Endothelial Cell Antigens Blind Panel Analysis

EC5 Endothelial Cell Blind Panel analysis: Overview and summary

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In the Endothelial Cell Blind Panel 93 monoclonal antibodies (mAb) (E001–E095) were studied. Immunofluorescence flow cytometry of cultured cells was used to cluster all mAb in the Endothelial Cell Blind Panel [Kitayama *et al.*, EC7] and many of these mAb were also studied in the central Workshop Blind Panel. Antigens were immunoprecipitated from tumor necrosis factor (TNF)-stimulated human umbilical vein endothelial cells [Puri *et al.*, EC6]. Immunohistochemistry localized antigen expression in tissues [Hancock, EC8]. The CD Chairs and working group leaders [Springer and Kitayama, EC1, Tables 1 and 2] examined all mAb in the Endothelial Cell Blind Panel for reactivity to the antigens for which they were responsible. Additionally, CD31 and E-selectin transfectants were studied by Dr P. Pizcueta, and vascular adhesion protein (VAP) 1 transfectants by Dr S. Jalkanen. Binding to recombinant CD31 or CD34 molecules was examined by Dr H. Stockinger, and carbohydrate specificities were analyzed by Dr B. Kniep with a Panel of purified glycolipids.

mAb assigned to previously defined CD

CD31

Two mAb with uncharacterized specificities, E050 (RB10) and E076 (Moon-1) clustered by flow cytometry with the CD31 reference mAb E003 (V21). All three mAb bound to CD31 transfectants and to recombinant, purified CD31.

CD62E

E087 (IG9) immunoprecipitated a 125 000 M_r molecule from TNF-stimulated HUVEC and bound to the E-selectin transfectants just as did the reference CD62E mAb E005 (H18/7). E087 specifically stained HUVEC activated with TNFα or interleukin (IL) 1β. E087 is thus a CD62E mAb; however, it stained cells less brightly than did the reference mAb E005, and therefore did not group with it in the Endothelial Cell Panel dendrogram.

CD90

The mAb E046 (5E10), E054 (V45), and A011 (F15-42-1-5) were confirmed to recognize Thy-1 (CD90) with transfectants. These mAb brightly stained high endothelial venules, and focally other endothelial cells, epithelium, and smooth muscle cells (SMC). Flow cytometry on cultured cells showed bright staining of fibroblasts and myeloid cells, and little reactivity with endothelial cells.

CD105

Two mAb submitted with unknown specificity E075 (CLE-4) and E088 (F430C-5) resembled the CD105 reference mAb E008 (44G4) in having a strong reaction with endothelial cells, THP-1 and some stromal cell lines in flow cytometry, and had pan-endothelial reactivity by immunohistochemistry. Immunoprecipitation of a molecule of 90 000 M_r in reduced and 180 000 M_r in non-reduced sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and reactivity with transfectants confirmed their specificity for CD105.

CD109

The mAb E063 (23/5F6), E064 (39/6C3), and E079 (TEA 2/16) were found to be specific for CD109. All of them showed strong reactivity to KG1a and endothelial cells, and stained some stromal and myeloid cell lines. The pattern was identical to the CD109 standard, E010 (8A3). These mAb were pan-endothelial cell in immunohistochemistry, although E079 showed additional staining to some epithelium and SMC.

mAb assigned to new clusters in the Endothelial Cell Panel

CD140a (PDGF receptor α) and CD140b (PDGF receptor β)

The platelet-derived growth factor (PDGF) receptor α and β isoforms were specifically recognized by mAb. E022 (16A1), E031 (alpha-R1), and E090 (PR292) reacted with CD140a, and E023 (28D4) and E089 (PR7212) reacted with CD140b as shown with transfectants. The CD140b isoform but not the CD140a isoform was well enough expressed to be detectable by flow cytometry. mAb to CD140b showed

