EC1 Endothelial Cell Antigens: Section report

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Organization

The Endothelial Cell Section was first included in the Fifth Workshop because of the growing recognition of the physiological importance of endothelial cell antigens in development, host immune responses and maintenance of homeostasis. Furthermore, many endothelial cell antigens are shared with platelets, which have had a Section in the Workshop for some time, and many endothelial surface molecules interact with surface molecules on leucocytes. In the last Workshop chaired by John Harlan, three new CDs, CD105 (endoglin), CD106 (VCAM-1), and CDw109 were assigned. A total of 57 monoclonal antibodies (mAb) was studied. We recruited a total of 126 mAb, and targeted ten different well-characterized endothelial cell antigens as "pre-CDs" (Tables 1 and 2). Eight new CDs were assigned. Five different mAb Panels were studied: an Endothelial Blind Panel, a CD105 Panel, a CD106 Panel, a CDw109 Panel, and a tissue factor (CD142) Panel.

Since there is little tradition in the endothelial cell community of participation in the leucocyte Workshop, submissions were actively recruited. Letters were sent to 151 experts in the field of endothelial cell biology for suggestions on Blind Panel antibodies, target antigens, and contributing laboratories, and to ask for participation in the Workshop. The literature was searched for mAb to endothelial surface molecules. Letters soliciting donation of mAb were sent to 153 mAb producers with the "Antibody Submission Form" in April 1995. Some laboratories sent the "Antibody Submission Form" to the Endothelial Cell Workshop Committee in response to advertisements and mailings from the Central Workshop Committee. Finally, 95 mAb were accepted in July 1995. The donors of the mAb were sent screw cap conical 0.15-ml tubes (USA Scientific) and 1 ¼ in. × 9/16 in. labels (Lion Labels, South Easton, MA) with Workshop codes and asked to divide in equal aliquots of 15 ml of ascitic fluid with a titer > 1:1000 or 15 ml of 1 mg/purified mAb/ml. A total of 93 mAb (two mAb were withdrawn) were submitted to the Endothelial Cell Panel by October 1995. The Panel included 10 reference mAb and 83 mAb reported to react with previously unclustered or unknown surface molecules.

Table 1 Endothelial Panels

Panel	New CD	Chair	mAb
Endothelial Panel		Timothy Springer	EC001–EC097
CD105		Michelle Letarte	EC101–EC109
CD106		Walter Newman	EC111–EC112
CDw109	CD109	Robert Sutherland	EC121–EC128
Tissue factor	CD142	James Morrissey	EC131–EC142

Table 2 Working Groups

Working Group	New CD	Leader
CDw90 (Thy-1)	CD90	Rachael Clark
PDGF receptor α	CD140a	Charles Hart
PDGF receptor β	CD140b	Charles Hart
Thrombomodulin	CD141	Phil Bird
Angiotensin-converting enzyme	CD143	Sergei Danilov
VE-cadherin	CD144	Elisabetta Dejana
S-ENDO/Muc 18	CD146	Françoise Dignat-George, David Simmons
Neurothelin/basigin	CD147	Hannes Stockinger
P-glycoprotein		Isamu Sugawara
MECA-79		Ellen Berg

PDGF, platelet-derived growth factor.

The Endothelial Cell Blind Panel antibodies were distributed to 48 laboratories for evaluation in November 1995, and an additional 30 sets of mAb were contributed to the Central Blind Panel. Two mAb (E096 (5G9), E097 (8C7)) were additionally accepted and submitted after the distribution of Endothelial Cell Panels, which were sent to the Central Blind Panel study. All mAb were sent blinded with information on species and subclass of each mAb. Fluorescein isothiocyanate (FITC)-anti murine IgG (courtesy of Caltag Inc.) and calibration beads (courtesy of Flow Cytometry Standards Inc.) were provided so that the flow results could be standardized. The Chairs of the CD105, CD106, CDw109, and tissue factor (CD142) Panels distributed mAb in the same way.

The results of flow cytometric, immunoprecipitation, immunohistochemical, transfectant, and other studies are summarized in the following chapters. The CD Chairs (Table 1) and working group leaders (Table 2) used transfectants, recombinant molecules, or purified molecules to test all mAb in the Workshop on the antigen specificity for which they were responsible. By combined efforts, the specificity of most mAb could be assigned. The characteristics of antibodies are summarized in order of Workshop code (Table 3) and specificity (see Kitayama and Springer, EC5, Table 1). The important contributions of the antibody submitters and evaluators are gratefully acknowledged in Table 4.

Table 3 Antibodies in the Endothelial Cell Section

Workshop mAb Code	Clone Name	Donor	Species	Isotype	Other WS Code*	Characterization	Reference
E001	Mouse IgM	Coulter Corp.	Mouse	M		Control antibody	
E002	Mouse IgG1	Coulter Corp.	Mouse	G1		Control antibody	
E003	V21	Clark	Mouse	G2a		CD31	
E004	581	Gaudernack	Mouse	G1κ	V:M027	CD34	
E005	H18/7	Kawahara	Mouse	G2a	V:BP212, E049, S045	CD62E (E-selectin)	[1]
E006	G1	McEver	Mouse	G1	V:E018, P057, BP200	CD62P (P-selectin)	[2]
E007	21-43	Francis	Mouse	G1		von Willebrand factor	[3]
E008	44G4	Letarte	Mouse	G1	VI:BP222, BP270	CD105 (endoglin)	
E009	2G7	Newman	Mouse	G1	VI:BP223	CD106 (VCAM-1)	[4]
E010	8A3	Sutherland	Mouse	G2κ	VI:BP224	CD109	[5,6]
E011	11B1.G4	Ashman	Mouse	G2a	VI:BP225, N-L071, P048	CD151 (PETA-3)	
E012	14A2.H1	Ashman	Mouse	G1	V:BP312, E017, P005, VI:P049	CD151 (PETA-3)	[7–9]
E013	1A4	Bird	Mouse	G1κ	VI:BP226	CD141 (thrombomodulin)	[10–12]
E014	Sy12	Bovin	Mouse	G	VI:BP227	Unique	[13]
E015	67A4	Bühring	Mouse	G1		E-cadherin	
E016	HTF1-7B8	Carson	Mouse	G1κ		CD142 (tissue factor)	[14]
E017	M72	Clark	Mouse	M	VI:BP244, A125	CD13?	
E018	7Z1	Clark	Mouse	M	VI:BP245	Unique	
E019	V34	Clark	Mouse	M	VI:BP246	Unknown glycolipid	
E020	BV9	Dejana	Mouse	G2a		CD144 (VE-cadherin)	[15,16]
E021	BV6	Dejana	Mouse	G2a	VI:BP228	CD144 (VE-cadherin)	[16,17]
E022	16A1	Bühring	Mouse	G1		CD140a (PDGFR-α)	
E023	28D4	Bühring	Mouse	G2a		CD140b (PDGFR-β)	
E024	BU96	Hardie	Mouse	G2b	VI:B038, BP247	Peripheral myelin protein PMP-22	
E025	TMmAb20	Ishii	Mouse	G1		CD141 (thrombomodulin)	[18]
E026	104-9	Jalkanen	Rat	G1		Weak reactivity	
E027	10G7	Koch	Mouse	G3	V:BP226, M11, VI:BP248	Negative	[19–21]
E028	8H2	Koch	Mouse	G1	VI:BP249	CD39-like	[19–21]
E029	8D7	Koch	Mouse	G1	V:BP211, E029, E048, M13, VI:BP250	Unique	[19,20, 22–25]
E030	4A11	Koch	Mouse	M	V:BP205, E030, E031, F102, VI:BP251, A101	$Lewis^y$	[26–28]
E031	alpha-R1	LaRochelle	Mouse	G2a	VI:BP229	CD140a (PDGFR-α)	[29,30]
E032	VI-C7	Muller	Mouse	G1	VI:BP230	CD142 (tissue factor)	[31–33]
E033	hec1	Muller	Mouse	G2a		CD144 (VE-cadherin)	[34]
E034	HTF-K108	Nakamura	Mouse	G1κ		CD142 (tissue factor)	[35]
E035	HTF-K180	Nakamura	Mouse	G1κ		CD142 (tissue factor)	[36]
E036	7E9	Paulie	Mouse	G3	VI:BP252	CDw145	[37–39]
E037	P7A5	Paulie	Mouse	G2a	VI:BP253	CDw145	[40]
E038	B148.4	Perussia	Mouse	G1	VI:BP254	CD93?	[41]
E039	LIA 1/14	Sánchez-Madrid	Mouse	G1	VI:BP255	CD93?	
E040	SN12	Seon	Mouse	G1	VI:BP256	Similar to A115, A124, MGC-24-like	
E041	MRK16	Sugawara	Mouse	G2a	VI:BP231	P-glycoprotein	[42]
E042	TEA 1/31	van Agthoven	Mouse	G1κ		CD144 (VE-cadherin)	[43]
E043	B-B2	Vermot-Desroches	Mouse	G2b	VI:BP232	CD138 (syndecan-1)	
E044	B-B4	Vermot-Desroches	Mouse	G1	VI:BP233	CD138 (syndecan-1)	
E045	KA-4	Aoki	Mouse	G		CD141 (thrombomodulin)	[44–46]

