# Nonhuman Primates Express Human Leukemia-Associated Antigens

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**Serologic studies using four murine monoclonal antibodies specific for the common acute lymphoblastic leukemia antigen (CALLA) and five monoclonal antibodies specific for the gp24 surface antigen indicate that these leukemia-associated antigens are present on cells of comparable tissues in man and in four nonhuman primates. As in man, adherent cell populations obtained from skin, lung, and bone marrow of *Macaca fascicularis, M mulatta, M nemestrina,* and *Papio cynocephalus* react with these antibodies. Similarly, granulocytes from both man and these nonhuman primates bind CALLA- and gp24-specific antibodies. Radioimmune precipitation experiments confirm the identity of these antigens. Our studies suggest that nonhuman primates can be used to screen serologic reagents to leukemia-associated antigens for potential toxic effects on normal tissues prior to their use in man. Similarly, nonhuman primates could be employed to assess the possible role of antigen-positive stromal cells in the reconstitution of bone marrow following transplantation.**

MONOCLONAL ANTIBODIES to the common acute lymphoblastic leukemia antigen (CALLA) have been used alone[1] or with monoclonal antibodies to a 24-kD surface glycoprotein (gp24)[2] in efforts to rid marrow of malignant cells prior to autologous bone marrow transplantation for acute lymphoblastic leukemia (ALL). In addition, monoclonal antibodies to CALLA have also been used for passive serotherapy in this disease.[3] Since serologic and immune precipitation studies have demonstrated the presence of a CALLA-like molecule on adherent normal cell populations from human lung, skin, and bone marrow, on a small population of nonadherent cells in normal bone marrow, and on polymorphonuclear leukocytes,[4-7] the possible toxicity of CALLA-specific antibodies and conjugates on normal tissues must be evaluated. Similarly, both serologic and immune precipitation studies have demonstrated the presence of gp24 on normal cell populations from human lung and bone marrow[8,9] (J.M. Pesando, submitted for publication). Serologic studies have also identified gp24 on polymorphonuclear leukocytes.

The purpose of our present studies was to determine whether or not the CALLA and gp24 antigens are present on cells of corresponding normal tissues from nonhuman primates. Adherent cell populations from cultured specimens of lung, skin, and bone marrow from four nonhuman primates (*Macaca fascicularis, M mulatta, M nemestrina,* and *Papio cynocephalus*) were tested for the expression of these antigens using a panel of four monoclonal antibodies specific for CALLA and five monoclonal antibodies specific for gp24. Polymorphonuclear leukocytes from these four species were also studied for expression of these two antigens both because of their ready availability and because they could be assayed immediately upon collection, without the need for prior growth in tissue culture.

## MATERIALS AND METHODS

### *Antibodies*

Four CALLA-specific monoclonal antibodies—αCALLA-1 (J5), αCALLA-2 (J13), αCALLA-3 (24.1), and αCALLA-4—were employed in these studies. The first three of these antibodies have been described previously.[5,6,10] αCALLA-4 was raised by immunizing BALB/c mice with leukemic cells from the CALLA-positive ALL cell line Laz 221. It identifies a 100,000-dalton surface glycoprotein on CALLA-positive leukemic cells and shows an identical reactivity profile with the other three antibodies against cells from a panel of 32 acute leukemias and 22 hematopoietic cell lines. Modulation of the expression of CALLA by J5 antibody (αCALLA-1) on reactive cell populations inhibits binding of αCALLA-4. The CALLA-specific 24.1 antibody (αCALLA-3) was the gift of Dr Marcus Braun, Fred Hutchinson Cancer Research Center.

Five monoclonal antibodies specific for the αgp24 antigen were employed—αgp24-1 (J2), αgp 24-2 (J9), αgp24-3, αgp24-4, and αgp24-5. One of these antibodies (αgp24-1) has been described elsewhere,[11] while a detailed description of the remaining antibodies is the subject of another report (J.M. Pesando, submitted for publication). All of these antibodies identify a 24,000-dalton surface protein by radioimmune precipitation, and sequential immune precipitation studies confirm that they identify the same monomorphic antigen. They demonstrate identical patterns of reactivity against all human cell populations tested. All antibodies were obtained as ascites from tumor-bearing mice at a concentration of approximately 7 mg/mL and were ultracentrifuged and filtered (0.45 μm Millex, Millipore, Bedford, Mass) before use.

