Immunoglobulin and T Cell Receptor Gene Configuration in Acute Lymphoblastic Leukemia of Infancy

By Carolyn A. Felix, Gregory H. Reaman, Stanley J. Korsmeyer, Gregory F. Hollis, Patricia A. Dinndorf, John J. Wright, and Ilan R. Kirsch

We examined immunoglobulin (Ig) heavy chain, \textit{\alpha} light chain, and T cell receptor (TCR) \textit{\gamma} and \textit{\beta} gene configuration in the leukemic cells from a series of infants aged <1 year with acute lymphoblastic leukemia (ALL). Each of these 11 cases demonstrated leukemic cell surface antigens that have been correlated with a B cell precursor phenotype. Of the 11, lymphoblasts of 4 retained the germline configuration of both Ig and TCR loci, whereas 7 had rearranged the Ig heavy chain gene. Two of these seven showed light chain gene rearrangement. TCB \textit{\beta} chain rearrangement had occurred in only one of the 11 patients' tumors. No TCR \textit{\gamma} chain rearrangements were identified. These results are in contrast to earlier studies of B cell precursor ALL in children in which Ig heavy chain gene rearrangements were evident in every case and \textasciitilde 40\% showed Ig light chain rearrangement as well. In addition, 45\% of cases of B cell precursor ALL of children had rearranged their \textit{\gamma} TCR genes, and 20\% had rearranged \textit{\beta}. These data suggest that ALL in infancy represents an earlier stage of B cell development than is found in B cell precursor ALL of children. ALL in the infant age group has been associated with the worst prognosis of all patients with ALL. This study suggests that the disease in infants differs not only clinically, but also at the molecular genetic level, from the disease in children.

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MATERIALS AND METHODS

Following informed consent of the parents, leukemic cells were obtained by Ficoll-Hypaque density-gradient centrifugation of peripheral blood mononuclear cells. Acute leukemias were initially defined as lymphoblastic based on French-American-British (FAB) morphology and as well as phenotypic screening for B cell and T cell-associated surface antigens by fluorescent microscopy as previously described. Monoclonal antibodies used included HLA-DR (4.1), B4 (CD19), Common ALL antigen (CALLA) (BA-3, CD10), OKT11(CD2). All leukemic cells were also examined for myeloid-associated antigens with the monoclonal antibodies L4F3, F122 and a cocktail of 1G10(CD15), F122 and 5Fl(CDw14). Megakaryocyte differentiation antigens were examined with the 13.17 and C7E10 monoclons. All had been examined with the 12-8 monoclonal, which recognizes a 115-kd molecule present on hematopoietic colony-forming cells and their precursors. At the time of this study, surface immunoglobulin (Slg) and 3A12 were also examined (Table 1). Patients were further characterized by sex, age in months, WBC and platelet counts at diagnosis, FAB morphology of lymphoblasts, and the presence or absence of meningeal disease, massive organomegaly, and relapse.

Immunoglobulin and TCR gene configuration. High-mol-wt DNA was extracted from leukemic cells as well as peripheral blood total WBCs from normal individuals. Ten micrograms of this genomic DNA was digested to completion with 3 to 5 U/\muL of DNA of the restriction endonuclease BamHI (Bethesda Research Laboratories) in all cases, and EcoRI (Bethesda Research Laboratories) and HindIII (Boehringer Mannheim) when ample DNA was available, size-fractionated on 0.8\% agarose gels, and transferred to nitrocellulose paper. Human Ig gene probes were used to assess the status of immunoglobulin heavy (Ig H) as well as \textit{\alpha} light chain genes. A human genomic 6.0-kb BamHI-HindIII fragment containing the entire Ig heavy chain joining (J\textit{H}) region was used to assess rearrangements of the Ig heavy chain locus. This fragment detected a 17-kb BamHI fragment in the germline form. Some rearranged forms were detected in some samples using a 3'JH probe, a genomic fragment of the J\textit{H} and 3' flanking region of the same human DNA. Ig H chain gene configuration was confirmed by hybridization of the 3'JH probe with EcoRI or HindIII-digested genomic DNA. \textit{\alpha} gene rearrangements were also detected in BamHI-digested genomic DNA by hybridization with a 2.5-kb germline EcoRI constant (C\textsubscript{x}) region-containing DNA probe prepared by a nick-translation method, washed at 52°C in 0.1 \times 0.15 mol/L of NaCl/0.015 mol/L of NaCitrate (SSC) and 0.1% sodium dodecyl sulfate (SDS), and autoradiographed. Blots hybridized with an entire TCR \textit{\gamma} gene cDNA were washed at 42°C.

