Monoclonal Antibody Definition of T Cell Acute Leukemia: A Pediatric Oncology Group Study

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Leukemic blasts from 774 children with newly diagnosed acute lymphocytic leukemia (ALL) have been phenotyped by microcytotoxicity testing with a panel of monoclonal antibodies and heteroantisera as part of a Pediatric Oncology Group classification study of acute leukemia. One hundred twenty-two cases, or 16%, were designated as T cell leukemia based on the reactivity of blasts cells with previously well-characterized antisera (PT) against a T lymphocyte-associated antigen. Using this antisera-based definition as a standard, we looked for a monoclonal antibody combination that would be a suitable substitute. An algorithm calling for reactivity with either monoclonal antibody 3A1 or Leu-1 was a 92% sensitive and 97% specific predictor of PT reactivity. Only 27 of 755 cases of leukemia were incorrectly classified using this algorithm. Subsequently, Ficoll-Hypaque-separated bone marrow cells from 118 additional patients with ALL (21 of whom had T cell ALL) were stained by immunofluorescence using a combination of directly fluoresceinated 3A1 and Leu-1. Reactivity of 20% or more of the cells with this antibody combination was a 100% sensitive and 94% specific indicator of T cell ALL defined by PT positivity; with a higher cutoff value for positive values, or the use of supplemental tests, even this small number of false-positives could be eliminated. We conclude that this monoclonal antibody combination is a satisfactory replacement for our heteroantisera definition of T cell ALL.

Several investigators have described different monoclonal antibodies including 3A1,7 Leu-9,9 and WT1,9 which react with a 40,000-dalton mol wt T cell-associated antigen. This 40,000-dalton antigen, like TLAA, has been shown to be present on all E-rosette-positive T cell ALL; it has also been found on cases of E-rosette-negative leukemia.10 The occurrence of monoclonal antibody-defined T cell-associated antigens on some E-rosette-negative ALL suggests that these leukemias are also of T cell lineage. Proof of this
conjecture obviously requires a standard for definition of T cells other than E-rosette formation. The experience of the POG with the PT antisera suggests that this might be a useful standard. Since July 1981, all cases of ALL studied by the POG have been tested with a battery of monoclonal antibodies as well as the PT heteroantisera. We thus can ask what monoclonal antibody or antibodies can be used to replace the antisera definition of T cell leukemia. In this report we describe the results of phenotyping 774 pediatric patients with ALL, using T cell monoclonal antibodies and PT antisera. We find that reactivity with monoclonal antibody 3A1 and/or Leu-1 is both a highly sensitive and highly specific predictor of PT positivity.

**MATERIALS AND METHODS**

**Patient Samples**

Bone marrow samples from all untreated patients with ALL entered on the POG ALInC 13 classification protocol were sent to the Duke University reference laboratory for membrane antigen phenotyping studies. In addition, E-rosette testing was done at the institution of origin, and patients were entered on the T cell leukemia protocol either by virtue of E-rosette positivity (>40% of blasts forming rosettes) or PT antigen positivity at Duke University. At least 60% replacement of marrow by leukemic cells was required; specimens that were heavily contaminated with peripheral blood lymphocytes were not considered T cell leukemia on the basis of the evaluable as defined above and are included in this analysis. Samples on 774 of 978 children submitted were inadequate for processing. Samples from 743 children were used in this step, since 31 samples were missing values for at least one monoclonal. In a univariate analysis, all five monoclonal antibodies tested were significantly (P < .0001) related to PT positivity. The final multiple regression model included DU-SKW-1, 3A1, and 17F12, with both OKT3 and OKT4 failing the test of significance (P > .3) after adjusting for the final model. Simply stated, the logistic multiple regression model suggested that a leukemic cell be called PT positive (T cell) if at least two of the three (DU-SKW-1, 3A1, or 17F12) monoclonal antibodies were found to be positive. The results of this classification scheme on our data set are summarized in Table 1. This model is 83.5% sensitive and 99.2% specific. The false-positive rate is 5% and the false-negative rate is 3%.

**Microcytotoxicity Testing**

Ficoll-Hypaque-separated bone marrow specimens were phenotyped by microcytotoxicity testing as previously described, using a large panel of heteroantisera and monoclonal antibodies. Data presented in this report are based on analysis with antisera against TLAA, and the following monoclonal antibodies: 3A1, against the 40,000-dalton mel wT cell-associated antigen, provided by Dr Barton Haynes; L17F12 provided by Dr R. Levy (and, in some experiments, the equivalent monoclonal antibody Leu-1, purchased from Becton Dickinson); DU-SKW-1, an antibody against a different epitope of the same antigen recognized by Leu-1; and OKT3, OKT4, OKT8 (Orthomune, Inc, Piscataway, NJ), and Leu-6 (Becton Dickinson, Sunnyvale, Calif). No monoclonal antibody against the E-rosette receptor was used because none is satisfactory in cytotoxic assays. An antibody assay was considered positive if it reacted by ≥40% over background with a target leukemia. In most cases, this reactivity was 70% to 80% above background.

