CONCISE REPORT

Serologic Characterization of a Monkey Antiserum to Human Leukemic Myeloblasts

By T. Mohanakumar, Michael A. Baker, D. A. K. Roncar, and Robert N. Taub

We have raised a monkey antiserum that is selectively reactive with human leukemic myeloblasts by immunization with a glycoprotein antigen (AMLSGA) released from myeloblasts in short-term culture. Antimyeloblast activity can be demonstrated using complement-dependent cytotoxicity or indirect immunofluorescence. Selective antimyeloblast activity is retained following absorption with leukemic lymphoblasts or lymphocytes, nonleukemic lymphocytes, neutrophils, or mononuclear cells from nonleukemic bone marrow. Anti-AMLSGA antisera are not reactive with B-cell-enriched cell populations, and antimyeloblast activity is not reduced by absorption with la-positive cells. Anti-AMLSGA is a useful reagent for identification of human leukemic myeloblasts.

HETEROANTISERA to human leukemic cells have been raised in rabbits, nonhuman primates, mice, or in patients receiving immunotherapy. Activity against histocompatibility antigens may reduce the utility of such antisera in spite of extensive absorption prior to use. We have recently isolated and partially characterized a myeloblastic leukemia-associated cell surface glycoprotein antigen (AMLSGA) from components released from the myeloblast surface. In this article we report that a monkey antiserum to AMLSGA is selectively reactive with human leukemic myeloblasts, but is unreactive with nonleukemic cells from peripheral blood or bone marrow.

MATERIALS AND METHODS

Cells

Normal and leukemic mononuclear cells were obtained from heparinized blood using Ficoll-Hypaque density gradient centrifugation (1.077 g/cm, 400 g for 20 min at room temperature).

Enriched T- or B-lymphocyte preparations were obtained either by selective rosetting of T lymphocytes using sheep erythrocytes, or by removing adherent B lymphocytes by absorption on a flask coated with affinity-purified goat anti-human Fab. The details of T- and B-cell enrichment procedures have been published earlier.

Other test cells used in this study included an established lymphoblastoid cell line with T-cell properties (HSB) and another with B-cell properties (SB); a PH+ myeloblastic cell line (K562) initiated from a patient with chronic myelogenous leukemia, and a histiocytic cell line (U937) originated from a donor with histiocytic lymphoma.

Separation of cells by discontinuous density gradient centrifugation was performed according to the method of Winchester et al. with some modification.

Antigen

Isolation of radioiodinated myeloblastic leukemia-associated cell surface antigen (AMLSGA) has been described in detail. Briefly, peripheral blood myeloblasts from a patient with acute myeloblastic leukemia were radiolabeled by the lactoperoxidase-catalyzed surface radiolabelling technique and incubated in a nutrient medium at 37°C. Radioactive materials shed from viable cells into the supernatant at 24 hr were purified by gel filtration and by DEAE-cellulose chromatography. The DEAE-cellulose eluate contained a major peak in which radioactivity and protein levels were coincident. This glycoprotein material (mol wt 75,000–80,000 daltons) was specifically reactive in a coprecipitation assay with defined antimyeloblast alloantisera obtained from leukemic patients receiving immunotherapy. No reactions were seen either with antisera to HLA-A and B locus antigens or to HLA-DR antigens.

Preparation of Antiserum

Heterologous anti-AMLSGA serum was prepared by injecting monkey (M. speciosa) intravenously and intradermally (50 μg) 3 times, 14 days apart between immunizations. For intradermal injections, the antigen, suspended in phosphate-buffered saline (PBS) pH 7.2, was mixed with an equal volume of Freund's complete adjuvant (H37Ra). The antiserum described in this report was obtained 7 days after the third inoculation with antigen.

An antiserum to normal human B-cell antigens (la-like) was produced in a rabbit by immunizing with papain-digested cell membrane obtained from the B-cell line (SB), as described by Greier and Cresswell with minor modifications.

Absorption of Antisera

Immune and the preimmune sera were heat inactivated at 56°C for 30 min and then absorbed twice for 20 min at 4°C with an equal volume of cells (pooled normal human platelets, leukocytes, bone marrow cells, or myeloblasts). The rabbit antiserum to purified B-cell glycoprotein did not require absorptions.

Microcytotoxicity Assay

A standard Amos modification of the microtechnique described by Mittal et al. was used for unfractionated cell preparations, and...
the incubation time with complement was extended to 2 hr when enriched B and T lymphocytes were used as targets. Selected nontoxic rabbit complement was used throughout.

**Immunofluorescence**

The reactivity of antisera to target cells was determined by indirect immunofluorescence. Cells (2 × 10⁶) were incubated for 30 min at 4°C with 50 μl of antisera or preimmune control sera. After the incubation, the test cells were washed with PBS and incubated another 30 min at 4°C with 50 μl of fluorescein isothiocyanate-conjugated protein A (Pharmacia Fine Chemicals, Piscataway, N.J.). The cells were then washed and mounted with PBS-glycerol under a coverslip, and at least 200 cells incubated with either immune or preimmune sera were examined under a Zeiss microscope III equipped with a mercury lamp and dark field condenser.

