

NOVEL SURFACE ANTIGEN EXPRESSED ON DIVIDING  
CELLS BUT ABSENT FROM NONDIVIDING CELLS\*

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All cells, including human hemopoietic cells and lines derived from them, possess numerous surface proteins. In most human leukocytes and lymphoid cell lines several dozen proteins can be detected in membrane preparations, but so far only those proteins encoded by the various HLA loci have been extensively characterized. Virtually nothing is known about the great majority of lymphocyte cell surface proteins. In this paper, we provide preliminary characterization of a previously undescribed antigen that is prominent on the surface of all dividing blood cells examined and describe its distribution on a variety of normal and transformed human cells.

**Materials and Methods**

The cells examined were as follows: B lymphoblastoid lines: McB, Ramos, Laz 263, Mu, SB, JY, SzB, 22B, and MoB; T lymphoblastoid lines: CEM, HSB, Molt 4, YT4E, HPB-ALL, HPB-MLT, TALL-1, RPMI 8402, and MoT; null lymphoid lines: REH and Nalm 1. Other lines examined were the macrophage line U937, the granulocyte line HL-60, and the erythroleukemia line K562. Blood cells examined included erythrocytes, granulocytes, monocytes, peripheral blood lymphocyte (PBL) B cells, PBL T cells, PBL cells from three Sézary syndrome patients, thymocytes obtained during cardiac surgery on infants, and PBL T cells stimulated either with phytohemagglutinin (PHA) (10  $\mu\text{g}/\mu\text{l}$ ; Difco Laboratories, Detroit, Mich.) or irradiated allogeneic B cells. PBL B and T cells were purified by passing fresh lymphocytes over columns that contained Sepharose conjugated to antibodies directed against human IgG Fab fragments. Cells passing through the column were allowed to sit at 37°C for 1 h and nonadherent cells were taken as T cells. Cells adhering after vigorous washing were used as purified monocytes. B cells were eluted from the column with a solution of human IgG, and E-rosetting was carried out with neuraminidase-treated sheep erythrocytes to remove any contaminating T cells. The non-blood cells examined included human embryonic lung fibroblasts (Flow Laboratories, Inc., Rockville, Md.) and a human melanoma cell line, SK-MEL-37, syngeneic with the B cell line Mu. All cells were cultured in RPMI-1640 medium that contained 10% fetal calf serum.

*Labeling.*  $10^7$  cells were cultured for 6–13 h with 0.5 mCi [ $^{35}\text{S}$ ]methionine (New England Nuclear, Boston, Mass.) in methionine-free RPMI-1640 that contained 10% dialyzed fetal calf serum. Typically, cell lines incorporated 60–80%, and unstimulated PBL T cells 8–15%, of the [ $^{35}\text{S}$ ]methionine. Surface radioiodination was carried out on  $2 \times 10^7$  healthy, washed cells using 0.5 mCi  $^{125}\text{I}$ , and Iodogen (Pierce Chemical Co., Rockford, Ill.) as a catalyst in accordance with the manufacturer's suggestions. Labeled cells were washed extensively and extracted in 1% Nonidet P-40, 2 mM phenylmethylsulfonyl fluoride and 10 mM Tris-HCl, pH 7.4, for 1 h on ice. Extracts were centrifuged for 10 min at 12,000 *g*, then used fresh or stored frozen.

*Antisera.* Sera prepared in New Zealand white rabbits (and immunogens) were as follows: A99 (CEM membranes) (1); C51 (PBL from a Sézary syndrome patient);  $\alpha\text{OD}$  (T cells from a T cell ALL), given by Dr. L Chess (2); cALLa (common ALL PBL cells) absorbed to recognize

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the cALL antigen, given by Dr. M. Greaves (3). Antisera were absorbed with either PBL cells (B cells, T cells, or unseparated) or B or T lymphoblastoid lines.

**Radioimmunoprecipitation.** Radioimmunoprecipitations were carried out and analyzed as described elsewhere (4). Normally 1,000–10,000 cpm were loaded for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Counts loaded are not listed in figure captions because so many factors (e.g., length of exposure, film type, and photographic processing) affect the intensity of bands as they appear in figures. Radioactive markers (particularly methylated phosphorylase [93,000 mol wt] and bovine serum albumen [69,000 mol wt]) were used to determine molecular weights. Fluorography was carried out using Enhance (New England Nuclear) and results were visualized on Kodak XRP, XR, or SB film (Eastman Kodak Co., Rochester, N. Y.) exposed at  $-70^{\circ}\text{C}$ .