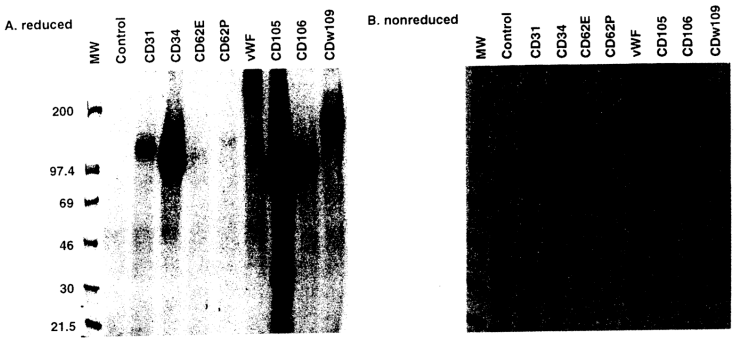

Fig. 1 SDS-PAGE of antigens immunoprecipitated from radioiodinated HUVEC. The HUVEC were cultured on collagen-coated flasks and were stimulated with TNFα (10 ng/ml) for 6 h. Cells were incubated in phosphate-buffered saline (PBS) containing 5 mM EDTA to detach them from the flask, and washed twice with PBS. Typically 5×10^6 cells were labelled with 4 mCi ^{125}I, using Iodogen, and lysed with 2 percent (W/V) Triton X-100, phenylmethylsulfonyl fluoride (PMSF), benzamidine, pepstatin and iodoacetamide. The lysates were dialyzed in 0.1 percent NP-40 and precleared using 187.1 (rat mAb against mouse kappa chain) adsorbed to protein A-Sepharose. Precipitation was carried out by mixing 1.5 μl of ascites or 3 μl of purified mAb or 15 μl of culture supernatant with cell lysate for 1 h at 4 °C followed by 5 min of incubation after the addition of mAb 187.1. Protein A-Sepharose was then added to the complex and the mixture incubated for 4 h at 4 °C. After extensive washing with 0.5 percent Triton X-100, 0.5 percent sodium deoxycholate, 0.01 percent SDS, 10 mM Tris-HC1, 150 mM NaC1 (pH 8.0), the samples were resuspended in SDS sample buffer for gel electrophoresis (non-reducing conditions) or sample buffer supplemented with 10 mM dithiothreitol (reducing conditions), heated to 90 °C and subjected to 5–15 percent gradient SDS-PAGE according to Laemmli. Immunoprecipitates with anti-CD31 mAb E003 (V21), CD34 mAb E004 (581), CD62E mAb E005 (H18/7), CD62P mAb E006 (G1), von Willebrand factor (vWF) mAb E007 (21/43), CD105 mAb E008 (44G4), CD106 mAb E009 (2G7), and CD109 mAb E010 (8A3).

with ^{125}I. Immunoprecipitates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing and non-reducing conditions.

The apparent molecular weights (M_r) of endothelial cell antigens precipitated by reference antibodies (Fig. 1) under reducing and non-reducing conditions, respectively were: CD31, 130 000 and 130 000; CD34, 115 000 and 115 000; CD62E, 125 000 and 135 000; CD62P, 150 000 and 155 000; von Willebrand factor (vWF), 252 000 and 250 000; CD105, 94 000 and 180 000; CD106, 118 000 and 92 000; and CD109, 175 000 and 180 000. Workshop antibodies were similarly analyzed and the antibodies that gave similar gel

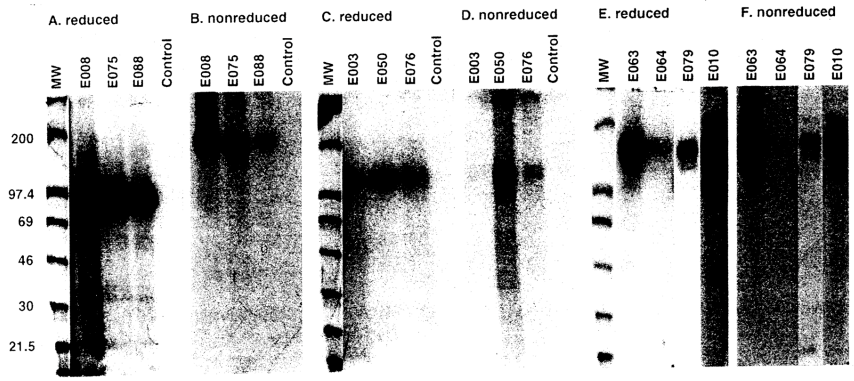

Fig. 2 Comparison of reference antigens with the immunoprecipitated structures. Immunoprecipitations and SDS-PAGE analysis were as in Fig. 1. (A) and (B) Endoglin (CD105) mAb; (C) and (D) CD31 mAb; (E) and (F) CD109 mAb.

patterns were run side by side on gels as much as possible. Several antibodies were grouped to known and unknown specificities on the basis of the molecular weight analysis.