**RESULTS**

In complement-dependent cytotoxicity testing, the monkey anti-AMLSGA was reactive with cells from leukemic patients but was unreactive with nonleukemic peripheral blood or bone marrow cells (Table 1). Absorption of anti-AMLSGA with leukemic myeloblasts from patients with acute myeloblastic or chronic myelocytic leukemia removed all antileukemic activity. Absorption with leukemic lymphoblasts or leukemic lymphocytes removed activity against ALL or CLL cells but did not remove antimyeloblast activity. Anti-AMLSGA was unreactive with B lymphocytes, including those of an identical twin to a patient with AML, and absorption with B lymphocytes did not reduce antimyeloblast activity. In contrast, the rabbit anti-Ia was reactive with all nonleukemic B lymphocytes. Antileukemic activity was not reduced by absorption of anti-AMLSGA with the enriched mononuclear fractions of nonleukemic marrow or with neutrophils from nonleukemic patients.

Anti-AMLSGA was cytotoxic to cell line K562 with myeloid properties, cell line U937 with myeloid and histiocytic properties, cell line SB with lymphoblastoid and B-cell properties but was unreactive with T-cell line HSB. In testing with indirect immunofluorescence, anti-AMLSGA was reactive with leukemic myeloblasts (84% ± 13%) and leukemic lymphoblasts (53% ± 16%), but was unreactive with leukemic lymphocytes (4% ± 4%) or nonleukemic B lymphocytes (3% ± 4%). Immunofluorescent reactivity with nonleukemic marrow (6% ± 3%) was similar to that seen with preimmune sera (4% ± 4%, p = 0.3). Anti-AMLSGA absorbed with leukemic lymphoblasts retained immunofluorescent reactivity with leukemic myeloblasts (65% ± 14%), but was unreactive with leukemic lymphoblasts (4% ± 2%).

**DISCUSSION**

We have raised an antiserum in monkeys reactive with leukemic myeloblasts using soluble compounds shed from the leukemic cell surface. The acute myeloblastic leukemia cell surface glycoprotein antigen (AMLSGA) has been partially characterized. The antiserum that we have raised to this antigen is highly reactive with leukemic myeloblasts from patients with AML or CML and, following appropriate absorption, is unreactive with histocompatibility antigens including Ia antigens, normal marrow fractions, leukemic lymphoblasts, and leukemic lymphocytes. Absorptions with normal peripheral blood cells, bone marrow cells, lymphoblasts, or CLL cells does not remove activity against myeloblasts.

Although the antiserum is selectively reactive with leukemic myeloblasts, it is not necessarily leukemia specific. The antigens recognized may be present in a

**Table 1. Cytotoxic Reactivity of Monkey Anti-AMLSGA With Leukemic and Nonleukemic Target Cells**

<table>
<thead>
<tr>
<th>Serum</th>
<th>Absorbed With†</th>
<th>Leukemic Cells</th>
<th>Lymphs</th>
<th>Nonleukemic Marrow‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AML</td>
<td>CML†</td>
<td>ALL</td>
<td>CLL</td>
</tr>
<tr>
<td>Monkey anti-AMLSGA</td>
<td>Unabsorbed</td>
<td>11/13</td>
<td>8/8</td>
<td>6/9</td>
</tr>
<tr>
<td>AML</td>
<td>0/5</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>CML†</td>
<td>0/5</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>ALL</td>
<td>4/4</td>
<td>2/2</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>CLL§</td>
<td>4/4</td>
<td>2/2</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>B cells</td>
<td>4/4</td>
<td>2/2</td>
<td>2/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Nonleukemic</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>marrow</td>
<td>3/3</td>
<td>2/2</td>
<td>2/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>3/3</td>
<td>2/2</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Rabbit anti-Ia</td>
<td>Unabsorbed</td>
<td>7/7</td>
<td>2/8</td>
<td>8/9</td>
</tr>
</tbody>
</table>

*Number reactive/number tested. Greater than 20% cell lysis above control serum values was considered reactive.

†All sera were absorbed with pooled human platelets and red cells.

‡Enriched mononuclear cell fraction.

§Selected from patients reactive with anti-AMLSGA.
cell population occurring infrequently in normal blood or marrow, or may be well masked on the cell surface of nonleukemic cells. Leukemic blast cells carry oncofetal antigens or antigens characteristic of specific phases of the cell cycle, and these may be recognized by the antiserum. Selective reactivity against myeloblasts may be explained by cell surface glycoprotein changes characteristic of leukemic cells. Nevertheless, the anti-AMLSGA should aid in further isolation of antigen fractions from the leukemic cell surface.

An antiserum highly selective for myeloblasts may be valuable in understanding tissues of origin of leukemic cells and may be clinically useful in classifying undifferentiated leukemias or detection of leukemic cells in remission or preleukemic marrow. Studies to test the clinical value of this antiserum are in progress.

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