was studied in 73 cases of selected B- and T-acute leukemias. Chronic B-cell proliferations and established cell lines were also studied. We show evidence that a human RAG2 homologous to the murine gene is expressed and, as observed in the mouse, that RAG1 and RAG2 are in most cases transcribed concordantly. Moreover, we show that RAG mRNAs are expressed in almost all early human pre-B leukemic cells.

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but not in cells from sIg-positive acute lymphoblastic leukemia (ALL) (Burkitt's type) or cells from sIg' chronic B-cell proliferations. We also show that RAG expression is present in most immature (ie, CD3') ALLs and that, more surprisingly, RAG are also expressed in some CD3' ALLs that express αβ or γδ antigen receptors.

These data are consistent with the hypothesis that RAG are involved in the in vivo control of V(D)-J recombination in humans. They allow correlation of RAG activity with some aspects of allelic exclusion and will increase our understanding of the mechanisms of genetic alterations occurring in immature lymphoid cells.

MATERIALS AND METHODS

Cells. Diagnosis of lymphoid proliferations was performed by standard morphologic and phenotypic criteria. Cryopreserved cells obtained from peripheral blood (PB) samples or, in some cases, from bone marrow (BM) aspirates or lymph nodes biopsies were used. Only those cases with a high percentage of malignant cells were included in the study (see Tables 1 and 2 for acute leukemias). Surface phenotype, detection of sIg, and intracytoplasmic chains were performed as described. The surface phenotype of the malignant cells was determined by standard indirect immunofluorescence and cytofluorographic analysis using a panel of monoclonal antibodies (MoAbs) that recognize T-associated or T-restricted antigens (anti-CD1 [OKT6], CD2 [OKT11], CD3 [Leu4], CD4 [Leu3a], CD5 [Leu1], CD7 [Leu9], CD8 [OKT8]; OKT MoAbs from Ortho [Raritan, NJ], Leu MoAbs from Becton Dickinson [Sunnyvale, CA] and B-restricted antigens (CD19 [B4], CD20 [B1], CD22 [To15]; B MoAbs from Coultronics [Margency, France], To15 MoAb from Dakopatts a/s [Glostrup, Denmark]). A number of other antibodies were also used (anti-CD10 [J5; Coultronics]; OKT9, OKT10, CD13 [My7; Coultronics]; CD33 with some aspects of allelic exclusion and will increase our understanding of the mechanisms of genetic alterations occurring in immature lymphoid cells.

Table 1. RAG Expression in T-Lineage ALL Cells (29 Cases)

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*The percentage of blast cells (determined by morphologic examination) and the approximate percentage of pre-B and B cells (determined using the CD19 MoAb by indirect immunofluorescence) are shown.

† , >30% positive cells; +/−, 20% to 30%; −, <30%; "", low antigen density. For the definition of the differentiation stages, see Materials and Methods.

‡, level similar to that observed in undiluted Reh RNAs; +/-, level similar to 10⁻² dilution of Reh RNAs; (+/-), low transcription level that may be due to malignant cells or alternatively (but less probably) to persisting normal pre-B cells; −, undetectable transcription or level lower than that observed in 10⁻² dilution of Reh RNAs. For details see Materials and Methods.
zymes as Some of the cases studied here have been ing cases were identified with a unique number used in these of the neoplastic cells reacted with the MoAb. The phenotype of due to malignant cells or alternatively (but less probably) to persisting normal pre-B cells; included in previous contributions from our group. The correspond-

ments. A cell population was considered positive if more than 30% of the neoplastic cells reacted with the MoAb. The phenotype of the acute leukemia samples is summarized in Tables 1 and 2. Studies of Ig/TCR gene rearrangements were performed by Southern blotting using appropriate probes and restriction en-

Table 2. RAG Expression in B-Lineage ALL Cells (44 Cases)

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Abbreviation: ND, not done.