### *Cell Populations*

Sterile samples of primate lung, skin, and bone marrow obtained from animals sacrificed for experimental purposes at the Regional Primate Research Center at the University of Washington were cut

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into small pieces and placed in RPMI medium with 20% fetal bovine serum (FBS) in a humidified atmosphere containing 7% $CO_2$ at 37 °C. Adherent monolayers were harvested by trypsinization for fluorescence-activated cell sorter (FACS) analysis after four weeks. Cell viability was greater than 95%. Most of these cells appeared either flat and angulated or spindle-shaped. A cryopreserved ampule of *M mulatta* cells was obtained through the American Type Culture Collection.

Granulocytes from peripheral blood samples from the aforementioned primates were isolated by Ficoll-Diatrizoate (LSM, Bionetics, Kensington, Md) density gradient centrifugation using standard procedures. Red cells were selectively removed from granulocyte-enriched pellets by hypotonic lysis in hemolytic buffer (0.155 mol/L ammonium chloride, 0.012 mol/L sodium bicarbonate, and 0.110 mol/L EDTA at pH 7.4). Wright-Giemsa staining confirmed that greater than 90% of the cells so obtained had the morphological appearance of granulocytes. Cell viability was greater than 95%, as measured by trypan blue exclusion.

Laz 221[12] is a CALLA- and gp24-positive human ALL cell line grown in RPMI with 10% FBS. These cells were used as positive controls in all immunofluorescence experiments.

## *Immunofluorescence Analysis*

Binding of monoclonal antibodies to target cell populations was determined using a standard indirect immunofluorescence assay[13] and monitored on the fluorescence-activated cell sorter (FACS-IV, Becton-Dickinson, Mountain View, Calif), with fluorescence displayed in the logarithmic mode. Prior to immunofluorescence analysis, both adherent cells and granulocytes were suspended in phosphate-buffered saline (PBS) containing 5% human AB serum to block Fc receptor sites. Monoclonal antibodies were used at a 1:1,000 dilution of ascites fluid (0.5 μg) to ensure antibody excess. Fluoresceinated goat antibodies to mouse IgG were purchased from Meloy Laboratories (Springfield, Va). All procedures were performed at 4 °C to inhibit antibody-induced modulation of CALLA.

## *Identification of Antigens*

Cell surface radiolabeling with $^{125}I$ and immune precipitation of detergent-solubilized cell extracts were performed using standard methods, as described previously.[14] Antibodies bound to protein A coupled to Sepharose were used to extract specific antigen from radiolabeled cell extracts, followed by extensive washing with a detergent-containing buffer. Antigens were resolved by electrophoresis on a 7% to 15% gradient sodium dodecyl sulfate-polyacrylamide slab gel (SDS-PAGE) and visualized by autoradiography. A ratio of 1 mCi of $^{125}I$ to 50 × $10^6$ hematopoietic cells and 20 × $10^6$ adherent cells was used for radiolabeling.

## RESULTS

All CALLA-specific monoclonal antibodies tested reacted with adherent cell populations from all four species of primates. This reactivity is recorded in Table 1 and Fig 1. It is of note that not all target cell populations within each species were reactive. Adherent cells from lung tissue of both *P cynocephalus* and *M mulatta* were reactive, while those from *M nemestrina* and *M fascicularis* were not. Adherent cells of bone marrow from *M mulatta* but not *P cynocephalus* or *M nemestrina* were reactive, while those from *M fascicularis* were not available for testing. In contrast, adherent cells from skin of fetal and adult *M nemestrina, M fascicularis,* and *P cynocephalus* were all reactive, while those from *M mulatta* were not tested.