Continued on next page

Table 3 — *Continued*

Workshop mAb Code	Clone Name	Donor	Species	Isotype	Other WS Code*	Characterization	Reference
E046	5E10	Lansdorp	Mouse	G1		CD90 (Thy-1)	
E047	MECA-79	Butcher	Rat	M	VI:BP546	Peripheral node addressin	[47,48]
E048	PNAdJG-1	Butcher	Rat	G	VI:BP547	Peripheral node addressin	
E049	RB8	Butcher	Mouse	G	VI:BP257	Unique	
E050	RB10	Butcher	Mouse	G		CD31	
E051	RB11	Butcher	Mouse	G	VI:BP258	Like P-50, A103	
E053	JG2	Butcher	Rat	G	VI:BP549	VAP-1	
E054	V45	Clark	Mouse	G1	VI:BP234	CD90 (Thy-1)	
E055	BNH-9	Delsol	Mouse	M	V:A139, VI:BP259, N-L003	H type 1 + H & Y type 2 blood group	[49–51]
E056	BNF-13	Delsol	Mouse	G2a	VI:BP260	H type 1 + H & Y type 2 blood group	[49]
E057	F4-35H7	Dignat-George	Mouse	G1	VI:BP235, A111	CD146 (S-ENDO/Muc 18)	[52–54]
E058	F439E	Dignat-George	Mouse	G1	VI:A113	CD146 (S-ENDO/Muc 18)	[52–54]
E059	F432G-3	Dignat-George	Mouse	G1	VI:A112	CD146 (S-ENDO/Muc 18)	[52–54]
E060	RF3	Hirano	Mouse	G2a	VI:BP236, N-L117	CD157 (BST-1)	[55–57]
E061	BEC7	Hirano	Mouse	G1	VI:N-L118	CD157 (BST-1)	
E062	AAA1	Knapp	Mouse	G1	VI:BP237	CD147 (basigin/neurothelin)	[58,59]
E063	23/5F6	Knapp	Mouse	G2a		CD109	
E064	39/6C3	Knapp	Mouse	G1		CD109	
E065	541/10B2	Knapp	Mouse	G1		CD146 (S-ENDO/Muc 18)	
E066	541/2E5	Knapp	Mouse	G1		CD146 (S-ENDO/Muc 18)	
E067	OJ79	Simmons	Mouse	G1		CD146 (S-ENDO/Muc 18)	
E068	OJ91	Simmons	Mouse	G1		CD146 (S-ENDO/Muc 18)	
E070	38.13	Wiels	Rat	M	IV:B5, V:CD77.1, VI:BP548	CD77?	[60–62]
E071	64C7	Cass	Mouse	G	VI:BP236	(Glut-1)†	[63]
E072	C3	Kumar	Mouse	G	VI:BP262	CD49c?	
E073	CLE6	Kumar	Mouse	G	VI:BP263	?	
E074	CLE-1	Kumar	Mouse	G	VI:BP264	?	
E075	CLE-4	Kumar	Mouse	G		CD105 (endoglin)	
E076	Moon-1	Malavasi	Mouse	G1		CD31	
E077	TEA 1/34	Sánchez-Madrid	Mouse	G2a		CD146 (S-ENDO/Muc 18)	
E078	TEA 1/8	Sánchez-Madrid	Mouse	G2a	VI:BP265	CD13?	
E079	TEA 2/16	Sánchez-Madrid	Mouse	G1	VI:BP266	CD109	
E080	TEA 2/5	Sánchez-Madrid	Mouse	G1	VI:BP267	Similar to 6T-047, 6T-105	
E081	B-K4	Vermot-Desroches	Mouse	G1	VI:BP268	CD13?	
E082	10H4	David	Mouse			Unique	
E083	65D4	Cass	Mouse	G	VI:BP269	(Glut-1)†	
E084	9B9	Danilov	Mouse	G1	VI:BP239	CD143 (angiotensin conv. enz.)	[64–66]
E085	i2H5	Danilov	Mouse	G1		CD143 (angiotensin conv. enz.)	[64,65]
E086	3A5	Danilov	Mouse	G1		CD143 (angiotensin conv. enz.)	[64,65]
E087	IG9	Berman	Mouse	G3		CD62-E (E-selectin)	[67]
E088	F430C-5	Dignat-George	Mouse	G2a	VI:A025	CD105 (endoglin)	[52]
E089	PR7212	Hart	Mouse	G1	VI:BP240	CD140b (PDGFRβ)	[68,69]
E090	PR292	Bowen-Pope	Mouse	G1		CD140a (PDGFRα)	[70]
E091	C4A9.2.3	Klickstein	Mouse	M	V:E057	?	
E092	55-7H1	Gamble	Mouse	G1κ		CD144 (VE-cadherin)	
E093	C219	McCabe	Mouse	G2a		P-glycoprotein	
E094	4 E3	Arceci	Mouse	G2a		P-glycoprotein	[71]

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Table 3 Antibodies in the Endothelial Cell Section — *Continued*

Workshop mAb Code	Clone Name	Donor	Species	Isotype	Other WS Code*	Characterization	Reference
E095	JSB-1	Scheper	Mouse	G1	VI:BP241	P-glycoprotein	[72]
E096	5G9	Bernfield	Mouse	G2a	VI:BP242	(Syndecan-4)†	
E097	8C7	Bernfield	Mouse	G1	VI:BP243	(Syndecan-4)†	
E101	K22-2H7	Boyd	Mouse	G1		CD105 (endoglin)	
E102	43A3	Bühring	Mouse	G1		CD105 (endoglin)	
E104	IMMU-40.19	Hirn	Mouse	M		CD105 (endoglin)	
E105	TEA 3/17	Sánchez-Madrid	Mouse			CD105 (endoglin)	
E106	F430C-5	Dignat-George	Mouse	G2a	VI:E088,A025	CD105 (endoglin)	[52]
E107	29-G8	Rokhlin	Mouse	Mκ		CD105 (endoglin)	[73]
E108	SN6	Seon	Mouse	G1		CD105 (endoglin)	[74–76]
E109	SN6h	Seon	Mouse	G1		CD105 (endoglin)	[74,76]
E111	Hu 8/4	Cybulsky	Mouse	G1		CD106 (VCAM-1)	
E112	51-10C9	Vadas	Mouse	G1κ		CD106 (VCAM-1)	
E121	D2	Finberg	Mouse	G1		CD109	[77]
E122	LDA1	Suciu-Foca	Mouse	G		CD109	[78]
E123	7D1	Sutherland	Mouse	G1κ		CD109	[5,6]
E124	40B8	Bühring	Mouse	G1	V:BP175 and C023	CD109	
E125	1B3	Bernstein	Mouse			CD109	
E126	59D6	Bühring	Mouse	M		CD109	
E127	8A1	Sutherland	Mouse	G1		CD109	
E128	7C5	Sutherland	Mouse	G1		CD109	
E131	MTFH-1	Morrissey	Mouse	G1		CD142 (tissue factor)	
E132	TF9-5B7	Morrissey	Mouse	G1		CD142 (tissue factor)	[79]
E133	TF9-10H10	Morrissey	Mouse	G1		CD142 (tissue factor)	[79]
E134	HTF-K4	Nakamura	Mouse	G1		CD142 (tissue factor)	
E135	HTF-K14	Nakamura	Mouse	G1		CD142 (tissue factor)	
E136	HTF-K108	Nakamura	Mouse	G1		CD142 (tissue factor)	
E137	HTF1-7B8	Carson	Mouse	G1		CD142 (tissue factor)	[80]
E138	TF8-5G9	Edgington	Mouse	G1		CD142 (tissue factor)	[79]
E139	TF10-1D10	Edgington	Mouse	G1		CD142 (tissue factor)	
E140	III-D8	Albrecht/Luther	Mouse	G1		CD142 (tissue factor)	[81]
E141	V-D8	Albrecht/Luther	Mouse	G1		CD142 (tissue factor)	[81]
E142	VI-C7	Albrecht/Luther	Mouse	G1	VI:BP230	CD142 (tissue factor)	[81]

*V, Fifth Workshop; VI, Sixth Workshop; BP, Blind Panel (note actual Blind Panel codes do not start 'BP'); VCAM-1, vascular cell adhesion molecule 1; PETA-3, platelet-endothelial cell tetraspan antigen 3; PDGFR-α/β, platelet-derived growth factor α/β; angiotensin conv. enz., angiotensin-converting enzyme.
†(): specificity assigned by donor.

Table 4 Donors and Evaluators of the Endothelial Cell Blind Panel

Donor/ Evaluator	Institution	Department	City	Country	Workshop Codes and Clone Names
Agis	University of Vienna	Department of Internal Medicine I	Vienna	Austria	
Aoki	Saitama Medical School	1st Dept. of Medicine	Saitama	Japan	E045(KA-4)
Arceci	Children's Hospital Medical Center	Hematology/Oncology	Cincinnati, OH	USA	E094(4 E3)
Armetta	Facolta' di Medicina e Chirurgia	Instituto di Patologia Generale e Oncologia	Napoli	Italy	
Ashman	Institute of Medical & Veterinary Science	Division of Haematology	Adelaide	Australia	E011(11B1.G4), E012(14A2.H1)
Autschbach	German Cancer Research Center	Department of Applied Immunology	Heidelberg	Germany	
Berg	Stanford University School of Medicine	Department of Pathology	Stanford, CA	USA	
Berman	Albert Einstein College of Medicine	Department of Pathology, Forchheimer 516	Bronx, NY	USA	E087(IG9)
Bernabeu	Centro de Investigaciones Biologicas		Madrid	Spain	
Bernfield	The Children's Hospital, Harvard Medical School	Joint Program in Neonatology	Boston, MA	USA	E096(5G9), E097(8C7)
Berti	University of Milan	Institute of Dermatologic Sciences	Milan	Italy	
Bird	Monash University	Department of Medicine	Box Hill	Australia	E013(1A4)
Bovin	Russian Academy of Sciences	Shemyakin Institute of Bioorganic Chemistry	Moscow	Russia	E014(Sy12)
Bowen-Pope	University of Washington	Department of Pathology	Seattle, WA	USA	E090(PR292)
Boyd	Walter and Eliza Hall Institute of Medical Research	Lion's Cancer Rersearch Laboratory	Victoria	Australia	E101(K22-2H7)
Bühring	Medizinische Klinik II	FACS-Labor	Tübingen	Germany	E015(67A4), E022(16A1), E023(28D4), E102(43A3), E124(40B8)
Butcher	Stanford University School of Medicine	Department of Pathology, L235	Stanford, CA	USA	E047(MECA-79), E048(PNAdJG-1), E049(RB8), E050(RB10), E051(RB11), E053(JG2)
Callard	University of London	Institute of Child Health	London	UK	
Carson	University of Nebraska Medical Center	Dept. of Pathology and Microbiology	Omaha, NE	USA	E016(HTF1-7B8)
Cass	University of Alberta	Department of Biochemistry	Edmonton, Alberta	Canada	E071(64C7), E083(65D4)
Charron	Hôpital Saint Louis	Laboratorie d'Immunologie et d'Histocompatibilité	Paris	France	
Clark	Harvard Medical School	Center For Blood Research & Department of Immunology	Boston, MA	USA	E003(V21), E017(M72), E018(7Z1), E019(V34), E054(V45)
Cybulsky	Brigham and Women's Hospital		Boston, MA	USA	E111(Hu 8/4)
Danilov	University of Illinois at Chicago	Laboratory of Peptide Research, Department of Pharmacology	Chicago	USA	E084(9B9), E085(i2H5), E086(3A5)
David	Catholic University of Leuven	Center For Human Genetics	Leuven	Belgium	E082(10H4)
de Landazuri	Hopital de la Princessa	Servicio de Immunologia	Diego de Leon	Spain	
Dejana	Instituto Di Richerche Farmacologiche Mario Nergi	Vascular Biology Laboratory	Milan	Italy	E020(BV9), E021(BV6)

