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Structural heterogeneity of human Pgp-1 and its relationship with p85

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Pgp-1 is a major cell-surface phosphoglycoprotein (relative mass approximately 95 000) that has been identified in mouse and human cell lines and tissues (Isacke et al. 1986, Trowbridge et al. 1982, Hughes et al. 1983). Although monoclonal antibodies (mAb) that recognize this glycoprotein were initially raised against plasma membranes from NIH/3T3 fibroblast cells (Hughes and August 1981), its designation of Pgp-1 (phagocyte glycoprotein-1) was based on its marked abundance on macrophages, monocytes, and polymorphonuclear cells (Colombatti et al. 1982). Despite that the function of Pgp-1 is unknown, the mouse homolog is a useful differentiation antigen in that it is expressed on prothymocytes in the bone marrow (Trowbridge et al. 1982) and on a proportion of mouse thymocytes, including cells thought to be the immediate descendants of bone marrow prothymocytes (Lesley et al. 1985a, Hyman et al. 1986). Many cells found in an adult mouse thymocyte subpopulation enriched in Pgp-1$^+$ cells contain an unrearranged T-cell antigen receptor β-chain gene (Trowbridge et al. 1985). Further, Pgp-1 is expressed on more than 90% of fetal thymocytes on days 13 and 14 of gestation before the fraction of Pgp-1$^+$ cells declines to adult levels shortly before birth (Lesley et al. 1985b). In contrast to the mouse, 50–60% of human thymocytes express Pgp-1. The Pgp-1$^+$ subpopulation in the human comprises primarily medullary thymocytes, although some Pgp-1$^+$ cells scattered throughout the cortex can be detected by immunofluorescence staining (Isacke et al. 1986).

p85 is a human glycoprotein with an approximate relative mass of 85 000 which was initially isolated from human chronic lymphocytic leukemia cells using mAb 50B4 and 50E6 (Letarte et al. 1985). More recently, the p85 glycoprotein was shown to carry all the antigenic determinants identified with mAb independently obtained by several different groups (Letarte 1986). These mAb include F10-44-2, which defines a T-lymphocyte-granulocyte-brain antigen (Dalchau et al. 1980); A1G3, which defines a human medullary thymocyte antigen (Haynes et al. 1983); and A3D8, which defines an erythrocyte antigen (Telen et al. 1983).

Here we show that there are striking and unusual differences in the apparent relative mass of Pgp-1 expressed on cultured cell lines derived from a variety of human tissues. We further present evidence that establishes that Pgp-1 and p85 are closely related if not identical.

The possibility that Pgp-1 and p85 are related was first suggested by experiments in which both antigens were immunoprecipitated from a variety of human cell lines by their respective mAb. As shown in Figure 1, a single major species (M_r 85 000–95 000) was immunoprecipitated from AG1523 diploid human fibroblasts by the Pgp-1-specific mAb E1/2 as observed in previous studies of human Pgp-1 (Isacke et al. 1986). Species of similar relative mass to Pgp-1 were immunoprecipitated from AG1523 cells when p85-specific mAb was used. However, both Pgp-1-specific and p85-specific mAb immunoprecipitated a major species (M_r 145 000) and two less prominent species having relative masses in the range of 185 000–215 000 from the human colonic epithelial cell line HT29 (Fig. 1). Hence, as shown in Figure 1, four of the five cell lines we tested expressed as major species either a lower relative mass form (M_r 85 000–95 000) (diploid fibroblasts AG1523 or laryngeal epidermoid carcinoma HEp2) or a major higher relative mass form (M_r 145 000) (colon carcinoma HT29 or vulvar epidermoid carcinoma A431). In contrast, HUT78, a cutaneous T-cell lymphoma cell line, showed both the M_r 85 000–95 000 and 145 000 forms. Further, multiple additional M_r species are present in immunoprecipitates from HT29, HEp2, and A431 cells. A significant amount of radioactivity in immunoprecipitates of A431 cells remained at the top of the resolving gel (Fig. 1). This was reproducible and is