**Table 1. Presence of Human Leukemia-Associated Antigens on Nonhematopoietic Cells of Primates***

| | Anti-CALLA Antibodies | | | | Anti-gp24 Antibodies | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Source of Cells | CALLA-1 (J5) | CALLA-2 (J13) | CALLA-3 (24.1) | CALLA-4 | gp24-1 (J2) | gp24-2 (J9) | gp24-3 | gp24-4 | gp24-5 |
| *M nemestrina* (adult) | | | | | | | | | |
| Lung | − | − | − | − | + | + | + | + | + |
| Skin | + | + | + | + | + | + | + | + | + |
| Bone marrow | NT† | NT | NT | NT | NT | NT | NT | NT | NT |
| *M nemestrina* (151-day fetus) | | | | | | | | | |
| Lung | − | − | − | − | + | + | + | + | + |
| Skin | + | + | + | + | + | + | + | + | + |
| Bone marrow | − | − | − | − | + | + | + | + | + |
| *M fascicularis* | | | | | | | | | |
| Lung | − | − | − | − | + | + | + | + | + |
| Skin | + | + | + | + | + | + | + | + | + |
| Bone marrow | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| *M mulatta* | | | | | | | | | |
| Lung | + | + | + | + | + | + | + | + | + |
| Skin | NT | NT | NT | NT | NT | NT | NT | NT | NT |
| Bone marrow | + | + | + | + | + | + | + | + | + |
| *P cynocephalus* | | | | | | | | | |
| Lung | + | + | + | + | + | + | + | + | W‡ |
| Skin | + | + | + | + | + | + | + | + | + |
| Bone marrow | − | − | − | − | + | + | + | + | W |

*Binding of antibodies was detected using a standard indirect immunofluorescence assay, as described in the text. Background fluorescence was determined by incubating cells with a nonreactive antibody. Samples were scored as seropositive if the majority of cells stained more intensely with test than with control antibodies. Most samples were obtained from a single animal of a given species.

†NT, not tested.

‡W, weak reactivity.

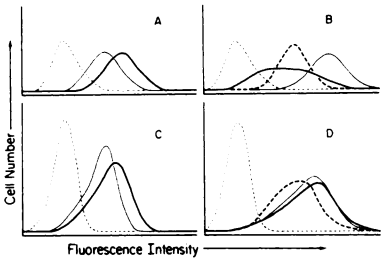


**Fig 1. Expression of human leukemia-associated CALLA and gp24 antigens on adherent cells from skin and on granulocytes of *M fascicularis*. Cell number is plotted against fluorescence intensity, and 4,000 cells were counted in each sample. (A and B) Binding of αCALLA and αgp24 antibodies to adherent cells of skin, respectively; (C and D) binding of these same antibodies to circulating blood granulocytes. Binding of a nonreactive control antibody is indicated by the thin dashed curve in each panel. In A and C, the curves for binding of antibodies αCALLA-1, -2, and -4 are superimposable and are indicated by a single, thick solid line. That for αCALLA-3 is indicated by a thin solid line. In B and D, binding of antibodies αgp24 -1, -2, and -3 is indicated by thick solid, thin solid, and thick dashed curves, respectively. Binding of antibodies αgp24-4 and -5 is not shown.**

Although cells having different morphologies were frequently observed in these adherent cell populations, the majority of cells in a specific culture had similar morphologies and demonstrated comparable reactivities with the antibodies being tested (Fig 1). Multiple cell populations were not readily demonstrable by FACS analysis. Our failure to demonstrate serologic reactivity on cells of comparable tissues from all four of these primates most likely reflects inherent differences in the phenotype of the predominant cell type growing in tissue culture rather than the absence of the antigen on all cells of that tissue for a specific primate. Since most samples were obtained from single animals, we cannot exclude the possibility of individual variation within species. We were unable to correlate the expression of CALLA with a specific cellular morphology in these studies. Similar heterogeneity in the expression of CALLA by cultures of bone marrow-derived adherent cells obtained from different normal human donors has also been observed (J.M. Pesando, unpublished observations, September 1983).

