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# Human leucocyte surface glycoprotein CDw44 and lymphocyte homing receptor are identical molecules

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The antigen CDw44 is a glycoprotein with a relative mass ($M_r$) of approximately 80 000 which is expressed on most leucocytes, weakly on erythrocytes, and which also occurs on other cell types such as neurons, but is absent from the surface of immature thymocytes. It was defined during the collaborative studies of the Third International Workshop on Human Leucocyte Differentiation Antigens as the antigen recognized, e. g., by mAbs A1G3 (Haynes et al. 1983), F10-44-2 (Dalchau et al. 1980), or 106-4D5 (Borche et al. 1987) and distinct from a major leucocyte surface sialoglycoprotein of similar $M_r$ called CD43 (Cobbold et al. 1987). Recently, identity of the CDw44 antigen with a leucocyte surface glycoprotein Pgp-1 has been shown (Omary et al. 1988). Several other broadly expressed leucocyte surface antigens of similar $M_r$ have been described, e. g., lymphocyte homing receptor (Jalkanen et al. 1987), DAF (Davitz et al. 1987), ICAM-1 (Dustin et al. 1987), or Cromer-related blood group antigen (Spring et al. 1987). In this communication we report that one of the mAbs produced recently in our laboratory, MEM-85, recognizes an antigen identified as both CDw44 and homing receptor; thus, we demonstrate the identity of these molecules.

The MEM-85 hybridoma was obtained by fusion of the Sp2/0-Ag14 myeloma with spleen cells of a mouse immunized repeatedly with PB MNC of a patient suffering from an large granular lymphocyte (LGL)-type leukemia, using standard techniques. The mAb (IgG1, pI=7.6–8.1) reacted with all PB MNC as well as with granulocytes and cell lines such as CEM, MOLT-4, ARH-77, HEL, or HL-60, while other cell lines such as Jurkat, Nalm-6, Daudi, and KM 3 were only weakly positive or negative, as observed by cytofluorometry or fluroescence microscopy (not shown). It immunoprecipitated an antigen of apparent $M_r$ of approximately 80 000 (nonreduced) or 85 000 (reduced) from the detergent lysate of surface radioiodinated PB MNC (Fig. 1).

In order to study the relationship between this antigen and similar molecules described before, we purified it by immunoaffinity chromatography on immobilized mAb MEM-85 and used this purified preparation for SDS-PAGE and Western blotting. The nitrocellulose replicas were immunoperoxidase stained with standard mAbs F10-44-2 and 33-3B3 directed against the CDw44 antigen (Dalchau et al. 1980; R. Vilella, unpublished data), mAbs Hermes-1 and Hermes-3 reactive with the lymphocyte homing receptor (Jalkanen et al. 1987), IA10 recognizing DAF (Davitz et al. 1987), BRIC 128 directed against the Cromer-related blood group antigen (Spring et al. 1987), and a CD43-specific mAb MEM-59 (Štefanová et al. 1988). Under these conditions (using nonreduced samples for SDS-PAGE), the isolated antigen zone was specifically stained with mAb MEM-85, both CDw44-specific mAbs, and both mAbs recognizing the homing receptor (Fig. 2). Only mAb Hermes-3 also reacted with the reduced antigen (Fig. 2d). Monoclonal antibody IA10 reacted weakly with a 67 000 zone both in the original lysate and after passing through the MEM-85 column, but not with the material eluted from this immunosorbent; similarly, BRIC 128 weakly stained a triplet of zones between $M_r$ of 60 000–67 000 in the lysates (results not shown), indicating that these antigens are different from CDw44 and homing receptor.

Monoclonal antibodies MEM-85, 33-3B3, and Hermes-1 competed with each other for binding to viable MOLT-4 cells, while Hermes-3 apparently recognized a different epitope (Fig. 3). After modulation (capping) of the antigen on MOLT-4 cells by mAb MEM-85 (performed as described by Hořejší et al. 1988), the staining of the cells outside the cap with mAbs 33-3B3, Hermes-1, and Hermes-3 was lost, while staining with an CD43-specific mAb MEM-59 was unaffected (observed by immunofluorescence microscopy, results not shown),

*Abbreviations used in this paper:* mAb, monoclonal antibody; SDS-Page, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate; PB MNC, peripheral blood mononuclear cells; DAF, decay accelerating factor; PBS, phosphate-buffered saline

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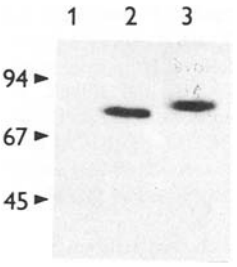


**Fig. 1.** SDS-PAGE of nonreduced (lanes 1 and 2) and reduced (lane 3) samples of the antigens immunoprecipitated from the surface radioiodinated PB MNC by an irrelevant negative control (lane 1) and MEM-85 (lanes 2, 3). PB MNC were prepared by a modification of the method of Bøyum (1968), surface radioiodinated by the lactoperoxidase technique, lysed in PBS containing 1% Nonidet P-40 and protease inhibitors, immunoprecipitated according to Tamura and co-workers (1984), and analyzed by SDS-PAGE on a 10% gel in the Laemmli buffer system. Positions of the $M_r$ standards ($\times 10^{-3}$) are indicated

