AS7/8 Adhesion structure subpanels 7 and 8, β_3, β_4, β_7 integrins and novel functional antigens: CD51, CD61, CD103, and CD104

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Subpanel 7

Subpanel 7 contained antibodies against β integrin families other than the β_1 or β_2 families and against other molecules thought to be adhesion structures. Literature references to these monoclonal antibodies (mAb) are listed in Table 3 of the Adhesion Structure Section report [Springer *et al.*, AS1]. mAb included in this Subpanel were to CD51 (integrin α^V), CD61 (integrin β_3), and integrin subunits targeted for clustering at this workshop: α^E, β_4, and β_7. We accepted 29 mAb for the Subpanel. On the basis of our preliminary studies, 22 of these mAb were included in the Blind Panel. The Subpanel was sent to 81 laboratories for blind evaluation. Many laboratories returned data, including 28 flow cytometric studies, 12 immunohistochemical studies, 6 immunoprecipitation studies, and 8 functional studies. These studies along with data from our laboratory and the Blind Panel were used to cluster the antibodies (Table 1).

CD51 α^V

The clustering of CD51 mAb was based on flow cytometry, immunoprecipitation, enzyme-linked immunosorbent assay (ELISA), and immunohistochemistry. mAb S236 (AMF7), S245 (13C2), and S262 (LM142) react with both $\alpha^V\beta_3$ and $\alpha^V\beta_5$ but not $\alpha^{IIb}\beta_3$. They immunoprecipitated $\alpha^V\beta_5$ from epithelial cells (Fig. 2). These three antibodies therefore, cluster to CD51 (α^V).

CD51/CD61 $\alpha^V\beta_3$

S246 (23C6) and S263 (LM609) clustered together in flow cytometric studies, showing a reactivity distinct from that of CD51 mAb. They reacted with cells expressing $\alpha^V\beta_3$, but not cells known to be positive for $\alpha^V\beta_5$ or platelets positive for with $\alpha^{IIb}\beta_3$. They immunoprecipitated $\alpha^V\beta_3$ well (Fig. 1) but showed little immunoprecipitation of $\alpha^V\beta_5$ (Fig. 2). By ELISA and immunoprecipitation they were reactive to $\alpha^V\beta_3$but not $\alpha^{IIb}\beta_3$[Honda *et al.*, P 7.1]. The immunohistochemistry of these two antibodies showed consistently the same pattern, which was different from that of the CD51 antibodies [Zutter, AS7/8.1]. S246 (23C6) and S263 (LM609) are therefore specific for the CD51/CD61 ($\alpha^V\beta_3$) complex, and do not react with α^V or β_3 associated with other integrin subunits.

CD61 β_3

The clustering of CD61 antibodies was based on flow cytometry, immunoprecipitation, and ELISA. The clustering of CD61 was difficult because none of the antibodies in Subpanel 7 gave equivalent staining of cells expressing both CD41/CD61 and CD51/CD61 except for S258 (C5-1) which was a rat mAb and gave weak staining. In the Blind Panel the reference CD61 mAb P022 (Y-2/51) did give good staining of both CD61 integrins. S239 (CLB-thromb/1) and S249 (AP5) stained CD41/CD61 but only weakly stained CD51/CD61. S249 (AP5) did cluster near the other CD61 mAb in the Blind Panel. S249 (AP5) immunoprecipitated CD41/CD61 from platelets but was weak or ineffective in immunoprecipitating CD51/CD61 (Fig. 1); however, in ELISA studies S239 (CLB-thromb/1) and S249 (AP5) clearly bound to both CD41/CD61 and CD51/CD61. Therefore, they were clustered as CD61 mAb that favour CD41/CD61. S250 (AP6) was present on activated but not resting platelets, as previously reported, and did not cluster to other CD61 antibodies. S250 (AP6) immunoprecipated CD41/CD61 but failed to immunoprecipitate CD51/CD61. S234 (7G2) immunoprecipitated both but appeared to be better at precipitating CD41/CD61 than CD51/CD61. In ELISA, both AP6 and 7G2 bound to CD41/CD61 and CD51/CD61. Therefore, they appear to be CD61 mAb that are reactive to a CD61 epitope exposed on only a subset of CD61 molecules. S258 (C51) clustered as a CD61 mAb and immunoprecipitated both CD51/CD61 and CD41/CD61. On ELISA it bound to both CD41/CD61 and CD51/CD61. It has been clustered

