2. The mAb tested recognize epitopes that do not play a relevant role in CD144 biological activity.
3. The mAb do not have easy access to biologically relevant epitopes, once the junction is organized.

The specificity of endothelial cell recognition was confirmed for all the mAb tested in the Panel.

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EC14 CDw145 Workshop Panel report

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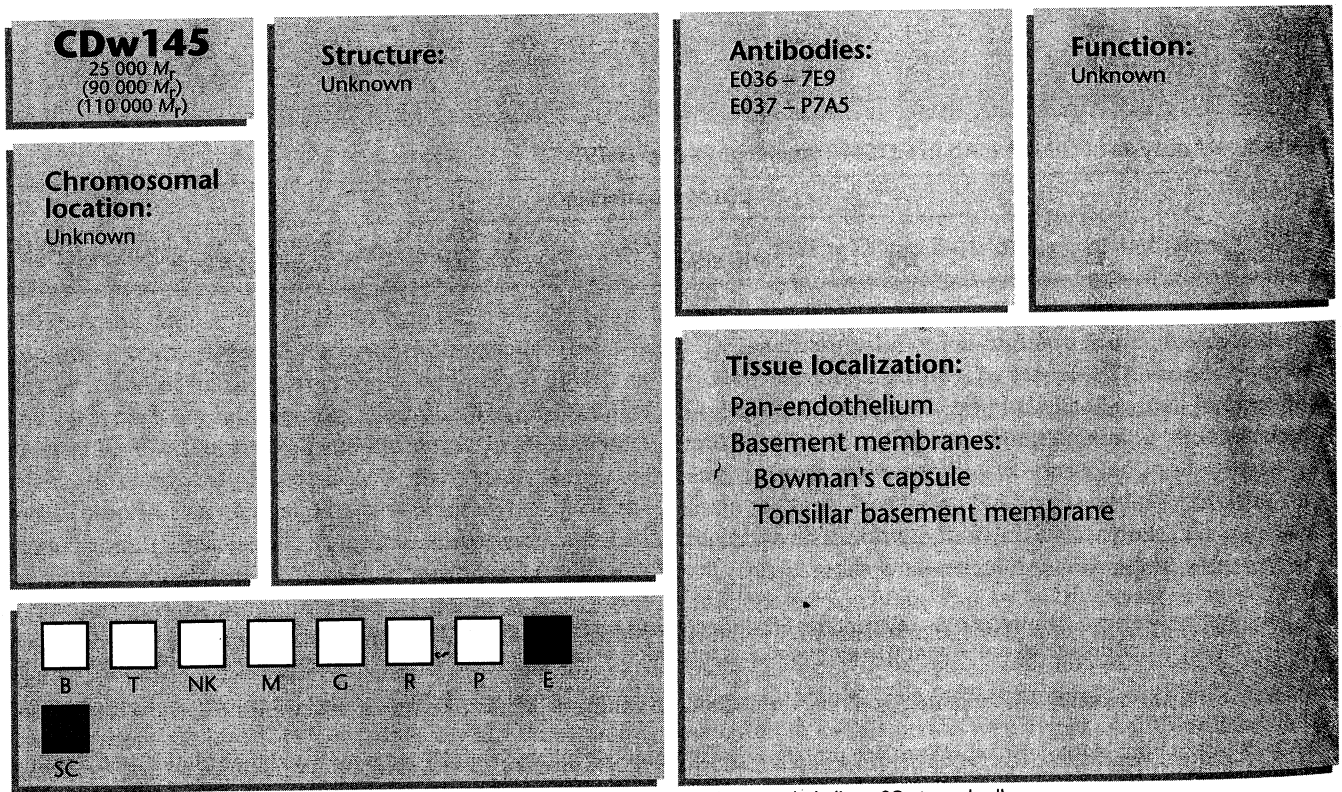

B, B cell; T, T cell; NK, NK cell; M, monocyte; G, granulocyte; R, red blood cell; P, platelet; E, endothelium; SC, stromal cell

negative weakly positive positive

Two mouse monoclonal antibodies (mAb) from the Endothelial Cell Blind Panel, E036 (7E9, IgG3) and E037 (P7A5, IgG1), that were originally raised as mAb to urinary bladder carcinoma [1,2], constitute a new endothelial-based cluster, based upon analysis by flow cytometry, immunoprecipitation and immunohistology.

Both mAb stained human umbilical vein endothelial cells (HUVEC) brightly and some stromal cell lines moderately. Staining intensities on HUVEC were slightly decreased by activation with tumor necrosis factor α, interleukin 1β, and phorbol myristate acetate. None of the hemopoietic cell lines tested by flow cytometry reacted with these mAb. Immunoprecipitation from ^{125}I-labelled HUVEC showed one intense band of 25 kDa, and two additional bands of 90 and 100 kDa in reducing and non-reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Immunoperoxidase analysis of paraformaldehyde-lysine-periodate fixed cryostat sections of normal human kidney, liver, lung, spleen, tonsil and gut showed that both mAb gave strong labelling of endothelial cells (EC) in all tissues tested, as well as staining of specific basement membranes (Bowman's capsule, tonsillar basement membrane). Within each tissue, both mAb gave strong, uniform staining of EC, including that of arteries, arterioles, capillaries, high endothelial and regular venules, and veins, whereas other cell types were unstained. Neither mAb stained corresponding formalin-fixed and paraffin-embedded tissue sections, and in both cases, vessels within sections of kidneys subject to acute inflammation (renal allograft rejection), or atherosclerotic arteries, showed preservation or even enhanced immunoreactivity. Thus, this pair of mAb can best be summarized as pan-EC markers.

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EC15 CD146 (S-ENDO/Muc 18) Workshop Panel report

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CD146, the S-ENDO/Muc 18 antigen (Ag) was initially identified with Muc 18 monoclonal antibody (mAb) as a marker of melanoma progression (1,2,3). cDNA cloning and sequencing revealed that the Ag is a member of the immunoglobulin superfamily named melanoma cell adhesion molecule (MCAM) [4,5]. More recently, S-ENDO/Muc 18 Ag was defined as a molecule constitutively expressed in all types of human endothelial cells [6,7,8,9].

Reactivity on transfectants and purified Ag

Twentyeight mAb were recruited and distributed to six laboratories for analysis by different techniques (Table 1). These mAb were screened on Muc 18 transfectants by flow cytometry (laboratories 1 and 4) and on Muc 18 Fc protein by enzyme-linked immunosorbent assay (ELISA) (laboratory 5). Sixteen mAb showed a strong reactivity in flow cytometry as well as in ELISA, whereas Muc BA 18.4, E1-4E3, and E078 (TEA 1/8) presented a weaker reactivity (Table 2). Disparate results obtained for E066 (541/2E5), E067 (OJ79), E068 (OJ91), 1, MN15, MN18, MN19 can be due to differences in sensitivity of the method and/or variability of antigen conformation leading to differences in mAb binding.

Tissue expression

On normal tissues (umbilical cord, striated muscle, lymph node) most of the mAb reacted with endothelial cells whatever the vessel type (arteries, venules, high endothelial venules). The expression of the molecule was not restricted to the endothelium since vessel wall, Schwann cells and occasionally striated muscle fibers showed positive labelling with some antibodies. In lymph node, a noticeable finding was the weak staining of the germinal center (laboratory 2). On several pathological tissue sections (lymph node metastasis of malignant melanoma, glioma, hemangiopericytoma, cavernoma), most of the mAb tested were