Continued on next page

Table 4 Donors and Evaluators of the Endothelial Cell Blind Panel—*Continued*

Donor/ Evaluator	Institution	Department	City	Country	Workshop Codes and Clone Names
Delsol	Centre Hospitalier Universitaire Purpan	Laboratiore d'Anatomie et de Cytologie Pathologiques	Toulouse	France	E055(BNH-9), E056(BNF-13)
Dignat-George	Faculté de Pharmacie	Laboratoire d'Hématologie et d'Immunohématologie	Marseille	France	E057(F4-35H7), E058(F439E), E059(F432G-3), E088, E106(F430C-5)
Fainboim	Hospital ce Clinicas	Lab. Immunogentica	Buenos Aires	Argentina	
Fanslow	Immunex Corporation		Seattle, WA	USA	
Faure	Groupe de Recherche en Immunopathologie	Laboratoire d'Immunologie Faculté de Medicine	Vandoeuvre	France	
Finberg	Dana-Farber Cancer Institute		Boston, MA	USA	E121(D2)
Francis	Royal Prince Alfred Hospital	Haematology Department	Camperdown NSW	Australia	E007(21-43)
Franke	Justus-Liebig University	Department of Pathology	Giessen	Germany	
Fuggle	University of Oxford	Nuffield Department of Surgery	Oxford	UK	
Gamble	Institute of Medical & Veterinary Science	Hanson Centre for Cancer Research	Adelaide	Australia	E092(55-7H1)
Gattei	Centro di Riferimento Oncologico	The Leukemia Unit	Aviano	Italy	
Gaudernack	The Norwegian Radium Hospital	Institute for Cancer Research	Oslo	Norway	E004(581)
Haraldsen	University of Oslo, Institute of Pathology	Laboratory for Immunohistochemistry and Immunopathology	Oslo	Norway	
Hardie	University of Birmingham Medical School	Department of Immunology	Edgbaston, Birmingham	UK	E024(BU96)
Harlan	University of Washington Harborview Medical Center	Hematology	Seattle, WA	USA	
Hart	ZymoGenetics, Inc.		Seattle, WA	USA	E089(PR7212)
Heidaran	National Institutes of Health		Bethesda, MD	USA	
Hirano	Biomedical Research Center	Division of Molecular Oncology	Osaka	Japan	E060(RF3), E061(BEC7)
Hirn	Immunotech		Marseille	France	E104(IMMU-40.19)
Hollenbaugh	Bristol-Myers Squibb		Seattle, WA	USA	
Horton	University College London Medical School	Department of Medicine	London	UK	
Ishii	Teikyo University	Department of Biochemistry, Faculty of Pharmaceutical Sciences	Kanagawa	Japan	E025(TMmAb20)
Jalkanen, Markku T.	University of Turku	Centre for Biotechnology	Turku	Finland	E026(104-9)
Jalkanen, Sirpa T.	University of Turku	MediCity Research Laboratory	Turku	Finland	
Jonker	Biomedical Primate Research Center		Rijswijk	The Netherlands	
Kamoun	University of Pennsylvania	Pathology and Laboratory Medicine	Philadelphia, PA	USA	
Kawahara	Becton Dickinson Cellular Imaging Systems		San Jose, CA	USA	E005(H18/7)
Klickstein	Brigham and Woman's Hospital		Boston, MA	USA	E091(C4A9.2.3)

Knapp	University of Vienna	Institute of Immunology	Vienna	Austria	E062(AAA1), E063(23/5F6), E064(39/6C3), E065(541/10B2), E066(541/2E5)
Kniep	Gasellschaft für Biotechnologische Forschung		Braunschweig	Germany	
Koch	Northwestern University Medical School		Chicago, IL	USA	E027(10G7), E028(8H2), E029(8D7), E030(4A11)
Kumar	Christie Hospital	Clinical Research Lab	Manchester	UK	E072(C3), E073(CLE-6), E074(CLE-1), E075(CLE-4)
Lansdorp	Terry Fox Laboratory		Vancouver, British Columbia	Canada	E046(5 E10)
LaRochelle	National Cancer Institute	Laboratory of Cellular and Molecular Biology	Bethesda, MD	USA	E031(alpha-R1)
LeBien	University of Minnesota		Minneapolis, MN	USA	
Letarte	The Hospital for Sick Children	Division of Immunology & Cancer Research	Toronto, Ontario	Canada	E008(44G4)
Lo Pardo	Hospital A-Cardarelli	Servizio di Immunoematologia	Naples	Italy	
Malavasi	University of Toronto	Laboratory of Cell Biology	Torino	Italy	E076(Moon-1)
Malizia	OSP. V. Cervello	Divisione de Medicina	Palermo	Italy	
McCabe	Centocor		Malvern, PA	USA	E093(C219)
McEver	University of Oklahoma HSC	Department of Medicine	Oklahoma City, OK	USA	E006(G1)
Molinari	Facolta' di Medicina e Chirurgia	Instituto di Patologia Generale e Oncologia	Napoli	Italy	
Monk	University of Sheffield	Department of Molecular Biology & Biotechnology	Sheffield	UK	
Morrissey	Oklahoma Medical Research Foundation	Cardiovascular Biology Research	Oklahoma City, OK	USA	
Müller	Universitätsklinikum Dresden	Institut für Pathologie	Dresden	Germany	E032(VI C7)
Muller	The Rockefeller University		New York, NY	USA	E033(hec1)
Nakamura	Kyoto University	Primate Research Institute	Inuyama City, Aichi	Japan	E034(HTF-K108), E035(HTF-K180)
Newman	Leukosite		Cambridge, MA	USA	E009(2G7)
Parish	Australian National University	Division of Cell Biology	Canberra	Australia	
Parravicini	Ospedale "L. Sacco"		Milano	Italy	
Parums	Royal Postgraduate Medical School	Department of Histopathology	London	UK	
Patarroyo	Karolinska Institutet	Microbiology and Tumorbiology Center	Stockholm	Sweden	
Paulie	Stockholm University	Department of Immunology	Stockholm	Sweden	E036(7E9), E037(P7A5)
Perussia	Thomas Jefferson University		Philadelphia, PA	USA	E038(B148.4)
Pignatelli	Royal Postgraduate Medical School	Department of Histopathology	London	UK	
Pinto	Centro di Riferimento Oncologico	The Leukemia Unit	Aviano	Italy	
Pizcueta	Fundacio Privada Clinic per la Recerca		Barcelona	Spain	
Rokhlin	University of Iowa 118 IIL	Department of Pathology	Iowa City, IA	USA	E107(29-G8)
Ross	University of Edinburgh Department of Surgery	Lister Research Laboratories	Edinburgh	UK	

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Table 4 Donors and Evaluators of the Endothelial Cell Blind Panel—*Continued*

Donor/ Evaluator	Institution	Department	City	Country	Workshop Codes and Clone Names
Sánchez-Madrid	Hospital de la Princesa	Servicio de Inmunologia	Madrid	Spain	E039(LIA 1/14), E077(TEA 1/34), E078(TEA 1/8), E079(TEA 2/16), E080(TEA 2/5), E105(TEA 3/17)
Santoni	University "La Sapienza"	Department of Experimental Medicine and Pathology	Rome	Italy	
Scheper	Free University Hospital	Department of Pathology	Amsterdam	The Netherlands	E095(JSB-1)
Sedlak	Cancer Research Institute	Department of Molecular Immunology	Spitalska	Slovakia	
Seon	Roswell Park Cancer Institute	Molecular Immunology	Buffalo, NY	USA	E040(SN12), E108(SN6), E109(SN6h)
Shaw	National Institute of Health		Bethesda, MD	USA	
Simmons	Imperial Cancer Research Fund, Institute of Molecular Medicine	Cell Adhesion Laboratory	Headington, Oxford	UK	E067(OJ79), E068(OJ91)
Skubitz	University of Minnesota	Department of Medicine	Minneapolis, MN	USA	
Stockinger	University of Vienna	Institute of Immunology	Vienna	Austria	
Suciu-Foca	Columbia University	Department of Pathology	New York, NY	USA	E122(LDA1)
Sugawara	Saitama Medical School	Department of Pathology, Saitama Medical Center	Kawagoe, Saitama	Japan	E041(MRK16)
Sunder-Plassmann	University of Vienna	Department of Nephrology	Vienna	Austria	
Sutherland	The Toronto Hospital	Oncology Research Laboratory	Toronto, Ontario	Canada	E010(8A3), E123(7D1)
Tedder	Duke University Medical Center	Department of Immunology	Durham, NC	USA	
Vadas	Institute of Medical & Veterinary Science	Hanson Centre for Cancer Research	Adelaide	Australia	E112(51-10C9)
van Agthoven	Immunotech SA		Marseille	France	E042(TEA 1/31)
van Den Oord	University Hospital St Rafael	Department of Pathology, Laboratory of Histochemistry and Cytochemistry	Leuven	Belgium	
Vermot-Desroches	Centre de Transfusion	Dpt Diaclone	Besançon	France	E043(B-B2), E044(B-B4), E081(B-K4)
Vidal-Vanaclocha	Universidad del Pais Vasco	Dept. de Biologia Celular y Ciencias Morfologicas	Vizcaya	Spain	
Voland	University of California San Diego	Cancer Center	La Jolla, CA	USA	
Wang	PharMingen		San Diego, CA	USA	
Wiels	Institut Gustave Roussy	Laboratoire de Biologie des Tumeurs Humaines	Villejuif	France	E070(38.13)

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Endothelial Cell Antigens Blind Panel Analysis

EC5 Endothelial Cell Blind Panel analysis: Overview and summary

JOJI KITAYAMA and TIMOTHY A. SPRINGER

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

positive staining of HUT78, mesothelial cells, and JMN (mesothelial cell line) in flow cytometry.

CD141 (thrombomodulin)

E013 (1A4), E025 (TMmAb20), and E045 (KA-4) were confirmed to be specific for CD141 with transfectants. The mAb gave relatively weak but significant staining to endothelial cells and monocyte/macrophages, and the antigen expression in EC was downregulated by treatment with TNFα or IL-1β. All the mAb detected in 100-kDa band in immunoprecipitation.

CD142 (tissue factor)

Four mAb to tissue factor (CD142) were in the Endothelial Cell Blind Panel (E016 (HTF1-7B8), E032 (VI-C7), E034 (HTF-K108), and E035 (HTF-K180)), and 12 mAb (E131–E142) were in the tissue factor Panel. All the mAb reacted with endothelial cells, monocyte and monocytic cell lines in flow, and showed some additional staining to keratinocytes, vascular adventitia, and various epithelia in immunohistology. Immunoprecipitation detected a band of 45 kDa.

CD143 (angiotensin-converting enzyme)

The mAb E084 (9B9), E085 (i2H5), and E086 (3A5) were submitted to angiotensin-converting enzyme (ACE, CD143) from one laboratory. The mAb immunoprecipitated a band of 170 kDa and were clustered by flow cytometry in the Endothelial Cell and Blind Panels. Specificity was confirmed by binding to purified ACE in enzyme-linked immunosorbent assay (ELISA). Immunohistochemical studies revealed that the staining intensity of these mAb varied among arterial endothelial cells, venous endothelial cells and capillaries in various organs, and epithelium was positive in kidney.

CD144 (VE-cadherin)

VE-cadherin (CD144) is a cell–cell adhesion molecule of the cadherin family specifically expressed in endothelial cells. This molecule was characterized with mAb E020 (BV9), E021 (BV6), E033 (hec1), E042 (TEA 1/31), and E092 (55-7H1) as a 135 000 M_r molecule with specificity for endothelial cells. The mAb did not stain any cultured cell types except HUVEC, and showed specific staining of endothelium in tissue sections, although the staining intensities of E021 and E033 were relatively weaker than that of the three other mAb in flow cytometry. The specificities of these five mAb were confirmed with transfectants.

