E6.29 Identification of Workshop Endothelial Section mAb that recognize novel glycosyl phosphatidylinositol-anchored antigens

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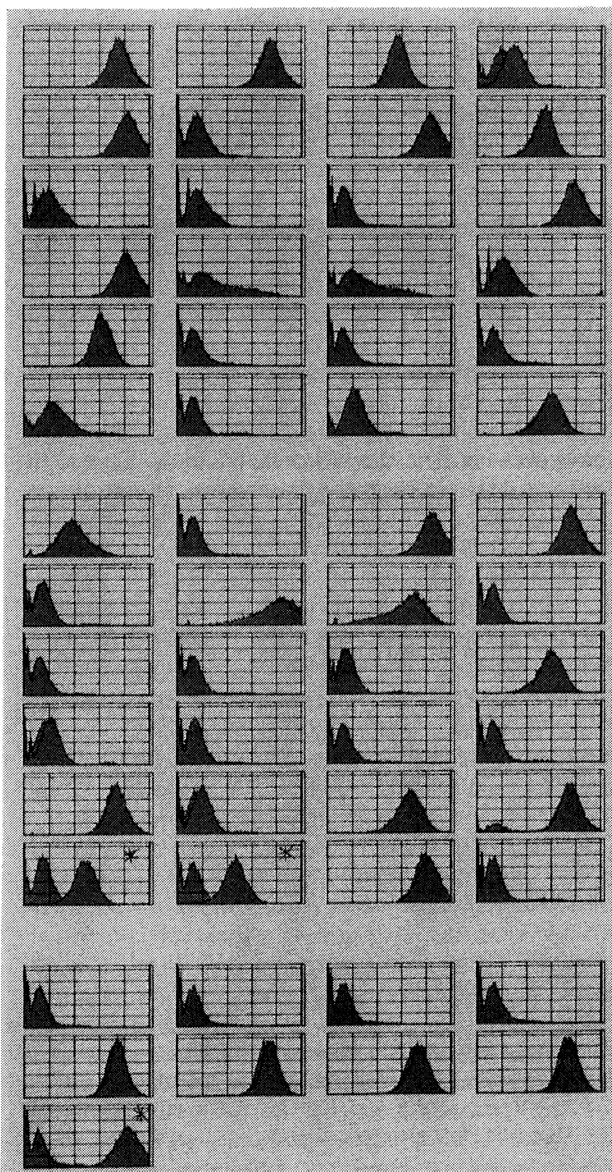

The glycosyl phosphatidylinositol (GPI) anchor is a posttranslational modification of newly synthesized proteins in which the polypeptide chain is cleaved at a signal sequence near the COOH-terminal end of the protein and a GPI moiety is transferred to the new COOH-terminal residue [1]. It is well recognized that many GPI-anchored antigens are able to mediate a mitogenic signal [2] and this signalling function may occur in part via $p56^{lck}$ [3; L. F. Thompson *et al.*, abstract T075 at the Fifth Workshop]. Previously identified GPI-anchored antigens on endothelium include the complement regulatory proteins CD55 (DAF) and CD59 (MIRL) and the CD2 counter-receptor CD58 (LFA-3) [Klickstein and Springer, AS1.4]. To identify monoclonal antibodies (mAb) in the Workshop Endothelial Section that recognize novel GPI-anchored antigens, human umbilical cord vein endothelial cells (HUVEC) with and without phosphatidyl-specific phospholipase C (PI-PLC) treatment were analysed by indirect immunofluorescence and flow cytometry with the 57 mAb of the panel. All mAb in the Endothelial Section that were submitted to the Blind Panel were further analysed on three additional cell lines and their GPI-anchored deficient counterparts [Klickstein *et al.*, AS1.7] and 19 additional cell lines analysed by our laboratory as part of the Blind Panel study.

Three mAb, E045 (8A3, BP210), E046 (7D1, BP055), and E057 (C4A9, BP218), were found to stain

Fig. 1 *(opposite)* Flow cytometric analysis of HUVEC with and without PI-PLC treatment. Each panel shows the two histograms, + and − PI-PLC. A single histogram seen in a panel indicates no change with PI-PLC, and two histograms in a panel (with asterisks) indicates PI-PLC sensitivity. Numbering is E001 in the upper left, then numbering continues in order across and down. E045, E046, and E057 show a significant change with PI-PLC.