Antibodies E008 (44G4), E075 (CLE-4), and E088 (F430C-5) all precipitated endoglin (CD105) (Fig. 2A and B). Human endoglin is a disulfide-linked homodimeric transmembrane glycoprotein of subunits of 95 000 M_r and is present at high levels on HUVEC [1].

Antibodies E003 (V21), E050 (RB10), and E076 (Moon-1) precipitated the CD31 antigen (Fig. 2C and D).

Antibodies E010 (8A3), E063 (23/5F6), E064 (39/6C3), and E079 (TEA 2/16) all precipitated CD109, a major structure of 170 000 M_r and a minor structure of 150 000 M_r (Fig. 2E and F). CD109 is a monomeric glycoprotein and contains two N-linked Endo-H-sensitive glycans. Peptide mapping of the minor band at 150 kDa has shown that it is closely related to the 170 000 M_r glycoprotein and may be derived by degradation or post-translational modifications [2].

CD146 or S-ENDO/Muc 18 is a 125 000 M_r member of the immunoglobulin gene superfamily and is strongly expressed on endothelial cells. Seven antibodies, E057 (F4-35H7), E058 (F439E), E059 (F432G-3), E065 (541/10B2), E066 (541/2E5), E067 (OJ79), and E077 (TEA 1/34) recognized this antigen of 125 000 M_r (Fig. 3A and B).

Three antibodies, E013 (1A4), E025 (TMmAb20), and E045 (KA-4) precipitated a 100-kDa band corresponding to thrombomodulin (CD141) (Fig. 3C and D).

Four antibodies to VE-cadherin (CD144), E021 (BV6), E033 (hec1), E042 (TEA 1/31), and E092 (55-7H1) all recognized a major structure of 120 000 M_r (reduced and non-reduced) (Fig. 3E and F).

Three antibodies to angiotensin-converting enzyme (CD143), E084 (9B9), E085 (i2H5), and E086 (3A5) immunoprecipitated an antigen of 160 000 M_r (reduced) and 168 000 M_r (non-reduced) (Fig. 3G and H).

Platelet-endothelial cell tetraspan antigen 3 (PETA-3, CD151) is a member of the tetraspan family. Two antibodies to CD151, E011 (11B1.G4) and E012 (14A2.H1) recognized a structure of 28 000 M_r (reduced and non-reduced) (Fig. 3I and J).

Antibodies E055 (BNH-9) and E056 (BNF-13) react with blood group H-associated carbohydrate antigens and recognized broad bands at 125 and 90 (Fig. 3K and L).

The antibodies E017 (M72), E078 (TEA 1/8), and E081 (B-K4) that appear to be CD13-like, precipitated two bands of 210 and 145 kDa under reducing and non-reducing conditions (Fig. 3 M and N); however, the structure at 210 kDa was not consistently present in different precipitations.

Antibodies to tissue factor (CD142), E016 (HTF1-7B8), E032 (VI-C7), E034 (HTF-K108), and E034 (HTF-K180) were weak precipitators, except E034 which strongly precipitated a 45 000 M_r structure.

Unclustered mAb are shown in Fig. 4A and B. E014 (Sy12) precipitated three bands at 56, 43, and 25 kDa. mAb

