
EC7 Endothelial Cell Blind Panel: Flow cytometric analysis report

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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) 1 β , 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 TNF α , IL-1 β 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 TNF α or IL-1 β 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 TNF α and IL-1 β (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

1. Thioulouse, J. *Computer Applications in the Biosciences* 5, 287–92 (1989).

Cell Line	1	2	3	4	5	6	7	8	9	10	11
Mesothelial Cells	+	+	+	+	+	+	+	+	+	+	+
Mesangial Cells	+	+	+	+	+	+	+	+	+	+	+
KU-812	+	+	+	+	+	+	+	+	+	+	+
HMC-1	+	+	+	+	+	+	+	+	+	+	+
DKRT	+	+	+	+	+	+	+	+	+	+	+
SK-IHEP-1	+	+	+	+	+	+	+	+	+	+	+
Fibroblast	+	+	+	+	+	+	+	+	+	+	+
FS1/23	+	+	+	+	+	+	+	+	+	+	+
KGB-2	+	+	+	+	+	+	+	+	+	+	+
IMN	+	+	+	+	+	+	+	+	+	+	+
Ax36M	+	+	+	+	+	+	+	+	+	+	+
A431	+	+	+	+	+	+	+	+	+	+	+
MG63	+	+	+	+	+	+	+	+	+	+	+
RD3/5	+	+	+	+	+	+	+	+	+	+	+
293	+	+	+	+	+	+	+	+	+	+	+
HeLa	+	+	+	+	+	+	+	+	+	+	+
THP-1	+	+	+	+	+	+	+	+	+	+	+
SKW	+	+	+	+	+	+	+	+	+	+	+
JY	+	+	+	+	+	+	+	+	+	+	+
Jurkat	+	+	+	+	+	+	+	+	+	+	+
HUT78	+	+	+	+	+	+	+	+	+	+	+
HEL	+	+	+	+	+	+	+	+	+	+	+

Continued on next page

Table 1 Endothelial Cell Panel Flow Cytometry Studies—Continued

	HUVEC	ECV304	ECRP24	EAhy992	VEEC	HUVEC*	HNVEC*	Granulocyte	Monocyte	Lymphocyte	PIA Blast	Platelet	HL-60	K51a
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.	-	±	-

Table 1 Endothelial Cell Panel Flow Cytometry Studies—Continued

	HUVEC	ECV304	ECRF24	Fahy932	IVEC	HUVEC*	HLN2C*	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 TNF α /ml, 10 U IL-1 β /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 TNF α /ml, 10 U IL-1 β /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-