CDw145

The mAb E036 (7E9) and E037 (P7A5) are of a different subclass and from the same laboratory. They show strong staining to endothelial cells both in cultured cells and tissue section, and additional reactivity to some epithelial and stromal cells. Immunoprecipitation detected one strong band of 25 kDa, and two weak bands of 90 kDa and 110 kDa. These mAb were independently clustered in the dendrograms of the Endothelial Cell Panel and central Blind Panel, and thus provisionally designated as CDw145.

CD146 (S-ENDO/Muc 18)

The S-ENDO mAb (E057 (F4-35H7), E058 (F439E), E059 (F432G-3), mAb to Muc 18 (E067 (OJ79) and E068 (OJ91), and three mAb of unknown specificity (E065 (541/10B2), E066 (541/2E5), and E077 (TEA 1/34)) were found to react with the same molecule of 118 000 M_r. E068 was positive by immunoprecipitation but not by flow cytometry or immunohistochemistry. CD146 is highly expressed in HUVEC, and mildly on stromal cells such as HeLa, RD3/5, and KGB2. The mAb clustered in the Endothelial Cell and Blind Panels. Immunostaining of tissue sections showed clear staining of all types of endothelium, the vascular wall, melanoma cells and follicular dendritic cells. The mAb were all reactive with recombinant Muc 18-Fc chimera.

CD147 (basigin/neurothelin)

mAb E062 (AAA1), N-L082 (UM-8D6), N-L018 (HI197), N-L109 (HIM6), N-L155 (H84), 6T-064 (UM-8D6), and P58 (UM-8D6) bound to recombinant basigin/neurothelin (CD147). The mAb E062 and two samples of UM-8D6 clustered in the Blind Panel, and defined CD147. In addition to reactivity with endothelial cells, all the mAb broadly stained hemopoietic cells and stromal cells slightly to moderately.

mAb assigned to new clusters in other Panels

CD138 (syndecan-1)

mAb to syndecan-1 (CD138) B-B2 (E043, B092), B-B4 (E044, B093), MI15 (B005), and 1D4 (B102) were submitted to both the Endothelial Cell and B-cell Sections. The mAb E043 and E044 were grouped by flow cytometry and immunohistochemistry (Table 1); the CD report is in the B-cell Section [Nijdenes *et al.*, BC29].

CD151 (PETA-3)

The mAb 11B1.G4 (E011, P48) and 14A2.H1 (E012, P49) to the tetraspanner PETA-3 (CD157) were submitted to both the Endothelial Cell and Platelet Sections. The mAb were clustered in the Endothelial Cell Panels by flow cytometry and immunoprecipitation of a 28-kDa band. CD151 is described in the Platelet Section [Ashman *et al.*, PL14].

Table 1 Specificity of mAb in the Endothelial Cell Blind Panel

Specificity	Workshop Code	Evaluation Method	Evaluator	Flow Clustering	$M_r(\times 10^{-3})$ R/NR	Immunohistochemistry
CD31	E003, E050, E076	Transfectant, recombinant protein	Pizcueta, Stockinger	CD31	130/130	Strong pan-EC
CD34	E004	Recombinant protein	Stockinger	CD34	115/115	Strong pan-EC
CD62E, E-selectin	E005, E087	Transfectant, recombinant protein	Pizcueta	Group 10	125/135	
CD62P, P-selectin	E006				150/155	Focal EC, platelet focal-EC and epi, SMC
CD90, Thy-1	E046, E054, A011	Transfectant	Clark	Thy-1		
CD105, endoglin	E008, E075, E088	Transfectant, recombinant protein	Letarte	CD105 (endoglin)	90/180	Strong pan-EC
CD106, VCAM-1	E009		Newman	Group-10	118/92	No EC labelling, BC
CD109	E010, E063, E064, E079	Transfectant	Sutherland	CD109	170/180	pan-EC, (E079: additional focal epi, SMC)
CD138, syndecan-1	E043, E044			Group-3		Focal-EC and epi
CD140a, PDGF-receptor α	E022, E031, E090	Fusion protein	Hart		180	No labelling
CD140b, PDGF-receptor β	E023, E089	Fusion protein	Hart	PDGF receptor β	180	Focal-EC
CD141, thrombomodulin	E013, E025, E045	Transfectant	Bird, Morrissey	Thrombomodulin	100	Pan-EC, Mϕ, various epi, SMC
CD142, tissue factor	E016, E032, E035, (E034)*	Transfectant	Morrissey		45	Focal-EC, tubules, SMC, fibro (E034:restricted to SMC)
CD143, angiotensin-converting enzyme	E084, E085, E086	Purified protein	Danilov	Group-12	160/168	Focal EC and proximal tubules
CD144, VE-cadherin	E020, E021, E033, E042, E092	Transfectant	Dejana	Group-12	120/120	Weak pan-EC
CDw145	E036, E037			Group-5	25, (90, 110)	Strong pan-EC, focal epi
CD146, S-ENDO/Muc 18	E057, E058, E059, E065, E066, E067, E077, (E068)†	Transfectant, recombinant protein	Dignat-George, Simmons	S-ENDO/Muc 18	125/125	Pan-EC, SMC (E068: no labelling) (E077: Focal EC, SMC)
CD147, basigin/ neurothelin	E062, N-L082, N-L108, N-L109, N-L155, 6T-064, P-58	Recombinant protein	Stockinger			Focal EC and epi
CD151, PETA-3	E011, E012	Transfectant	Ashman	PETA-3	28/28	Pan-EC, various epi, SMC, focal EC, and epi
CD157, BST-1	E060, E061, M051	Transfectant	Hirano	BST-1		
vWF	E007				252/250	Pan-EC, platelet
CD13-like	E017, E078, E081				145, 210/145, 210	Focal-EC and various epi
CD77?	E070	Purified glycolipid	Kniep			Focal EC
CD93?	E038, E039			Group-9	110/90	Pan-EC
H type 1 and H & Y type 2 blood groups	E055, E056	Purified glycolipid		Group-1b	90, 125/90, 125	Strong pan-EC, focal epi

Continued on next page

Table 1 Specificity of mAb in the Endothelial Cell Blind Panel—*Continued*

Specificity	Workshop Code	Evaluation Method	Evaluator	Flow Clustering	$M_r(\times 10^{-3})$ R/NR	Immunohistochemistry
Lewis^y-containing glycolipid	E030	Purified glycolipid	Kniep	Group-1a	No bands	Strong pan-EC, focal epi
Peripheral node addressin	E047, E048	Purified molecule	Berg			HEV
P-glycoprotein	E041, E093, E094, E095					Weak focal EC and epi
Unknown glycolipid	E019	Purified glycolipid	Kniep			No EC, focal epi
VAP-1	E053	Transfectant	Jalkanen			Focal EC, HEV, SMC, fibro
	E015					Various epi
	E018					Focal-EC and various epi
	E024				30, (50)/ 50	Pan-EC, focal epi, conn.tissue
	E026					Focal epi
	E027					No labelling
	E028				77/75	Pan-EC, SMC
	E029					Strong pan-EC, focal epi
	E040				100	Weak pan-EC, focal epi
	E049					Focal large vessel EC
	E051					Focal EC
	E071				55	Focal EC and epi
	E072				130/130, 145	Pan-EC, focal epi, SMC
	E073			Group-12	130/130, 145	Strong pan-EC
	E074			Group-12	52/55	Pan-EC, focal epi
	E080				50, (120)	Focal EC, focal epi, SMC
	E083					Focal EC and epi, SMC
	E091					No labelling

Flow clustering group number was determined from the dendrogram shown in Kitayama *et al.* [EC7, Fig. 1].
Immunoprecipitation: Immunoprecipitation was performed in HUVEC stimulated with 100 μg TNFα/ml for 6 h. Molecular weights were expressed as reduced/non-reduced. Faint bands are shown in parenthesis.
Immunohistochemical clustering: EC, endothelial cells; epi, epithelial cells; SMC, smooth muscle cells; fibro, fibroblasts; Mϕ, macophrage or dendritic cells; BC, Bowman's capsule. VWF, von Willebrand's factor; for other abbreviations, see the text.
*mAb recognizes denatured tissue factor protein.
†Thought to recognize an intracellular determinant.

Endothelial Cell Antigens Blind Panel Analysis

EC5 Endothelial Cell Blind Panel analysis: Overview and summary

JOJI KITAYAMA and TIMOTHY A. SPRINGER

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

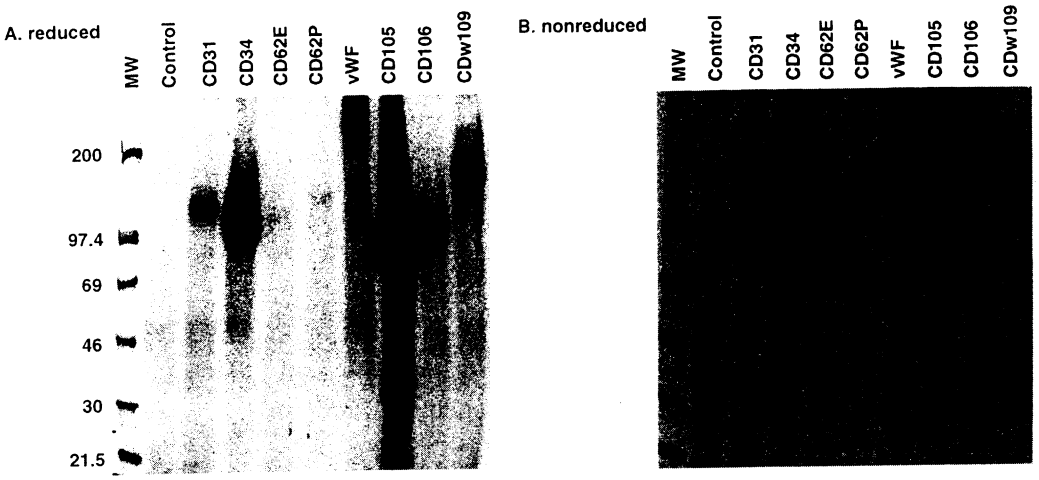

Fig. 1 SDS-PAGE of antigens immunoprecipitated from radioiodinated HUVEC. The HUVEC were cultured on collagen-coated flasks and were stimulated with TNFα (10 ng/ml) for 6 h. Cells were incubated in phosphate-buffered saline (PBS) containing 5 mM EDTA to detach them from the flask, and washed twice with 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

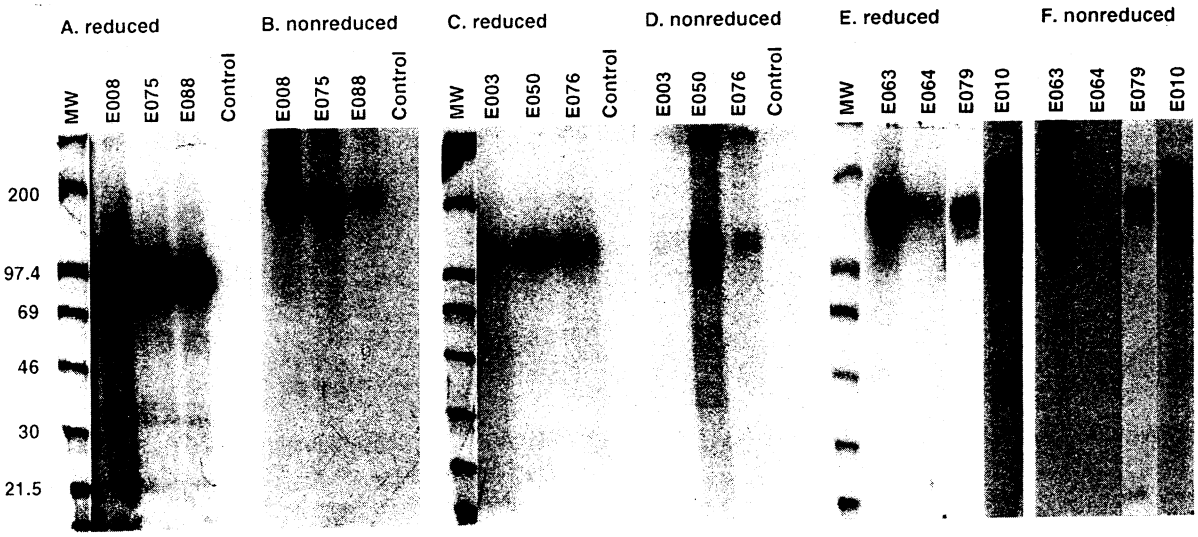

Fig. 2 Comparison of reference antigens with the immunoprecipitated structures. Immunoprecipitations and SDS-PAGE analysis were as in Fig. 1. (A) and (B) Endoglin (CD105) mAb; (C) and (D) CD31 mAb; (E) and (F) CD109 mAb.