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ACUTE LYMPHOBLASTIC LEUKEMIA OF INFANCY

Table 1. Genotypes and Phenotypes of ALLs of Infants

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<th>Patient No.</th>
<th>Ight Chain</th>
<th>TCR γ</th>
<th>TCR β</th>
<th>HLA-DR</th>
<th>B4</th>
<th>CALLA</th>
<th>Sig</th>
<th>3A1</th>
<th>T11</th>
<th>LAF3</th>
<th>1G10/5F1</th>
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Phenotypic data (except for Sig and 3A1) were previously tabulated in Dinndorf and Reaman. The germline configuration of the Ig H chain locus was confirmed for patients 4 and 11 using HindIII and/or EcoRI digests in addition to BamHI digests probed with the 3'γJ probe. The germline configuration of the γ TCR locus was found in every case by probing BamHI-digested DNA with the Cγ2, Jγ1, and an entire TCR γ cDNA and was further confirmed by hybridization of EcoRI blots with the Jγ1, γ probe for patients 4, 5, 10, and 11. The β TCR locus was studied by hybridization of BamHI-digested DNA with the Cβ probe in all cases, and hybridization of EcoRI-digested DNA with the same probe for patients 4, 5, 10, and 11.

Numbers to the right of Genotype columns represent the number of alleles. + represents <10% of cells showing reactivity, + represents >50%, and specific values (percentages) are given for the 10% to 50% range.

Abbreviations: R, rearranged; D, deleted; G, germline.

RESULTS

Ig gene configuration in ALL of infancy. Comprehensive genotyping of ALL of infancy reveals two patterns of Ig gene configuration in the tumors in this series of patients; one with rearranged Ig genes (Fig 1A), and one with germline Ig genes (Fig 1B).

Two-thirds of the cases studied (7 of 11) have rearranged Ig H chain genes, and the majority of these (4 of 7) have rearranged both alleles (Fig 1A, Table 1). In one of these cases, rearrangement of one allele with deletion of the other is evident (patient 2). The leukemic cells of patients 1, 7, and 9 rearrange a single allele of the Ig H chain locus (Table 1). Two of the 7 with Ig H chain gene rearrangement have progressed to k L chain gene rearrangement (Table 1).

The seven ALL cells that have rearranged Ig genes also express the B cell differentiation antigens HLA-DR and B4 and lack phenotypic evidence of T cell, myeloid, and megakaryocytic lineage (Table 1), suggesting that these cases represent precursors committed to the B cell lineage at both the genotypic and phenotypic levels. The majority of these leukemias (6 of 7), including the tumor cells of patient 5 with rearranged Ig H chain and κ light chain genes, are CALLA negative. The ALL cells of patient 7, which also have progressed to light chain rearrangement, have the B cell-associated surface antigens HLA-DR and B4 on the majority of cells as well as limited expression of CALLA (11%) (Table 1). Close examination reveals that the rearranged κ L chain band is less intense than the rearranged Jκ band (Fig 2). Perhaps a subclone of the tumor cell population has developed this additional rearrangement. This population may overlap or may be identical to the population expressing CALLA.

TCR gene configuration in ALL of infancy. None of the 11 infants' leukemic cells in this series have rearranged γ TCR genes when analyzed by hybridization of BamHI-digested DNA with the probes Cγ2, Jγ1, and an entire TCR γ cDNA. In 4 of the 11 cases (patients 4, 5, 10, and 11), the germline configuration of the γ TCR locus was further...
confirmed by hybridization of EcoRI-digested DNA with the Jγ1,3 probe (Figs 1A and B and 2; Table 1).