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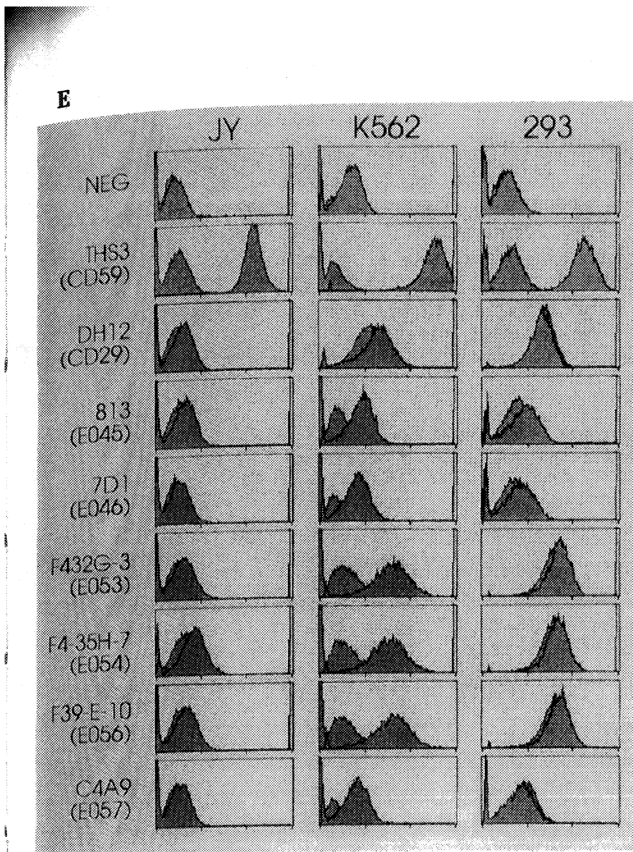

Fig. 2 Flow cytometric analysis of normal and GPI-anchor-deficient cell lines. The cell lines are described in Klickstein *et al.* [AS1.7]. Each panel shows the results of an antibody with the indicated normal cells and the corresponding GPI-anchor-deficient cells. Two distinct histograms indicate a GPI-anchored antigen. CD59 is a known GPI-anchored antigen and CD29 is a transmembrane-anchored antigen.

HUVEC brightly and the reactivity was sensitive to PI-PLC treatment (Fig. 1). E045 and E046 defined the cluster CDw109 and E057 did not cluster with any other mAb. The CDw109 antigen was present at low levels on normal K562 and 293 cells, but not on the corresponding GPI-anchor deficient cells, or on PI-PLC-treated 293 cells, confirming the presence of a GPI anchor (Fig. 2). The E057 mAb did not stain any of these cell lines. Interestingly, E053 (F435H-3), E054 (F435H-7), and E056 (F439E-10), three mAb that recognize the endothelial antigen S-ENDO 1, stained the normal K562 clone weakly and did not stain the GPI-anchor-deficient cells suggesting the presence of a GPI anchor (Fig. 2). Because GPI-anchored antigens on K562 cells are not sensitive to PI-PLC, this result could not be confirmed with PI-PLC, and the

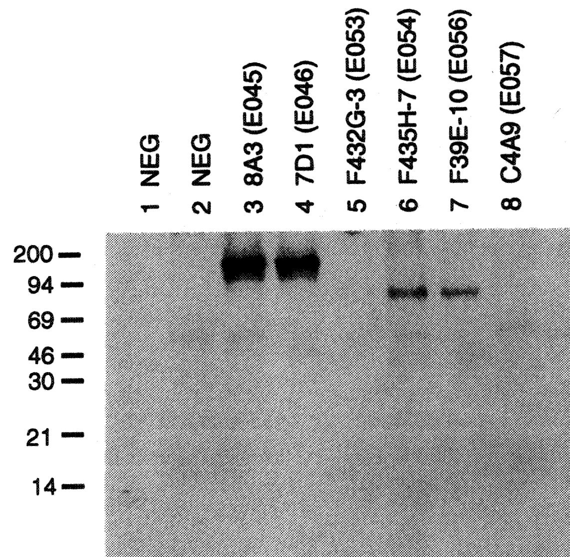

Fig. 3 Immunoprecipitation of ^{125}I surface labelled HUVEC with Endothelial Section mAb that recognize GPI-anchored antigens. Lanes 1 and 2 are negative controls with IgG and IgM primary mAb, respectively.

possibility that the GPI-anchor-deficient K562 clones were missing the S-ENDO 1 antigen on the basis of an altered state of differentiation or an independent mutation was not ruled out. The S-ENDO 1 antigen was not GPI-anchored on 293 cells.

To further examine the antigens recognized by mAb E045, E046, E053, E054, E056, and E057, ^{125}I-labelled HUVEC lysates were immunoprecipitated with 2 μl of undiluted mAb and goat anti-mouse IgG + IgM Sepharose and analysed by 4–15 per cent gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions and autoradiography. The two CDw109 mAB, E045 and E046, precipitated a single band at 150 M_r and S-ENDO 1 mAb, E053, E054, and E056, precipitated a 95 M_r band (Fig. 3). No specific bands were seen with mAb E057 in this experiment, or with [^{35}S] cystein + methionine biosynthetic labelling (data not shown).

Thus, CDw109 and the E057 (C4A9) antigens were identified as novel GPI-anchored antigens on endothelial cells. The S-ENDO 1 antigen is not GPI-anchored on HUVEC or 293 cells, but may be GPI-anchored on the erythroleukaemia line K562. The function of these newly identified

cell surface endothelial antigens remains to be determined.

Acknowledgements

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References

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