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

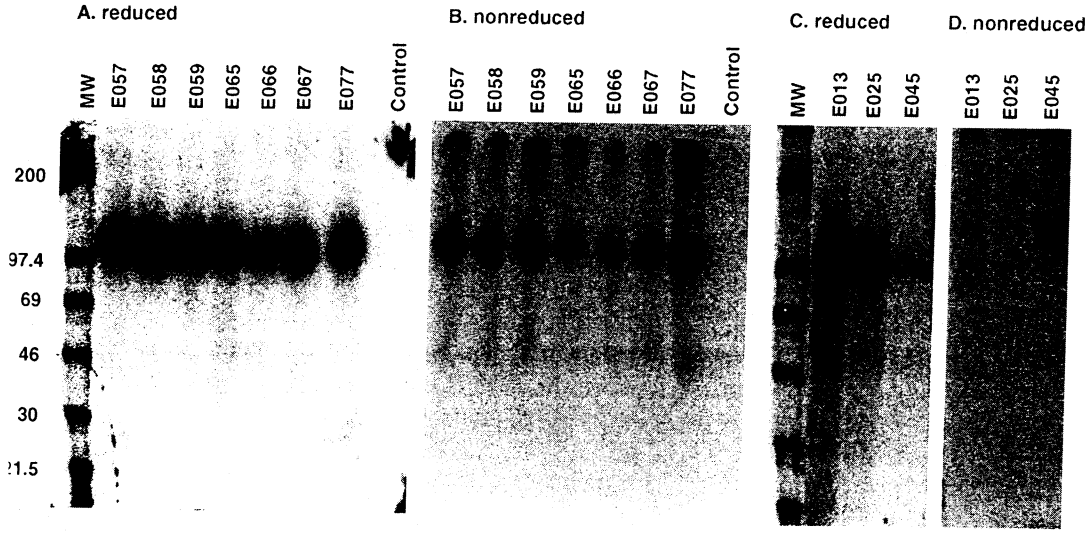

Fig. 3 A–D: Side-by-side comparisons of immunoprecipitated antigens. Procedures are as described in Fig. 1. (A) and (B) S-ENDO/Muc 18 (CD146) mAb; (C) and (D) thrombomodulin (CD141) mAb. *Continued on next page.*

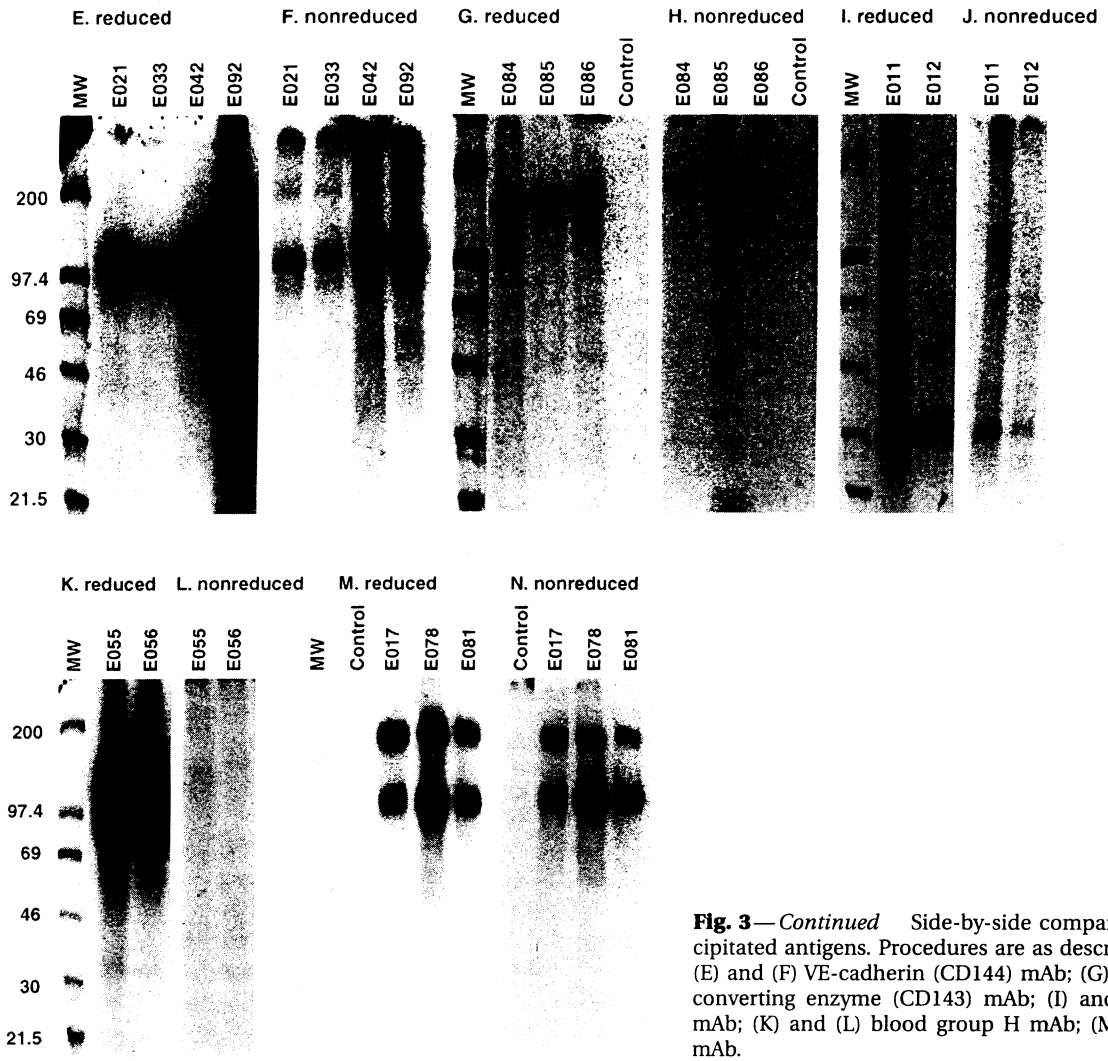

Fig. 3 — *Continued* Side-by-side comparisons of immunoprecipitated antigens. Procedures are as described in Fig. 1A and B. (E) and (F) VE-cadherin (CD144) mAb; (G) and (H) angiotensin-converting enzyme (CD143) mAb; (I) and (J) PETA-3 (CD151) mAb; (K) and (L) blood group H mAb; (M) and (N), CD13-like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

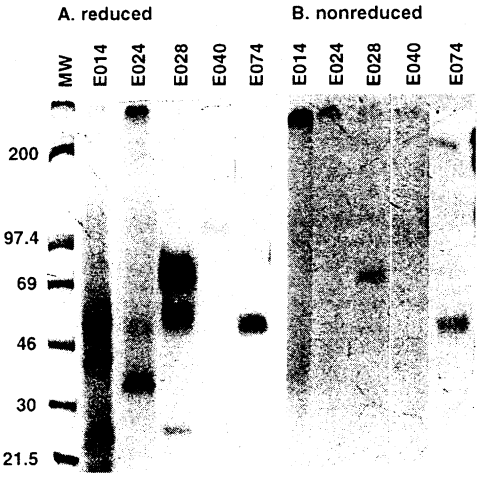

Fig. 4 SDS-PAGE analysis of the antigens immunoprecipitated by the Endothelial Cell Panel of monoclonal antibodies with unique specificities (procedures are as described in Fig. 1).

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).

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EC7 Endothelial Cell Blind Panel: Flow cytometric analysis report

JOJI KITAYAMA, MARK RYAN, RACHAEL CLARK, and TIMOTHY A. SPRINGER

Reactivity and clustering of the Blind Panel

Flow cytometric analysis of the Endothelial Cell Blind Panel was performed in 18 laboratories and another laboratory used an enzyme-linked immunosorbent assay (ELISA) assay. The reactivities are summarized in Table 1. According to the molecules equivalent of soluble fluorochrome (MESF) of 28 different cell types including cytokine-activated human umbilical cord vein endothelial cells (HUVEC) examined in our laboratory, a dendrogram was constructed using the "MacMu" and "MacDendro" programs [1] (Fig. 1). The dendrogram identified 16 groups of monoclonal antibodies (mAb), in addition to group 17, which included the negative mAb. Group 1 was the most distant from the negative control. These groups when combined with other criteria were of great utility in assigning specificities.

Modification of Blind Panel antigen expression in HUVEC by inflammatory cytokines

Treatment of HUVEC with inflammatory cytokines or phorbol myristate acetate (PMA) significantly modified the reactivities of some of the Panel mAb (Table 2). All laboratories detected the enhancement of staining intensity in E009 (2G7, VCAM-1, CD106). The ration of enhancement was prominent with tumor necrosis factor (TNF) α, interleukin (IL) 1β, and PMA (Stimulation Index, SI: 17.0–73.7), and smaller with IL-4, IL-13 and interferon (IFN) γ (SI: 1.58–6.32). The reactivity of E087 (IG9) and E005 (H18/7) mAb to E-selectin (CD62E) was strongly increased by TNFα, IL-1β and PMA (SI: 3.42–115) but not by IL-4 or IFN-γ. P-selectin (CD62P) recognized by E006 (G1) and the CD13-like antigen recognized by E017 (M72) were moderately upregulated by IL-4 and IL-13 (SI: 1.79–2.47), but not by the other cytokines. The E016 (H7F1-7B8), E032 (VI-C7), and E035 (HTF-K180) mAb to tissue factor, CD142, were modulated similarly to CD62E, although the enhancement by TNFα or IL-1β was less than for E-selectin (SI: 1.29–12.6). Binding of E013 (1A4), E025 (TMmAb20), and E045 (KA-4) mAb to thrombomodulin, CD141, were decreased by TNFα and IL-1β (SI: 0.17–0.70), increased by PMA (SI: 1.42–19.4), and not altered by IL-4, IL-13 and IFN-γ. The reactivities of E084 (9B9), E085 (12H5), and E086 (3A5) to angiotensin-converting enzyme (CD143), were markedly enhanced by PMA stimulation (SI: 10.1–16.9), but not affected by the cytokines. In addition, treatment with PMA increased the reactivity of E036 (7 E9) and E037 (P7A5) mAb to CDw145 and of E038 (B148.4) and E039 (LIA 1/14) mAb to a CD93-like antigen by more than twofold.

