

**DISTRIBUTION OF COMMON ACUTE LYMPHOBLASTIC
LEUKEMIA ANTIGEN IN NONHEMATOPOIETIC TISSUES***

BY RICHARD S. METZGAR,‡ MICHAEL J. BOROWITZ, NANCY H. JONES, AND
BARRY L. DOWELL

*From the Departments of Microbiology and Immunology and Pathology, Duke University Medical Center,
Durham, North Carolina 27710*

An antigen that is present on the cells from many patients with acute lymphoblastic leukemia (ALL) and some patients with chronic myelocytic leukemia (CML) in blast crisis (1-6) has been called the common ALL antigen (CALLA). The antigen was initially serologically defined by Greaves (1), and, by immunoprecipitation with xenoantisera, the antigen was reported to be a 95,000-100,000 mol wt glycoprotein (4, 7). More recently (8), a monoclonal antibody (J-5) was generated to CALLA by Ritz et al. The murine monoclonal antibody also precipitated a 95,000-100,000 mol wt antigen that demonstrated identical serological specificity to the xenoantisera of Greaves when tested on uncultured cells from leukemic patients (8, 9). There were, however, some reported specificity differences between the J-5 monoclonal and the various polyclonal antisera in the detection of CALLA on marrow cells of nonleukemic patients and on T-ALL cells and cell lines. The J-5 monoclonal antibody usually failed to react with bone marrow cells from normal donors, cells from T-ALL patients, or with T-ALL cell lines (8), whereas the polyclonal xenoantisera in some instances showed some degree of cross-reactivity with these cell types (3-5, 10-12). However, no other cells or tissues tested had detectable levels of CALLA, and thus the J-5 monoclonal reagent had apparent value as a diagnostic reagent, and more recently (13) it has been used in passive serotherapeutic studies. This report describes the detection of CALLA on certain cell types of nonhematopoietic adult and fetal tissues, as determined by immunoperoxidase staining with the J-5 monoclonal antibody. Our study indicates that this antigen is unlikely to be a true differentiation antigen specific for lymphoid cells.

Materials and Methods

Monoclonal Antibody. The J-5 monoclonal antibodies were graciously provided as ascitic fluid by Dr. Jerome Ritz of the Sidney Farber Cancer Institute, Boston, Mass. The J-5 antibodies have been routinely used in our laboratory for membrane phenotyping of marrow, blood, or lymphoid tissue cells from patients with leukemia and lymphoma by cytotoxicity and immunofluorescence (14). Control ascites fluid was obtained from BALB/c mice injected intraperitoneally with the P3 × 63Ag 8 myeloma cell line (P3).

Collection of Tissue. Portions of various normal tissues were removed during the course of surgery and processed within 2 h of their removal. In addition, a 20-wk-old fetus from a prostaglandin-induced abortion was obtained ~6 h after delivery, and a full autopsy was performed. Portions of most tissues were reserved to be processed, as described below.

* Supported by grants CA 08975 and CA 11265 from the National Cancer Institute. Dr. Dowell is a Special Fellow of the Leukemia Society of America.

‡ To whom reprint requests should be addressed.

Tissue Processing. Tissue was cut into 2- to 4-mm² pieces and embedded in blocks containing a solution of 7.5% gelatin. The blocks were snap-frozen by submersion in isopentane that had been precooled in liquid nitrogen and were stored at -70°C. Sections were cut at a thickness of 5-7 µm on a microtome-cryostat, thaw-mounted onto gelatin-coated slides, and immediately fixed in acetone at -20°C for 15-30 sec. Slides were either stored at -70°C or used immediately for immunoperoxidase staining.

Immunoperoxidase Testing. Sections were stained using a modified indirect immunoperoxidase procedure (15). After preincubation of the tissue sections with 10% nonimmune goat serum, they were incubated for 60 min at room temperature with 1:1000 diluted J-5 or P3 ascitic fluid. A second 45 min incubation was performed with a 1:50 dilution of horseradish peroxidase-conjugated goat anti-mouse IgG (N. L. Cappel Laboratories, Inc., Cochranville, Pa.). Slides were developed by incubation for 15 min with a solution of diaminobenzidine (0.5 mg/ml) and hydrogen peroxide (0.001%) in 0.05 M Tris buffer at pH 7.6 and were counterstained with hematoxylin.