Similarly, ALL cells from 10 of the 11 infants have a germline configuration of the β TCR when BamHI-digested DNA is hybridized with the Cγβ probe (Fig 1A and B; Table 1). In 4 of these 10 cases, the germline configuration of the β TCR was confirmed by hybridization of EcoRI blots with the same Cγβ probe (patients 4, 5, 10, and 11). The one exception (patient 7, Fig 2) has rearranged both Ig H chain and κ L chain genes, as well as the β TCR locus. These leukemia cells, as mentioned above, demonstrate HLA-DR and B4 positivity and some CALLA, but lack any definitive T cell surface antigens, suggesting a B cell precursor lineage commitment.

ALLs germline for both Ig and TCR genes. One-third of ALLs of infancy (4 of 11) in this series have not rearranged either their Ig H chain, κ L chain, TCR γ, or TCR β genes (Table 1, Fig 1B). In two of these four cases (patients 4 and 11), ample DNA was available for confirmation of the germline configuration of the Ig H chain locus with the

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**Fig 1.** (A) The lymphoblasts of patient 10 show the pattern of rearranged Ig genes and germline TCR genes. In this case, both alleles of the Ig heavy chain are rearranged and the κ light chain gene remains in the germline form. Similar patterns of rearranged Ig genes were found in 7 of 11 ALLs of infants in the series examined. (B) The lymphoblasts of patient 11 show the germline configuration of the Ig heavy chain locus, and the κ light chain locus as well as the TCR γ and β genes. This pattern was found in 4 of 11 cases in the series of ALLs of infants examined.

**Fig 2.** The lymphoblasts of patient 7 show rearrangement of the Ig heavy chain locus, and κ light chain locus and in addition show rearrangement of the β TCR locus. The rearranged κ light chain band is less intense than the rearranged Jκ band, suggesting that a subclone of the tumor cell population has developed this additional rearrangement.
restriction enzymes *Hind*III and/or *EcoR*I in addition to *Bam*HI. Each of these four leukemias expresses surface markers that have been correlated with early B lineage commitment (HLA-DR and/or B4). Two also express CALLA (patients 4 and 6). They lack the 3A1 and T11 T cell differentiation antigens, and do not express myeloid- or megakaryocyte-associated markers (Table 1). This pattern of surface markers and genes indicates that these cells may be very immature lymphoid precursors. Consistent with this, the tumor cells of patient 11 were positive when examined with the 12-8 monoclonal antibody, which recognizes a stem cell-associated antigen. This group with germ line configuration of Ig and TCR genes suggests that the acquisition of the HLA-DR and B4 markers occurs early in lymphoid development, before the rearrangement of either Ig or TCR genes.

**Comparison of genotypes of ALLs of infants and ALLs of children by statistical analysis.** We examined whether genotypic differences existed between ALLs of infants aged <12 months and ALLs of children. By Fisher’s exact test, the proportion with rearranged Ig genes was statistically significantly lower (*P* < .05) in ALLs of infants (7 of 11) than in ALLs of children (25 of 25). In addition, the proportion of infants’ ALLs with rearranged γ chain TCR genes (none of 11) was significantly lower (*P* < .05) than in ALLs of children (8 of 17).

We found no differences (*P* > .2) between these two groups in the proportion with Ig heavy chain gene rearrangements that also rearranged light chains, nor in the proportion that rearranged their β TCR genes (*P* > .2).

**Correlation of genotype with clinic and biologic features of ALL of infancy.** We tabulated clinical and biologic features and corresponding patterns of rearranged or germ line Ig genes for this series of infants with ALL (data not shown). By the Wilcoxon two-sample test, there were no apparent differences in distribution of age in months, WBC, and platelet count at diagnosis or in percentage of blasts with L2 FAB morphology between infants with ALLs with germ line ν rearranged Ig genes. Nor were there differences between infants with ALLs with germ line ν rearranged Ig genes in sex, or proportions with meningeal involvement, massive organomegaly, or relapse when assessed by Fisher’s exact test.