Reference

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Table 1 Endothelial Cell Panel Flow Cytometry Studies

	HUVEC	ECV304	ECRF24	Eahy.926	IVEC	HUVEC*	HLNEC*	Granulocyte	Monocyte	Lymphocyte	PHA Blast	Platelet	HL-60	KG1a
Lab	**1,2,3**	**1,2,3**	**4**	**2,4**	**5**	**6**	**6**	**1,4,7,8**	**1,4,7,8**	**1,4,7,8**	**8**	**1,7**	**1**	**1,8**
Code														
E001	−	−	−	−	−	−	−	−	−	−	−	−	−	−
E002	−	−		−	−	−	−	−	−	−	−	−	−	−
E003	+++	−	+++	+++	+++	+++	+++	++	++	±/+	±	++	+	++
E004	±/+	−	−	−	+++	+	+	−	−	−	−	±	−	++
E005	−	−	−	−	−	−	−	−	−	−	−	−	−	−
E006	−/±	−	−	−	−	−	−	−	−/+	−	−	±	−	−
E007	−	−	−	−	+	+++	+++	−	−	−	−	−	−	−
E008	++	+	+++	++	+++	+++	+++	−	−/±	−	±	−	−	−/+
E009	−/±	−	±	±	+	−	−	−	−	−	−	−	−	−
E010	±/++	−/++	++	−/++	+	+++	+++	−/+	−/±/+	±	+	±	−	++
E011	+++	+++	+++	+++	+++	+++	++	+	+/++	±	++	++	++	++
E012	+/++	+/++	++	++	++	+	+	±	±/+	±	+	+	+	+
E013	+/++	−/++	++	+/++	++	++	++	−	±/+	−	−	−	−	−
E014	−/±	−	−	−	−	++	++	−	−	±	+	−	±	±
E015	−	−	−	−	−	−	−	−	−	−	−	−	−	−
E016	±/+	+	−	−	−	++	+	−	−	−	−	−	−	−
E017	++	−	++	++	+	++	++	+	±/+	−/±	−	−	++	+
E018	−	−	−	−	+	−	−	−	−	−/±	−	−	−	−
E019	−	−	−		−	−	−	−	−	−	−	−	−	−
E020	+	−	+	++/+	+	++	++	−	−	−	−	−	−	−
E021	±/+	−	+	−	−	+++	++	−	−	−	−	−	−	−
E022	−	−	−	−	−	+	−	−	−	−	−	−	−	−
E023	−	−	−	−	−	+	+	−	−	−	−	−	−	−
E024	++	+/+++	+	++	+++	++	−	−/±	−	−	−	−	±	−
E025	±/+	−/++	−	+/++	++	+	+	±	±/+	−/±	−	−	−	−
E026	−	−	−	−/++	−	−	+	−	−	−	−	−	−	−
E027	−/±	−	−	−/+	−	−	+	−	−	−	−	−	−	−
E028	−/±	−	−	−/±	−	++	+	±	±/+	±	±	−	−	−
E029	−	±	±	−/±	+	+	+	−	−/±	−	−	−	−	−
E030	++	+	+	++	+++	++	++	±/+	±/+	−/+	±	+	−	−
E031	−/±	−	−	−	−	+	−	−	−/±	−	−	−	−	−
E032	−/±	−/±/+	−	−	−	+	−	−	−	−	−	−	−	−
E033	+	−	+	+	−	+	−	−	−	−	−	−	−	−
E034	−/±	−	−	−	+	+	+	−	−/±	−	−	−	−	−
E035	−/±	+	−	−	−	+	+	−	−	−	−	−	−	−
E036	++/+++	±/+++	+++	+++	+++	++	++	−	−	−	−	−	−	−
E037	++/+++	−/+++	+++	+++	++	+++	++	−	−	−	−	−	−	−
E038	+/++	−/±	±	++	+	+	−	−/+	±/+	−	−	−	−	+
E039	+/++	−	+	++	+	++	−	−/+	±/+	−	−	−	−	+
E040	±	−/±	+	+	+	+	+	−/±	−/±	−	±	−	−	±/+
E041	−	−	+	+	−	+	+	−	−/+	−	−	−	±	−/±
E042	+/++	−	++	++	−	+	+	−	−	−	−	−	−	−
E043	−/±	−/+	+	−/+	−	+	+	−	−	−	−	−	±	−
E044	−/±	−/++	+	++/N.D.	−	+	−	−	−	−	−	−	+	−

HEL	HUT78	Jurkat	JY	SKW	THP-1	HeLa	293	RD3/5	MG63	A431	Ax36M	JMN	KGB-2	FS1/23	Fibroblast	SK-HEP-1	DKKT	HMC-1	KU-812	Mesangial Cells	Mesothelial Cells
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	9	9	10	11
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+	+	±	–	–	++	–	–	–	–	–	–	–	–	–	–	–	–	–	++	–	–
±	–	±	–	–	±	–	–	–	–	–	–	–	–	±	–	+	–	–	–	–	–
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±	–	–	–	–	+	±	–	±	–	–	–	–	±	±	+	+	+	–	–	±	±
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±	+	±	±	–	+	±	–	±	+	–	+	+	+	±	±	+	++	++	++	+	±
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±	±	±	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	++	–	–
–	–	–	±	–	±	±	–	±	–	–	±	–	±	±	±	+	++	–	+	–	±
+	–	–	–	–	+	±	–	±	+	–	–	–	±	+	+	N.D.	N.D.	+	++	–	±
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	N.D.	N.D.	–	–	–	–
–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	N.D.	N.D.	–	–	–	–
±	–	–	–	–	–	±	–	–	–	–	–	–	±	±	±	–	–	–	–	–	–
±	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	+	–	–	–	–	±	–	–	–	–	±	+	–	–	±	–	–	–	–	+	–
–	±	–	±	–	+	++	–	++	+	–	±	+	+	++	+	+	+	+	+++	–	±
–	–	±	±	–	+	–	–	–	–	–	–	–	±	±	±	+	±	–	+	–	–
–	–	–	–	–	–	–	–	–	±	–	–	–	–	–	–	N.D.	N.D.	–	–	–	–
–	–	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	+	–	++	–	–	±	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–
±	±	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	±	–	±
++	++	–	±	–	–	±	–	–	–	–	–	–	–	–	–	N.D.	N.D.	–	+++	–	–
–	–	–	±	–	–	–	–	–	±	–	–	–	–	±	±	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	±	–	±	–	±	+	++	–	–	–	±
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	±	–	–	–	–	–
–	–	–	–	–	±	±	–	±	–	–	±	±	±	±	±	+	++	–	–	–	±
±	–	–	–	–	–	+	–	+	+	–	+	+	+	+	+	+++	++		+	±	±
±	–	–	–	–	–	+	–	+	+	–	+	+	+	+	+	+++	++		+	±	±
–	–	–	±	–	+	–	–	–	–	–	–	–	–	–	–	–	–		±	–	–
–	±	–	±	–	+	–	–	–	–	–	–	–	–	–	–	–	–		±	–	–
–	–	±	+	–	–	–	–	–	–	–	±	–	±	±	–	+	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	±	–	–	–	–		+	–	–
±	–	–	–	–	–	–	–	–	–	–	±	–	–	–	–	–	–	–	–	–	–
–	+	–	+	–	±	±	±	±	±	–	–	–	+	+	±	++	±	–	–	–	–
–	+	–	+	–	±	±	±	–	±	–	–	–	+	+	±	+++	++	++	–	–	–

Continued on next page

Table 1 Endothelial Cell Panel Flow Cytometry Studies—*Continued*

	HUVEC	ECV304	ECRF24	Eahy.932	IVEC	HUVEC*	HLNEC*	Granulocyte	Monocyte	Lymphocyte	PHA Blast	Platelet	HL-60	KG1a
Lab	**1,2,3**	**1,2,3**	**4**	**2,4**	**5**	**6**	**6**	**1,4,7,8**	**1,4,7,8**	**1,4,7,8**	**8**	**1,7**	**1**	**1,8**
Code														
E045	±/+	−	±	+	+	+	+	−	±	−	−	−	−	−
E046	−/±	−	−	−/+	−	+	+	−	−	−	±	−	−	−
E047	−	−	−	−	−	+	+	−	−	−/±	−	−	−	−
E048	−	−	−	−/+	−	+	+	−	−	−/±	−	−	−	−
E049	−/±	−	−	−	−	−	−	−	−	−	−	−	−	−
E050	++/+++	−	+++	+++	+++	+++	++	++	++	+	++	+	+	±/+
E051	−/±	−	−	−	+	+	+	−	−	−	−	−	−	−
E053	−	−	+	−/+	−	+	−	−	−	−/±	−	−	−	−
E054	−	−	±	−/+	−	−	−	−	−	−	−	−	−	−
E055	+++	++	+++	+++	+++	+++	+++	+/++	+/++	−/±	±	±	−	±
E056	+++	++	+++	+++	+++	++	++	±	+	−/±	±	−/±	−	−
E057	+++	−/++	+++	+++	+++	+++	+++	−	±	−	±	−	−	−
E058	+++	−/++	+++	+++	+++	+++	+++	−	−	−	±	−	−	−
E059	+++	−/++	+++	+++	++	+++	+++	−	−	−	±	−	−	−
E060	±/+	−	−	+	+	+	+	++	+/++	−	−	−	±	−
E061	±/+	−	−	+	+	++	++	++	±/++	−	−	−	±	−
E062	+/++	−/++	+	±/++	+	++	+	−/++	+/++	±	+	±	−	+/++
E063	±/+/++	−/++	±	+	+	++	++	±	±/+	−/±	+	−	−	+
E064	−/+	−/+	+	−/+	+	++	++	−	±	−/±	±	−	−	+
E065	+++	−/+	+++	+++	+++	+++	+++	−	−/±	−/±	±	−	−	−
E066	++/+++	−/++	+++	+++	+++	+++	+++	−	−/±	−	±	−	−	−
E067	++/+++	−/+	+++	+++	+	+++	+++	−	±	−	±	−	−	−
E068	−/±	−	+	−/+	−	+	+	−	−/±	−	−	−	−	−
E070	−	−	+	+	−	+	+	−	−	−/±	−	−	−	−
E071	−/±	−/±	+	−/++	+	+	+	−	−/±	−	−	−	−	−
E072	+	−/+++	±	+++	+++	+	++	−	−	±	+	−	−	±/+
E073	±/+	−/±	+	++	+	++	++	−	−	−/±	−	−	−	−
E074	+	−/±	−	++	++	++	++	−	−/±	±	−	−	−	±
E075	++/+++	−/±	++	−/++	++	+++	+++	−	−/±	−/±	+	−	−	−/+
E076	++/+++	−/+++	+++	+++	++	+++	+++	++	++	+	++	++	±	+/++
E077	+++	−/++	++	+++	+++	+++	+++	−	−	−	+	−	−	−
E078	++/+++	−/±	+++	++	++	+++	+++	++	±/+	−	±	−	−	+
E079	+	−/++	+++	+/+++	−	++	++	±	−/+	−	±	−	−	+
E080	±/+	−/±	+	−/+	+	+	++	−/±	−/±	−	−	−	−	−
E081	+/++	−	−	−/++	−	+	+	−/+	−/±	−	−	−	−	+
E082	−	−	++	−	−	+	+	−	−	−	−	−	−	−
E083	−/±	−	−	−	−	++	++	−	−	−	−	−	−	−
E084	+	−	−	−	−	++	+	−	−	−	−	−	−	−
E085	+/++	−	−	−	−	++	+	−	−	−/±	−	−	−	−/±
E086	+	−	−	−	−	+	+	−	−	−/±	−	−	−	−
E087	−	−	−	−/+	−	−	−	−	−	−	−	−	−	−
E088	+++	−	+++	+++	−	N.D.	N.D.	−	±	−	N.D.	−	±	−