*+, >30% positive cells; +/−, 20% to 30%; −, <30%. Differentiation stages according to Nadler et al.31

†+, level similar to that observed in undiluted Reh RNAs; +/−, level similar to 10−2 dilution of Reh RNAs; (+/−), low transcription level that may be due to malignant cells or alternatively (but less probably) to persisting normal pre-B cells; −, undetectable transcription or level lower than that observed in 10−2 dilution of Reh RNAs. For details see Materials and Methods.

Expression of Rag1 and Rag2 in B-Lineage ALL Cells. The phenotype of the acute leukemia samples is summarized in Tables 1 and 2. Studies of Ig/TCR gene rearrangements were performed by Southern blotting using appropriate probes and restriction en-

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RNA isolation. Extraction of total mRNA was performed by the guanidium-cesium chloride method as described.29 The cell pellet was lysed by vortexing into 6 mol/L guanidium isothiocyanate, 5
mmol/L sodium citrate, 0.1 mol/L β-mercaptoethanol, and 0.5% sarcosyl. The lysis was then centrifuged over a gradient of 5.6 mol/L cesium chloride at 77,000g for 16 hours at 20°C. RNA was precipitated in 70% ethanol and resuspended in diethylpyrocarbonate-treated water.

**Oligonucleotides.** All RAG1 oligonucleotides were derived from the human RAG1 sequence,13 (RAG1: RAG1A = CAGGGTTTGTGGAGCTTCC, RAG1B = GCCGTCTCAGAAGCTTCTA, RAG1T = AAGTATAGGATAGGGGAAAA.) RAG1B was synthesis for the detection of PCR cDNA; RAG1 and RAG1B were used for PCR amplification; RAG1T was used for the detection of PCR products. PCR amplification using RAG1A and RAG1B lead to the production of a 364-bp fragment. When this fragment is used as a probe, a 7-kb message is detected in pre-B cell RNA by Northern blotting (data not shown), consistent with previous reports.15

mR2A and mR2B were derived from the murine RAG2 sequence.16 (RAG2: mR2A = AGATTGAGGACTTCGTTG, mR2B = CCCTTTAGATAGGACAGGGAGCC.) hr2A, hr2B, and hr2C were synthesized after denaturing by standard methods.28 The mixture was loaded onto a 3% agarose gel, run in Tris/Borate/EDTA (TBE) buffer, and stained in ethidium bromide.

The cDNA product was obtained by PCR from DNA using RAG1A-RAG1B primers. hr2A and hr2B were used for PCR. hr2B was used for PCR amplification; RAG1T was used for the detection of PCR products. PCR amplification using RAG1A-RAG1B lead to the production of a 364-bp fragment. When this fragment is used as a probe, a 7-kb message is detected in pre-B cell RNA by Northern blotting (data not shown), consistent with previous reports.15

Oligonucleotides were also derived from the actin gene sequence (AC1: TCATCTCAGAAGATCC; AC2: TCTGTTGAGTCGAGGC.) AC1 and AC2 were used as amplifiers. AC3 was used as probe.

cDNA synthesis. One microgram of total RNA was heated at 92°C for 1 minute in a 20-μL mixture containing Tris-HCl pH 8.3, 50 mmol/L; MgCl2, 6 mmol/L; diithiothreitol, 10 mmol/L; KCl, 40 mmol/L; dATP, dGTP, dCTP, dTTP, 0.2 mmol/L; and 100 ng of the 3' primer (RAG1B, RAG2B or AC2) and subsequently chilled in ice for 3 minutes. Ten units of avian myeloblastosis virus reverse transcriptase (Boehringer Mannheim, Maylan, France) was added. cDNA synthesis was performed at 37°C for 1 hour.