Table 1 Specificities of Workshop Adhesion Structure Subpanel 7 and 8 mAb

Workshop mAb		Donor	Species	Isotype	Flow cytometry	Immuno-histochemistry	Immuno-precipitation	Species cross-reactivity	Reported functional activity	Characterization
Code	Clone name									
CD51 α^V										
S236	AMF7	van Agthoven/ deVries	Mouse	IgG1	Yes	Yes	Yes		Yes*	CD51
S245	13C2	Horton	Mouse	IgG1	Yes	Yes	Yes	Bovine		CD51
S262	LM142	Cheresh	Mouse	IgG1	Yes	Yes	Yes	Bovine	Yes†	CD51
CD51/CD61 $\alpha^V\beta_3$										
S246	23C6	Horton	Mouse	IgG1	Weak	Weak	Weak		Yes	CD51/CD61 complex
S263	LM609	Cheresh	Mouse	IgG1	Yes	Yes	Yes	Bovine	Yes†	CD51/CD61 complex
CD61 β_3										
S239	CLB-thromb/1	von dem Borne/ Admiraal	Mouse	IgG1	CD41/CD61 > CD51/CD61		CD41/CD61 > CD51/CD61			CD61, α-dependent
S249	AP5	Kunicki	Mouse	IgG1κ	CD41/CD61 > CD51/CD61	Yes CD41/ CD61	CD41/CD61 > CD51/CD61	Porcine		CD61, α-dependent
S234	7G2	Brown	Mouse	IgG1	Weak		Yes			CD61 subset
S250	AP6	Kunicki	Mouse	IgMκ	CD61 subset	Yes	CD41/CD61	Bovine		CD61 subset
S258	C5.1	Butcher	Rat	IgG1	Weak CD61	Yes	Yes	Bovine		Weak CD61
CD103 α^E										
S237	F3F7	Cerf-Bensussan	Mouse	IgG1	Yes	Yes	Yes		Yes	CD103
S238	F4F1	Cerf-Bensussan	Mouse	IgG1	Yes	Yes	Yes		Yes	CD103
S242	LF61	Falini	Mouse	IgG1	Yes		Yes			CD103
S256	Ber-ACT 8	Stein/Dürkop/ Schwarting	Mouse	IgG1κ	Yes	Yes	Yes		Yes	CD103
S257	HML-1	van Agthoven/ Cerf-Bensussan	Mouse	IgG2a	Yes	Yes	Yes		Yes	CD103
CD104 β_4										
S235	UM-A9	Carey	Mouse	IgG2a	Yes	Yes	Yes		Yes	CD104
S247	439-9B	Kennel/Falcioni	Rat	IgG2b	Yes	Yes	Yes			CD104
S248	450-11A1	Kennel	Mouse	IgG1	? Some cells	Yes	Yes			CD104, cytoplasmic

(continued)

Table 1 *(continued)*

Workshop mAb Code	Clone name	Donor	Species	Isotype	Flow cytometry	Immuno-histochemistry	Immuno-precipitation	Species cross-reactivity	Reported functional activity	Characterization
β_7										
S253	BP6	Pulford	Mouse	IgG	Some lymphocytic leukaemias	Yes	Yes $\alpha^E\beta_7$		Yes‡	? β_7 subset
S254	ACT-1	Shaw/Lazarovits	Mouse	IgG1	Only $\alpha^4\beta_7$	Yes	Yes $\alpha^E\beta_7$ and $\alpha^4\beta_7$	Porcine	Yes§	$\beta_7\alpha^4$-dependent
Clustered to other CD										
S231	PEN3	P. Anderson/ Vivier	Mouse	IgG1	Yes		Yes			CD11b
S232	PEN2	P. Anderson/ Vivier	Mouse	IgG2a	Yes	Yes	Yes	Porcine		CD11b
S233	A8	Aversa	Mouse	IgG1	Yes	Yes	Yes	Bovine		CD100
S241	10.1.2	Corte/Giunta	Mouse	IgG1	Yes		Yes			CD49c
S244	GRV1	Garrido	Mouse	IgG1$\varkappa$	Yes		Yes			CD98
S271	B6H12	Brown	Mouse	IgG1	Yes					CD47
S272	CBR5D.1	Diamond/ Springer	Mouse	IgG1	Yes		Yes			CD43
S273	1D6	Levy	Mouse	IgG1	Yes	Yes				CD81
S274	5A6	Levy	Mouse	IgG1	Yes	Yes				CD81
S276	Mo2PT501	Navarrete/ Pathan	Mouse	IgG1$\varkappa$	Yes					CD48
Unclustered										
S240	FB12	Zocchi	Mouse	IgG1						Weak or negative
S251	J3-119	Pesando	Mouse	IgG2a	Yes	Yes	Yes, 105 kDa reduced		CD6 ligand	
S252	J4-81	Pesando	Mouse	IgG1	Yes	Yes	Yes, 105 kDa reduced		CD6 ligand	
S255	LAM2	Stahel/Lehmann	Mouse	IgM						Weak or negative
S275	G28-8	Clark/Ledbetter	Mouse	IgG1	Yes		Yes, 97 kDa reduced			

*For details see Flora and Gregory, AS7/8.9.
†Unpublished Workshop report by Wyss-Coray *et al.*
‡Unpublished Workshop report by Poignard *et al.*
§Information supplied by Asjo.

