The E Rosette-Associated Antigen of T Cells Can Be Identified on Blasts From Patients With Acute Myeloblastic Leukemia

By Joseph Mirro, Gamal R. Antoun, Theodore F. Zipf, Susan Melvin, and Sanford Stass

Monoclonal antibody T-11, considered specific for the sheep erythrocyte rosette–associated antigen of T cells, reacted with leukemic blasts from four of 23 patients with morphologic and cytochemical criteria for acute myeloblastic leukemia (AML). Although 83%, 87%, 88%, and 96% of the blasts from these patients reacted with T-11, only one patient demonstrated a small percentage of heat-stable E rosettes (5%). Antibody 9.6, which also reacts with the E rosette–associated antigen, was tested on blasts from two of the T-11–positive patients and was also strongly reactive (96% and 98%). Dual staining of blasts from these two patients demonstrated a small number of cells that simultaneously expressed the E rosette–associated antigen and myeloid–associated cytochemistries (myeloperoxidase [MPO] and Sudan black B). Additionally, leukemic blasts were identified that simultaneously expressed the E rosette–associated antigen and contained Auer rods. Antibody OKT-11 immunoprecipitated a 48,100-dalton glycoprotein from these leukemic blasts that is similar in molecular weight to that previously determined for the T cell surface protein (Tp50), thus providing strong evidence that this molecule can be found in some cases of AML. Because cells simultaneously expressing both the E rosette–associated antigen and MPO were identified, it would appear likely that leukemic blasts with only the E rosette–associated antigen or only MPO arose from the same progenitor. Our findings further demonstrate that the epitopes identified by antibodies OKT-11, T-11, and 9.6 are not always associated with, or sufficient for, 37°C E rosette formation and can be found on blasts from patients with AML.

Monoclonal Antibodies OKT-11 and 9.6 are widely accepted as specific for T cells, since they reportedly detect an antigen important in sheep erythrocyte rosette (ER) formation. ER formation and OKT-11 antibody binding have a high direct correlation, and ER formation is blocked by antibodies OKT-11 and 9.6. These two antibodies have been reported to react only with leukemic blasts having a T cell phenotype, and their reactivity has correlated with ER formation. These monoclonal antibodies bind to a polypeptide of approximately 50,000 dalton (Tp50) and have become widely accepted as pan–T cell reactive. We report here four patients with acute myeloblastic leukemia (AML) by standard criteria whose leukemic blasts reacted strongly with antibody OKT-11. When blasts from two of these patients were tested, they were also strongly reactive with antibody 9.6. Three of these patients had blasts that were negative for heat-stable (37°C) ER formation, whereas the fourth patient’s blasts demonstrated an extremely low percentage of E rosettes.

By using a new technique for simultaneously analyzing immunologic and cytochemical characteristics of individual cells, we have further demonstrated 9.6 reactivity with Auer rod–positive and myeloperoxidase (MPO)–positive leukemic blasts. In addition, using antibody OKT-11, we have immunoprecipitated a 48,100-dalton glycoprotein from two of our patients on which cells were available. The molecular weight of this protein is similar to that on T cells and T-ALL blasts (Tp50). Thus, (a) OKT-11, T11, or 9.6 reactivity does not always correlate with ER formation; (b) 9.6 can be found on MPO-positive blasts; and (c) Tp50 can be present in AML.

Materials and Methods

Patients

Twenty-three consecutive patients with AML, from whom adequate numbers of leukemic cells could be obtained after informed consent, were studied. This investigation was part of a protocol-controlled cell profile analysis approved by the institution’s Clinical Trials Committee. Patients with Philadelphia chromosome–positive chronic myelocytic leukemia in myeloblastic crisis were excluded from this investigation.

Cytochemical Studies

Leukemic blasts from blood and bone marrow of the 23 patients were studied before therapy by Wright-Giemsa and standard cytochemical reagents, including MPO, Sudan black B (SBB), chloroacetate esterase (CAE), and alpha naphthyl butyrate esterase (ANB) as previously described. When greater than 3% of blasts reacted, the cytochemical test was considered positive.

