AS1.1 Adhesion Structure Subpanel 1, E rosetting/GPI anchor: CD2, CD48, CD55, CD58, CD59, CD99, and CDw108

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Subpanel 1 of the Adhesion Section included 31 monoclonal antibodies (mAb) recognizing cell surface antigens involved in rosette formation between human erythrocytes (E^{hu}) and T cells, as well as existing or novel glycosylphosphatidylinositol (GPI)-anchored antigens (Table 1). References in the literature to the mAb can be found in Table 3 of the Section Report [Springer *et al.*, AS1]. Some of the antigens studied in this panel were also studied in the T-cell Section. Prior to distribution to the 53 evaluating laboratories, pre-cluster analyses were performed to confirm mAb specificity, titre, and the presence or absence of a GPI anchor. First, Jurkat, JY, and K-562 cells were analysed by indirect immunofluorescence and flow cytometry for the presence of positive staining with mAb. Second, ^{125}I-labelled JY or Jurkat lysates were immunoprecipitated with mAb and the immunoprecipitates analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions and autoradiography to confirm the reported M_r of the antigens. Third, a panel of three pairs of normal and GPI-anchor-deficient cell lines [Klickstein *et al.*, AS1.7] were indirectly stained with mAb and analysed by flow cytometry to determine if the antigens were GPI-anchored. Thirty-four laboratories returned data, including 24 flow cytometric analyses, three immunohistochemical studies, and 11 functional assays. The specificity of each mAb was confirmed by at least one participant by binding to transfected cells or purified protein (Table 1). Epitope mapping was performed for CD58 and CD59 with the Fifth Workshop mAb (Table 1) and for CD48 with non-Workshop mAb [Henniker and Bradstock, AS1.14].

mAb were screened for the ability to block neuraminidase-treated E^{hu} binding to purified T cells or T-cell lines [Menu *et al.*, T11.4; van Kemenade, Unpublished Workshop report] and for the ability to stimulate or inhibit T-cell proliferation [Pickl *et al.*, T6.9; van Kemenade, unpublished Workshop report]. A subset of CD58 and CD2 mAb completely inhibited E^{hu} rosetting to Jurkat cells and a subset of CD55 and CD59 mAb partially inhibited this (Table 2) [Menu *et al.*, T11.4]. In contrast, mAb to CD2, CD58, and CD59 only partially inhibited E^{hu} rosetting with peripheral blood mononuclear cells (Table 2). Proliferation of peripheral blood T cells in response to a variety of stimuli was primarily inhibited by mAb to CD2 or CD58, and to a lesser extent by many other mAb (Table 2).

Several laboratories screened mAb for binding to normal, phosphatidylinositol-specific phospholipase C-treated and GPI-anchor-deficient cells [Solomon *et al.*, AS1.12; Henniker and Bradstock, AS1.14; Klickstein *et al.*, AS1.7; Schubert *et al.*, AS1.10] and identified all CD48, CD55, & CD58, and CD59 mAb in the subpanel. These studies also recognized two mAb, contributed by Hořejší's group that are entirely GPI-anchored and form a new cluster, CDw108. Finally, these studies confirmed that the CD99 (E2) and CD2 antigens are not GPI-anchored.

The mechanism by which mAb to GPI-anchored antigens are able to stimulate cellular proliferation is an active area of investigation, and mAb recognizing several GPI-anchored antigens were found to coprecipitate a protein kinase activity [Angelisová *et al.*, AS1.8]. This coprecipitating activity may contain p56^{lck} and other kinases related to the T-cell antigen receptor (TCR) because mAb to CD73 fail to stimulate proliferation in TCR- or p56^{lck}-deficient Jurkat cells transfected with the CD73 cDNA; however, these mAb stimulate vigorous proliferation in normal Jurkat cells [Resta *et al.*, T26].

In the following pages, a cluster report for each of CD48, CD55, CD58, CD59, and CDw108 briefly summarizes current structural and functional information and introduces the original reports that follow. Cluster reports for CD2 and CD99 (E2) are located in the T-cell Section [Denning, T11; Gélin *et al.*, T3].