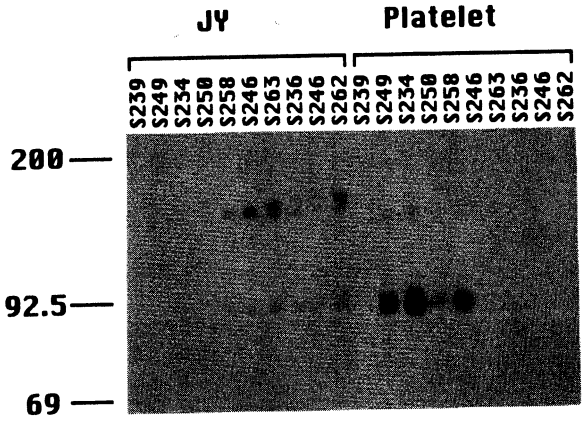

Fig. 1 Immunoprecipitation of CD51/CD61 from ^{125}I-surface-labelled JY cells and CD41/CD61 from ^{125}I-surface-labelled platelets. Immunoprecipitates were formed with the indicated mAb, mAb 187.1 directed against the mouse kappa chain, and protein A Sepharose, and subjected to 7% SDS-PAGE under non-reducing conditions.

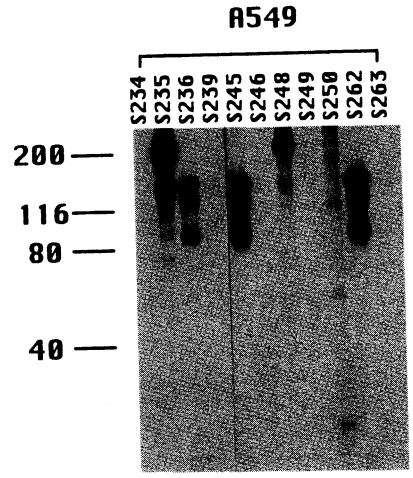

Fig. 2 Immunoprecipitation of CD51/β_5 and CD49f/CD104 from ^{125}I-surface-labelled A539 cells. Immunoprecipitates were formed with the indicated mAb, and subjected to 7% SDS-PAGE under non-reducing conditions as described in Fig. 1. This experiment was carried out by Drs Jana Bodorova and Martin Hemler.

as a weak CD61 antibody, although the choice of second antibody may play a role in the reactivity of this rat mAb.

CD103 α^E

The clustering of CD103 was based on flow cytometry and studies on intestinal intraepithelial lymphocytes (iIEL) and JY cells. mAb S237 (F3F7), S238 (F4F1), S242 (LF61), S256 (Ber-ACT-8), and S257 (HML-1) all stained iIEL cells in immunohistochemistry and were clustered closely in the Blind Panel. All five antibodies immunoprecipitated $\alpha^E\beta_7$ from iIEL (Fig. 3). These mAb were negative on JY cells that express $\alpha^4\beta_7$ by flow cytometry and by immunoprecipitation.

CD104 β_4

The clustering of mAb to the integrin β_4 subunit as CD104 was based on flow cytometry, immunohistochemistry, and immunoprecipitation. mAb S235 (UM-A9) and S247 (439-9B) clustered closely in flow cytometry. They stained epithelial cells from the skin and thymus as well as some monocytic and B-cell lines. Surprisingly, S248 (450-11A1) to the cytoplasmic domain of β_4 stained a subset of the cells stained by the other mAb. On histology all three antibodies stained skin in a similar pattern. All three antibodies were able to immunoprecipitate β_4 (Fig. 2 and data

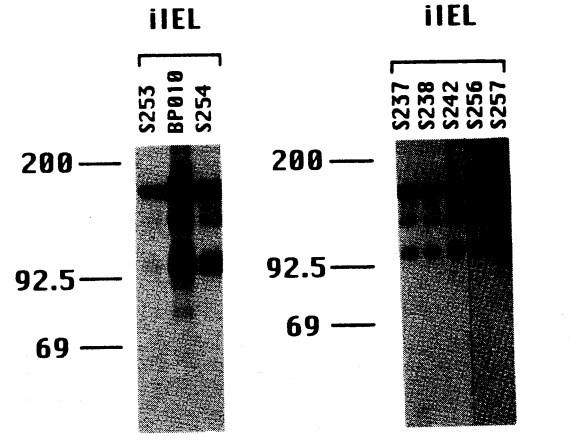

Fig. 3 Immunoprecipitation of CD103/β_7 from ^{125}I-surface-labelled, cultured intestinal intraepithelial lymphocyte (iIEL) cells. Immunoprecipitates formed with the indicated Adhesion Structure and Blind Panel mAb were subjected to 7% SDS-PAGE under non-reducing conditions. This experiment was carried out by Karen Cepek and Dr Michael Brenner.

not shown). β_4 transfectants studied by V. Quaranta confirmed that S235 (UM-A9), S247 (439-9B), and S205 (AA3), a Subpanel 6 mAb, are all specific for the β_4 subunit [Hemler *et al.*, AS6.7].

