Monoclonal antibody 7.1, which recognizes the chondroitin sulfate proteoglycan molecule NG2, was used to screen prospectively blast cells from 104 consecutive children at initial presentation with acute lymphoblastic leukemia (ALL). Reactivity with this antibody was found in 9 cases (8.6%), of whom 5 had a t(4;11)(q21;q23) and 4 had a t(11;19)(p13;q23). None of the NG2+ cases had either translocation. Southern blot analysis disclosed MLL gene rearrangement in only the 9 cases with 7.1 reactivity plus the t(4;11)(q21;q23) or t(11;19)(q23;p13) translocation. MLL gene rearrangements were not detected in 89 patient leukemic samples that did not express NG2, including 7 patients with del(11)(q23) or inv(11)(p13q23). As expected from the association with t(4;11) and t(11;19), NG2+ cases were significantly more likely to be infants, to have hyperleukocytosis and central nervous system involvement, to be CD10-, and to express myeloid-associated antigens CD15 and CD65. Des that short follow-up duration, 3 of the NG2+ cases have relapsed while the other 101 patients remain in remission. Thus, blast cell surface expression of NG2 is useful for identifying patients with ALL having t(4;11) or t(11;19) translocations that are associated with poor prognosis, especially in the infant age group.

IN RECENT YEARS, studies have shown an association of nonrandom chromosomal translocations, such as t(9;22) and t(4;11), with clinical outcome in patients with acute lymphoblastic leukemia (ALL). More recently, some investigations have shown that blasts with certain translocations tend to express particular combinations of surface differentiation antigens. For example, blasts of B-lineage ALL with the t(4;11)(q21;q23) or t(11;19)(q23;p13) usually lack the CD10 protein and atypically express the CD15 myeloid-associated antigen, and those of pre-B ALL with the t(1;19)(q23;p13) express CD19, CD10 and lack CD34. Recently Smith et al. described a 220- to 240-kD cell-surface protein recognized by a murine monoclonal antibody (MoAb) named 7.1. The antigen recognized by MoAb 7.1 was subsequently determined to be the human homologue of the rat NG2 molecule, a chondroitin sulfate proteoglycan molecule. The NG2 molecule recognized by MoAb 7.1 was found to be expressed on the blasts of some patients with acute myeloid leukemia but not by normal hematopoietic cells. Patients whose leukemic blasts expressed this chondroitin sulfat proteoglycan experienced poorer clinical outcomes. In this study of childhood ALL, we found that leukemias with t(4;11) and t(11;19) involving the MLL gene on chromosome 11q23 had blasts that reacted with MoAb 7.1, whereas leukemias without these reciprocal translocations or with chromosome 11q23 abnormalities not involving the MLL gene failed to express the NG2 molecule.

MATERIALS AND METHODS

Patients. Of 117 children with newly diagnosed ALL admitted consecutively to St Jude Children’s Research Hospital from 1992 to 1994, complete immunophenotyping and cytogenetic analyses were available on 104 patients who comprise the reported cohort. Informed consent was obtained from all patients or their guardians, and the investigations were approved by the institution’s clinical trials review committee.

Bone marrow (BM) and spinal fluid studies. BM cells were stained by standard techniques, including Wright-Giemsa, myeloperoxidase, and α-naphthyl butyrate esterase. The diagnosis of ALL was classified as follows: CNS-I, no detectable blasts; and CNS-3, ≥5 leukocytes/µL with detectable blasts; and CNS-3, ≥5 leukocytes/µL with detectable blasts or cranial nerve palsies.