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Table 1 Specificities of the Adhesion Structures Subpanel 1 mAb

Workshop mAb					Specificity determined by				
Code	Clone name	Donor	Species	Isotype	ELISA*	Epitope mapping†			GPI-anchored‡
CD48									
S014	MEM-102	Hořejší	Mouse	IgG1	CD48	CD48			Yes
S018	MEM-124	Hořejší	Mouse	IgM	CD48	CD48			Yes
S028	6.28	Thorley-Lawson	Mouse	IgG3	CD48	CD48			Yes
S276	Mo2PT501	Navarette/Pathan	Mouse	IgG1	CD48	NT			Yes
					CD55 transfected cells§				GPI-anchored‡
CD55									
S016	MEM-118	Hořejší	Mouse	IgM	CD55				Yes
S031	IA10	Whitlow	Mouse	IgG2a	CD55				Yes
					Flow cytometry¶	Cross-blocking‖			GPI-anchored‡
CD58									
S002	BRIC5	Anstee/Judson	Mouse	IgG2a	CD58	CD58, a			Partially
S024	TS2/9	Springer	Mouse	IgG1	CD58	CD58, a			Partially
S029	AICD58	van Agthoven	Mouse	IgG2a	CD58	CD58, b			Partially
					Flow cytometry¶	ELISA*	Epitope mapping† Fletcher	Epitope mapping† Anstee	GPI-anchored‡
CD59									
S003	BRIC229	Anstee/Judson	Mouse	IgG2b	CD59	CD59	CD59, a	CD59, a	Yes
S006	p282	Bernard	Mouse	IgG1	CD59	CD59	CD59, c	CD59, b	Yes
S010	BRA10G	Chorvath	Mouse	IgG2b	CD59	CD59	CD59, a	CD59, a	Yes
S011	YTH53.1	Hale/Waldmann	Rat	IgG2b	CD59	CD59	CD59, a	CD59, a	Yes
S012	MEM-43/5	Hořejší	Mouse	IgG2b	CD59	CD59	CD59, b	CD59, b	Yes
S013	MEM-43	Hořejší	Mouse	IgG2a	CD59	CD59	CD59, a	CD59, b	Yes
S019	MEM-125	Hořejší	Mouse	IgM	CD59	CD59	CD59, b	CD59, b	Yes
S022	C2G4	Klickstein/Springer	Mouse	IgM	CD59	CD59	CD59, b	CD59, b	Yes
					Flow cytometry¶ Zola	Flow cytometry¶ Boumsell			GPI-anchored‡
CD99									
S007	D44	Bernard	Mouse	IgG2b	CD99R				No
S008	L129	Bernard	Mouse	IgM	NT	CD99R			No
S009	0662	Bernard	Mouse	IgG3	NT	CD99			No
S020	MEM-131	Hořejší	Mouse	IgM	CD99R	CD99R			No
S023	12E7	Levy	Mouse	IgG1	NT	CD99			No
S027	O13	Rettig	Mouse	IgG1	NT	CD99R			No
					Binding to CD2-Ig fusion protein**	Flow cytometry¶			GPI-anchored‡
CD2									
S004	D66	Bernard	Mouse	IgM	CD2				No
S005	GT2	Bernard	Mouse	IgG1	CD2	CD2			No
S025	TS1/8	Springer	Mouse	IgG1	CD2	CD2			No
S026	TS2/18	Springer	Mouse	IgG1	CD2	CD2			No

(Continued)

Table 1 *(Continued)*

Workshop mAb					Specificity determined by		
Code	Clone name	Donor	Species	Isotype	ELISA*	Epitope mapping†	GPI-anchored‡
					Specificity		GPI-anchored‡
CDw108							
S015	MEM-121	Hořejší	Mouse	IgM	CDw108		Yes
S017	MEM-150	Hořejší	Mouse	IgM	CDw108		Yes
					Specificity		GPI-anchored‡
Other mAb							
S030	148-2D12	Vilella	Mouse	IgG1	CD100		No
S001	BRIC214	Anstee/Judson	Mouse	IgG1	CD44		No
S033	2/1A4.1	Klickstein/Springer	Mouse	IgG1	CD16		Yes

