dendritic cells, −/(+), surface and crypt epithelium (basal part), −/+, vascular endothelium, −/+; S110 (TP1/15), extrafollicular compartment, −/(+), plasma cells, +, vascular endothelium, +.

In normal and neoplastic colonic tissues, epithelium, autonomic nervous tissue, smooth musculature, and vascular endothelium have been investigated. CAM expression in normal and neoplastic colonic epithelium as tested using the Workshop mAb was a rare and inconsistent phenomenon. In *normal mucosa* mAb S090 (CBR-IC3/4), S093 (WDS 3.A9), and S106 (By44) weakly stained epithelium in two of six cases. One of the unclustered mAb, S102 (4A11), strongly stained normal epithelium in all cases. In *solitary adenomas* the following mAb focally stained adenomatous epithelium in one of five cases: S081 (BRIC79), S088 (CBR-IC3/2), S089 (CBR-IC3/3), S091 (CBR-IC3/5), S092 (CBR-IC3/6), S093 (WDS 3.A9), S108 (TP1/24), S109 (HP2/19), S112 (KS128), S113 (152-2D11), S114 (140-11), and S115 (101-1D2). One of the unclustered mAb, S102 (4A11), stained adenomatous epithelium in three of five cases. In *FAP adenomas* only one unclustered mAb, S102 (4A11), showed weak staining in two of five cases. In *colorectal carcinomas* the following mAb showed weak and focal staining in two of eight cases: S081 (BRIC79), S088 (CBR-IC3/2), S089 (CBR-IC3/3), S092 (CBR-IC3/6), S093 (WDS 3.A9), S108 (TP1/24). In *liver metastases* of colorectal carcinomas mAb S081 (BRIC79), S088 (CBR-IC3/2), S108 (TP1/24), S112 (KS128), and S115 (101-1D2) showed weak and focal staining in one of five cases.

In colorectal tissues the following mAb stained *vascular endothelium*: S082 (F10.2), S083 (1304.100.4), S085 (CBR-IC2/1), S086 (CBR-IC2/2), S094 (CBR-IC1/3), S095 (CBR-IC1/4), S096 (CBR-IC1/11), S097 (CBR-IC1/12), S098 (7F7), S100 (8-4A6), S102 (4A11; inconsistent staining), S105 (MAY.029), S107 (RR1/1), S110 (TP1/15), and S116 (YH370). mAb S115 (101-1D2) stained *smooth musculature* of the gut wall. Staining of *neural structures* in colorectal tissues was not observed.

The flow cytometric data of colon carcinoma cell lines are listed in Table 1. In contrast to carcinomas *in situ*, the colon carcinoma cell lines were recognized by mAb of Groups 2 and 3; this especially applied for cell lines HT-29 and SW480. Group 1 mAb that inconsistently and often focally stained carcinomas *in situ* reacted with a subgroup of tumour cells of SW480. The unclustered mAb S102 (4A11) was found to recognize all five cell lines, although in various amounts and intensities. CAM expression in normal and neoplastic colonic epithelium is a rather rare and inconsistent phenomenon using the Workshop mAb. mAb clustering was mainly achieved on the basis of tonsillar tissue. Group 1 comprised mAb recognizing CD50 on the one hand and ICAM-3 on the other. Our results support the view that CD50 is ICAM-3 [1].

Acknowledgement

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AS5 Adhesion Structure Subpanel 5, leucocyte integrins: CD11a, CD11b, CD11c, and CD18

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The leucocyte integrin family consists of three heterodimeric molecules that are composed of a common β subunit (CD18) in complex with one of three distinct α subunits, CD11a, CD11b, and CD11c [1]. These molecules, known, respectively, as LFA-1, Mac-1, and p150,95, play a critical role in cell–cell adhesion in