**Lectin-binding Analyses.** Lectin-binding analyses were carried out using lectins from wheat germ, lentil, and ricin conjugated to Sepharose beads (Vector General Inc., Woodland Hills, Calif.). Aliquots of labeled extracts were mixed with 0.1 ml washed, pelleted beads and the mixture agitated at  $4^{\circ}\text{C}$  in lectin buffer (10 mM Tris-HCl, pH 7.8, 0.14 M NaCl, 0.1 mM  $\text{MnCl}_2$ , 0.1 mM  $\text{CaCl}_2$ , and 0.2% Brij 97) for 1 h. Beads were then washed extensively and bound material eluted with the appropriate sugar (5%) in lectin buffer. Eluates were analyzed by SDS-PAGE both with and without immunoprecipitation.

**Fluorescence Analysis.** Healthy cells ( $5 \times 10^6$ ) were washed and incubated at  $4^{\circ}\text{C}$  for 1 h with either normal rabbit serum or A99 antiserum. They were then washed three times with ice-cold RPMI-1640 and reacted for a further 45 min with fluoresceinated goat anti-rabbit IgG at  $4^{\circ}\text{C}$ . After three additional ice-cold washes, cells were examined for fluorescence either by light microscopy (with incident ultraviolet illumination) or by the fluorescence-activated cell sorter (FACS) (BD FACS Systems, Mountain View, Calif.).

## Results

**Presence of the Antigen on Hemopoietic Cell Lines.** Even after absorption with B cell lines, most antisera prepared against various human T cells and T cell lines precipitated numerous bands from radiolabeled T cell lines. Exceptions to this were the antisera  $\alpha\text{OD}$  and A99 each of which, after limited absorption with syngeneic B cell lines, reacted primarily with a major peptide in  $^{35}\text{S}$ -labeled extract from the T cell line CEM at an apparent mol wt of 90,000 (reduced) (Fig. 1 a and b, 90 K). The A99 antiserum reacted less strongly with an additional band at  $\sim 250,000$  mol wt present on most cell lines. Several other sera prepared against human T cells also precipitated a 90,000-mol wt peptide from CEM. Immunoprecipitations carried out with  $\alpha\text{OD}$ , A99, C51, or the other antisera on other T cell lines (Molt 4, HSB, YT4E, HPB-ALL, HPB-MLT, TALL-1, MoT, and RPMI 8402) and B lymphoid lines (McB, SB, Ramos, 22B, MoB, Laz 263, and Mu [Fig. 1 c]) showed that the antigen was expressed on all of them. Furthermore, it was also present on the macrophage line U937, the granulocytic line HL-60, the erythroleukemic line K562, and the null-cell lines Nalm 1 and REH (Fig. 1 e). If the antisera were absorbed on a T cell line, no reactivity against the 90,000-mol wt protein present on B cell lines remained. After thorough absorption of all the antisera (including A99 and  $\alpha\text{OD}$ ) with B lymphoblastoid cell lines (1 vol antiserum; 4 vol packed Laz 263, McB, or 22B cells), all reactivity against the 90,000-mol wt antigen on CEM or HSB was abolished. These results confirmed that the same antigen was present on both B and T cell lines. Both [ $^{35}\text{S}$ ]methionine- and  $^{125}\text{I}$ -labeled cell extracts gave similar results. Iodination generally labeled the protein more heavily than [ $^{35}\text{S}$ ]methionine.

Because the 90,000-mol wt peptide was present on all available hemopoietic cell lines, human embryonic lung fibroblasts were examined as an example of a non-lymphoid-transformed cell type, and human melanoma cells (SK-MEL-37) as a non-lymphoid-transformed cell type. The antigen was strongly expressed on the melanoma