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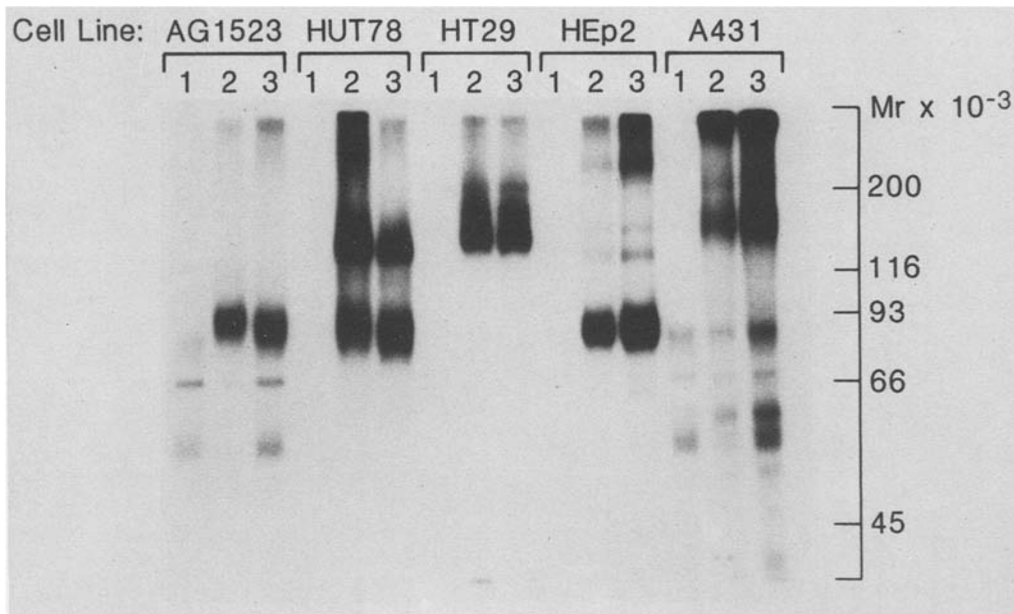

Fig. 1. Heterogeneity of Pgp-1 and p85 glycoproteins isolated from several human cell lines. Cell lines used for the study were: AG1523 (NIA Aging Cell Repository, Institute for Medical Research, Camden, New Jersey), HUT78 (Gootenberg et al. 1981), HT29 (Fogh et al. 1977), HEp2 (Moore et al. 1955), and A431 (Fabricant et al. 1977). Radiolabeling with ^{125}I and immunoprecipitation was carried out as described previously by Isacke and co-workers (1986). Iodinated cells were solubilized using 1% Nonidet P40 in phosphate-buffered saline containing 400 KIU/ml aprotinin, 10 μM leupeptin, 10 μM pepstatin, and 0.5 mM phenylmethyl sulfonylfluoride. MAb E1/2 (Isacke et al. 1986) and 50B4 (Letarte et al. 1985) were used for immunoprecipitating Pgp-1 and p85, respectively. Immunoprecipitated material was analyzed using an 8% SDS-polyacrylamide gel under reducing conditions (Laemmli 1970). Autoradiography was carried out using an intensifying screen (Laskey and Mills 1977). Cell lines used for each immunoprecipitation are indicated at the top of the figure. Lanes 1, control immunoprecipitations whereby the addition of a specific antibody was omitted. Lanes 2 and 3, immunoprecipitation using mAb 50B4 (anti-p85) and mAb E1/2 (anti-Pgp-1), respectively. Relative mass markers are shown in kilodaltons. (The hybridoma lines used in this study are available to interested investigators)

likely to represent species so large that they are unable to enter the resolving gel.

Because the striking similarity in the size of Pgp-1 and p85 on the various cell lines suggested the antigens may be related, immunodepletion studies were performed to examine this question more directly. As shown in Figure 2d and e, Pgp-1-specific and p85-specific mAb reciprocally immunodeplete each other. Control lanes (Fig. 2f–j) show that Pgp-1 and p85 immunodeplete themselves as expected, yet do not significantly interfere with the immunoprecipitation of the transferrin receptor (TrR), an unrelated glycoprotein. Hence, epitopes recognized by Pgp-1-specific and p85-specific mAb reside on the same glycoprotein species. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under nonreducing conditions (Fig. 2k–m) showed that the high and low relative mass forms of Pgp-1 (or p85) are not disulfide bonded, as is the case for the TrR (Omary and Trowbridge 1981, Fig. 2m). However, both forms show a slightly faster migration under nonreducing conditions. The identity of Pgp-1 and p85 is also supported by peptide mapping using the Cleveland technique (Cleveland et al. 1977). As shown in Figure 3, *Staphylococcus aureus* V8 digests of radioiodinated Pgp-1 and p85, immunoprecipitated by their corresponding mAb, were indistinguishable.

