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Biochemical Characterization of an Antigen Associated with Acute Lymphoblastic Leukemia and Lymphocyte Precursors

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A. Introduction

Acute lymphoblastic leukemia (ALL) associated antigen (ALLA) is expressed on the surface of cells from patients with the common (or non-T, non-B) variant of ALL as well as in some cases of "undifferentiated" leukemias and chronic myeloid leukaemia (CML) in "lymphoid" blast crisis (Greaves and Janossy 1978).

This antigen has also been detected serologically on a small proportion of normal bone marrow cells (Greaves et al. 1978, 1980; Janossy et al. 1978) and is suggested to be a normal differentiation antigen of "early" hemopoietic cells which are possibly restricted to lymphoid development (Greaves and Janossy 1978; Greaves et al. 1980). Despite the lack of absolute leukemic specificity, antisera to ALLA have been useful for differential diagnosis of acute leukemia. The biochemical characteristics of this structure have been analyzed in detail using the cell line Nalm-1.

B. Results and Discussion

I. Integrity of ALLA

The ALLA, precipitated by rabbit antisera, is a single glycosylated polypeptide of approximately 100,000 daltons (gp 100) containing no intrachain disulfide linkages as judged by SDS-polyacrylamide gel electrophoresis (PAGE) under reducing and nonreducing conditions (Sutherland et al. 1978). A monoclonal anti-ALL (J-5) precipitates a similar if not identical molecule from leukemic cells (Ritz et al. 1980).

The lectin binding characteristics of gp 100 are somewhat unusual in that only 50% binds to lentil lectin, whereas 100% binds to *Ricinus communis* lectin (Fig. 1).

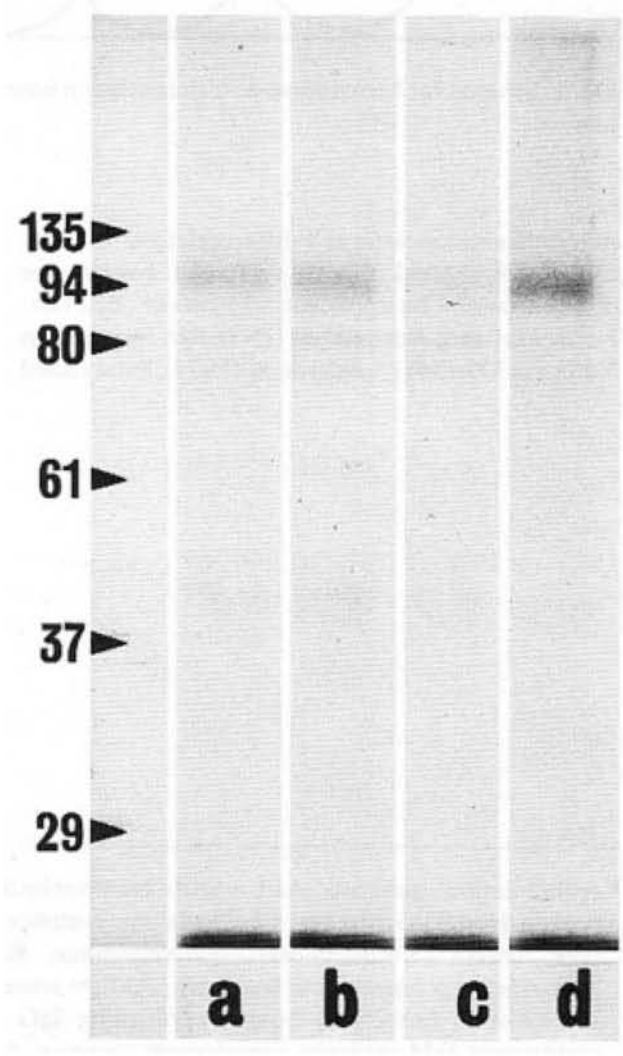

Fig. 1. SDS-PAGE of immunoprecipitates from NP-40 extracts of ^{35}S-methionine labeled Nalm-1 cells using 5 µl of rabbit anti-ALL. *a*, LcH$^+$ fraction; *b*, LcH$^-$ fraction; *c*, LcH$^-$ and RCA$^-$ fraction; *d*, RCA$^+$ fraction

Two-dimensional isoelectric focusing/SDS-PAGE of the lentil binding and lentil nonbinding forms of gp 100 over a narrow pH range gave identical homogeneous spots with a pI of 5.1–5.5. Neuraminidase treatment of both forms of gp 100 resulted in a shift of approximately 0.6 of a pH unit towards the basic end of the gel.

II. Is Carbohydrate Involved in the Antigenic Site of gp 100?

Treatment of cells with tunicamycin (20 μg/ml) and examination of immunoprecipitates from extracts on SDS-PAGE resulted in the disappearance of gp 100 with the concomitant appearance of a molecule at 75K. This was precipitable using rabbit antisera and a monoclonal anti-ALL (Ritz et al. 1980). Digestion of cell extracts with a mixture of glycosidases also gave an immunoprecipitable band of lower molecular weight. This evidence plus the heat lability of gp 100 suggests that carbohydrate, while possibly constituting up to 25% of the molecular weight, is not involved in the antigenic site.