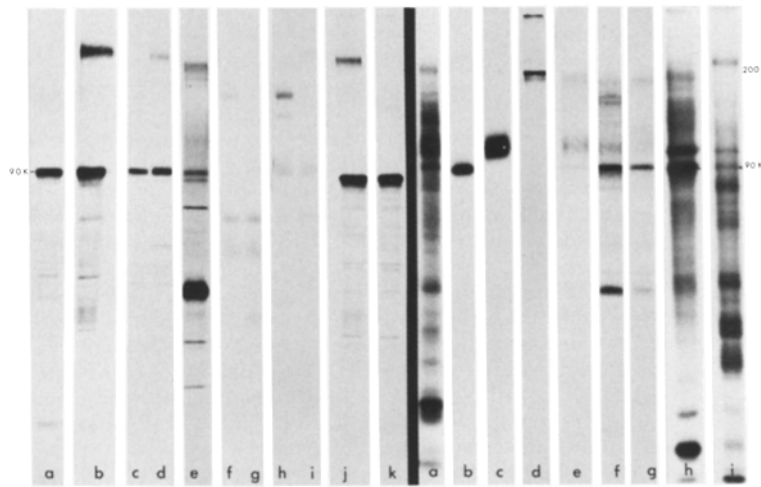


FIG. 1 (left) and FIG. 2 (right). Radioimmunoprecipitations analyzed on SDS-PAGE gradient gels under reducing conditions unless otherwise indicated. Fig. 1: (a)  $^{35}\text{S}$ -CEM  $\times$   $\alpha\text{OD}$ ; (b)  $^{35}\text{S}$ -CEM  $\times$  A99; (c)  $^{35}\text{S}$ -Mu  $\times$   $\alpha\text{OD}$ ; (d)  $^{35}\text{S}$ -SK-MEL-37  $\times$   $\alpha\text{OD}$ ; (e)  $^{35}\text{S}$ -REH  $\times$  unabsorbed C51; (f)  $^{125}\text{I}$ -PBL T  $\times$   $\alpha\text{OD}$ ; (g)  $^{125}\text{I}$ -PBL T  $\times$  A99; (h)  $^{125}\text{I}$ -PBL B  $\times$   $\alpha\text{OD}$ ; (i)  $^{125}\text{I}$ -PBL B  $\times$  A99; (j)  $^{35}\text{S}$ -PHA-stimulated PBL T  $\times$  A99; and (k)  $^{35}\text{S}$ -PHA-stimulated PBL T  $\times$   $\alpha\text{OD}$ . 90 K, 90,000 mol wt.

FIG. 2. (a)  $^{125}\text{I}$ -Nalm 1 non-immunoprecipitated; (b)  $^{125}\text{I}$ -Nalm 1  $\times$   $\alpha\text{OD}$ ; (c)  $^{125}\text{I}$ -Nalm 1  $\times$  acALL; (d)  $^{35}\text{S}$ -CEM  $\times$   $\alpha\text{OD}$  (not reduced); (e)  $^{35}\text{S}$ -CEM eluted from wheat germ lectin  $\times$  C51 (unabsorbed); (f)  $^{35}\text{S}$ -CEM eluted from lentil lectin  $\times$  C51 (unabsorbed); (g)  $^{35}\text{S}$ -CEM eluted from ricin lectin  $\times$  C51 (unabsorbed); (h)  $^{125}\text{I}$ -CEM non-immunoprecipitated; and (i)  $^{125}\text{I}$ -YT4E non-immunoprecipitated. 200 K, 200,000 mol wt; 90 K, 90,000 mol wt. The 92,500-mol wt standard, methylated phosphorylase B, has a mobility between the novel 90K protein and the common ALL antigen (95,000 mol wt).

line (Fig. 1 d). A much weaker band was precipitated from human embryonic lung fibroblasts.

*Distribution of the Antigen on Blood Cells.* Radioimmunoprecipitation from iodinated PBL B and PBL T cells failed to detect the 90,000-mol wt antigen (Fig. 1 f-i). On one occasion, a very small amount of 90,000-mol wt protein was seen in a [ $^{35}\text{S}$ ]methionine-labeled preparation of PBL T cells (see below). When  $^{125}\text{I}$ -labeled extracts were used, a weak band was sometimes present in the same region of the gel, but it was also seen on precipitations obtained with antisera from which all activity against the 90,000-mol wt band had been absorbed. This nonspecific contaminant band was generally broader and of slightly larger size (91,000-93,000 mol wt) than the 90,000-mol wt peptide (Fig. 1 h and i). Weak bands of 52,000 and 68,000 mol wt were present on gels of  $^{125}\text{I}$ -labeled PBL T cells immunoprecipitated with A99 or  $\alpha\text{OD}$  but not on PBL B cells (Fig. 1 f-i).  $\alpha\text{OD}$  and A99 have previously been shown to react with PBL T cells but not with PBL B cells (1, 2). Antisera absorbed with PBL T or PBL B cells retained the ability to recognize the 90,000-mol wt antigen from CEM extract, thus confirming that PBL B and T cells lack the antigen.

