Table 1 CD18 and CD50 (ICAM-3) mAb block HTLV-I-induced syncytium formation

Workshop mAb		Specificity	% Inhibition* syncytium formation
Code	Clone name		
S146	MAY.035	CD11a	3.9
S148	25-3-1	CD11a	−15.7
S158	TS2/14	CD11a	−30.4
S160	TS2/4	CD11a	−12.1
S161	TS2/6	CD11a	−14.8
S147	MAY.017	CD18	6.5
S162	**TS1/18**	CD18	**54.7**
S163	BL5	CD18	11.6
S164	CLB-LFA1/1	CD18	30.2
S081	**BRIC79**	ICAM-3?	**55.7**
S087	CBR-IC3/1	ICAM-3	12.4
S088	CBR-IC3/2	ICAM-3	−1.0
S089	CBR-IC3/3	ICAM-3	−13.4
S091	CBR-IC3/5	ICAM-3	3.2
S092	**CBR-IC3/6**	ICAM-3	**63.2**†

*mAb were used at a dilution of 1:100. mAb producing more than 50% inhibition of syncytium formation are shown in bold face.
†mAb S092 (CBR-IC3/6) completely inhibited (100%) syncytium formation at 1:50 dilution.

counted the number of syncytia by a phase-inverted microscope after 18 h cocultivation. At first, we analysed the expression of adhesion molecules on both T-cell lines using a flow cytometer. ILT-8M2 cells expressed adhesion molecules including CD11a, CD18, CD54, ICAM-2, ICAM-3, CD43, and CD50. MOLT-4 cells expressed CD11a, CD18, ICAM-2, ICAM-3, CD43, CD44, and CD50 on the cell surface. Next, the assay of inhibition of syncytium formation was examined by the cocultivation of ILT-8M2 and MOLT-4 in the presence or absence of monoclonal antibodies (mAb) against the adhesion molecules. As the syncytium formation was markedly inhibited by a very low concentration of sodium azide (0.005 per cent), the syncytium formation was thought to be dependent on energy. Thus the blocking study was performed using mAb in the absence of sodium azide. As shown in Table 1, more than 50 per cent inhibition of syncytium formation was detected by some of the mAb against CD18 and ICAM-3 at a dilution of 1:100. In particular, S092 (CBR-IC3/6) was able to inhibit the syncytium formation completely at a dilution of 1:50.

Our present results suggest that adhesion molecules such as CD18 and ICAM-3 may play a critical role in HTLV-I-induced syncytium formation.

Reference

1. Ida, H., Kurata, A., Eguchi, K., Yamashita, I., Nakashima, M., Sakai, M., Kawabe, Y., Nakamura, T., and Nagataki, S. *Antiviral Res.* **23**, 143 (1994).

AS5.9 Subunit specificity and epitope mapping of Mac-1 and p150,95 mAb using chimeric CD11b × CD11c transfectants

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The leucocyte integrins Mac-1 (CD11b/CD18) and p150,95 (CD11c/CD18) are adhesion molecules that mediate neutrophil aggregation and adherence of monocytes and neutrophils to endothelium and the extracellular matrix [1]. There is increasing evidence that a major recognition site for ligand binding (or for adhesion) is located in the I domain of the integrin α^M (CD11b) and α^x (CD11c) subunits [2,3]. In this study, we have characterized the specificity of monoclonal antibodies (mAb) for Mac-1 and p150,95, and localized their epitopes to specific domains within the α subunits of these molecules.

To cluster CD11/CD18 antibodies, all 52 mAb in the Adhesion Structure Subpanel 5 were examined by flow cytometry using cell lines CHO and K-562 stably transfected with LFA-1, Mac-1, p150,95, or ICAM-1 as a control (Fig. 1). CD18 mAb stained cells bearing

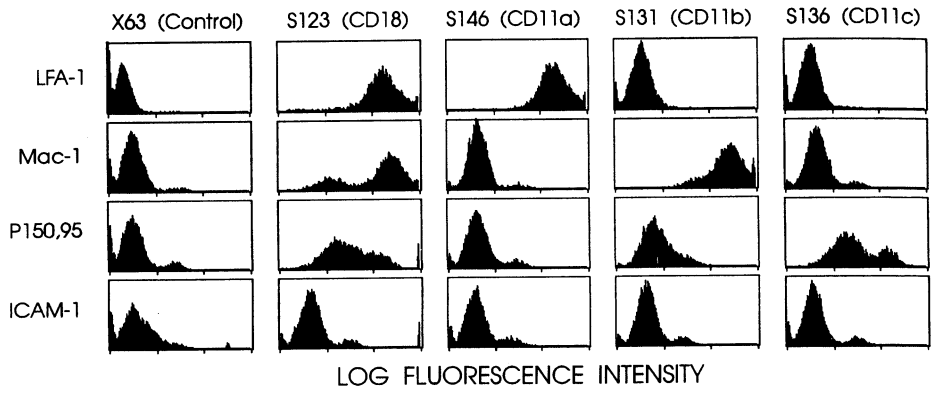

Fig. 1 Fluorescent staining of CHO transfectants expressing the LFA-1, Mac-1, p150,95 and ICAM-1 antigens by Subpanel 5 mAb. Transfected CHO cells ($5-10\times10^6$/ml in 50 μl) grown in αMEM medium supplemented with 10 per cent fetal calf serum (FCS) and 100 μM methotrexate [2] were labelled with an equal volume of 1/50 diluted Adhesion Subpanel 5 mAb or P3X63 IgG1 control mAb, according to the Workshop protocol.