Pre-CD β_7

β_7 could not be clustered in this Workshop. Both β_7 antibodies submitted to the Workshop, S253 (BP6) and S254 (ACT-1), immunoprecipitated $\alpha^E\beta_7$ from iIEL (Fig. 3). S253 (BP6) was almost completely negative in flow cytometry, including on iIEL, but reacted with iIEL in tissue sections [Zutter, AS 7/8.1). S254 (ACT-1) reacts with a subpopulation of lymphocytes consistent with recognition of $\alpha^4\beta_7$ and did not stain $\alpha^E\beta_7$ iIEL in flow cytometry or in tissue sections. Therefore, S254 (ACT-1) appears to bind to β_7 only when associated with the α^4 subunit in intact cells, and can recognize solubilized $\alpha^E\beta_7$, whereas S253 (BP6) binds to an epitope on $\alpha^E\beta_7$ that is not present on intact cells but is present on cells in tissue sections or on $\alpha^E\beta_7$ after solubilization.

Antibodies clustered to other CDs

Five mAb were clustered to other panels based on the Blind Panel result and follow-up studies. mAb S231 (PEN3) and S232 (PEN2) are directed against CD11b. S241 (10.1.2) binds to CD49c. S244 (LF61) was clustered to CD98 (4F2). S233 (A8) was clustered to CD100. S233 immunoprecipitated a 280–300 kDa band in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing conditions [Cepek *et al.*, Wong *et al.*, unpublished Workshop reports] and under reducing conditions two bands at 150–140 and at 70–80 kDa [Kissansen *et al.*, unpublished Workshop report] (Fig. 4).

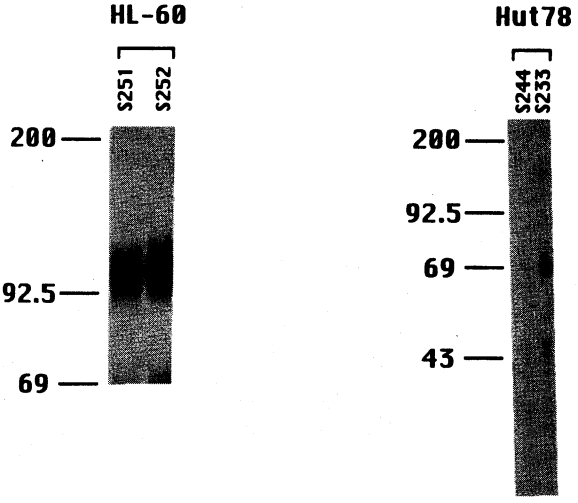

Fig. 4 Immunoprecipitation of other antigens. ^{125}I-surface-labelled HL-60 or HUT-78 lysates were subjected to immunoprecipitation and SDS-PAGE as described in Fig. 1.

Unclustered antibodies

Four Subpanel 7 mAb remain unclustered. S240 (FB12) and S255 (LAM2) were weak or negative. S251 (J3-119) and S252 (J4-81) immunoprecipated a 105-kDa protein on non-reducing and reducing SDS-PAGE (Fig. 4) that is present on almost all stromal cells tested, B-cells, monocytes, and T cells. By screening the Adhesion Structure Panel in a functional assay, Patel *et al.* [AS7/8.13] found that S252 (J4-81) recognizes the receptor for CD6.

Subpanel 8

Subpanel 8 contained six mAb that could not otherwise be catagorized, but had effects on cell function. All six mAb were included in the Blind Panel. This Subpanel was sent to 75 laboratories for blinded evaluation. Many laboratories returned data, including 17 flow cytometric studies, 7 immunohistochemical studies, 3 biochemical studies, and 13 functional studies. These studies along with data from our laboratory and the Blind Panel were used to cluster the antibodies.

Antibodies clustered to CDs

Five mAb were clustered to other CDs. S271 (B6H12), which recognizes an integrin-associated protein, was clustered to CD47. S272 (CBR5D.1) was clustered to CD43. S273 (1D6) and S274 (5A6) were clustered to CD81 (TAPA). S276 (Mo2PT501) was tentatively clustered to CD48.

Unclustered antibody

S275 (G28-8) has remained unclustered after being submitted to its third Workshop. During the Workshop it was found to be expressed on some B-cell lines, monocytes, and the RD rhabdomyosarcoma cell line.

Acknowledgements

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