Immunologic marker studies. BM cells were separated on a 1.077 g/mL density-gradient for analysis. Cell-surface antigens were detected by a standard indirect immunofluorescence method using a panel of antibodies that react with antigens of the CD2 (Leu 5a), CD3 (Leu 4), CD4 (Leu 3a), CD5 (Leu 1), CD7 (Leu 9), CD9 (BA-2), CD10 (J5), CD13 (Leu M7), CD14 (Leu M3), CD15 (My1), CD19 (Leu 12), CD20 (Leu 16), CD22 (Leu 14), CD24 (BA-1), CD33 (Leu M9), CD34 (HPCA-1/My10), CD41a (anti-gpIb/IIa), CD45 (HLE-1/2D1), CD65 (VIM-2), and anti–HLA-DR. Appropriate titters of MoAb 7.1 were determined using HeLa cell line. In all experiments, isotype-matched murine IgGs and anti–β2-microglobulin were used as negative and positive controls. Leukemic cells were also tested for surface and cytoplasmic Iggs (sdg μ, κ, λ, and clg μ) with goat-antihuman IgGs conjugated to fluorescein or rhodamine (Southern Biotechnology, Birmingham, AL). Fluorescence activity was analyzed by an EPICS Elite flow cytometer (Coulter, Hialeah, FL), for surface antibody studies and a Zeiss epifluorescent microscope (Carl Zeiss, Oberkochen, Germany) for clg μ analysis. Histograms of fluorescent intensity were based on a log scale. The percent-
age of positive cells was obtained by counting the number of events of fluorescent intensity greater than the isotype-matched negative control. A leukemic sample was considered positive for antigen expression if ≥25% of cells expressed a fluorescent intensity greater than 95% of the intensity of cells stained with the isotype-matched control antibody. Additionally, histograms of tested MoAbs were superimposed on the histograms of negative controls for detection of weak reactivities of leukemic cells. Immunophenotyping data was used to classify cases of ALL as T cell (CD3+), B cell (lgM+ plus κ+ or λ+), transitional pre-B (lgM+, slgμ+, κ+, and λ+), pre-B (lgM+, slgμ+), or early pre-B (CD19+, CD22+, slgμ+, slgλ+).5–8

MoAb 7.1. The MoAb 7.1 is a murine MoAb of IgG1 isotype. Hybridomas were prepared from mice immunized with a BM stromal cell line established by infection with SV-40.5–8 No reactivity is found with surface proteins of normal marrow hematopoietic cells, peripheral blood leukocytes, red blood cells, or platelets.5–8 Leukemic cell lines including KG1, KG1a, HEL, Jurkat, K562, Raji, HL-60, U937, Daudi, Nalm6, and CEM do not react with MoAb 7.1.5–8 Similarly, no reactivity is detected with the RS4;11 and MV4;11 cell lines that carry t(4;11) translocations. However, MoAb 7.1 does react with a variety of cell lines of nonhematopoietic origin, including HeLa cells.5–8

Cytogenetic studies. Immediately after collection, BM specimens were processed for chromosomal analysis by the direct method of Williams et al.11 A modified trypsin-Wright technique was used for chromosome banding. Chromosome abnormalities were described according to conventions of the International System for Human Cytogenetic Nomenclature (ISCN-91).12

Southern blot analysis. Genomic DNA was extracted from BM of leukemic patients at diagnosis. Aliquots (10 μg) of high-molecular-weight DNA were digested with BamHI, HindIII, and Sac I, separated by electrophoresis on 0.8% agarose gels, and blotted onto nylon membranes. A 32P-labeled probe (Oncor, Gaithersburg, MD) derived from a 0.74-kb BamHI cDNA fragment of the MLL gene was used for all hybridizations.13 Hybridization and posthybridization washes were performed under high stringency conditions. All blots included a control DNA with 2 germline MLL genes and DNA from the RS4;11 cell line that contains 1 germline and 1 rearranged MLL gene.13 Nylon membranes were exposed to Kodak XAR-5 film (Eastman Kodak, Rochester, NY) at -70°C for 5 days.

Statistical analysis. Differences in distribution of laboratory study features were tested by the two-tailed Fisher's exact test.

RESULTS

Surface antigen studies. MoAb 7.1 reacted with the leukemic blasts of 9 (8.6%) of 104 cases of childhood ALL. In these 9 patient marrow samples, the 7.1 antibody reacted with 28% to 95% blasts (median, 86%). The fluorescent intensity of antibody reactivity varied. The reactivity of 7.1 with blasts of several cases produced histograms curves that were partially superimposed on curves of the isotype negative control antibody and other leukemia cases demonstrated histogram curves of 7.1 reactivity of more intense fluorescence than negative control curves (Fig 1). In the remaining 95 cases, less than 4% of cells in leukemic marrow samples reacted with MoAb 7.1 and no shifts of intensity of MoAb 7.1 histogram curves compared with the isotype negative control curves were observed.