Table 1 Characterization of Subpanel 5 mAb

Workshop mAb					Characterization	
Code	Clone name	Donor	Species	Isotype	Transfection*	Epitope mapping†
CD11a						
S125	F8.8	Bloem	Mouse	IgG1	CD11a	N or I-domain
S140	NKI-L16	Figdor/van Kooyk	Mouse	IgG2a	CD11a	?
S145	S6F1	Morimoto/Raynor	Mouse	IgG1	CD11a	N-terminus
S146	MAY.035	Ohashi	Mouse	IgG1	CD11a	I-domain
S148	25-3-1	Olive	Mouse	IgG1	CD11a	I-domain
S149	CBR LFA-1/10	Petruzzelli/Springer	Mouse	IgM	CD11a	N-terminus
S150	CBR LFA-1/9	Petruzzelli/Springer	Mouse	IgM	CD11a	N or I-domain
S151	CBR LFA-1/1	Petruzzelli/Springer	Mouse	IgG1	CD11a	I-domain
S152	CBR LFA-1/4	Petruzzelli/Springer	Mouse	IgM	CD11a	?
S154	CBR LFA-1/3	Petruzzelli/Springer	Mouse	IgG1	CD11a	ND
S158	TS2/14	Springer	Mouse	IgG1	CD11a	I-domain
S159	TS1/22	Springer	Mouse	IgG1	CD11a	I-domain
S160	TS2/4	Springer	Mouse	IgG1	CD11a	N-terminus
S161	TS2/6	Springer	Mouse	IgG1	CD11a	I-domain
S163	BL5	van Agthoven/Brochier	Mouse	IgG1	CD11a	ND
S167	G-25.2	Warner/Clayberger/ Krensky	Mouse	IgG2a	CD11a	Divalent cation repeats
S169	YTA-1	Yodoi	Mouse	IgG1	CD11a	Divalent cation repeats
CD11b						
S127	CBRM1/13	Diamond/Springer	Mouse	IgG1	CD11b	I-domain
S128	CBRM1/1	Diamond/Springer	Mouse	IgG2b	CD11b	I-domain
S129	CBRM1/20	Diamond/Springer	Mouse	IgG1	CD11b	Divalent cation repeats [3]
S130	CBRM1/23	Diamond/Springer	Mouse	IgG2a	CD11b	C-terminus
S131	CBRM1/29	Diamond/Springer	Mouse	IgG1	CD11b	I-domain
S132	CBRM1/34	Diamond/Springer	Mouse	IgG1	CD11b	I-domain
S141	Bear-1	Figdor	Mouse	IgG1	negative	?
S172	CC1.7	Ando	Mouse	IgG1	CD11b	N-terminus?
S173	TMM1	Ando	Mouse	IgG1	negative	?
S174	TMG6-5	Ando	Mouse	IgG1	CD11b	I-domain
S231	PEN3	Anderson/Vivier	Mouse	IgG1	CD11b	ND
S232	PEN2	Anderson/Vivier	Mouse	IgG2a	CD11b	ND
CD11c						
S133	CBRp150/2C1	Diamond/Springer	Mouse	IgG2a	CD11c	C-terminus
S134	CBRp150/3C1	Diamond/Springer	Mouse	IgG1	CD11c	?
S135	CBRp150/2E1	Diamond/Springer	Mouse	IgG2b	CD11c	C-terminus
S136	CBRp150/4G1	Diamond/Springer	Mouse	IgG2a	CD11c	C-terminus
S138	BL-4H4	Fiebig	Mouse	IgG1	CD11c	I-domain
S143	BU-15	Hardie/Johnson	Mouse	IgG1	CD11c	?
S144	3.9	Hogg	Mouse	IgG1	CD11c	I-domain
S156	KB90	Pulford	Mouse	IgG1	CD11c	C-terminus
S157	KB23	Pulford	Mouse	IgG	CD11c	Divalent cation repeats?
S171	S-HCL3/Leu M5	Schwarting	Mouse	IgG2b	CD11c	?

(continued)

Table 1 *(continued)*

Workshop mAb					Characterization	
Code	Clone name	Donor	Species	Isotype	Transfection*	Epitope mapping†
CD18						
S123	6.7	Bensussan/Boumsell	Mouse	IgG1	CD18	
S137	CBRM1/19	Diamond/Springer	Mouse	IgG2a	CD18	
S147	MAY.017	Ohashi	Mouse	IgG1	CD18	
S153	CBR LFA-1/7	Petruzzelli/Springer	Mouse	IgG1	CD18	
S155	CBR LFA-1/2	Petruzzelli/Springer	Mouse	IgG1	CD18	
S162	TS1/18	Springer	Mouse	IgG1	CD18	
S164	CLB-LFA1/1	van Lier	Mouse	IgG1	CD18	
S166	L130	Warner/Lanier	Mouse	IgG1,k	CD18	
Reference and other mAb						
S126	7E3	Coller/Centocor	Mouse	IgG1	CD41	
S139	BL-GCE/G3	Fiebig	Mouse	IgG1	CD43	
S122	BRIC 235	Anstee/Judson	Mouse	IgG2b	CD44?	
S168	DH12.8F5 or L129	Warner/Buck	Mouse	IgG2a,k	CD44	
S121	ICO-60	Anatoly	Mouse	IgG1	CD50	
S142	NCAM	Figdor	Mouse	IgG1	CD56	
S170	F4F1	Cerf-Bensussan	Mouse	IgG1	CD103	