*For CD48 mAb an enzyme-linked immunosorbent assay (ELISA) was performed by Ianelli and Thorley-Lawson using purified, recombinant CD48 protein. For CD59 mAb an ELISA was performed by Knapp using purified CD59 protein.
†For CD48 mAb epitope mapping was performed by Henniker using fluorescein isothiocyanate (FITC)-conjugated mAb and ^{125}I-labelled mAb. Two groups performed epitope mapping for CD59 mAb. Fletcher's group [AS1.13] used FITC-conjugated CD59 mAb 2/24 for this purpose. In their data: a, cross-blocks 2/24 completely; b, cross-blocks partially; c, does not cross-block. Anstee's group used mAb S003 (BRIC229). In their data: a, cross-blocking mAb; b, non-cross-blocking mAb.
‡Pooled results of Alfinito, Finberg, Henniker, Klickstein, and Schmidt.
§Studies by Klickstein's group on CD55 transfectant COS and L cells. Note that S031 (IA10) recognizes an endogenous COS-cell antigen, probably monkey CD55.
¶For CD58 and CD59, Perry and Bierer carried out flow cytometry of a CHO cell transfectant. For CD99 Zola's group and Boumsell's group both carried out flow cytometry of E2 transfectants. For CD2, Bierer's group carried out flow cytometry of a human CD2-transfected murine T-cell hybridoma.
||Cross-blocking of ^{125}I-labelled CD58 mAb BRIC227. a, Cross-blocking mAb; b, non-cross-blocking mAb. Study carried out by Anstee's group.
**Study by Knapp's group of mAb binding to CD2 Ig fusion protein captured on Immunobeads.

Table 2 Functional studies of Adhesion Structure Subpanel 1 mAb

Workshop mAB		% Inhibition of					Binding of rCD2–dextran–FITC ligand to B-LCL¶	PBMC proliferation**	
		E^{hu} rosette formation*		Elutriated lymphocyte proliferation†	Peripheral blood T-cell proliferation‡	CD2+CD2R stimulated PBMC proliferation§		Stimulation‖	Costimulation with CD28 mAb**
Code	Clone name	+Jurkat	+PBMC						
CD2									
S004	D66	20	26	52	28	22		Yes	No
S005	GT2	14	20	Stimulatory	11	13		Yes	No
S025	TS1/8	100	77	99	67	100		No	Yes
S026	TS2/18	100	76	92	65	100		No	Yes
CD48									
S014	MEM-102	0	NT	NT	47				
S018	MEM-124	5	10	19	25				
S028	6.28	14	3	51	3				

(Continued)

Table 2 *(Continued)*

Workshop mAB		% Inhibition of						PBMC proliferation**	
		E[hu] rosette formation*		Elutriated lymphocyte proliferation†	Peripheral blood T-cell proliferation‡	CD2 + CD2R stimulated PBMC proliferation§	Binding of rCD2–dextran–FITC ligand to B-LCL¶		Costimulation with CD28 mAb**
Code	Clone name	+ Jurkat	+ PBMC					Stimulation	
CD55									
S016	MEM-118	50	12	78	17				
S031	IA10	50	4	55	35				
CD58									
S002	BRIC5	100	63	95	61		99		
S024	TS2/9	100	66	88	48		98		
S029	AICD58	29	15	77	7		55		
CD59									
S003	BRIC229	25	1	13	32				
S006	p282	38	14	70	20				
S010	BRA10G	20	7	40	NT				
S011	YTH53.1	31	0	76	18				
S012	MEM-43/5	12	5	58	33				
S013	MEM-43	30	12	80	47				
S019	MEM-125	34	85	9	28				
S022	C2G4	53	76	44	NT				
CD99									
S007	D44	5	6	50	NT				
S008	L129	42	5	35	10				
S009	0662	3	9	26	8				
S020	MEM-131	0	9	40	29				
S023	12E7	7	5	63	15				
S027	O13	5	6	16	11				
CDw108									
S015	MEM-121	12	10	28	34				
S017	MEM-150	5	4	34	14				
Other mAb									
S030	148-2D12	14	13	64	7				
S001	BRIC214	40	9	0	NT				
S033	2/1A4.1	0	0	18	12				

*The group of Perry and Bierer studied the inhibition of E[hu] rosette formation with the Jurkat T-cell line and Van Kemenade studied the inhibition of E[hu] rosette formation with peripheral blood mononuclear cells (PBMC).
†A study by Van Kemenade of % inhibition of elutriated lymphocyte proliferation stimulated by an irradiated Epstein–Barr virus (EBV)-transformed B-cell large-cell lymphoma (B-LCL).
‡A study by Kamoun of % inhibition of peripheral blood T-cell proliferation stimulated with irradiated, activated T cells.
§A study by Knapp of % inhibition of OKT11 + VIT13 (CD2 + CD2R)-stimulated proliferation of PBMC.
¶A study by Warren of % inhibition of binding of rCD2–dextran–FITC ligand to a B-LCL.
||A study by Knapp of stimulation of PBMC proliferation.
**A study by Knapp of costimulation with CD28 mAb of PBMC.