Of the 9 cases that reacted with 7.1 antibody, 5 were early pre-B, 2 pre-B, 1 transitional pre-B, and 1 T-lineage ALL (Table 1). MoAb 7.1 reactivity was significantly associated with absence of CD10 expression (7 of 8 MoAb 7.1+ cases v 12 of 95 negative cases, P < .001). The MoAb 7.1 reactivity correlated strongly with expression of CD15 (6 of 8 MoAb 7.1+ v 12 of 95 MoAb 7.1− cases, P = .003) and CD65 (7 of 8 v 8 of 95, P < .001). Insufficient blasts were available from case 7 for studies of CD13, CD15, CD33, CD34, and CD65. No correlations were identified between MoAb 7.1 reactivity and expression of CD9, CD24, CD13, CD33, and CD34, although CD24 was often weakly expressed by MoAb 7.1+ blasts.

Cytogenetic and Southern blot analyses. As shown in
Table 1. Clinical and Laboratory Features of Nine Patients With Leukemic Blasts Reacting With MoAb 7.1

| Case | Clinical Status | Age (yr) | Sex | CNS | WBC (× 10³/L) | FAB | IP | 7.1* | CD10 | CD13 | CD15 | CD33 | CD34 | CD65 | Course |
|------|----------------|----------|-----|-----|--------------|-----|----|------|-----|-----|-----|-----|-----|-----|-------|--------|
| 1    | Dx             | 12.5     | M   | CNS-2 | 383 | L2 | EPB | + (91) | -   | -   | +   | -   | +   | -   | +     | CCR, 14+ mo |
| 2    | Dx             | 15.5     | M   | CNS-2 | 573 | L2 | TPB | + (72) | -   | -   | -   | +   | +   | -   | +     | CCR, 12+ mo |
| 3    | Dx             | 0.2      | F   | CNS-3 | 491 | L1 | EPB | + (52) | -   | -   | +   | -   | +   | -   | +     | CNS-2 |
| 4    | Dx             | 0.2      | F   | CNS-2 | 330 | L1 | EPB | + (85) | -   | -   | +   | -   | +   | -   | +     | CNS-2 |
| 5    | Rlp            | 9.6      | M   | CNS-2 | 581 | L1 | EPB | + (95) | -   | -   | +   | -   | +   | -   | +     | CCR, 23+ mo |
| 6    | Rlp            | 16.6     | M   | CNS-1 | 6.2 | L1 | T   | + (79) | -   | -   | +   | -   | -   | +   | -     | Relapse, 13 mo |
| 7    | Dx             | 0.2      | M   | CNS-2 | 8.7 | L1 | PB  | + (90) | -   | -   | +   | -   | +   | -   | +     | CNS-2 |
| 8    | Dx             | 0.6      | M   | CNS-3 | 67.7 | L1 | PB  | + (28) | -   | -   | +   | -   | +   | -   | +     | Relapse, 9 mo |
| 9    | Dx             | 2.0      | M   | CNS-2 | 7.3 | L2 | EPB | + (90) | -   | -   | +   | -   | +   | -   | +     | CCR, 5+ mo |

Abbreviations: Dx, diagnosis; Rlp, relapse; CNS, central nervous system leukemia; CNS-1, no disease; CNS-2, <5 leukocytes/µL with blasts in CSF; CNS-3, >5 leukocytes/µL with blasts in CSF; WBC, white blood cell count; IP, immunophenotype; EPB, early Pre-B ALL; PB, Pro-B ALL; TPB, transitional Pre-B ALL; M/Mo, myelomonocytic; ND, not done; CCR, complete continuous remission.

* Percent of leukemic blasts reacting with MoAb 7.1 given in parentheses.

Table 2, blasts of all 9 MoAb 7.1* ALL specimens had reciprocal translocations involving chromosome 11, band q23 (t(4;11)(q21;q23) in 5 cases and t(11;19)(p13;q23) in 4). None of the cases with t(1;19)(q21;p13) (n = 7), t(9;22)(q34;q11) (n = 2) or t(8;14)(q24;q32) (n = 2) had MoAb 7.1 reactivity. Although none of the other 95 patients had t(4;11) or t(11;19) translocations, 7 of these cases showed other 11q23 abnormalities (cases 10 through 16, Table 2). Because of the association of MLL gene with t(4;11) and t(11;19), 13,14-20 DNA was extracted from leukemic blasts and probed for breaks of the MLL gene in all 16 patients with abnormalities as well as 82 cases with no detectable 11q23 chromosomal defects. Strikingly, breaks of the MLL gene were found in only the 9 patients whose blasts reacted with MoAb 7.1 (P < .001; Fig 2, Table 3).