In contrast to the results with CALLA, adherent cells from all tissues tested from these four primates expressed the gp24 antigen as judged by their reactivity with a panel of five monoclonal antibodies specific for gp24 (Table 1 and Fig 1). The reduced binding of antibody αgp24-5 to adherent cells from lung and bone marrow of *P cynocephalus* relative to that of the other four antibodies suggests that it may recognize a unique antigenic determinant. Simultaneous binding of αgp24-5 and these other gp24-specific antibodies to antigen-positive human cell populations could not be demonstrated, suggesting that the ability of antibodies to bind to cells of different species is a more sensitive test for the identity of the epitopes that they recognize. Adverse steric interactions between bound immunoglobulin molecules are avoided in the latter system.

All CALLA-specific monoclonal antibodies tested reacted with polymorphonuclear leukocytes from man and all four of these primates (Table 2 and Fig 1). Once again, all cells in these samples reacted comparably with the different anti-CALLA antibodies. Polymorphonuclear leukocytes from cebus monkey (but not lemur) also reacted with these antibodies suggesting that CALLA first appeared in a common ancestor of Old World and New World monkeys. Similarly, monoclonal antibodies specific for the gp24 antigen bound to samples of polymorphonuclear leukocytes from all primates tested, though only weak binding to cells from some human donors was noted. αGp24-5 failed to show significant binding to granulocytes of *P cynocephalus*. Binding of αgp24-5 to granulocytes from cebus monkey and lemur was also not detected, despite binding of the other four gp24-specific antibodies to these samples.

Radioimmune precipitation experiments were performed using αCALLA and αgp24 antibodies to exclude the possibility that identical antigenic determinants might be shared by otherwise dissimilar macromolecules (Figs 2 and 3). These studies confirm that similar molecular species are identified by these antibodies on the ALL cell line Laz 221, human

**Table 2. Presence of Human Leukemia-Associated Antigens on Granulocytes From Primates***

| | Anti-CALLA Antibodies | | | | Anti-gp24 Antibodies | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Source of Cells | CALLA-1 (J5) | CALLA-2 (J13) | CALLA-3 (24.1) | CALLA-4 | gp24-1 (J2) | gp24-2 (J9) | gp24-3 | gp24-4 | gp24-5 |
| *M nemestrina* | + | + | + | + | + | + | + | + | + |
| *M fascicularis* | + | + | + | + | + | + | + | + | + |
| *M mulatta* | + | + | + | + | NT† | NT | NT | NT | NT |
| *P cynocephalus* | + | + | + | + | + | + | W‡ | + | − |
| Cebus monkey | + | + | + | + | + | + | + | + | − |
| Lemur | − | − | − | − | + | + | + | + | − |

*Binding of antibodies was detected as described in the text and in Table 1. For *M nemestrina, M fascicularis, M mulatta,* and *P cynocephalus,* granulocytes from at least two individuals were tested for expression of CALLA.

†NT, not tested.

‡W, weak binding.

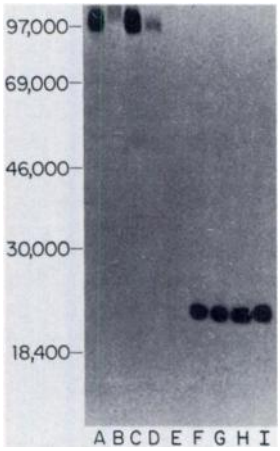


**Fig 2. Presence of CALLA and gp24 antigens on primate cells. Results obtained with the ALL cell line Laz 221 are shown in lanes A and F, with human granulocytes in lanes B and G, with granulocytes of *M fascicularis* in lanes C and H, and with adherent cells from skin of *M fascicularis* in lanes D and I. Cells were surface-labeled with $^{125}$I before immune precipitation of cell extracts with αCALLA-1 (lanes A through D) or αgp24-2 (lanes F through I). Molecular weight markers labeled with $^{14}$C-acetate from New England Nuclear (Boston) include myosin (200,000 daltons), phosphorylase B (97,000 daltons), albumin (69,000 daltons), ovalbumin (46,000 daltons), carbonic anhydrase (30,000 daltons), and lactoglobulin (18,000 daltons).**