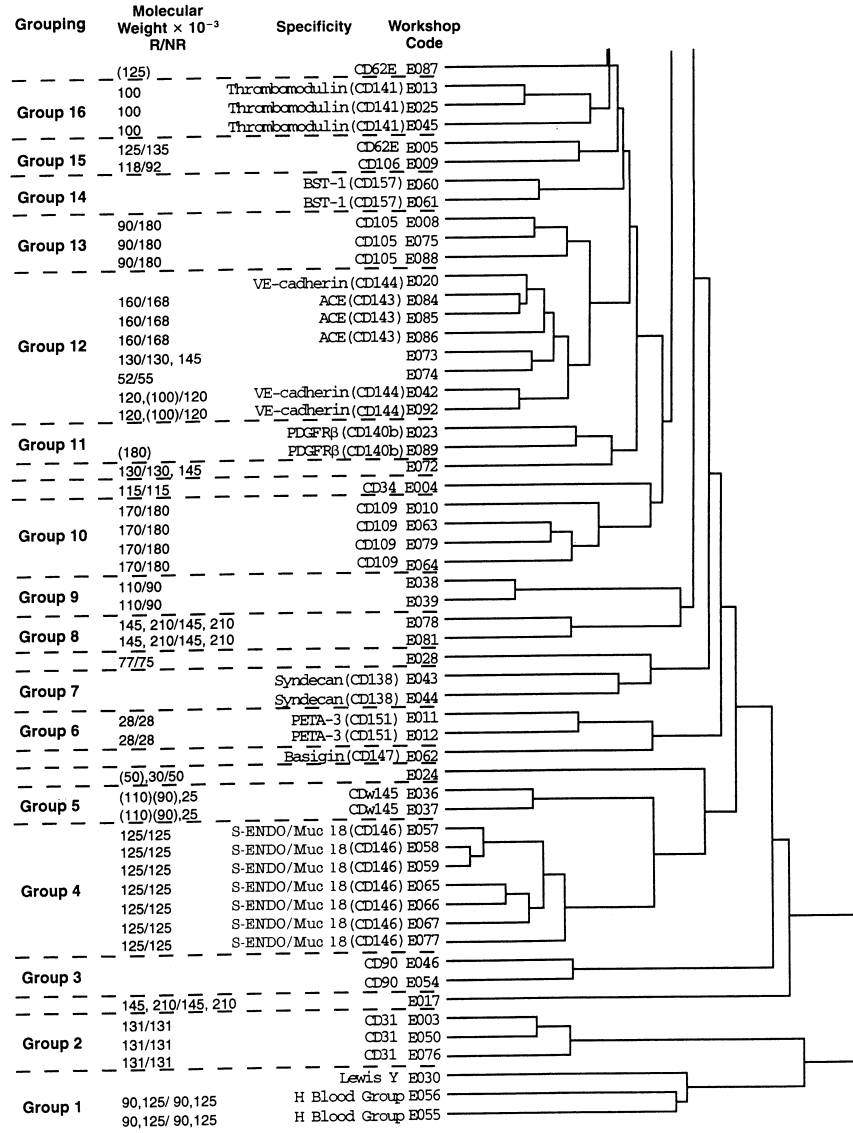


Fig. 1 The dendrogram of the 93 mAb in the Endothelial Cell Panel. Molecular weight ($\times 10^{-3}$) determined by immunoprecipitation with TNF α -activated HUVEC is shown. The remaining 38 mAb were clustered with negative controls (E001, E002) in group 17. ACE, angiotensin-converting enzyme; BST-1, bone marrow stromal cell antigen 1; PDGFR β , 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)					
		TNF α 1,2,3,6	IL-1 β 2	IL-4 1,3	IFN- γ 1	IL-13 3	PMA 2
E003							
E005		↑ 15.2-115*	↑ 114*				↑ 1.60
E006							↑ 22.7*
E008			↓ 0.64†	↑ 1.79-2.47		↑ 1.85	
E009		↑ 25.1-73.7	↑ 17.0*	↓ 2.13-5.70	↑ 1.58	↑ 6.32	↑ 1.36*, ↓ 0.62†
E011							↑ 14.5*
E012							↑ 1.30*
E013		↓ 0.40-0.66	↓ 0.43				↓ 0.70
E016		↑ 1.36-4.00	↑ 12.6†				↑ 2.25
E017			↓ 0.68*	↑ 1.40-1.67		↑ 1.73	↑ 19.4*
E025		↓ 0.56-0.70	↓ 0.37				
E030		↑ 1.18-2.21	↑ 1.41				↑ 3.86
E032		↑ 1.31-2.37*	↑ 2.30*				↓ 0.39
E033			↓ 0.54*				↑ 12.2*
E035		↑ 1.29-3.10*	↑ 3.30		↓ 0.60		↑ 1.90
E036							↑ 12.4
E037		↓ 0.70-0.73	↓ 0.63			↓ 0.69	↑ 2.45†
E038		↑ 1.77-2.05				0.74	↑ 2.31†
E039		↑ 1.79-2.21	↑ 1.30	↓ 0.66-0.73		↓ 0.69	↑ 2.34
E042			↓ 0.54*	↓ 0.69-0.73		↓ 0.69	↑ 2.69
E045		↓ 0.40-0.79	↓ 0.17				↑ 1.31
E050					↓ 0.64		↑ 1.42
E057		↑ 1.15-1.31					↑ 1.50
E058		↑ 1.15-1.78	↓ 0.66				↑ 1.37†
E059		↑ 1.31-1.77					↑ 1.41†
E062			↓ 0.36*, ↑ 1.35				↑ 1.41†
E065			↓ 0.26*				↓ 0.50
E066			↓ 0.51*				↑ 1.41†
E067			↓ 0.57*				
E068							↑ 1.42†
E073					↑ 1.34		
E076							↑ 1.43
E077							↓ 0.66*
E078							↑ 1.40†
E081				↑ 1.24-1.61		↑ 1.51	
E083			↓ 0.66*				
E084					↑ 1.36		
E085							↑ 10.6
E086							↑ 16.9
E087		↑ 3.42-28.4*	↑ 25.2*				↑ 10.1
E092							↑ 8.60*
							↑ 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.