**DISCUSSION**

These data reveal that the clinical difference between ALL of infancy and ALL of childhood is correlated, in general, with distinctive patterns of Ig and TCR gene configurations. This may also be correlated with phenotypic differences in the leukemic infant population where CALLA positivity frequency is decreased.

Within this group of ALLs of infants, genotypic characterization suggests three subsets of lymphoid cells. One genotypic pattern is represented by a subset (4 of 11) that possesses germ line configurations of both Ig and TCR genes. These cells express surface markers correlated with early B cell lineage (HLA-DR and/or B4), and lack surface antigens of other lineages, suggesting that they might be B cell precursors at a stage prior to Ig gene rearrangement. Interestingly, one case of ALL in this subset (patient 11) reacts with the markers HLA-DR and B4, as well as the stem cell monoclonal 12-8, and may therefore represent a very early stage in lymphoid development. We have seen this germ line pattern before in two children with ALL bearing some T cell-associated antigens, another group in which a poor outcome often occurs.

Cells from only one infant in this series (patient 7) have rearranged their Ig genes as well as the β TCR locus. These cells may be examples in lymphoid development when both Ig and TCR genes are accessible to a putative common recombinase. This ALL is also interesting because it is an example of β chain rearrangement without γ. We have seen this pattern before in a rare T-ALL, as well as in some B cell precursor ALLs of children. Whereas we found 45% of B cell precursor ALL cells of older children to possess rearranged γ TCR genes, no γ TCR gene rearrangements were found in the 11 ALLs of infants in this group. This statistically significant finding indicates another molecular genetic difference between the disease in infants and the disease in children.

Finally, six of eleven ALLs of infants show rearranged Ig genes and germ line TCR genes. Usually only Ig heavy chain genes rearrange without light chain gene rearrangement. These data suggest that ALL in infancy represents an earlier stage of B cell development than ALL of childhood.

The extensive phenotypic characterization of this group of ALL cells of infants has been presented before and is consistent with an early B lymphoid origin. Within the B cell precursor ALL series in children, a developmental hierarchy of Ig gene rearrangements in which all cases possess rearranged Ig heavy chains and ~40% had progressed to the later developmental step of k followed by λ light chain rearrangement, was noted. This sequence was coordinate with an ordered expression of the B cell-associated surface antigens in which HLA-DR and B4 were present on the most immature cells, followed by CALLA and then B1 on the most mature cells. In this context, we correlated genotypic and phenotypic data in our analysis of tumor cells from infants with ALL (Table 1). Based on this schema, we found several aberrant phenotypes. For example, CALLA expression that has been correlated with a stage of B cell precursor development after rearrangement of Ig heavy chain genes but before light chain activation was found on lymphoblasts of two infants with the germ line Ig heavy and light chain gene pattern (patients 4 and 6), but not on the cells of another with rearranged k L chain genes (patient 5). The tumor cells of patient 4 that express B4 and CALLA do not express HLA-DR. The cells of patient 6 were more positive for CALLA than for HLA-DR or B4. Thus, in this infant population, the order of Ig gene activation and coordinate B cell-associated surface antigen expression is not invariant. These “aberrant” phenotypes may reflect variability of normal lymphocyte development, assuming that differentiation in leukemic cells faithfully mimics normal differentiation. Alternatively, they may reflect events that result from malignant transformations.

The T cell phenotype has not been described in ALL in infancy and was not found in this series either. Its incidence
may be less than the 20% found in older children, as study of a larger series of infants with ALL suggested.

This molecular analysis of ALL in infancy extends our understanding of the genetic stages of lymphoid maturation in that it reveals cells with germline Ig and TCR loci that possess B cell-associated antigens and lack phenotypic markers of other lineages. Moreover, this study reveals ALL of infancy to generally represent an earlier stage of lymphoid development than do the ALL cells of children. The distinctive molecular genetic patterns of this leukemia may be reflected in its biologic and clinical behavior.

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