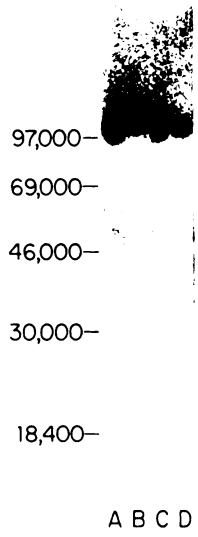


**Fig 3. Presence of CALLA on primate cells. Cells were surface labeled with $^{125}$I, and antigens detected by αCALLA-1 were isolated and visualized as described in the text. Samples are as follows: (A) ALL cell line Laz 221; (B) human granulocytes; (C) granulocytes of *M fascicularis;* and (D) adherent cells from skin of *M fascicularis*. Bands having molecular weights of approximately 100,000 daltons are seen in lanes A, C, and D, while once again, the antigen isolated from human granulocytes has a slightly higher molecular weight.**

granulocytes, granulocytes from *M fascicularis*, and adherent cells from the skin of *M fascicularis*. Molecules identified by αCALLA-1 (J5 antibody) on Laz 221 cells and on granulocytes and adherent skin cells of *M fascicularis* have molecular weights of approximately 100,000 daltons. In contrast, that identified by αCALLA-1 on human granulocytes has a molecular weight of approximately 110,000 daltons, in agreement with the findings of Braun et al.[6] The basis for the difference in the molecular weight of the antigen detected by αCALLA-1 on granulocytes as compared to other human hematopoietic cells is currently under investigation. The αgp24-2 antibody identifies antigens having a molecular weight of approximately 24,000 daltons on all four cell types.

## DISCUSSION

For several years, monoclonal antibodies have been used clinically in efforts to eradicate malignant cells and to manipulate the immune response for therapeutic purposes.[1-3,15-18] Monoclonal antibodies represent a diverse group of new pharmacologic reagents, but they have not been tested for toxicity in animals because of their apparent species specificity. Our serologic and immune precipitation studies indicate that the leukemia-associated CALLA and gp24 antigens are expressed by comparable normal cells of man and nonhuman primates. These observations suggest that nonhuman primates can be used to screen both conjugated and unconjugated serologic reagents to leukemia-associated antigens for potential adverse side effects on normal tissues prior to their use in man. Similarly, the recent demonstration of cross-reactivity of monoclonal antibodies to human T cell and myeloid antigens with appropriate primate hematopoietic cells[19-21] suggests that primates may also serve as important models for evaluating the toxicities of serologic reagents to other human tumor-associated antigens.

Gp24-specific monoclonal antibodies react with adherent cells obtained from every sample of primate and human bone marrow tested. CALLA-specific antibodies react with several but not all of these samples. These same adherent cells presumably include the hematopoietic stromal cells that are required for long-term culture of marrow cells in vitro.[22,23] Therefore, successful efforts to purge bone marrow of leukemic cells using gp24-specific monoclonal antibodies prior to autologous transplantation would most likely eliminate these adherent cells as well as leukemic ones. Whether or not the removal of such stromal cells from treated marrow would affect subsequent engraftment and hematopoiesis would depend on the efficacy of the cytolytic process employed and on whether or not the chemoradiotherapy preparative regimens also eliminated stromal cells in the patient. At least one report in the literature suggests that

stromal cells become progressively donor in origin with time after allogeneic bone marrow transplantation.[24] Our present studies suggest that nonhuman primates could be employed to assess the possible role of antigen-positive stromal cells in bone marrow reconstitution and engraftment.

Finally, it should be noted that despite the presence of a CALLA-like molecule on human granulocytes, CALLA has not been demonstrated on normal or malignant myeloid blasts by ourselves or others, despite study of over 700 patients with acute nonlymphocytic leukemia (ANL)[5,13,25] (J.M. Pesando, unpublished observations, March 1984). Therefore, antibodies to this antigen retain their considerable operational utility as reagents with which to discriminate between ALL and ANL.

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