Immunophenotyping

Antibody reactivity of the leukemic blasts was assessed by a standard indirect immunofluorescence assay. Each patient was assessed with monoclonal antibodies that identify cells of nonlymphoid lineage—anti-My-1, SJ-D1, MCS-1, and MCS-2—and T-11, T-11, and 9.6.
Simultaneous Cytochemical and Surface preparations were used to confirm ER formation by centrifuge column purified antibody 9.6 was covalently bound to fluorescent and I3'3 (anti-CALLA). Additional studies on these leukemic blasts with antibodies 9.6, T-101,2 OKT-3 (Ortho), blasts was assessed blasts.

When indicated, further antibody reactivity on frozen leukemic blasts was assessed with antibodies 9.6, T-101,12 OKT-3 (Ortho), and J51 (anti-CALLA). Additional studies on these leukemic blasts included testing for HLA-DR antigen, cytoplasmic immunoglobulins, and surface immunoglobulins. Terminal deoxynucleotidyl transferase (TdT) was assayed as described previously.14

Simultaneous Cytochemical and Surface Antigen Analysis

Dual staining of individual leukemic blasts for cytochemical reactivity and surface antigen expression was performed by a recently described technique.15 Briefly, protein A-Sepharose CL-4B column purified antibody 9.6 was covalently bound to fluorescent microspheres (Covalent Technology Corp, Ann Arbor, Mich) as previously published.16 To prevent phagocytosis of the microspheres, leukemic blasts were fixed in formalin (0.18% wt/vol) for 12 minutes at 22 °C. The blasts were then stained for surface antigens using the 9.6-conjugated microspheres in an immunofluorescence assay17 and examined by epifluorescence microscopy. Blasts were considered positive with antibody 9.6 if they had more than three surface-bound microspheres per cell.18 After the immunofluorescence assay, leukemic blasts having 9.6-conjugated microspheres attached were cytcentrifuged, 550 × g for seven minutes (Shandon Southern, Sewickley, Pa), onto glass slides and stained with MPO and SBB using standard techniques.5'5 Single cells were then analyzed by light microscopy and epifluorescence illumination for binding of 9.6-conjugated microspheres, cytochemical reactivity, and morphology.

Radiolabeling, Affinity Chromatography, Immunoprecipitation, and Gel Electrophoresis

Intact cells radiiodinated with 125I were solubilized, precleared, incubated with immunoadsorbent Sepharose CL-4B gel that had antibody OKT-11 covalently attached, and immunoprecipitated. These procedures have been described in detail elsewhere.17 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out under reducing conditions with a 4% acrylamide stacking gel and a 7% to 17% acrylamide separating gel (140 × 120 × 1.5 mm).

RESULTS

All 23 patients tested with antibody T-11 had greater than 3% MPO-positive blasts and therefore met standard criteria for the diagnosis of AML.6 None of the patients with adequate karyotypic analysis had a Philadelphia chromosome. Blasts from the four patients described in detail below had Auer rods and were reactive with at least one myeloid-associated monoclonal antibody (Table 1), further supporting the diagnosis of AML. These patients were negative (patients 1 through 3) or only weakly positive (patient 4) for CAE, which is consistent with our experience in AML patients that have less than 15% MPO reactivity.

Leukemic blasts from four of the 23 patients with AML reacted strongly with T-11 (or OKT-11) (Table 1). Nevertheless, the blasts from these patients, with the exception of patient 3, did not form heat-stable (37 °C) ERs; the blasts from patient 3 demonstrated only a small percentage (5%). The low percentage of ER formation in patient 3 was markedly discordant with the T-11 reactivity (96%). Blasts from patients 1 and 3 did form slightly more ERs at 4 °C (13% and 20%, respectively), but this was still markedly discordant with T-11 reactivity. The cells from all four patients were negative for CALLA and surface immunoglobulin (Table 1). However, patient 1 was positive for T-3 (87%), whereas patients 3 and 4 were positive for HLA-DR. TdT positivity was demonstrated in all three patients tested (Table 1). Both patients tested with a second antibody (9.6) to the ER-associated antigen were positive (Table 1).