Preparation of Kidney Membrane Glycoproteins. A segment of normal kidney containing both medulla and cortex was cut into small fragments, and cells were gently teased from the organ using 20-gauge needles. The cells were washed, and crude membrane fragments were prepared by hypotonic lysis and density gradient centrifugation, as previously described (16). The membrane fragments were solubilized for 15 min at 4°C in 2 ml of 0.5% (wt/vol) sodium deoxycholate (DOC) in 0.1 M borate buffer, pH 8.5, and centrifuged for 60 min at 100,000 g. The protein content of the supernate was estimated by reading absorbance at 280 and 260 nm, and 100 µg of supernate was labeled by the lactoperoxidase method of Collins et al. (17).

1 ml of the ¹²⁵I-labeled kidney antigen was preabsorbed for 15 min at 23° with 0.2 g formalin-fixed *Staphylococcus aureus* (Pansorbin; Calbiochem-Behring Corp., American Hoechst Corp., La Jolla, Calif.). An aliquot of this preabsorbed antigen was added to 100 µl of packed *Pisum sativum* lectin bound to Sepharose 4B, incubated 30 min at 23°, and washed 5 times with 10 ml of Tris-DOC buffer. The lectin-bound material was then eluted with 0.5 ml of 10% glucose in Tris-DOC buffer. The *P. sativum* lectin beads were a generous gift of Dr. R. Byrn (Duke University, Durham, N. C.).

Immunoprecipitation and Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Aliquots of *S. aureus* preabsorbed, lectin-bound kidney antigen were precipitated with J-5 and P3 ascites by previously described methods (18). The common ALL-derived cell line, 697, which we had previously shown to express CALLA, was radioiodinated with lactoperoxidase (17) and used as a positive control. The 697 cell line was kindly provided by Dr. A. Ragab (Emory University, Atlanta, Ga.).

Results

Sections of various tissues from a 20-wk-old fetus and from a number of normal adults were tested with the J-5 ascitic fluid. The following tissues demonstrated no significant immunohistochemical staining when compared to the P3 ascites control: fetal heart, liver, lung, pancreas, salivary gland, spleen, and thymus; and adult skeletal muscle, spleen, tonsil, salivary gland, small intestine, and colon. Certain adult and fetal tissues, however, showed significant staining. In the adult kidney, most but not all of the tubules stained intensely (Fig. 1). The large number of postively staining tubules and their lower nuclear density compared to the nonstaining tubules indicate that the CALLA-positive cells are in the proximal tubules. The pattern of staining in the tubules was apical, and sloughed or secreted antigenic material was present in the tubular lumens. In addition to the tubular staining, there was intense glomerular staining in a fairly linear pattern along glomerular basement membranes. A similar staining pattern was present in fetal kidney. Again, some but not all of the tubules stained, and there was intense staining of the cells forming the primitive glomeruli. The fetal small intestine also showed apical staining of the epithelial cells of the villi (Fig. 2), and occasionally some shedding of antigenic material was noted, but no staining of adult small intestine was seen.

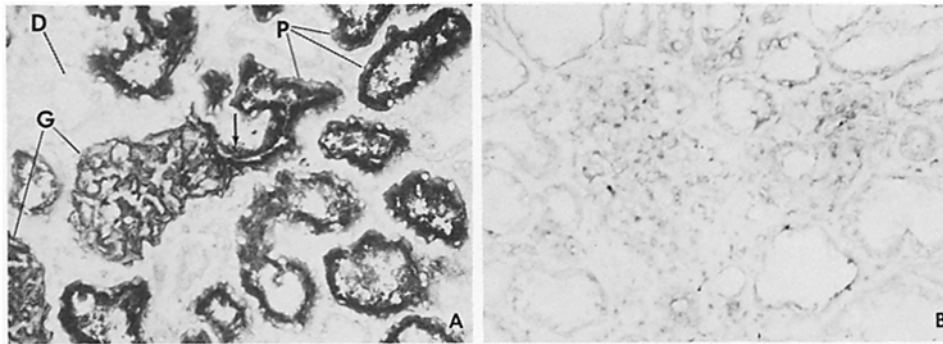


FIG. 1. Frozen section of normal kidney stained by immunoperoxidase with antibody J-5 (A) and with P3 ascites control (B). There is intense staining for J-5 in glomeruli (G) and on the surface and within the lumens of proximal tubules (P). Distal tubules (D) do not stain. The continuity between proximal tubules and glomerular epithelium is fortuitously illustrated in this section (arrow). Hematoxylin counterstain, $\times 250$.