The 90,000-mol wt protein could not be precipitated from  $^{125}\text{I}$ -labeled thymocytes, granulocytes, monocytes, or erythrocytes. If separated PBL T cells were stimulated allogeneically with irradiated B cells or with PHA, radioimmunoprecipitation revealed that the antigen had been induced and was abundant on the surface of the resulting blast cells (Fig. 1 j and k). A small percentage of dividing blast T cells could have accounted for the single observation of the 90,000-mol wt band in PBL T cells.

Metabolic labeling would amplify the contribution made by a few rapidly dividing cells in an otherwise resting population. Iodination would not be affected in this way by blast cells and 90,000-mol wt antigen was never detected on PBL cells by this method.

In addition to being present on cell lines, the antigen occurred on several leukemic T cells examined. It was present in the immunoprecipitates from the PBLs of three Sézary syndrome patients examined and must also have been present on the leukemic T cells used as the immunogens in the preparation of  $\alpha$ OD.

*Relationship to the Common ALL Antigen.* The common ALL antigen (cALLa), a protein of similar size (95,000 mol wt) (3), and the antigen described here were independently precipitated (Fig. 2 b and c). The distribution of the two antigens was also different. On Nalm 1 for example, the 90,000-mol wt antigen and cALLa were both strongly expressed (Fig. 2 a-c). On REH, the 90,000-mol wt antigen was weak (Fig. 1 e), whereas cALLa was strong (3); while the reverse was true on CEM. cALLa could not be detected on any B cell lines, as previously reported (3).

*Characterization of the Antigen.* The antigen appeared as a polypeptide of 90,000 mol wt under reducing conditions. Under nonreducing conditions, the band at 90,000 mol wt disappeared to be replaced by another at  $\sim$ 200,000 mol wt (Fig. 2 d). The protein may occur normally as a disulphide bonded dimer. Because the 90,000-mol wt antigen can be surface iodinated and antibodies against it are absorbed by intact cells, it is obviously situated on the cell surface. Lectin-binding experiments revealed that the antigen is a glycoprotein that binds to lentil and ricin but not significantly to wheat germ agglutinin (Fig. 2 e-g).

*Relative Abundance of the Antigen on Different Cell Lines.* Data from the FACS showed that CEM reacted more strongly with A99 than any other cell line tested. Other cell lines that reacted less strongly than CEM but were clearly positive included the null-cell line Nalm 1, the B cell line McB, and the granulocytic line HL-60. Two B cell lines (JY and SzB) and the null-cell line REH appeared essentially negative, similar to Laz 007 (1). However, radioimmunoprecipitation had previously revealed a weak 90,000-mol wt band on REH (Fig. 1 e). Radioimmunoprecipitation, a highly concentrative procedure, is more sensitive than immunofluorescence analysis.

The amount of 90,000-mol wt antigen expressed appeared to differ considerably from one line to another. This conclusion was supported by the immunoprecipitation data and non-immunoprecipitated, surface-iodination analyses as well as the FACS results. On surface-iodinated CEM, non-immunoprecipitated gel analysis showed the band at 90,000 mol wt to be one of the major three or four peptides visible (Fig. 2 h). Similar experiments revealed that the 90,000-mol wt band was a less significant protein on most other lines including some T cell lines (YT4E, Fig. 2 i).

No single class of cells (e.g., null cells, B cells, or T cells) expressed a consistent amount of the antigen. For instance, the null-cell line Nalm 1 possessed considerable amounts of the antigen, whereas the amount present on REH, another null-cell line, was very low (Fig. 1 e and Fig. 2 a and b). Most lines expressed considerably more 90,000-mol wt antigen than REH but less than CEM.

### Discussion

This paper describes a novel blast antigen of 90,000 mol wt, clearly distinct in size as well as cell distribution from the common ALL antigen discovered by Sutherland et al. (3) and from band 3, the 90,000-mol wt protein on erythrocytes (5) that was not precipitated by  $\alpha$ OD. Our data should be compared to several recent reports. Saito