III. Is gp 100 an Integral Membrane Protein?

As gp 100 is shed into the culture medium(s) and is also present in the sera of some leukemic patients (Kabisch et al. 1979; unpublished observations), it was of interest to establish whether or not it is an integral membrane protein.

Incubation of Nalm-1 cells with the lipophilic photoactivatable reagent, hexanoyl diiodo-N-(4-azido-2-nitrophenyl)-tyramine (Owen et al., to be published), was able to label Ia antigens but no labeling of gp 100 was evident. Charge shift electrophoresis (Helenius and Simons 1977) showed that the molecule possessed limited hydrophobicity, although this was no greater than that associated with the soluble proteins, bovine serum albumin, and lysozyme. Thus, although gp 100 possesses some hydrophobic regions, it is most likely not a true integral membrane protein. Subcellular fractionation of ^{125}I/lactoperoxidase-labeled cells, however, showed that gp 100 is strongly associated with the plasma membrane and not released into the medium as is the case with known peripheral proteins such as fibronectin (Owen et al., to be published).

IV. The gp 100 on Normal Bone Marrow Cells

Small numbers of cells in normal bone marrow react with anti-ALL (Greaves et al. 1978, 1980; Janossy et al. 1978; see also contribution by Greaves et al. in this book). Uninvolved bone marrow from three cases of children with rhabdomyosarcoma and two samples of marrow from patients in remission from leukemia were metabolically labelled with ^{35}S-methionine and immunoprecipitates of cell extracts examined by SDS-PAGE. A glycoprotein with a molecular weight of 100,000 daltons identical to that seen in leukemias and leukemic cells lines was detected. Rabbit antisera and monoclonal (J-5) antibody precipitated a similar band (Fig. 2). Peptide mapping will be required to establish

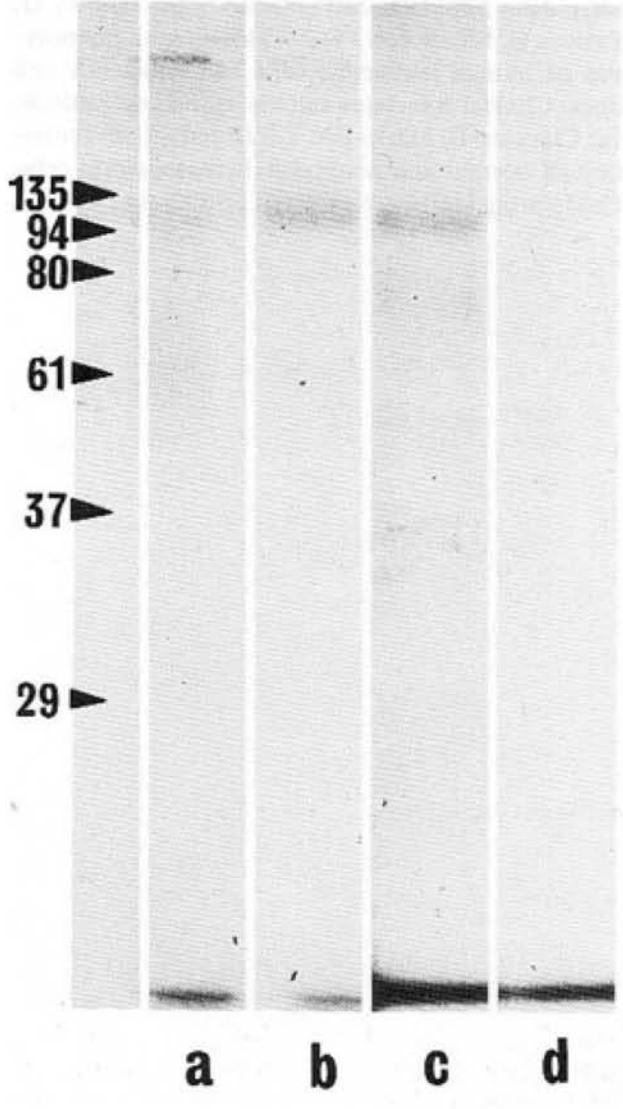

Fig. 2. SDS-PAGE of immunoprecipitates from ^{35}S-methionine labeled bone marrow cells. *a*, rhabdomyosarcoma and rabbit anti-ALL; *b*, rhabdomyosarcoma and monoclonal anti-ALL (J-5); *c*, remission marrow and rabbit anti-ALL; and *d*, remission marrow and normal rabbit serum

complete identity of gp 100 from normal marrow cells with that of leukemic cells. This data and other associated phenotypic features (see Greaves et al. pp 296–304) indicate that expression of gp 100 is likely to be a normal gene product reflecting the "target" cell for malignant transformation and/or the level of maturation arrest commonly seen in ALL.

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