**PCR amplification.** PCR27 was performed on the total cDNA reaction in 100 μL of Tris-HCl pH 8.3, 10 mmol/L; KCl, 50 mmol/L; MgCl2, 2 mmol/L; gelatin 0.01% (wt/vol); dATP, dGTP, dCTP, dTTP, 0.2 mmol/L; 100 ng of the 3' primer (RAG1A or RAG2A) and 2.5 U of Taq DNA polymerase were used. The first cycle consisted of 3 minutes at 92°C for denaturation, 1 minute at 55°C for annealing, and 1 minute at 72°C for elongation, followed by 28 cycles consisting of 1 minute at 92°C for denaturation, 1 minute at 55°C for annealing, and 1 minute at 72°C for elongation. The elongation time was increased by 2 seconds with each cycle; the 28th cycle elongation time was prolonged to 5 minutes to allow extension of incomplete DNA fragments.

**Detection of PCR products.** Ten microliters of the PCR reaction mixture was loaded onto a 3% agarose gel, run in Tris/Borate/EDTA (TBE) buffer, and stained in ethidium bromide.

Dot-blot hybridization of 10% of the products of PCR were precipitated ifi 70% ethanol and resuspended in diethylpyrocarbonate-treated water.

**Northern blots.** Northern blots were performed as previously described28 on mRNA purified on oligo-dT columns. RAG 1 probe was obtained by PCR from DNA using RAG1A-RAG1B primers. Probes were labeled by incorporation of P32-nucleotides with the Klenow polymerase using random hexamers.

**Strategy of the study.** Preliminary experiments showed that a large quantity of Poly-A RNA was necessary for the detection of RAG expression by Northern analysis (data not shown). This strategy appeared impractical for the analysis of a large number of cryopreserved samples. We therefore undertook to analyze RAG expression by PCR amplification of cDNA obtained by a reverse transcriptase (RT) reaction primed with specific oligonucleotides (RT-PCR).

To distinguish between illegitimate transcription226 and low-level transcription, we initially studied serial dilutions of RNA from the RAG+ cell line Reh into RNA from the RAG- cell line Hela. In almost all experiments, a low-level RAG transcription was detected in 10^-4 dilutions and in some cases RAG1 (but no RAG2) expression was observed in 10^-3 dilutions (Fig 2). The level of illegitimate transcription observed in Hela was undetectable or at least lower than that observed in 10^-7 dilutions (Fig 1 and data not shown). A level similar to that observed in 10^-3 dilutions (quoted...
Fig 2. Sensitivity of the RT-PCR method. Two different experiments (experiments 1 and 2) are shown. Lane 1, 1 μg of HeLa RNA. Lane 2, 1 μg of Reh RNA. Lane 3, 0.9 μg of HeLa RNA and 10⁻¹ μg of Reh RNA. Lane 4, 0.99 μg of HeLa RNA and 10⁻² μg of Reh RNA. Lane 5, 1 μg of HeLa RNA and 10⁻³ μg of Reh RNA. Lane 6, negative control (0 μg of RNA). (A) Detection of PCR-amplified products by dot-blot hybridization using RAG1, RAG2, and actin probes. (B) One-tenth of the PCR products (10 μL) run in an 3% agarose gel and stained by ethidium bromide. (-) Expected size of PCR products. (M) ex174 digested by Hae III.

It has been shown previously that most of the coding part of RAG is contained in only one exon. Sequences recognized by the primers used in this study were therefore located on the same exon. Because it was not possible to distinguish between amplified products derived from RNA and those corresponding to contaminating DNA on the basis of size, control reactions omitting the RT enzyme were performed in each case. Only samples with no contamination (signal lower than 10⁻¹) were included in the study.

To verify the quantity of RNA, a PCR reaction was performed on aliquots using primers directed against sequences of the β-actin gene. The usual procedures were performed to minimize the risk of carry-over. Controls samples without RNA were included in each experiment.

RESULTS

Transcription of a human gene (hRAG2) homologous to the murine RAG2. The existence of a human gene homologous to the murine RAG2 was suggested by three results: firstly, human or murine RAG1 cDNA only activates V-(D)J rearrangements poorly when used alone; secondly, human genomic DNA transfected into mouse fibroblasts activate recombination as efficiently as does murine genomic DNA containing RAG1 and RAG2; finally, a sequence homologous to mRAG2 has been detected in human DNA by Southern blotting with a murine RAG2 probe.