et al. (6) characterized a sialoglycoprotein from Molt 4 that has an estimated 95,000 mol wt, although cALLa is not present on Molt 4. They have not detailed the distribution of this protein on other cell lines and it differs from the protein described here in certain respects. The 90,000-mol wt protein binds to ricin and lentil but not WGA, whereas the 95,000-mol wt protein binds to ricin and WGA. Under non-reducing conditions, the 90,000-mol wt peptide is replaced by a band at 200,000 mol wt, whereas the 95,000-mol wt protein remains at the same molecular weight. Billings et al. (7) outlined serologically the distribution of a blast cell antigen on various lymphoid cells and cell lines. They used an antiserum prepared against CEM cells and studied its distribution by cytotoxicity. Although they examined certain cell types that we have not (CML, CLL, AML, and ALL cells) and we have looked at others they omitted (Sézary cells, granulocytes, thymocytes, and erythrocytes), there is a general similarity in the results. One point at which the results differ is that the 90,000-mol wt antigen is absent from monocytes but their antigen is present on these cells. Because they have not characterized the protein(s) their antiserum recognizes, it is not yet possible to say that the antisera react with the same antigen. It is worth noting that the A99 antiserum was made against CEM membranes also. Yin and Stossel (8) described a 91,000-mol wt protein named gelsolin isolated from lung macrophages of adjuvant-primed rabbits. This protein catalyzes the reversible breakdown of actin filaments in the presence of  $\text{Ca}^{++}$  and is thought to exist as a dimer in vivo. The protein is believed to occur in the peripheral part of the cytoplasm but it is unknown whether it is also present on the membrane. The 90,000-mol wt protein described here could be a human equivalent of this rabbit protein, although it has not been detected on monocytes from peripheral blood. Another possibility is that the 90,000-mol wt protein could represent the 100,000-mol wt glycoprotein found to be associated with malignancy in a variety of different tumors by Bramwell and Harris (9). Expression of the antigen may be enhanced on some malignant cells.

The A99 antiserum has recently been described as reacting with several T cell-specific components of CEM cell membranes (1). The A99 preparation used in the present study was not as thoroughly absorbed as that described previously. Among several proteins radioimmunoprecipitated by this antiserum under reducing conditions (1) were bands at 96,000 mol wt and 195,000 mol wt; the band at 96,000 mol wt disappeared under nonreducing conditions (1). It seems likely that this 96,000-mol wt band is the 90,000-mol wt blast antigen described here, and if so, it is definitely not T cell specific. A further possibility is that the 195,000-mol wt band represents incompletely reduced 90,000-mol wt protein.

Friedman et al. (2) reported that  $\alpha\text{OD}$  is cytotoxic for a subpopulation of PBL T cells thought to possess helper activity. It is possible that the weak bands precipitated by  $\alpha\text{OD}$  from PBL T cells between 52,000 and 60,000 mol wt and at 68,000 mol wt represent this reactivity. The A99 antiserum also recognizes these proteins and an additional weak band at 39,000 mol wt. None of these bands could be precipitated from PBL B cells by either antiserum.  $\alpha\text{OD}$  did precipitate some high molecular weight bands from both PBL T and PBL B cells that were not present in immune precipitates using A99 (Fig. 1 f, h).

These results obtained here suggest that this 90,000-mol wt antigen is present on all cell lines derived from transformed hemopoietic cells, although the amount of antigen present may vary considerably. Under normal in vivo circumstances, blast cells generated during infections such as infectious mononucleosis are probably the only hemopoietic cells expressing this antigen. In malignant disorders, it was expressed on

all leukemic blasts examined. Antisera against the antigen could provide a valuable tool for identifying leukemic blast cells. This protein may be purified for further study using a lentil-lectin column followed by an immunoaffinity column.<sup>1</sup>

### Summary

Radioimmunoprecipitation followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to study the distribution on human lymphoid cells of a previously undescribed surface antigen recognized by several heteroantisera. A glycoprotein with a 90,000 mol wt (under reducing conditions) was detected on all cell lines tested including T, B, null, and myeloid cell lines, although the amount of antigen present varied considerably. The antigen was absent from normal peripheral blood lymphocytes (PBL), B and T cells, monocytes, granulocytes, thymocytes, and erythrocytes. After stimulation with lectins or allogeneic B cells, the antigen was induced on PBL T cells. A limited number of leukemic T cells tested all expressed the antigen, as did a melanoma cell line and human embryonic lung fibroblasts. Hence, the antigen was present only on dividing lymphoid cells and absent from nondividing cells, but was also present on the two examples of dividing non-lymphoid cells tested. Under nonreducing conditions, the 90,000-mol wt band normally present disappeared to be replaced by another at ~200,000 mol wt. The glycoprotein bound to lectins from lentil and ricin, but not to wheat germ agglutinin. It could be readily labeled metabolically by [<sup>35</sup>S]methionine or by surface iodination, and appeared to be a major membrane protein on some cell lines.

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<sup>1</sup> We have recently found two monoclonal antibodies (OKT9 and 5E9) that recognize the 90,000-mol wt protein identified by the  $\alpha$ OD and A99 sera.