**Fluorescence Testing**

In a limited number of cases, aliquots of Ficoll-Hypaque-separated marrow blasts were stained by direct immunofluorescence using a “cocktail” of fluorescein-conjugated 3A1 (Dr Haynes) and Leu-1 (Becton Dickinson), and were analyzed on an Ortho Spectrum III flow cytometer. Fluorescein-conjugated mouse IgG (Becton Dickinson) was used as a negative control.

**Statistics**

A stepwise logistic multiple regression analysis was used to identify monoclonal antibodies that were significantly predictive of PT positivity. The monoclonal antibody OKT8 had a limited dispersion (very few positives) and had to be eliminated from consideration by the statistical procedure.

**RESULTS**

Of 774 cases of ALL studied for this report, 122 (16%) reacted with the PT antisera. These cases were extremely heterogeneous with respect to expression of the monoclonal antibody-defined T cell markers; no single monoclonal antibody reacted with as many cases as did PT antisera. Although monoclonal antibody 3A1 was the most frequently positive, in agreement with others’ observations that this is the most sensitive marker for T-ALL, 23 cases of PT-positive leukemia were negative for 3A1. Sixteen of these 23 cases were E-rosette negative. No other single monoclonal antibody was as good a predictor of PT positivity. The logistic regression model was used to identify a subset of the monoclonal panel that would be both sensitive and specific for predicting PT positivity. Samples from 743 children were used in this step, since 31 samples were missing values for at least one monoclonal. In a univariate analysis, all five monoclonal antibodies tested were significantly (P < .0001) related to PT positivity. The final multiple regression model included DU-SKW-1, 3A1, and 17F12, with both OKT3 and OKT4 failing the test of significance (P > .3) after adjusting for the final model. Simply stated, the logistic multiple regression model suggested that a leukemic cell be called PT positive (T cell) if at least two of the three (DU-SKW-1, 3A1, or 17F12) monoclonal antibodies were found to be positive. The results of this classification scheme on our data set are summarized in Table 2. This model is 83.5% sensitive and 99.2% specific. The false-positive rate is 5% and the false-negative rate is 3%.

Although this algorithm has very low false-positive and false-negative rates, it may not be appropriate for several reasons. First, from a biological standpoint, it might be expected that some cases of T cell leukemia might only express a single T cell antigen. Second, antibodies 17F12 and DU-SKW-1 recognize different epitopes of the same molecule, so an algorithm weighing each of them equally with another T cell antigen (3A1) may not be valid. Third, it is somewhat impractical to define a disease based on reactivity with two of three reagents. Accordingly, we next tested the algorithm “3A1’ or Leu-1’ predicts PT’.” In 19 patients, one or more of these tests was unsatisfactory, so that this algorithm uses 755 patients. The results of this model are summarized in Table 2. This algorithm
is 92% sensitive and 97% specific. Although the false-positive rate is higher than the full model at 14%, the false-negative rate is an extremely low rate of 1%. Using this algorithm in place of PT, 96% of patients would be correctly classified. In our data set Leu-1 and DU-SKW-1 had almost the same reactivity pattern and DU-SKW-1 used with 3A1 would have produced essentially the same results.

Based on this monoclonal antibody definition of T-ALL, we have begun screening leukemias by direct immunofluorescence using a cocktail with both 3A1 and Leu-1. The results of 118 cases tested so far are shown in Table 3. The 3A1/Leu-1 cocktail reacted with more than 20% of the cells in all 21 cases of leukemia that reacted by cytotoxicity with the PT antisera. However, in six of 96 cases of non-T cell leukemia, 3A1/Leu-1 stained more than 20% of the cells. When these six cases were reviewed in detail, it became apparent that the reactivity was due to an admixture of non-neoplastic peripheral blood T lymphocytes. In all six non-T cell cases, <40% of the cells were positive, while in all cases of T cell leukemia >40% of the cells were positive (Table 3).

**DISCUSSION**

The results of this study lead us to propose a simple monoclonal antibody-based definition of T cell ALL. Our previous experience with the PT heteroantisera has shown that patients with T antigen-positive ALL share a number of characteristics irrespective of whether or not their blasts form rosettes with sheep

**Table 1. Comparison of Monoclonal Antibody and Heteroantisera Reactivity Prediction by Logistic Regression Model**

<table>
<thead>
<tr>
<th>Actual (Heteroantisera)</th>
<th>Prediction (Monoclonal Antibody)</th>
<th>3A1</th>
<th>PT</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT-</td>
<td>96</td>
<td>19</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>PT</td>
<td>5</td>
<td>623</td>
<td>628</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>101</td>
<td>642</td>
<td>743</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity, 96/115, or 83.5%; specificity, 623/628, or 99.2%; predictive value (+), 96/101, or 95.0%; predictive value (-), 623/642, or 97.0%.