*Transfection of CHO cells [Luk *et al.*, AS5.9] or K-562 cells [Petruzzelli and Springer, personal communication] expressing CD18 with either CD11a, 11b, or 11c.
†Chimeric molecules generated between CD11b/CD11c and mouse × human CD11a [details in Luk *et al.*, AS5.9 and Huang and Springer, AS5.6].

the immune system and in the inflammatory response [2]. Details of their molecular structure and function can be found within each specific CD report.

Preclustering of the monoclonal antibodies (mAb) was based upon their reactivity with cell lines transfected with the common β subunit and each of the α subunits in both CHO cells and K-562 cells. In addition, several of the antibodies were analysed as part of the Blind Panel. Table 1 lists the mAb according to CD cluster results. Literature references to the mAb are listed in Table 3 of the Adhesion Structure Section report [Springer *et al.*, AS1]. The specificity of several of the mAb was modified. mAb S154 (CBR LFA-1/3) and S163 (BL5) were initially designated as CD18 but reacted with only LFA-1 transfectants and therefore were clustered as CD11a mAb. mAb S121 (ICO-60) was submitted as a CD18 mAb but clustered with CD50. Two antibodies, S231 (PEN3) and S232 (PEN2), were submitted in the unknown adhesion structure panel but clustered with CD11b and were found to react with only CD18/CD11b transfectants. Epitope mapping of the antibodies was performed using chimeric constructs of the CD11a, CD11b, and CD11c subunits and specificity is designated in Table 1. Details of these studies are presented within separate chapters of this book. The molecular weight of the species recognized by antibodies in this group and specific ability to immunoprecipitate and immunoblot are listed within each CD chapter.

Methods

Flow cytometry studies were submitted by 24 laboratories on a wide range of T- and B-cell lines, thymocytes, peripheral blood, and bone marrow. In addition, two groups analysed the ability of the antibodies to recognize structures on neutrophils, monocytes, lymphocytes, and bone marrow from cynomologus monkey, porcine, and bovine sources.

Immunohistochemistry was performed by seven laboratories on skin, liver, normal lymph nodes, lymph nodes with follicular hyperplasia, colon from patients with Crohn's disease and normal controls, tonsil cell extracts enriched for dendritic cells,

