T. Springer, in preparation]. All mAb were tested for their effect on binding to ICAM-1 and a representative group is presented in Table 1. The percentage of input cell binding is compared to that seen with phorbol ester stimulation and a control supernatant, X63. Many of the CD11a and CD18 antibodies, for example, S147 (MAY.017) and S161 (TS2/6), inhibit binding to ICAM-1. However, two mAb, S140 (NKI-L16) and S155 (CBR LFA-1/2), stimulated binding of all three cell lines to ICAM-1. One mAb, S137 (CBRM1/19), was able to stimulate binding of two of the cell lines.

The effect of a representative panel of antibodies on binding of cells to ICAM-3 is shown in Table 2. mAb S155 (CBR LFA-1/2) was able to enhance binding of both cell lines to ICAM-3, whereas the effect of S140 (NKI-L16) was seen only with the JY cells. mAb S137 (CBRM1/19) was also able to stimulate binding to ICAM-3.

These findings show that antibodies directed against both the α and β subunits of LFA-1 are able to induce binding to two of its ligands, ICAM-1 and ICAM-3, and to overcome the intracellular signalling defect found in the CD18/CD11a K-562 transfectants. S140 (NKI-L16) has previously been shown to recognize an activation epitope of LFA-1 and to stimulate binding to ICAM-1 and here is able to modulate binding of LFA-1 to both ICAM-1 and ICAM-3. The two CD18 mAb, S155 (CBR LFA-1/2) raised against purified LFA-1 and S137 (CBRM1/19) prepared using purified Mac-1, were both able to stimulate binding of LFA-1 bearing cells to ICAM-1 and ICAM-3. The mAb to both the α and β subunits can now be used to probe the structural and functional requirements for the activation of LFA-1.

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AS5.6 Domain localization and correlation with inhibition of function of Workshop CD11a mAb

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LFA-1 (CD11a/CD18) is important in many adhesion-dependent phenomena in immunity and inflammation [1]. LFA-1 has three counterreceptors, ICAM-1, -2, and -3, that are members of the immunoglobulin superfamily. The structural regions on LFA-1 that mediate the recognition of ICAMs are still undefined. LFA-1 is a heterodimer consisting of two subunits, α^L (CD11a) and β_2 (CD18). The extracellular domain of α^L has two prominent structural features: (1) there is a putative divalent-cation-binding region consisting of three EF-hand-like motifs; and (2) there is an insertion of about 200 amino acids (I domain) that is not present in many other integrins [2]. The I domain and EF-hand motifs have been implicated in ligand binding in other integrins [3,4].

To localize the ICAM-1 binding sites on human LFA-1, a set of nine CD11a chimeras between human and mouse α subunits with reciprocal exchanges of the N-terminal region, I domain, metal binding domain, and the remaining C-terminal portion were constructed

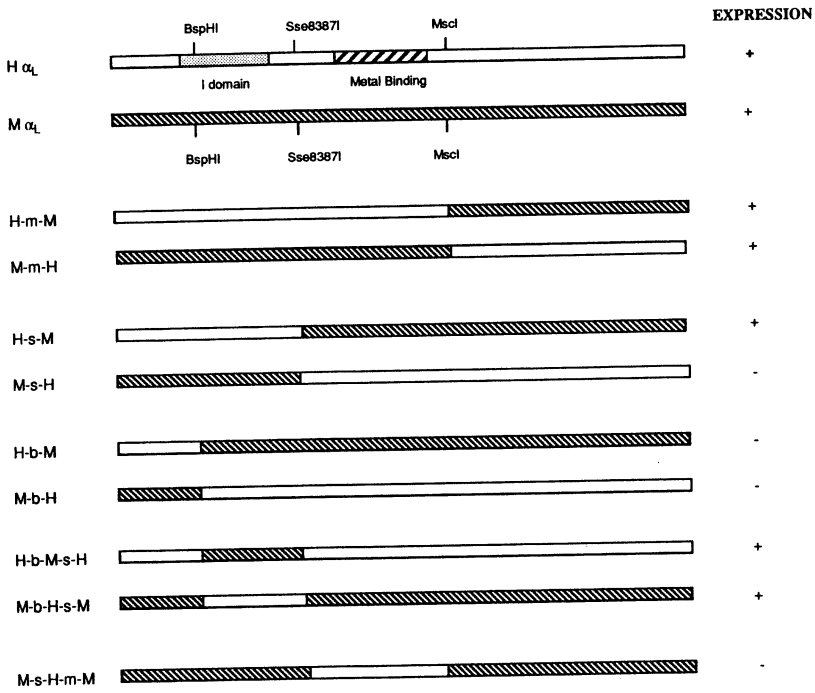

Fig. 1 Schematic structure of the human/mouse chimerical LFA-1 α subunits. Three restriction sites, s= type human LFA-1 were coexpressed with the human β_2 subunit in transfected COS cells and detected by staining with CD18 mAb and flow cytometry.

in the expression vector APrM8 (Fig. 1). In this report, we mapped the epitopes of Workshop mouse anti-human CD11a monoclonal antibodies (mAb) with these chimeras, and have preliminarily correlated epitope localization with ability to block LFA-1 binding to ICAM-1.