Because it has been reported previously that RAG1 and RAG2 are concordantly transcribed in murine lymphoid cells,¹⁴ we expected to detect expression of the human mRAG2 homologue gene from a RAG1-expressing human cell line. We first verified that RAG1 mRNA are detectable in a pre-B cell line, Reh, and then used RNA from these cells in an RT-PCR experiment using murine RAG2 primers. These primers (mR2A and mR2B) were selected to amplify a DNA segment that contains the acidic region previously described by Oettinger et al¹⁴ that was potentially important for the function of the gene (Fig 1).

A unique fragment was observed by electrophoresis whose size was consistent with that expected from the murine sequence. To verify that the RNA detected was homologous to murine RAG2 products, we then sequenced a 282-bp DNA fragment located in the PCR fragment. The nucleotide sequence shows a marked homology (243/282) to the murine gene (Fig 1). No frame shift was observed and no major alteration of the deduced amino acid sequence (homology 78/94 amino acids) was noted. Finally, we verified that the PCR product was recognized by an oligonucleotide complimentary to the human sequence (hRB). The oligonucleotides (hR2A, hR2B, hR2C) designed from the human sequence were used in all subsequent experiments on RAG2 expression.

These data were considered reasonable evidence for the existence of a human gene homologous to the murine RAG2.

Expression of hRAG1 and hRAG2 in T-lineage ALLs. No RAG expression was detected in PB mononuclear cells from healthy donors (data not shown), consistent with the situation in the mouse.¹³,¹⁴ We next investigated thymocytes obtained from a 2-year-old child. Abundant RAG1 and RAG2 messages were documented (data not shown). To delineate the stages of differentiation corresponding to RAG expression, we studied selected cases of T-ALLs that can be considered to be neoplastic expansions of cells frozen at discrete stages of thymic differentiation. Twenty-
nine samples encompassing the major stages of early T-cell differentiation were analyzed (Table 1). Representative experiments are shown in Fig 3.

Coordinate RAG1 and RAG2 expressions were observed in all cases. No correlation was found between the level of RAG expression and the type of analyzed sample (PB vs BM).

Clear RAG1 and RAG2 expression (quoted “+”) was found in 21 of 29 cases, including two cases of immature phenotype, 12 cases with common cortical phenotype, and five samples with a significant (>30%) percentage of CD3—αβ (T38r, T49) or γδ (T31, T31r, T66) neoplastic cells. Interestingly, the very high percentage of CD3—γδ expressing cells (96%) and of blast cells (95%) in the PB sample T31 suggested that TCR expression may coexist with RAG expression. We next compared RAG2 expression in undiluted and diluted T31 RNAs with that observed in dilutions of Reh RNAs. Expression in 10^-3 dilutions of T31 RNAs was clearly detectable, while no signal was observed in 10^-1 dilutions of Reh RNAs (Fig 4). This finding suggests that RAG expression is not due to rare CD3—ve neoplastic cells but occurred in at least a part of CD3—γδ cells in this particular case.

Among the cases with no or minimal expression, one was immature (T69), five were of the common cortical stage or expressed only low level of surface CD3 (T42r, T64, T73, T43r, T50), one expressed CD3—αβ (T61r), and one expressed CD3—γδ (T37) (Table 2). Although no CD19+ B-lineage cells could be detected in the T43r case (Table 2), it is difficult to be sure that the minimal RAG expression observed in the BM sample is due to malignant cells (95% of nucleated cells) or to normal BM pre-B cells. Clonal TCRγ rearrangements were documented in all of the RAG—ve cases (T69, T73, T50, T37; data not shown). This finding is consistent with the hypothesis that recombinase activity was in fact present in the precursors of these cells and suggests that some regulatory process was involved in the lack of RAG expression.

It was possible to compare in five cases RAG expression at presentation and during first relapse of the disease. In three cases (T41, T31, T38), identical findings were obtained. In the two last cases (T42, T43), RAG expression was clearly less intense during the relapse than at presentation.