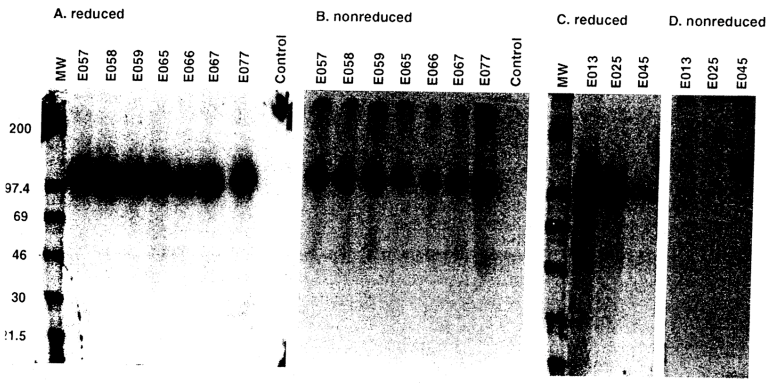

Fig. 3 A–D: Side-by-side comparisons of immunoprecipitated antigens. Procedures are as described in Fig. 1. (A) and (B) S-ENDO/luc 18 (CD146) mAb; (C) and (D) thrombomodulin (CD141) mAb. *Continued on next page.*

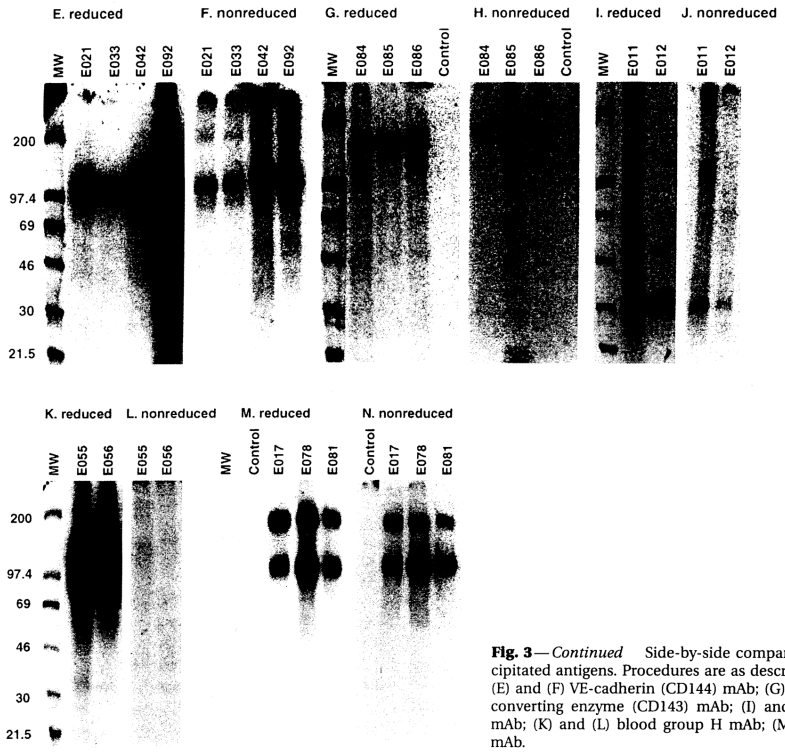

Fig. 3—*Continued* Side-by-side comparisons of immunoprecipitated antigens. Procedures are as described in Fig. 1A and B. (E) and (F) VE-cadherin (CD144) mAb; (G) and (H) angiotensin-converting enzyme (CD143) mAb; (I) and (J) PETA-3 (CD151) mAb; (K) and (L) blood group H mAb; (M) and (N), CD13-like mAb.

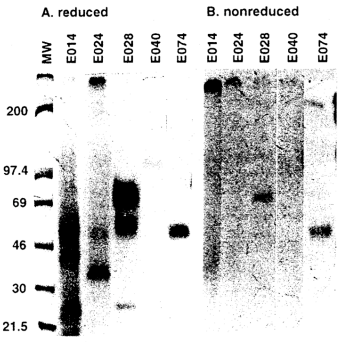

Fig. 4 SDS-PAGE analysis of the antigens immunoprecipitated by the Endothelial Cell Panel of monoclonal antibodies with unique specificities (procedures are as described in Fig. 1).

E024 (BU96) to peripheral myelin protein 22 precipitated a major band at 35 kDa and a minor band at about 50 kDa. E028 (8H2) precipitated a major structure at 77 kDa and a minor structure at 55 kDa, consistent with possible recognition of CD39. E040 (SN12) precipitated a 100 000 M_r structure. E074 (CLE-1) precipitated a structure of 55 000 M_r (reduced and non-reduced).

References

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