Despite the unusual phenotypic characteristics of these four patients, they were clinically indistinguish-
Leukemic myeloblasts from patient 2 containing an Auer rod and demonstrating simultaneous expression of the E rosette–associated antigen identified by attached antibody 9.6–conjugated microspheres (487 x original magnification).

Fig 2. Leukemic myeloblasts from patient 3 expressing the E rosette–associated antigen identified by attached antibody 9.6–conjugated microspheres. Arrow indicates blast with MPO-positive Auer rod and attached microspheres (487 x original magnification).

To prove that myeloblasts expressed the E–associated antigen, we performed single-cell, dual-labeling experiments using antibody 9.6 and blasts from patients 2 and 3 on which cells were available. We were able to identify MPO-positive and SBB-positive leukemic blasts that expressed the E–associated antigen in both patients. Figures 1 and 2 show myeloblasts from patients 2 and 3 with MPO-positive Auer rods that also expressed the E–associated antigen identified by the attached antibody 9.6-coated microspheres.

Antibody OKT-11 was used to immunoprecipitate the associated antigen from leukemic blasts of patients 1 and 3 under reducing conditions (Fig 3). In both instances, the molecular weight was determined to be approximately 48,100 dalton. This value is consistent with previous determinations and with our own measurements using normal T cells (data not shown).

DISCUSSION

These results indicate that monoclonal antibodies OKT-11, T-11, and 9.6 can react with leukemic myeloblasts that do not form ERs. This is evidence that the epitopes identified by OKT-11 and 9.6 antibody binding are not always associated with, or sufficient for, ER formation in acute leukemia. With dual staining in two patients, we were able to demonstrate 9.6 binding to a small percentage of MPO-positive, SBB-positive, and Auer rod–positive myeloblasts, indicating that these antibodies react with a surface polypeptide that may not be completely lineage specific. The molecule identified on the blasts of our patients by antibody binding and immunoprecipitation and SDS-PAGE is indistinguishable from the Tp50 found on T lymphoblasts. The fact that this molecule does not result in the formation of heat-stable (37 °C) ERs suggests an abnormality in the structure of the ER binding site. This could be due to a biochemical alteration of the protein. Alternatively, the Tp50 may be present in AML, but the epitope identified by T-11 or 9.6 is not usually exposed. This phenomena has been previously reported for another antigen. It would appear that the epitope of T-11 or 9.6 binding is not the site of heat-stable ER formation.

There are several possible mechanisms for the finding of ER-associated-antigen–positive myeloblasts. First, the leukemogenic event could occur in a pluripo-
tential stem cell\textsuperscript{21,22} with this phenotype. This is supported by the dual expression of ER-associated antigen on MPO- (and SBB-) positive blasts in two patients. It would appear that the majority of the leukemic blasts that expressed only ER-associated antigen or only MPO were progeny of the progenitor cell, which were capable of expressing both characteristics. Alternatively, these cells could represent a proliferation of rare normal myeloid cells, and thus OKT-11, T-11, or 9.6 reactivity would not be unique to lymphoid cells. So far, we have been unable to identify such normal hematopoietic cells. An additional possible mechanism for our findings is aberrant expression of gene products, such as a lymphoid marker (9.6/T-11) in an MPO- myeloblast or a myeloid marker (MPO) in a T lymphoblast. Such aberrant expression has been termed lineage infidelity,\textsuperscript{23} and is supported by the finding of a myeloid marker (VIM-D5) on lymphoid cells\textsuperscript{28} and lymphoid-associated markers, CALLA\textsuperscript{26} and TDT,\textsuperscript{29,30} on myeloblasts.

Thus, a spectrum of unexpected marker expression in acute leukemia is emerging. Our current study demonstrates that neither OKT-11, T-11, nor 9.6 reactivity can always be directly related to lymphoid lineage or ER formation in acute leukemia. The demonstration of Tp50 on leukemic blasts that simultaneously express MPO and SBB and that contain Auer rods extends our knowledge regarding the potential for gene expression in acute leukemia.

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