These data show that RAG expression is found in most T-ALLs and suggest that recombinase activity may coexist in some cases with CD3—TCR expression.

Expression of hRAG1 and hRAG2 in B-cell proliferations.

Expression of RAG1 and, at a more limited extent, of RAG2 has been previously studied by Northern analysis in murine-established cell lines. It was shown that these genes are expressed in pre-B but not in mature B cells, nor in the very immature LyD9 cells that are engaged in the B-cell lineage. RAG1 expression has also been previously documented in the human pre-B Namalva cell line.

To extend these data, we analyzed the expression of hRAG1 and hRAG2 in human B-cell proliferations.

Because it has previously been shown that RAG may be untranscribed in very immature pre-B cells and in mature B cells, we did not use random cases but selected 41 sIg—ALLs encompassing the major stages of early B-cell differentiation described by Nadler et al.11 Eleven Nadler stage II (CD19+CD10—CD20+), 11 Nadler stage III (CD19+CD10+CD20+), and 19 more mature Nadler stage IV (CD19+CD10+CD20+) cases were selected.

A clear RAG1 and/or RAG2 expression was detected in 38 cases (Table 2 and Fig 5). Although no rigorous quantitation can be made using our experimental procedure, expression of RAG compared with that of the β-actin gene was higher in some cases than in others. No evident

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**Fig 3.** Amplification of RNA from representative T-ALL patients using RAG1, RAG2, and actin primers. Lanes 1 and 8, negative control (0 μg RNA); 2 and 9, 10^-2 μg of Reh RNA; 3 and 10, 1 μg of Reh RNA; 4, T69; 5, T67; 6, T42r; 7, T60; 11, T54; and 12, T37. (A) Dot-blot hybridization using RAG1T (RAG1), hR2B (RAG2), and AC3 (actin) probes. (B) Ethidium bromide-stained agarose gel. (m) x174 digested by Hae III. (-) Expected size of PCR products.
Fig 4. Dilution experiment of T-ALL T31 RNA. Lane 1, negative control; 2, 1 μg HeLa RNA; 3, 10⁻¹ μg Reh RNA; 4, 10⁻² μg of Reh RNA; 5, 10⁻³ μg of T31 RNA; 6, 10⁻⁴ μg of T31 RNA; and 7, 1 μg of T31 RNA. (A) Ethidium bromide-stained agarose gel. (M) ex174 digested by Hae III. (−) Expected size of PCR products. (B) Dot-blot hybridization using the hR2B probe.

Fig 5. Amplification of RNA from representative B-lineage ALL patients using RAG1, RAG2, and actin primers. Lane 7, negative control (0 μg RNA); 6, 10⁻² μg of Reh RNA; 5, 1 μg of Reh RNA; 4, B38; 3, B41; 2, B44; and 1, B83. (M) ex174 digested by Hae III. (A) Detection of the amplification products by dot-blot hybridization with RAG1T (RAG1), hR2B (RAG2), and AC3 (actin) probes. (B) Detection of the PCR products on ethidium bromide-stained agarose gel. (M) ex174 digested by Hae III. (−) Expected size of PCR products.

In seven cases, however, partial dissociation was observed (B67, B68, B38, B72, B73, B74r, and B75).

In two cases (B45 and B77) no expression or minimal RAG expression was observed (Table 2). We analyzed the IgH locus and documented rearrangements in both cases with the JH probe (data not shown). We next verified that bi-allelic deletion of RAG1 and RAG2 has not occurred in the case with no detectable expression (B77) by performing PCR amplification on DNA extracted from these cells (data not shown). Interestingly, neither Cμ nor sIg could be detected in either case.

We then analyzed slg⁺ B-cell proliferations. Three slg⁺ ALLs (Burkitt's type) were studied. No RAG1 and RAG2 expression was found (Table 2). Nine B nodular lymphomas (BNL) and two B-chronic lymphocytic leukemias (B-CLL) were also analyzed, but no expression was found (data not shown).