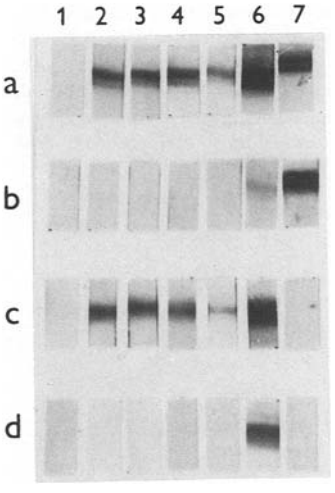


**Fig. 2a–d.** Western blotting of a crude MOLT-4 nonreduced detergent lysate before (**a**) and after (**b**) passing through a column of immobilized mAb MEM-85 and of nonreduced (**c**) and reduced (**d**) samples of the material specifically retained on the MEM-85 immunosorbent. Crude lysate was prepared by lysis of $5 \times 10^7$ MOLT-4 cells in 1 ml PBS containing 1% Nonidet P-40 and protease inhibitors and centrifugal removal of insoluble components. This lysate was either mixed 1:1 with 2× concentrated nonreducing sample solution for SDS-PAGE, boiled, and used for electrophoresis and Western blotting (**a**) or used for immunoaffinity chromatography. The material passed through the MEM-85 column was analyzed similarly as the crude lysate; the fraction eluted from the immunosorbent by glycine-NaOH buffer pH 11.5 containing 0.2% sodium deoxycholate was neutralized, concentrated by ultrafiltration, and analyzed similarly as the lysates. The primary mAbs used for staining of individual nitrocellulose strips were: an irrelevant mAb (1), MEM-85 (2), CDw44-specific mAbs F10-44-2 (3) and 33-3B3 (4), homing receptor-specific mAbs Hermes-1 (5) and Hermes-3 (6), and a CD43-specific mAb MEM-59 (7). Only the relevant part of the blot is shown (corresponding to the $M_r$ range of 65 000–100 000). Negative results obtained with mAbs IA10 and BRIC 128 are not shown

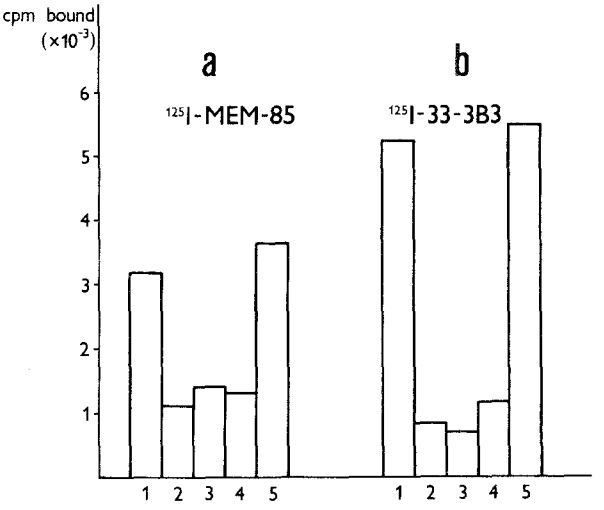


**Fig. 3a and b.** Blocking of $^{125}$I-MEM-85 (**a**) or $^{125}$I-33-3B3 (**b**) binding to MOLT-4 cells by other mAbs: column 1, a negative irrelevant control; column 2, MEM-85; column 3, 33-3B3; column 4, Hermes-1; column 5, Hermes-3. $10^6$ MOLT-4 cells were incubated with 100 µl of cell culture medium containing 10% bovine serum, 30 ng of the radioiodinated mAb (corresponding to approximately $10^5$ cpm), and approximately 5 µg of the competing unlabeled mAb. After washing with PBS the cell-associated radioactivity was measured

indicating that the determinants recognized by all these mAbs reside on the same membrane molecule. Taken together, all these results suggest that the antigen recognized by MEM-85 is identical to both CDw44 and the homing receptor. In addition, CDw44 also appears to be identical to the Pgp-1 glycoprotein (Omary et al. 1988). Hence, the thus far unknown biological receptor role of the molecules, up to now termed CDw44 and Pgp-1, is now established. It is of interest that the homing receptor is apparently a membrane lectin recognizing 6-phosphomannosyl structures on the surface of high endothelial venules (Stoolman et al. 1987); the mouse lymphocyte homing receptor was reported to have a usual structure: the polypeptide is branched by several ubiquitin peptide chains (Gallatin et al. 1986). Recently, we have detected a soluble form of the CDw44 glycoprotein in normal human sera which has $M_r$ identical to the membrane-bound antigen (I. Štefanová, I. Hilgert, R. Vilella, V. Horejsi, submitted for publication). This raises the question of the possible biological significance of such a soluble form of the CDw44/homing receptor. Further, it might be speculated that a rather widespread occurrence of 6-phosphomannosyl residues on the surfaces of various cells and microorganisms as well as on some soluble glycoproteins could indicate a more general role for the CDw44 glycoprotein in intercellular adhesion, and possibly also in the adhesion of leucocytes to some pathogenic microorganisms.

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