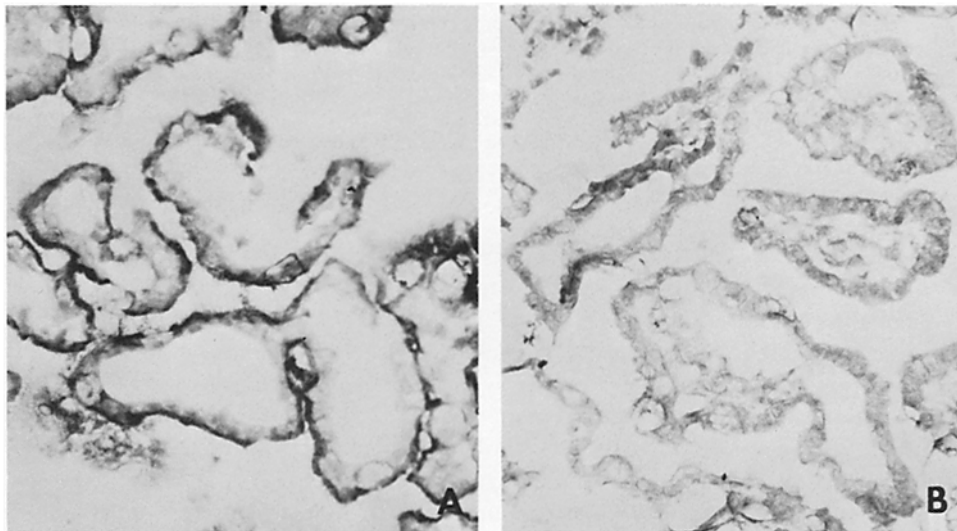


FIG. 2. Frozen section of fetal small intestine stained by immunoperoxidase with antibody J-5 (A) and with P3 ascites control (B). Several villi are shown cut in cross section, and the bowel lumen is represented by the empty space between villi. There is an intense rim of staining by J-5 along the luminal border. Hematoxylin counterstain, $\times 250$.

In contrast, a different pattern of staining was noted in the adult breast. Although there was no significant staining of the breast epithelium or the material within glandular lumens, there was staining around the myoepithelial cell layer of the breast (Fig. 3). This was particularly prominent around the breast ductal cells but was also seen to some extent around the acini of the breast. Interestingly, the myoepithelial cells of the salivary gland did not stain.

The J-5 ascites immunoprecipitated a molecule with an apparent $\sim 90,000$ mol wt from a lactoperoxidase-labeled kidney membrane glycoprotein preparation (Fig. 4, lane b). The molecule precipitated from the kidney migrated faster than that precipitated from the 697 cell line (Fig. 4, lane a).

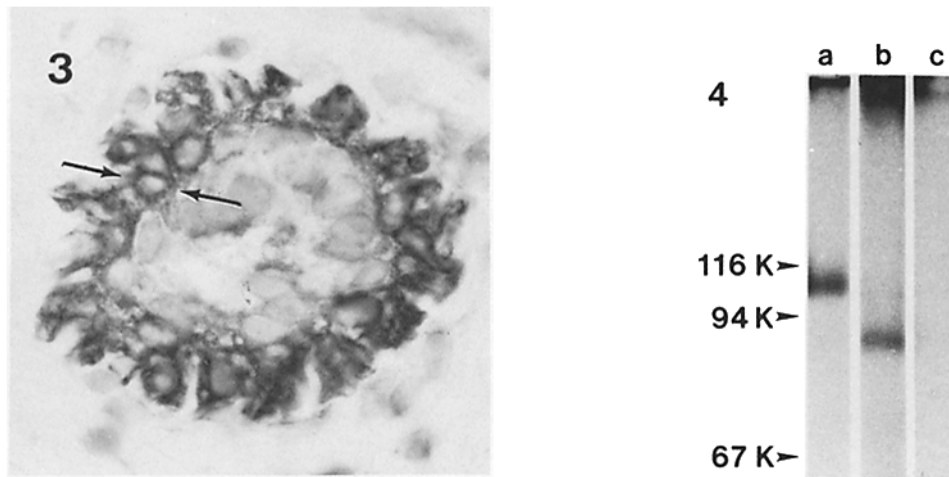


FIG. 3. Frozen section of normal breast stained by immunoperoxidase with antibody J-5. A ductule is cut in cross section illustrating central, nonstaining epithelial cells surrounded by a myoepithelial rim that is heavily stained (arrows). The myoepithelial cells are in turn well demarcated from the surrounding nonstaining stroma. Hematoxylin counterstain, $\times 680$.

FIG. 4. Immunoprecipitates of lactoperoxidase-labeled kidney membrane glycoproteins analyzed on SDS-PAGE. Immunoprecipitates were electrophoresed on 10% polyacrylamide gels under reducing conditions. The lactoperoxidase-labeled common ALL-derived cell line, 697, was precipitated with J-5 ascites (A). Kidney membrane glycoproteins were precipitated with J-5 (B) or P3 ascites (C). Arrows indicate the position of mol wt markers.