HEL	HUT78	Jurkut	JY	SKW	THP-1	Hela	293	RD3/5	MG63	A431	Ax36M	JMN	KGB-2	FS1/23	Fibroblast	SK-HEP-1	DKKT	HMC-1	KU-812	Mesangial Cells	Mesothelial Cells
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	9	9	10	11
−	−	−	−	−	+	−	−	−	−	−	−	−	−	±	±	±	±	−	−	−	−
+	++	+	±	−	±	−	±	−	+	−	−	−	−	−	+	+	+	−	−	+	+
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	N.D.	N.D.	−	−	−	−
−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	N.D.	N.D.	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
++	++	±	−	−	++	−	−	−	−	−	−	−	−	−	−	−	−	++	+++	−	−
±	+	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	++	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	N.D.	N.D.	−	−	−	−
±	++	+	−	−	±	−	−	−	±	−	−	−	−	−	+	±	±	−	−	+	+
++	++	±	−	−	−	++	±	±	−	+	+	−	±	±	+	N.D.	N.D.	+++	+++	−	−
++	++	−	−	−	±	±	±	−	−	−	±	−	−	−	−	N.D.	N.D.	++	+++	−	−
−	±	±	−	−	−	+	−	+	±	−	−	−	+	±	±	+	+++	−	±	+	−
−	±	±	−	−	−	+	−	+	±	−	−	−	+	±	±	+	+++	−	−	+	−
−	±	±	−	−	−	+	−	+	±	−	−	−	+	±	±	+	+++	+++	+	+	−
±	−	±	−	−	+	−	−	−	−	−	−	−	−	−	±	−	±	+	±	−	±
±	−	−	−	−	+	−	−	−	−	−	−	−	−	−	±	−	+	+	±	−	±
+	+	+	+	−	+	+	−	+	±	−	+	+	+	±	±	+	++	+++	+++	±	+
±	±	−	±	−	±	−	−	−	±	−	±	±	±	±	±	+	+	−	±	−	−
±	±	±	±	−	±	−	−	±	±	−	±	−	±	−	±	+	±	−	+	−	−
±	±	±	±	−	−	+	−	+	±	−	−	−	+	±	±	+	++	−	±	±,+	−
−	±	±	±	−	−	+	−	+	±	−	−	−	+	±	±	±	++	−	−	±	−
−	±	±	±	−	−	+	−	±	±	−	−	−	+	±	±	±	++	−	−	±	−
−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	±	±	−	−	−	±	−	−	−	−	−	−	−	−	N.D.	N.D.	−	−	−	−
−	−	−	±	−	±	±	−	−	−	−	−	−	−	±	−	+++	+	−	−	−	−
−	+	±	−	−	±	±	−	±	+	−	+	+	+	+	±	±	+++	−	−	+	±
−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	+	−	−	±	±
±	−	±	±	−	−	−	−	±	−	−	−	−	−	−	−	+	±	−	−	−	−
±	−	−	±	−	±	±	−	+	±	−	−	−	±	±	±	−	+	−	+	±	±
+	+	±	±	−	++	−	−	−	−	−	−	−	−	−	−	+	±	−	++	−	−
±	±	±	±	−	−	+	−	+	±	−	−	−	+	+	+	+++	+++	−	±	±	−
+	±	−	±	−	++	±	−	±	++	−	−	−	±	++	+	+	+++	+	++	±	±
±	±	±	−	−	±	−	−	−	±	−	−	−	±	−	−	±	+	−	++	−	−
−	±	−	±	−	−	−	−	±	−	−	−	−	−	−	−	−	±	−	−	±	±
+	−	−	−	−	+	−	−	−	+	−	−	−	±	+	+	N.D.	N.D.	±	+++	±	±
−	−	±	−	−	−	−	−	+	−	−	−	−	−	±	−	−	±	−	−	−	±
−	−	−	±	−	±	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
±	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
±	−	−	±	−	+	+	−	+	+	−	−	−	+	+	+	−	−	−	−	−	−

Continued on next page

Table 1 Endothelial Cell Panel Flow Cytometry Studies — *Continued*

	HUVEC	ECV304	ECRF24	Eahy.932	IVEC	HUVEC*	HLNEC*	Granulocyte	Monocyte	Lymphocyte	PHA Blast	Platelet	HL-60	KG1a
Lab	**1,2,3**	**1,2,3**	**4**	**2,4**	**5**	**6**	**6**	**1,4,7,8**	**1,4,7,8**	**1,4,7,8**	**8**	**1,7**	**1**	**1,8**
Code														
E089	−/±	−	−	−	−	−	−	−	−	−	−	−	−	−
E090	−/±	−	−	−	−	+	+	−	−	−	−	−	−	−
E091	+	±	−	−/+	−	+	+	−	−	−	−	−	−	−
E092	+/++	−	−	++	++	++	++	−	−	−	−	−	−	−
E093	−	−	−	−	−	−	−	−	−	−	−	−	−	−
E094	−	−	−	−	−	+	+	−	−	−	±	−	−	−/+
E095	−	−	−	−	−	−	−	−	−	−	−	−	−	−

N.D., not determined.

Laboratory 1, Springer: For HUVEC and ECV304, the MESF values were scored as: −, <40K; ±, 40K to 80K; +, 80K to 400K; ++, 400K to 1200K; +++, >1200K. For stimulation of HUVEC, 100 U TNFα/ml, 10 U IL-1β/ml, 10 ng IL-4/ml ng, or 200 U IFN-γ/ml were used for 24 h. For other cell types, the percentage of positive cells was scored as: −, <5; ±,5 to 20; +, 20 to 90; ++, >90. Jurkut and HUT78 (T-cell lines), JY and SKW3 (B-cell lines), THP-1 (monocytic cell line), HEL (erythroid cell line), HL-60, KG1a (myeloid cell lines), Fibroblast (from skin, lipopolysaccharide-activated), HeLa (epithelial cell carcinoma), 293-T17 (kidney epithelial cell), MG63 (osteosarcoma), RD2/3 (rhabdomyosarcoma), A431 (epidermoid cell carcinoma), JMN (mesothelioma), Ax36M (ovarian carcinoma), FS1/23 (renal epithelial cell), and KBG-2 (renal cell carcinoma, multidrug-resistant cell line).

Laboratory 2, Dignat-George: The MESF values were scored as: −, <20K; ±, 20K to 40K; +, 40K to 400K; ++, 400K to 1200K; +++, >1200K. HUVEC were stimulated with 15 ng TNFα/ml, 10 U IL-1β/ml, or 60 ng PMA/ml for 6 to 24 h.

Laboratory 3, Klein: The MESF values were scored as: −, <30K; ±, 30K to 50K; +, 50K to 400K; ++, 400K to 1200K; +++, >1200K. SK-HEP-1 was a cell line derived from ascites of liver adenocarcinoma, DKKT was a cell line generated from kidney embryonic tumor of X-

HEL	HUT78	Jurkat	JY	SKW	THP-1	HeLa	293	RD3/5	MG63	A431	Ax36M	JMN	KGB-2	FS1/23	Fibroblast	SK- HEP-1	DKKT	HMC-1	KU-812	Mesangial Cells	Mesothelial Cells
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	3	9	9	10	11
±	+	−	−	−	−	−	−	−	−	−	−	+	−	−	±	N.D.	N.D.	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	N.D.	N.D.	−	−	−	±
±	−	−	±	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
±	−	−	−	−	−	−	−	−	−	−	−	±	±	−	−	−	−	−	−	−	±
−	−		−	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	±	±

linked severe combined immunodeficiency disease patient. Both cells showed endothelial characteristics in culture. HUVEC were cultured with 50 ng TNFα/ml, 20 ng IL-4/ml, or 20 ng IL-3/ml for 6 to 48 h.

Laboratory 4, Vermot-Desroches: percentage of positive cells were scored as: −, <10; +, 10 to 50; ++, 50 to 90; +++, >90.

Laboratory 5, Suberbielle: percentage of positive cells were scored as: −, <5; +, 5 to 30; ++, 30 to 70; +++, >70. IVEC, HUVEC line transformed by simian virus 40 large T antigen.

Laboratory 6 (*), Pizcueta: Absorbance values examined by ELISA were scored as: −, <5 u; +, 5 u to 50 u; ++, 50 u to 200 u; +++, >200 u. HLNEC: human lymph node endothelial cell line. For stimulation of HUVEC, overnight incubation with 200 U TNFα/ml was used.

Laboratory 7, Shaw.

Laboratory 8, Wang: percentage of positive cells were scored as: −, <5; ±, 5 to 20; +, 20 to 90; ++, >90.

Laboratory 9, Wimazal: HMC-1 (mast cell line) and KU-812 (basophilic cell line). Percentage of positive cells were scored as: −, <10; ±, <20; +, < 50; ++, < 90; +++, > 90.

Laboratory 10, Sengølge and Sunder-Plassmann: percentage of positive cells were scored as: −, <10; ±, 11 to 40; +, 41 to 70; ++, >70.

Laboratory 11, Ross: percentage of positive cells were scored as: −, <10; ±, 11 to 40; +, >41.