A minor size difference was noted between the species immunoprecipitated by Pgp-1-specific and p85-specific mAb from AG1523 and HUT78 cells but not on the other cell lines tested (Figs. 1 and 2). This difference was reproducible in five experiments and involved all bands that were immunoprecipitated from a given cell line. As Pgp-1 and p85 mAb completely immunodeplete each other, the most likely explanation for this observation is that the two mAb have different affinities for the various molecular species which contribute to this diffuse relative mass band. This leads to quantitative differences in the glycoproteins immunoprecipitated.

Previous studies in which Pgp-1 and p85 have been independently characterized are generally compatible with the conclusion of the present work that they are closely related or identical. Both show a broad tissue and cell line distribution, including kidney, brain, and bone marrow (McKenzie et al. 1982, Quackenbush et al. 1985, 1986, Isacke et al. 1986). However, p85 has been reported to be expressed on brain white matter (Cruz et al. 1986, Quackenbush et al. 1985), whereas Pgp-1 was not detect-

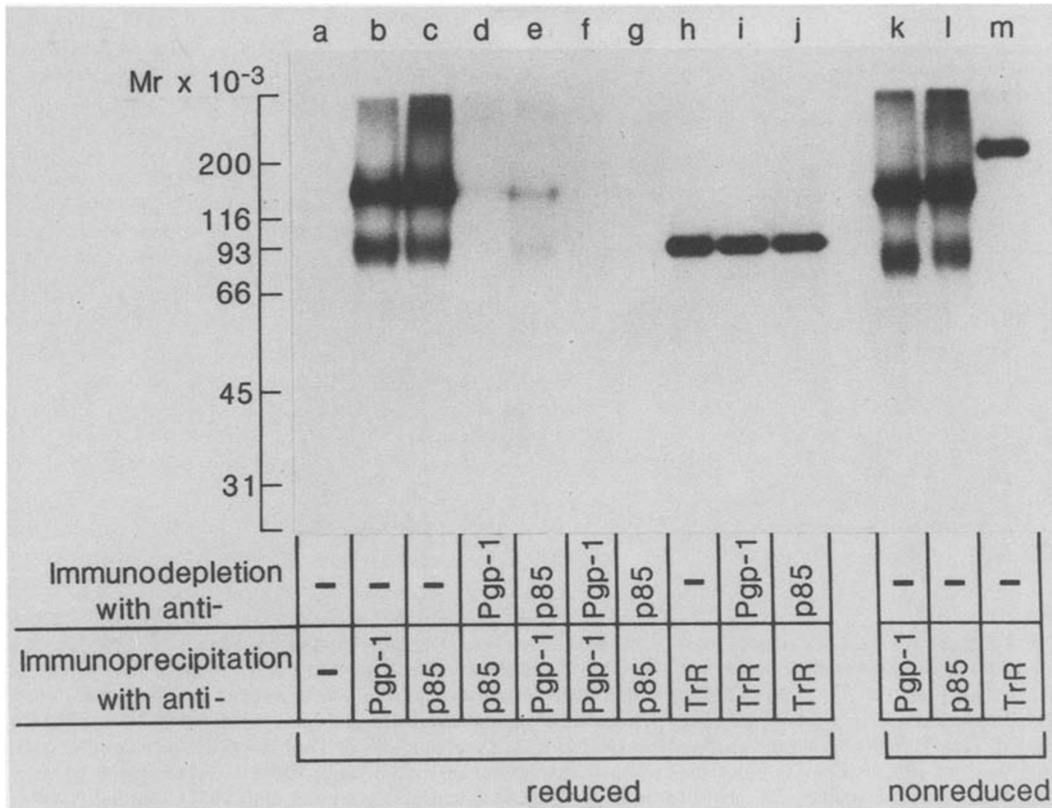

Fig. 2. Evidence, using immunodepletion, that Pgp-1 and p85 glycoproteins are identical. HUT78 cells were labeled with ^{125}I, followed by detergent solubilization as described in Figure 1. Immunodepletion was carried out by using the indicated antibody, then removing the immune complexes with fixed *S. aureus* bacteria (Pansorbin, Calbiochem). To ensure adequate immunodepletion, this was repeated two times. The remaining immunodepleted lysate was then immunoprecipitated using the indicated antibody followed by analysis by SDS-PAGE (8% acrylamide gel) under either reducing or nonreducing conditions. Transferrin receptor (TrR) was immunoprecipitated using monoclonal antibody B3/25 as described previously (Omary and Trowbridge 1981). Lane a represents control immunoprecipitation