Three restriction sites that naturally exist in human and mouse α chains at the same positions were used

Table 1 mAb epitopes on LFA-1 and inhibition of binding to ICAM-1

Workshop mAb		Binding to transfectants*							Inhibition of binding to ICAM-1 (% ± SEM)‡
Code	Clone name	H	HsM	HmM	MmH	HbMsH	MbHsM	Epitope†	
S145	S6F1	+	+	+	−	+	−	N	27.5 ± 6.3
S149	CBR LFA-1/10	±	±	±	−	±	−	N	13.6 ± 4.7
S160	TS2/4	+	+	+	−	+	−	N	29.2 ± 5.9
S125	F8.8	+	+	+	−	−	−	N or I	76.6 ± 3.6
S150	CBR LFA-1/9	+	+	+	−	−	−	N or I	37.3 ± 3.3
S146	May.035	+	+	+	−	−	+	I	82.4 ± 1.9
S148	25-3-1	+	+	+	−	−	+	I	45.3 ± 4.4
S151	CBR LFA-1/1	+	+	+	−	−	+	I	87.4 ± 1.3
S158	TS2/14	+	+	+	−	−	+	I	81.3 ± 6.4
S159	TS1/22	+	+	+	−	−	+	I	84.6 ± 6.9
S161	TS2/6	+	+	+	−	−	+	I	74.7 ± 2.2
S167	G-25.2	+	−	+	−	+	−	M	23.7 ± 2.7
S169	YTA-1	+	−	+	−	+	−	M	43.6 ± 4.1

*+, Most cells are stained with the mAb; ±, the mAb stained only a portion of cells positively; −, the mAb staining was not significantly different from that of the negative control.
†N, N-terminus to BspHI site; I, BspHI site to Se8387I site; M, Sse8387I site to MscI site.
‡The COS cells transfected with human LFA-1 were fluorescently labelled and tested for binding to purified ICAM-1 with or without mAb [5]. The data represent the average of at least three experiments.

to generate human/mouse chimeras. These sites were selected to bracket the N-terminal region, I domain, and divalent cation binding regions (Fig. 1). For all chimeras, H represents a region of the human LFA-1 alpha subunit, M represents a region of mouse LFA-1 alpha subunit, and b, s, and m refer to the restriction sites *Bsp*hI, *Sse*8387I, and *Msc*I. Complete digestion with one enzyme, followed by partial digestion with another, was required for generation of all of these chimeric constructs. The integrity of the chimeric α subunit cDNAs were verified by restriction mapping and by sequencing through the junctions.

COS cells transiently transfected with the human β_2 subunit plus a chimeric α subunit or human CD11a were stained with CD18 mAb S162 (TS1/18) and subjected to flow cytometry [3]. Five α subunit chimeras, H-m-M, M-m-H, H-s-M, H-b-M-s-H, and M-b-H-s-M, can associate with the CD18 β subunit and be expressed on the COS cell surface (Fig. 1). These chimeras were expressed as efficiently as wild-type human LFA-1 based on the percentage of positive cells and fluorescence intensity.

Epitope mapping of Workshop antibodies was undertaken by analysing the ability of these mAb to bind different chimeras. COS cells transiently cotransfected with human β_2 and wild-type or chimeric α^L cDNAs, or vector alone, were incubated with Workshop mAb, stained with a second fluorescein isothiocyanate (FITC)-labelled goat anti-mouse IgG antibody, and subjected to flow cytometry (Table 1). Epitopes were localized 5′ to the *Bsp*H1 site (N-terminal region, three mAb), between the *Bsp*H1 and *Sse*8387I sites (I domain, six mAb), between the *Sse*8387I and *Msc*I sites (putative metal binding region, two mAb), or to a site appearing to involve an interaction between the N-terminal region and I domain (two mAb) (Table 1).

The mAb were tested further for their ability to inhibit binding of LFA-1-transfected COS cells to purified ICAM-1. mAb to the I domain strongly inhibited LFA-1 binding to ICAM-1, with an average inhibition of 77 per cent (Table 1). mAb against the N-terminal region or metal-binding region did not inhibit ICAM-1 binding strongly. The average inhibition produced by these mAb was 27 per cent. mAb that mapped to the I domain were the strongest blockers of LFA-1 binding to ICAM-1. These results suggest that the I domain in LFA-1 has an important function in ligand binding.

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AS5.7 Induction of LFA-1-mediated adhesion to ICAM-1, -2, and -3 transfectants

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All antibodies directed against LFA-1 (CD11a/CD18) and against ICAM-1 (CD54), ICAM-2 (CD102), and ICAM-3 (CD50) of the Adhesion Structure Panel were screened for their capacity to induce adhesion of lymphocytes to L cells transfected with ICAM-1, -2, or -3. One monoclonal antibody (mAb) to CD11a S140 (NK1-L16) and one to CD18 S155 (CBR LFA-1/2) induced adhesion to ICAM-1, -2, and -3. Two anti-ICAM-3 mAB, S092 (CBR-IC3/6) and S109 (HP2/19), stimulated binding of lymphocytes to ICAM-3.

Previous reports have demonstrated that high-avidity binding of ICAM-1 requires activation of LFA-1 either by intracellular signals, by the addition of bivalent cations (manganese), or by activating mAb. In the present study we investigated the requirements for binding to ICAM-2 and ICAM-3. We generated stable L-cell transfectants that expressed one of the ICAM molecules at their cell surface. Similarly to binding to ICAM-1, binding of the other ICAMs required activation of LFA-1 (not shown). We screened the Adhesion Structure Panel for mAb that might stimulate LFA-1/ICAM interaction. For these experiments we used peripheral blood mononuclear