These data show that RAG1 and RAG2 are concordantly expressed in almost all early B-ALLs and not in more mature slg⁺ proliferations.

DISCUSSION

Somatic recombinations are of fundamental importance in the generation of the repertoire that allows lymphoid cells to specifically recognize antigens. RAG should be included among the major factors involved in the mechanism and/or the control of gene rearrangements. This was shown by transfection experiments showing that the products of both genes act coordinately to efficiently induce rearrangements of recombination substrates. The importance of RAG is also suggested by a high conservation of nucleotide and amino acid sequences among species. This was shown in the case of RAG1 by comparing murine and human sequences and is also true in the case of RAG2, as...
were rearranged did not express RAG. This lack of expression was found, however, in the present study and may indicate that antigen receptor expression is not per se directly involved in RAG transcription. Control of RAG expression may thus be one of the mechanisms involved in allelic exclusion. Some exceptions to the inverse correlation between antigen receptor expression and RAG transcription were found, however, in the present study and may indicate that the control of RAG transcription is complex. Firstly, we were unable to detect RAG expression in at least one sIg- case in which the IgH genes had rearranged. Similarly, three CD3-TCR- T-ALL cases in which the TCRγ genes were rearranged did not express RAG. This lack of expression may be due to an as yet poorly understood consequence of the leukemic process on cell differentiation that impairs the control of RAG transcription. Alternatively, this may indicate that antigen receptor expression is not per se directly involved in RAG transcriptional regulation. The same conclusion is also supported by the finding that RAG are expressed in at least one CD3-TCR-expressing ALL. These data should be interpreted in light of the suggestion that TCR gene allelic exclusion is not absolute at the DNA level. We have recently shown that two potentially functional TCRγ rearrangements can coexist in cells from T-ALLs. More significantly, others have shown that two TCRα rearrangements can be present in a CD3+ T-cell clone. Replacement of a functional TCRα rearrangement by another functional TCRα rearrangement was shown in a CD3αβ-expressing murine T-cell line obtained by retroviral transformation of thymocytes. If similar data are found in normal cells, this would imply that the ability to rearrange TCR genes is conserved in at least some CD3+ thymocytes and may be important for the selection of the T-cell repertoire.

RAG transcription in immature B- or T-leukemic cells is also potentially important in the understanding of oncogenesis in lymphoid cells. It is now well established that many nonrandom translocations involving Ig/TCR genes in lymphoid neoplasms involve breakpoints that occur near RSS and probably involve mechanisms similar to those leading to V-(D)-J rearrangements. These data were recently extended to chromosomal abnormalities occurring outside antigen receptor genes. For example, it was shown that deletions of the SCL/tal/TCL5 gene occurring in up to 30% of T-ALL involve heptamers identical to those found in RSS. Studies of nucleotide sequences at the chromosomal junctions have shown nucleotide deletions and additions very similar to those observed at V-(D)-J junctions. Therefore, it is possible that RAG products are involved in the pathogenesis of these chromosomal abnormalities.

From a less speculative point of view, the fact that neoplastic cells are frozen at a differentiation stage in which RAG are active may be important for practical reasons. Our group and others have recently shown that oligonucleotides directed against clone-specific V-(D)-J junctional sequences can be used to specifically detect malignant lymphoid cells even when they represent less than 1 of 105 cells. The use of such a strategy is hampered by oligoclonality and successive rearrangements. Oligoclonality is observed in the IgH locus in about 15% to 30% of B-lineage ALLs but is rarely found with TCR genes. Alteration of TCR rearrangement patterns occurs in about 15% of the cases if samples obtained at presentation and at relapse are analyzed (one example of such cases, T38, is included in this study and is described in more detail in Macintyre et al). There is presently no mean to detect those cases prone to clonal variation. It may be that high-level RAG expression is predictive of such an event. Quantitative PCR will be used in further studies to test this hypothesis.

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Expression of human recombination activating genes (RAG1 and RAG2) in neoplastic lymphoid cells: correlation with cell differentiation and antigen receptor expression

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