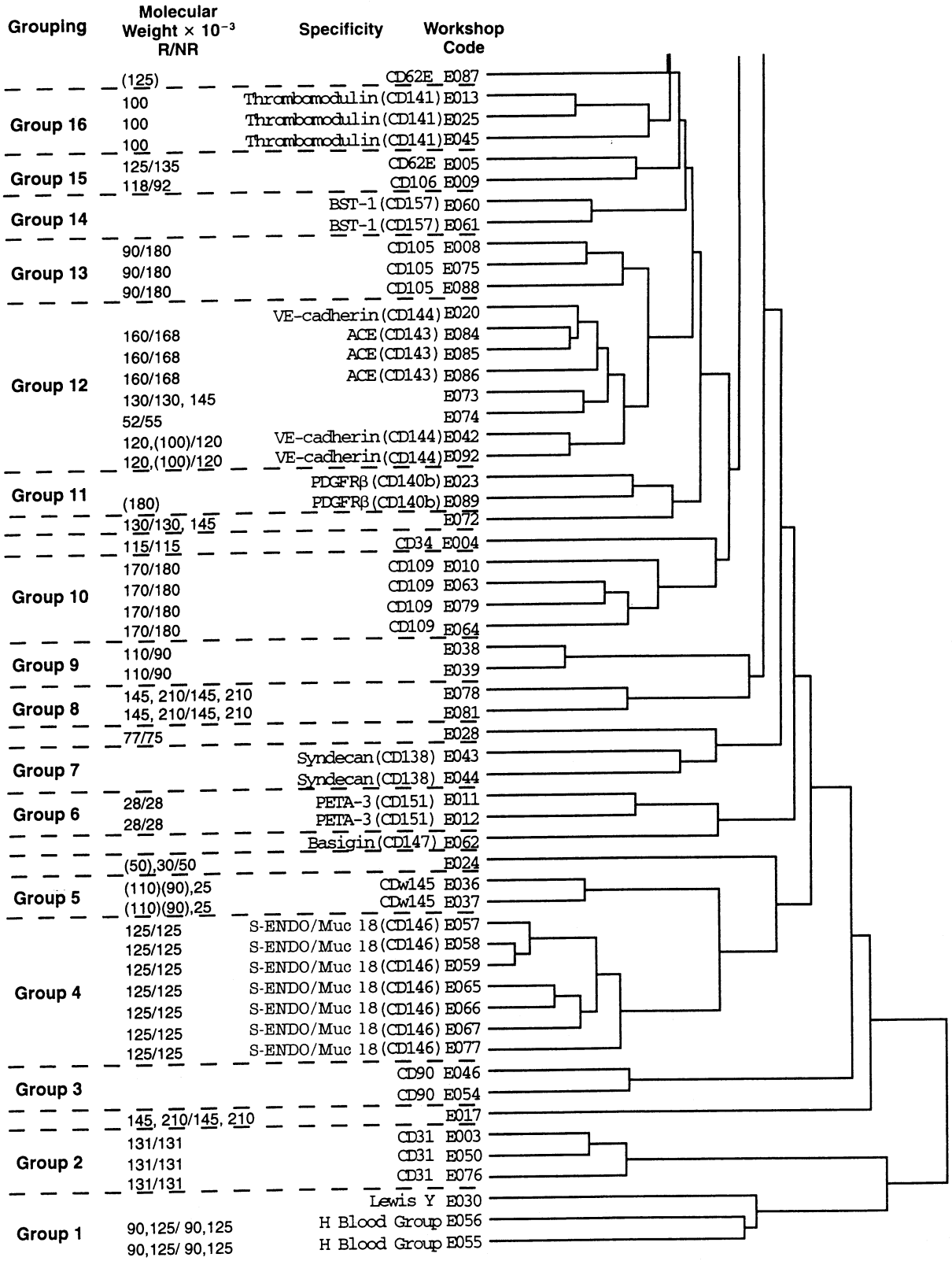

Fig. 1 The dendrogram of the 93 mAb in the Endothelial Cell Panel. Molecular weight ($\times 10^{-3}$) determined by immunoprecipitation with TNFα-activated HUVEC is shown. The remaining 38 mAb were clustered with negative controls (E001, E002) in group 17. ACE, angiotensin-converting enzyme; BST-1, bone marrow stromal cell antigen 1; PDGFRβ, platelet-derived growth factor receptor β; PETA-3, platelet endothelial cell tetraspan antigen 3; R, reduced; NR, non-reduced.

Table 2 mAb Reactivities with HUVEC Affected by Stimulation with Cytokines

Workshop Code / Stimulatory Mediator: Lab:	Stimulation Index (SI)					
	TNFα 1,2,3,6	IL-1β 2	IL-4 1,3	IFN-γ 1	IL-13 3	PMA 2
E003						↑ 1.60
E005	↑ 15.2–115*	↑ 114*				↑ 22.7*
E006			↑ 1.79–2.47		↑ 1.85	
E008		↓ 0.64†				↑ 1.36*, ↓ 0.62†
E009	↑ 25.1–73.7	↑ 17.0*	↓ 2.13–5.70	↑ 1.58	↑ 6.32	↑ 14.5*
E011						↑ 1.30*
E012						↓ 0.70
E013	↓ 0.40–0.66	↓ 0.43				↑ 2.25
E016	↑ 1.36–4.00	↑ 12.6†				↑ 19.4*
E017		↓ 0.68*	↑ 1.40–1.67		↑ 1.73	
E025	↓ 0.56–0.70	↓ 0.37				↑ 3.86
E030	↑ 1.18–2.21	↑ 1.41				↓ 0.39
E032	↑ 1.31–2.37*	↑ 2.30*				↑ 12.2*
E033		↓ 0.54*		↓ 0.60		↑ 1.90
E035	↑ 1.29–3.10*	↑ 3.30				↑ 12.4
E036					↓ 0.69	↑ 2.45†
E037	↓ 0.70–0.73	↓ 0.63			0.74	↑ 2.31†
E038	↑ 1.77–2.05		↓ 0.66–0.73		↓ 0.69	↑ 2.34
E039	↑ 1.79–2.21	↑ 1.30	↓ 0.69–0.73		↓ 0.69	↑ 2.69
E042		↓ 0.54*				↑ 1.31
E045	↓ 0.40–0.79	↓ 0.17		↓ 0.64		↑ 1.42
E050						↑ 1.50
E057	↑ 1.15–1.31					↑ 1.37†
E058	↑ 1.15–1.78	↓ 0.66				↑ 1.41†
E059	↑ 1.31–1.77					↑ 1.41†
E062		↓ 0.36*, ↑ 1.35				↓ 0.50
E065		↓ 0.26*				↑ 1.41†
E066		↓ 0.51*				
E067		↓ 0.57*				↑ 1.42†
E068				↑ 1.34		
E073						↑ 1.43
E076						↓ 0.66*
E077						↑ 1.40†
E078			↑ 1.24–1.61		↑ 1.51	
E081		↓ 0.66*				
E083				↑ 1.36		
E084						↑ 10.6
E085						↑ 16.9
E086						↑ 10.1
E087	↑ 3.42–28.4*	↑ 25.2*				↑ 8.60*
E092						↑ 1.56

Stimulation Index (SI) was calculated as MESF of stimulated HUVEC divided by MESF of unstimulated HUVEC, and the values were expressed for each SI that showed more than a 30 percent change in one of the laboratories. Each value represents the SI at 24 h incubation or SI of maximal modulation (* at 6 h, † at 12 h). Laboratories 1–6 were shown in the notes to Table 1.

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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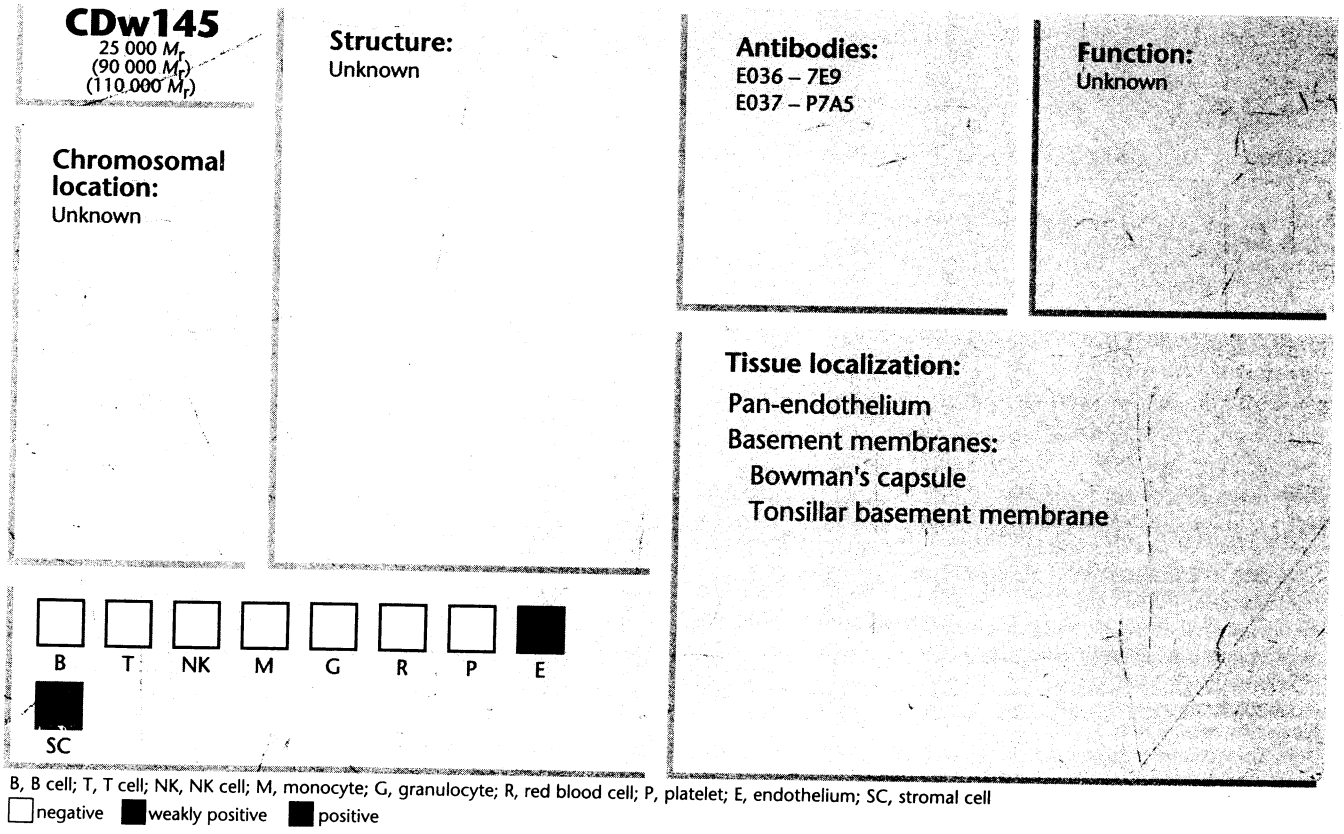

Two mouse monoclonal antibodies (mAb) from the Endothelial Cell Blind Panel, E036 (7E9, IgG3) and E037 (P7A5, IgG1), that were originally raised as mAb to urinary bladder carcinoma [1,2], constitute a new endothelial-based cluster, based upon analysis by flow cytometry, immunoprecipitation and immunohistology.

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