Discussion

The common ALL (CALLA) antigen was originally described (10, 11) as a leukemia-associated antigen, and the antigen defined by the xenogeneic polyclonal antisera was subsequently reported to be on a very small percentage of fetal liver and normal bone marrow lymphoid cells. However, this cross-reactivity was not unequivocally demonstrated with the J-5 monoclonal (8, 9). Thus, the idea of treating common ALL patients with the J-5 antibody seemed very attractive. This report demonstrates that the J-5 antigen is not restricted to common ALL cells but is also present on several nonhematopoietic tissues. In particular, it is present on proximal tubules and glomeruli of the kidney. Because the epithelial cells of the glomeruli are continuous with the proximal tubule, it is possible that the glomerular staining is on the podocyte and not the basement membrane. It is interesting that another common ALL antigen, P24 (19), as recognized by the monoclonal antibody DU-ALL-1 (20), is also present on kidney. However, in contrast to the antigen detected by the J-5-monoclonal, the P24 antigen is present on distal tubules and collecting ducts to a greater extent than on proximal tubules (unpublished data). The J-5 antigen is on the apical portion of the renal tubules and is in a similar location in the fetal small intestine, suggesting that CALLA may in some way be related to microvillial absorption. CALLA, however, was not detected in other glandular surfaces but was demonstrated on myoepithelial cells of the breast. Because J-5-reactive material was present in the lumen of the kidney tubules and along the microvilli of the fetal intestine, it seems that the antigen is shed *in vivo*, and this is consistent with the observation that CALLA is readily shed *in vitro* (7). The antigen was not detected on adult small intestine, although a systematic comparison of staining of different

portions of fetal and adult small bowel has not yet been completed. The function of CALLA is not yet evident from its tissue distribution.

The presence of a J-5-reactive molecule in kidney was confirmed by the immunoprecipitation of a molecule with an apparent ~90,000 mol wt from lactoperoxidase-labeled kidney membrane glycoproteins. The difference in migration of the antigen band from renal cells vs. that from the CALLA-positive cell line 697 could represent a true difference in polypeptide or carbohydrate composition of the molecule bearing the J-5-reactive determinant, or it could be due to the degradation of the molecule by the action of proteases or glycosidases during the processing and labeling of the renal cells. The treatment of Nalm-1 antigens with glycosidases or tunicamycin has been shown (9) to reduce the apparent mol wt of the CALLA molecule. Further biochemical and immunochemical analyses of kidney, common ALL cells, and other J-5 antigen-positive tissues are required to determine the structural relationship of these J-5-reactive molecules.

The detection of CALLA on some epithelial cells of kidney, breast, and intestine suggests that treatment of common ALL patients with the J-5 monoclonal antibody should be done with caution. Although the hematological toxicity is likely to be minimal because of the extremely restricted hematopoietic distribution of CALLA, it is important to evaluate patients receiving this therapy for renal and gastrointestinal toxicity.

Summary

The common acute lymphoblastic leukemia antigen (CALLA), as defined by J-5 murine monoclonal antibodies, was detected on renal tubular and glomerular cells from fetal and adult donors by an indirect immunoperoxidase technique. CALLA could also be detected on epithelial cells of the fetal small intestine and on myoepithelial cells of adult breast but not on myoepithelial cells of the salivary gland.

Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis of immunoprecipitated ¹²⁵I-labeled membrane antigens from dissociated renal cells demonstrated that the antigen migrated as a 90,000 mol wt antigen rather than the 98,000–100,000 mol wt antigen noted on CALLA-positive tissue culture cell lines. The data suggest that the determinant defined by the J-5 monoclonal antibody is neither a lymphoid cell-specific differentiation antigen nor a leukemia-specific antigen.

We thank Mr. William Boyarsky for the excellent photographic work and Mr. Frank Tuck for technical assistance.

Received for publication 20 July 1980.

References

1. Greaves, M. F., G. Brown, N. T. Rapson, and T. A. Lister. 1975. Antisera to acute lymphoblastic leukemia cells. *Clin. Immunol. Immunopathol.* 4:67.
2. Borella, L., L. Sen, and J. T. Casper. 1977. Acute lymphoblastic leukemia (ALL) antigen detected with antisera to E rosette-forming and non E rosette-forming ALL blasts. *J. Immunol.* 118:309.
3. Rodt, H., B. Netzel, E. Thiel, G. Jäger, D. Humm, R. Haas, D. Göetze, and S. Thierfelder. 1977. Classification of leukemia cells with T and O-ALL-specific antisera. In *Immunological Diagnosis of Leukemias and Lymphomas*. S. Thierfelder, H. Rodt, and E. Thiel, editors. Springer-Verlag, Berlin. 87.