ed on brain tissue in the studies of Isacke and colleagues (1986). The erythrocyte p85 antigen, defined by A3D8, appears to have its expression regulated by a rare Lutheran inhibitor gene, *In*(*Lu*) (Telen et al. 1983). Our finding of two major molecular forms using p85-specific mAb (M_r 85 000 and 145 000) in HUT78 cells differs from a previous study in which only one major species (M_r 80 000) was detected using mAb A1G3 (Haynes et al. 1983). Subsequently, epitopes recognized by mAb A1G3 and by p85-specific mAb 50B4 (used in this study) were shown to be distinct but to reside on the same molecule (Letarte et al. 1986). The discrepancy may be related to differences in antibody epitopes or to cell line variability in continuous culture.

The molecular basis of the unusual heterogeneity of Pgp-1 glycoprotein remains unclear. As only the high M_r form is found in immunoprecipitates from HT29 and A431 cells, it is almost certain that the other high M_r species express the antigenic determinant recognized by the mAb. A less likely alternative is that the high M_r species are noncovalently associated with the M_r 85 000–95 000 or with the 145 000 species and are not directly recognized by the mAb. It is possible the differences in apparent relative mass represent either different gene products or differential posttranslational modification of a single protein. It is unlikely that such large differences in apparent relative mass are generated by variation in the addition of high mannose or complex oligosaccharides or multiple small 0-linked sugar moieties. However, there is evidence that in the mouse, Pgp-1 is the core protein of a major proteoglycan species (T. August, personal communication) and differences in the addition of proteoglycan units in the various human cell lines could account for the observed structural variability. Interestingly, an 85 000–95 000 cell surface glycoprotein that may be related to tissue-specific homing of lymphocytes was recently reported to bear several N-linked glycans (Jalkanen et al. 1987).

In summary, the results reported in this paper demonstrate the striking structural heterogeneity of human Pgp-1 and establishes the molecular identity of Pgp-1 with another well-characterized cell surface antigen, p85.

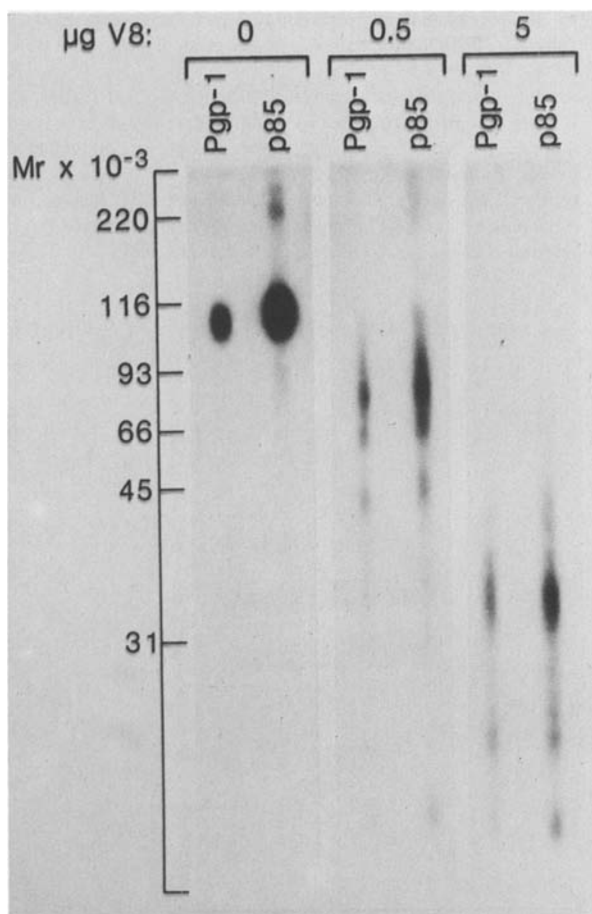

Fig. 3. Peptide mapping of Pgp-1 and p85 glycoproteins. Peptide maps were obtained as described by Cleveland and co-workers (1977). Briefly, immunoprecipitates were obtained from radioiodinated AG1523 cells using either Pgp-1-specific or p85-specific antibodies. After SDS-PAGE analysis, ^{125}I-labeled bands were excised from the gel and digested with *S. aureus* V8 protease. The digested fragments were resolved on 15% SDS-polyacrylamide gels

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