Table 2 Functional effects of Subpanel 5 mAb

Workshop mAb		% inhibition of aggregation*		Effect on ligand binding† ICAM-1					
Code	Clone name	B-cell lines	Granulocytes	Study 1‡	Study 2§	Study 3¶	Study 4‖	Fibrinogen‖	Cell killing†**
CD11a									
S125	F8.8	100	0	B	B	B			–
S140	NKI-L16	0	25	S	S	S			–
S145	S6F1	0	0	NT	–	–			–
S146	MAY.035	100	0	NT	B	B			B
S148	25-3-1	100	0	B	B	B			B
S149	CBR LFA-1/10	75	0	NT	B	–			–
S150	CBR LFA-1/9	0	0	NT	B±	B			–
S151	CBR LFA-1/1	100	0	B	B	B			B
S152	CBR LFA-1/4	0	0	NT	B±	–			–
S154	CBR LFA-1/3	50	0	–	–	–			–
S158	TS2/14	100	0	B	B	B			–
S159	TS1/22	100	0	B	–	B			B
S160	TS2/4	0	0	–	–	–			–
S161	TS2/6	100	0	B	B	B			B
S163	BL5	100	0	NT	–	B			B
S167	G-25.2	0	0	B	B±	–			–
S169	YTA-1	0	0	NT	B±	B			–
CD11b									
S127	CBRM1/13	0	75		–		B±	B±	–
S128	CBRM1/1	0	75		–		B	B	–
S129	CBRM1/20	0	0		–		–	–	–
S130	CBRM1/23	0	0		–		–	–	–
S131	CBRM1/29	0	75		–		B	B	–
S132	CBRM1/34	0	75		–		B±	B±	–
S172	CC1.7	0	0		–		NT	NT	–
S174	TMG6-5	0	50		–		NT	NT	–
S231	PEN3	NT	NT		NT		NT	NT	–
S232	PEN2	NT	NT		NT		NT	NT	–
CD11c									
S124	HC1/1	0	0		–	–			–
S133	CBRp150/2C1	0	0		–	–			–
S134	CBRp150/3C1	0	0		–	–			–
S135	CBRp150/2E1	0	0		–	–			–
S136	CBRp150/4G1	0	0		–	–			–
S138	BL-4H4	0	0		–	–			–
S143	BU15	0	0		–	–			B
S144	3.9	0	0		–	–			–
S156	KB90	0	0		–	–			–
S157	KB12	0	0		B±	–			B
S171	S-HCL3/Leu M5	0	0		B±	–			–
CD18									
S123	6.7	25	75	B	B	–			B
S137	CBRM1/19	0	0	NT	–	S			–

(continued)

Table 2 *(continued)*

Workshop mAb		% inhibition of aggregation*		Effect on ligand binding† ICAM-1					
Code	Clone name	B-cell lines	Granulocytes	Study 1‡	Study 2§	Study 3¶	Study 4‖	Fibrinogen‖	Cell killing†**
S147	MAY.017	100	88	NT	B	B			B
S153	CBR LFA-1/7	0	0	NT	B	–			–
S155	CBR LFA-1/2	50	0	S	S	S			–
S162	TS1/18	100	88	NT	B±	B			B
S164	CLB-LFA1/1	100	88	NT	B±	B			B
S166	L130	100	50	NT	B	B			B
Reference and other mAb									
S121	ICO-60	–	–	NT	–	B±			–
S122	BRIC235	–	–	NT	–	B±			–
S126	7E3	–	–	–	–	NT			–
S139	BL-GCE/G3	NT	NT	NT	NT	NT			–
S142	NCAM	–	–	NT	–	NT			–
S168	DH12.8F5/L129	–	–	NT	–	NT			–
S170	F4F1	–	–	NT	–	NT			–

*Study by M. Pattaroyo's group [unpublished Workshop report]. Inhibition of phorbol myristate acetate (PMA)-induced aggregation in lymphoblastoid B-cell lines and granulocytes. NT, Not tested; –, no effect.
†B, Blocking; B±, partial blocking; S, stimulatory; –, no effect; NT, not tested.
‡Study by C. Figdor's group. Effect of the mAb on lymphocyte cell line J136 binding to L cells expressing ICAM-1 with and without stimulatory mAb NKI-L16.
§Study by N. Hogg's group. Effect of binding of 2-week cultured peripheral blood lymphocytes to purified ICAM-1.
¶Study by Petruzzelli *et al.* [AS5.5]. Effect of mAb on JY-cell binding to purified ICAM-1 and ICAM-1 Fc.
‖See reference 3 for details.
**Study by P. I. Terasaki's group. Effect of mAb on the natural killing of K-562 cells. B, Blocking; –, no effect.

and synovium from patients with and without rheumatoid arthritis.

Functional studies were carried out by 12 groups and a summary of the results appears in Table 2. Three groups examined the effect of the mAb on both stimulating and inhibiting binding to T- and B-cell lines to ICAM-1. One group examined the ability of the antibodies to modulate neutrophil binding to ICAM-1 and fibrinogen. One group examined the ability of the mAb to inhibit aggregation of a B-lymphoblastoid cell line and granulocytes. Several groups examined the effect of the antibodies on binding or aggregation through other ligands for LFA-1, specifically, ICAM-2 and ICAM-3 [Binnerts *et al.*, AS5.7; Petruzzelli *et al.*, AS5.5]. One group examined the effect of the antibodies on natural killing of K-562 cells. Hořejší examined the effect of binding of these antibodies after stimulation of PBL with PMA and found that there was a marked increase in binding of one antibody, S172 (CC1.7).

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