**Table 2. Comparison of Monoclonal Antibody and Heteroantisera Definitions of T Cell Leukemia**

<table>
<thead>
<tr>
<th>Definitions of T Cell Leukemia</th>
<th>3A1 or Leu-1</th>
<th>3A1 and Leu-1</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT-</td>
<td>109</td>
<td>9</td>
<td>118</td>
</tr>
<tr>
<td>PT</td>
<td>18</td>
<td>619</td>
<td>637</td>
</tr>
<tr>
<td>Total</td>
<td>127</td>
<td>628</td>
<td>755</td>
</tr>
</tbody>
</table>

Sensitivity, 109/118, or 92%; specificity, 619/637, or 97%; predictive value (+), (3A1 or Leu-1) and PT-(3A1 or Leu-1), 109/127, or 86%; predictive value (-), (3A1 and Leu-1) and PT-(3A1 and Leu-1), 619/628, or 99%; efficiency, the percentage of patients correctly classified, 96%.

**Table 3. 3A1/Leu-1 Reactivity by Direct Immunofluorescence in ALL**

<table>
<thead>
<tr>
<th>(3A1/Leu-1)</th>
<th>20%</th>
<th>20%-40%</th>
<th>&gt;40%</th>
</tr>
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<tbody>
<tr>
<td>PT-</td>
<td>0</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>PT</td>
<td>90</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Sensitivity, 100%; specificity, 94%, with >20% positive as cutoff.

**RBCs.** For example, patients with PT-positive E-rosette-negative ALL are more likely to have mediastinal mass, a high WBC count, and poor prognosis than non-T cell leukemia patients. Thus, biologically and clinically it seems desirable to identify T cell ALL serologically, although a heteroantisera-based definition is impractical. In this work, we have shown that reactivity with either monoclonal antibody 3A1 or Leu-1 is essentially equivalent to reactivity with the PT heteroantisera: only 27 patients are incorrectly classified in a rigorous examination of 755 children with ALL. Neither of these antibodies alone, nor any other, was as good a predictor as this combination, although in a logistic regression model an algorithm that added DU-SKW-1, but required only two of three antibodies to be positive, was a slightly better predictor. Our current AlinC 13 study will accrue data for approximately two more years. On this subsequent data set we will prospectively test both the logistic regression model and the algorithm that uses only 3A1 and Leu-1. This independent set of data should provide a fair testing ground for both models.

*In the 27 misclassified cases, no special studies (or even repetition of the tests) were done at the time to try to resolve the discrepancy between the heteroantisera and monoclonal antibody reactivity. However, both the TLAA and the 40,000-dalton T cell antigen have been found on some myeloid leukemias. Although blasts in all 27 discrepant cases did not stain for myeloperoxidase or nonspecific esterase, it is possible that some of the cases represent unusual nonlymphoid leukemias. Work is currently in progress using monoclonal antibodies to myeloid-associated antigens in order to understand more about these presumed "early" leukemias.*

*Other investigators have reported monoclonal antibodies WT1 or Leu-9, directed against the same antigen represented by 3A1, as being 100% sensitive for detecting T cell ALL using an immunofluorescence assay. In these studies E-rosette positivity, reactivity with other T cell monoclonal antibodies, and, in a few cases, reactivity with a T cell antiserum were used as evidence for T cell leukemia. Vodinelich et al* found that antibody WT1 also reacted with 18 of 389 non-T cell ALL, but only with those of unusual phenotype.*
our hands, the specificity of 3A1 alone is similar to or slightly better than what Vodinelich et al reported for WT1, but the sensitivity is less than 100%. The reasons for this may be due to differences in methodology or perhaps due to differences in specificity of the particular heteroantisera used in defining T cell leukemia.

Because of the methodological differences between microcytotoxicity and immunofluorescence and because of the wider acceptance of the latter, we have prospectively tested our monoclonal antibody algorithm with more than 100 additional cases of ALL, using a cocktail of directly fluoresceinated Leu-1 and 3A1. Conventionally, in an immunofluorescence assay, reactivity of ≥20% of blast cells is considered the criterion for positivity. At this cutoff level, our data suggest that in a small number of cases peripheral T lymphocyte contamination might lead to an incorrect conclusion that a leukemia is of T cell origin. Using 40% as the cutoff value for positivity would eliminate the few false-positive results in our series, but the possibility exists that some cases of T-ALL might be missed. Although the likelihood of this is difficult to estimate without more data, in the series of Link et al, three of 23 cases of T-ALL had less than 50% reactivity of blasts with both Leu-1 and Leu-9. Alternatively, supplemental tests may help to resolve cases with intermediate T cell reactivity. Since T-ALL is essentially always negative for the Ia antigen by immunofluorescence and since the Ia antigen is almost always present on non-T-ALL, testing with a monoclonal antibody against the Ia antigen may help to exclude the problem of admixed peripheral blood in cases of non-T cell leukemia. No simple monoclonal antibody test can accurately identify all cases of T cell leukemia when only a small number of blasts is present with admixed normal peripheral blood and bone marrow. If used with appropriate caution in such difficult cases, however, a monoclonal antibody test using direct immunofluorescence with antibodies Leu-1 and 3A1 (or the commercially available equivalent, Leu-9) is a simple, easily standardized, and biologically accurate test for T cell ALL.

REFERENCES

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