Because there was a significant association of 7.1 reactivity with breaks in the MLL gene, cryopreserved leukemic cells obtained from 4 patients with t(4;11)-positive ALL at diagnosis, 1 patient with t(4;11)-positive ALL in relapse, Table 2.

Cytogenetic and MLL Gene Studies in 16 Patients With Chromosome 11q23 Abnormalities

<table>
<thead>
<tr>
<th>Case</th>
<th>Date of Study</th>
<th>Cytogenetics</th>
<th>MLL Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diagnosis</td>
<td>46,XY,t(4;11)(q21;q23),i(7)(q10)</td>
<td>R</td>
</tr>
<tr>
<td>2</td>
<td>Diagnosis</td>
<td>47,XY,+X,t(4;11)(q21;q23)/46,iderm,-X</td>
<td>R</td>
</tr>
<tr>
<td>3</td>
<td>Diagnosis</td>
<td>46,XX,t(4;11)(q21;q23)</td>
<td>R</td>
</tr>
<tr>
<td>4</td>
<td>Diagnosis</td>
<td>46,XX,t(11;19)(q23;p13)</td>
<td>R</td>
</tr>
<tr>
<td>5</td>
<td>Diagnosis</td>
<td>46,XX,t(11;19)(q23;p13)/46,iderm,t(2;3;der(11)(11;19);p12;p25;21)(q21;46,iderm,der(11)(p34)</td>
<td>R</td>
</tr>
<tr>
<td>6</td>
<td>Diagnosis</td>
<td>46,XY,del(4)(q21),del(9)(p13),dict(9;13)(p12;p12),t(11;19)(q23;p13)x2,+19,+20,+22,+mar1/48,iderm,+mar1,+mar2</td>
<td>R</td>
</tr>
<tr>
<td>7</td>
<td>Diagnosis</td>
<td>46,XY,del(4)(q21),del(9)(p13),dict(9;13)(p12;p12),t(11;19)(q23;p13)x2,+19,+20,+22,+mar1/48,iderm,+mar1,+mar2</td>
<td>R</td>
</tr>
<tr>
<td>8</td>
<td>Diagnosis</td>
<td>46,XY,t(4;11)(q21;q23)</td>
<td>R</td>
</tr>
<tr>
<td>9</td>
<td>Diagnosis</td>
<td>46,XY,t(4;11)(q21;q23),add(17)(q25)</td>
<td>R</td>
</tr>
</tbody>
</table>

MoAb 7.1*  
10  Diagnosis | 46,XY,inv(11)(p13q23) | G |
11  Diagnosis | 46,XY,del(11)(p33) | G |
12  Diagnosis | 46,X,-Y,t(12)(12;q23;p13),der(11)(11;3:11)(p11;q23),+mar47,iderm,+.X,der(11)(11?)(q32;7) | G |
15  Diagnosis | 46,XY,t(11;11)(q23;32),t(8;14)(q22;q32)/46,XY,der(11)(q14)* | G |
16  Diagnosis | 46,XY,inv(11)(p13q23)/47,iderm,+10 | G |

* Karyotype previously reported; R, rearranged MLL gene present; G, germline MLL genes.
NG2 PROTEIN AND MLL IN ALL

A

Control
RS4;11
1
2
3
4
5
6
7
8

B

Control
RS4;11
4
10
11

Fig 2. Southern blot of restricted DNA of BM from patients with abnormalities of chromosome 11q23. Lane labeled 'Control' contains DNA from a cell line with two germline MLL genes. Lane labeled 'RS4;11' contains DNA from RS4;11 cell line that has one germline and one rearranged MLL gene. Other lanes are labeled with patient case number. Blots were hybridized with a probe to the common restricted DNA of eight patients with reciprocal translocations of breakpoint region of the 'RS4;ll' contains DNA from RS4;ll cell line that has one germline and one rearranged DNA from a cell line with two germline abnormalities of chromosome 11q23. Lane labeled 'Control' contains normal cases 10 and 11, with inv(11)(p13q23) and del(11)(q231, respectively, is detected in case 4 with a t(11;19) and MoAb 7.1' leukemic blasts. had MoAb 7.1- blasts and show no rearrangement of an arrangements of the detected in all 6 cases, but only the leukemic blasts of the 90% of the cells displayed the immunophenotype (CD34+, CD19+, CD10-, CD15+) characteristic for ALL. Testing with MoAb 7.1 was found for leukemic blast FAB type and MoAb 7.1 activity. Although follow-up is too short for clinical outcome comparisons, 3 of the MoAb 7.1' patients have relapsed compared with none of the other patients. At relapse, leukemic cells from all three patients showed MLL gene rearrangements plus reactivity with MoAb 7.1 (Tables 1 and 2).