4. Billing, R., J. Minowada, M. Cline, B. Clark, and K. Lee. 1978. Acute lymphocytic leukemia-associated antigen. *J. Natl. Cancer Inst.* **61**:423.
5. Koshiba, H., J. Minowada, and D. Pressman. 1978. Rabbit antiserum against a non-T, non-B leukemia cell line that carries the Ph¹ chromosome (NALM-1): antibody specific to a non-T, non-B acute lymphoblastic leukemia antigen. *J. Natl. Cancer Inst.* **61**:987.
6. LeBien, T. W., R. L. Hurwitz, and J. Kersey. 1979. Characterization of a xenoantiserum produced against 3 M KCl-solubilized antigen obtained from a non-T, non-B (Pre-B) acute lymphoblastic leukemia cell line. *J. Immunol.* **122**:82.
7. Sutherland, R., J. Smart, P. Niaudet, and M. Greaves. 1978. Acute lymphoblastic leukemia associated antigen-II isolation and partial characterization. *Leukemia Res.* **2**:115.
8. Ritz, J., J. M. Pesando, J. Notis-McConarty, H. Lazarus, and S. F. Schlossman. 1980. A monoclonal antibody to human acute lymphoblastic leukemia antigen. *Nature.* **283**:583.
9. Newman, R. A., R. Sutherland, and M. F. Greaves. 1981. The biochemical characterization of a cell surface antigen associated with acute lymphoblastic leukemia and lymphocyte precursors. *J. Immunol.* **126**:2024.
10. Greaves, M. F., and G. Janosy. 1978. Patterns of gene expression and the cellular origins of human leukemias. *Biochem. Biophys. Acta.* **516**:193.
11. Janosy, G., G. E. Francis, D. Capellaro, A. H. Goldstone, and M. F. Greaves. 1978. Cell sorter analysis of leukemia associated antigens on human myeloid precursors. *Nature (Lond.)* **276**:176.
12. Roberts, M., M. F. Greaves, G. Janosy, R. Sutherland, and C. Pain. 1978. Acute lymphoblastic leukemia (ALL) associated antigen I. Expression in different haematopoietic malignancies. *Leukemia Res.* **2**:105.
13. Ritz, J., J. M. Pesando, S. E. Sallan, L. A. Clavell, J. Notis-McConarty, P. Rosenthal, and S. F. Schlossman. 1981. Serotherapy of acute lymphoblastic leukemia with monoclonal antibody. *Blood.* **58**:141.
14. Haynes, B. F., R. S. Metzgar, J. D. Minna, and P. A. Bann. 1981. Phenotypic characterization of cutaneous T-cell lymphoma-use of monoclonal antibodies to compare with other malignant T cells. *N. Engl. J. Med.* **304**:1319.
15. Nakane, P. K., and G. B. Pierce, Jr. 1967. Enzyme-labeled antibodies: preparation and application for the localization of antigens. *J. Histochem Cytochem.* **14**:929.
16. Anderson, J. A., and R. S. Metzgar. 1978. Detection and partial characterization of human T and B lymphocyte antigens of the HSB and SB cell lines. *J. Immunol.* **120**:262.
17. Collins, J. J., R. C. Montelaro, T. P. Denny, R. Ishizaki, A. J. Langlois, and D. P. Bolognesi. 1978. Normal chicken cells (chf⁻) express a surface antigen which cross-reacts with determinants of the major envelope glycoprotein (gp85) of avian myeloblastosis virus. *Virology.* **86**:205.
18. Dowell, B. L., J. M. Falletta, J. O. Moore, and R. S. Metzgar. 1980. Detection and partial characterization of human thymus-leukemia antigens. *J. Natl. Cancer Inst.* **65**:691.
19. Kersey, J. H., T. W. LeBien, C. S. Abramson, R. Newman, R. Sutherland, and M. Greaves. 1981. p24: a human leukemia-associated and lymphohemopoietic progenitor cell surface structure identified with monoclonal antibody. *J. Exp. Med.* **153**:726.
20. Jones, N. H., and R. S. Metzgar. 1981. Production of a novel monoclonal antibody (DU-ALL-1) to common acute lymphocytic leukemia cells. *Proc. Am. Assoc. Cancer Res.* **22**:1210.