DISCUSSION

MoAb 7.1 reacted with leukemic blasts from 9% of children with ALL. The reactivity of this antibody was concurrent with a translocation involving the MLL gene and was significantly correlated with infant age group, hyperleukocytosis, CNS leukemia, lack of CD10 expression, and atypical expression of CD15 and CD65 myeloid-associated antigens. This correlation is not surprising because these features are typical for lymphoblastic leukemias with either a t(4;11) or t(11;19). of the 9 MoAb 7.1' patients, 8 patients had blasts that reacted with MoAb 7.1. Other reciprocal translocations involving the MLL gene [e.g., t(6;11) and t(9;11)] were not available in this series of ALL. Thus, additional studies are needed to determine if MoAb 7.1 reactivity in ALL is associated with all rearrangements of a MLL gene or is restricted to the two reciprocal translocations studied in this investigation.

It has been determined that MoAb 7.1 recognizes a chondroitin sulfate proteoglycan molecule that is the human homologue of the rat NG2 molecule. Although chondroitin sulfate proteoglycan molecules have been shown to have functions in cellular adhesion and migration, the role of this molecule has not yet been defined. This protein has been detected on the surface of a cervical carcinoma cell line (HeLa) but not on normal marrow hematopoietic and peripheral blood cells. We have not observed any reactivity with MoAb 7.1 on cells of normal lymph node tissue (unpublished data, November 1995), but other normal tissues will have to

<table>
<thead>
<tr>
<th>No. of Cases of ALL With:</th>
<th>MoAb 7.1' Blasts</th>
<th>MoAb 7.1' Blasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLL genes rearranged</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>MLL genes germline</td>
<td>0</td>
<td>89</td>
</tr>
</tbody>
</table>
be screened with this antibody. Until more extensive examination of normal tissues including fetal tissues are completed, it remains possible that the NG2 molecule detected by MoAb 7.1 is a normal cellular protein whose expression is suppressed in hematopoietic tissues but is aberrantly expressed after malignant transformation in leukemias with the t(4;11) and t(11;19). Although NG2 expression by leukemic lymphoblasts is strongly correlated with a rearrangement of an MLL gene, rearrangement of this gene alone may not be sufficient for the aberrant expression of NG2. The RS4;11 and MV4;11 cell lines and leukemic blasts of a patient with ALL in relapse did not express NG2, even though all had evidence of an MLL gene rearrangement. Thus, aberrant NG2 expression by blasts of ALL may be dependent on additional regulatory factors or cellular interactions.

The unique reactivity of MoAb 7.1 with only neoplastic leukocytes and its specific association with chromosome 11q23 translocations involving the MLL gene suggest several important clinical uses. Firstly, we have shown a very high specificity of MoAb 7.1 reactivity for blasts with t(4;11) or t(11;19) translocations, which are associated with increased risk of treatment failure in infants and possibly older adolescents. Thus, this antibody may be useful for screening lymphoblastic leukemias which have unsuccessful or normal cytogenetic studies. Secondly, because NG2 is not found on normal hematopoietic cells, MoAb 7.1 may prove to be a more sensitive marker for minimal residual disease than other MoAbs or their combinations now in use. We have been able to detect small numbers of MoAb 7.1+ mononuclear cells in a remission BM sample from 1 of 3 patients diagnosed with t(4;11)+ ALL. In this patient, 0.5% of the marrow mononuclear cells expressed the NG2 protein 4 months after successful induction therapy, despite morphologic evidence of residual leukemia. This patient remains in clinical remission 4 years later but has not been restudied. Since a case of relapsed ALL with a t(4;11) translocation examined in this investigation had blasts that failed to react with MoAb 7.1, additional sequential studies of diagnosis, remission, and relapse samples are needed to see if there may be loss of MoAb 7.1 reactivity with persistent or recurrent leukemia.

ACKNOWLEDGMENT
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Human homologue of the rat chondroitin sulfate proteoglycan, NG2, detected by monoclonal antibody 7.1, identifies childhood acute lymphoblastic leukemias with t(4;11)(q21;q23) or t(11;19)(q23;p13) and MLL gene rearrangements

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