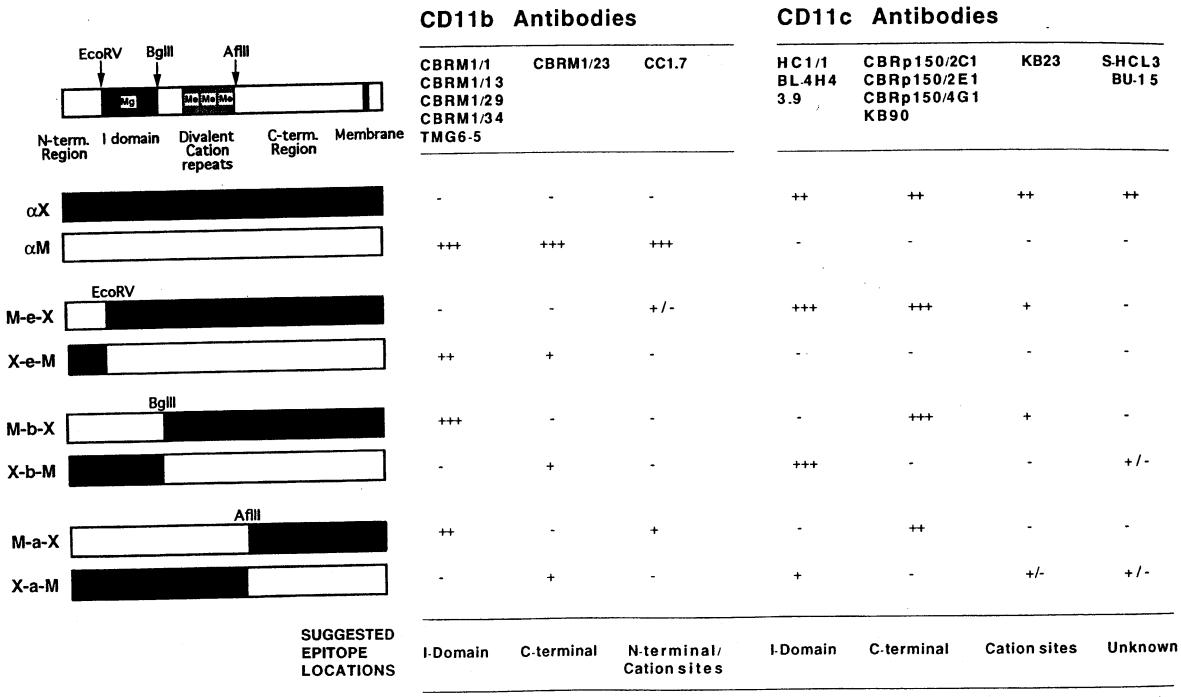

Fig. 2 Reactivity patterns of the CD11b and CD11c mAb against a panel of Mac-1 $\times$ p150,95 chimeric CHO transfectants. Construction of the chimeric Mac-1 and p150,95 α chains has been described previously [2]. Unique restriction sites were introduced by site-directed mutagenesis and used to create the chimeric α subunits. Stable expression of these chimeric molecules in association with the β subunit in CHO cells was obtained [2]. Immunofluorescence staining of these chimeric transfectants with the CD11b and CD11c mAb was carried out as described in Fig. 1. Binding of mAb to cells expressing the native and chimeric Mac-1 and p150,95 molecules was scored as follows: + + +, >90 per cent of the cells stained strongly; + +, >90 per cent of cells were positive but with a lower fluorescence intensity; +, a subpopulation of the cells stained positively; −, negative reaction.

all three CD11/CD18 complexes, but not ICAM-1. mAb specific for CD11a, CD11b, or CD11c stained cells bearing only a single type of CD11/CD18 complex. Representative patterns for each specificity are shown in Fig. 1, and the results are summarized in the Adhesion Structure Subpanel 5 Report [Petruzzelli *et al.*, AS5].

In a previous study, a series of Mac-1 × p150,95 chimeric α subunit cDNAs were constructed and expressed stably in association with the β subunit in CHO cells [2,4]. These chimeric transfectants were used to map the epitopes of the CD11b and CD11c antibodies in this Subpanel. Based upon the reactivity patterns in flow cytometry (Fig. 2), the results indicated that the majority of CD11 mAb tested were directed against the I domain of the integrin α chain. For the CD11b antibodies, five mAb, S128 (CBRM1/1), S127 (CBRM1/13), S131 (CBRM1/29), S132 (CBRM1/34), and S174 (TMG6-5) bound to the I domain, one mab S130 (CBRM1/23) to the extracellular C-terminal region, and mAb S172 (CC1.7) probably recognizes a conformational epitope involving both the N-terminal and divalent cation repeats. Three of the CD11c mAb, S124 (HC1/1), S138 (BL-4H4), and S144 (3.9), were mapped to the I domain, and four mAb, S133 (CBRp150/2C1), S135 (CBRp150/2E1), S136 (CBRp150/4G1), and S156 (KB90), to the C-terminal region. We have previously found that mAb to the I domain of Mac-1 block neutrophil homotypic aggregation and binding to fibrinogen, ICAM-1, and iC3b, whereas mAb to the C-terminal region have little effect on adhesive function [2]. Similarly, mAb to the I domain of p150,95 block iC3b binding [3]. The epitope mapping studies performed here will be of interest when compared to functional studies performed on the same mAb elsewhere in the Workshop. For example, all five of the mAb found to block phorbol ester-induced homotypic adhesion of polymorphonuclear leucocytes [Salcedo and Patarroyo, unpublished Workshop report] map to the I domain of CD11b.

References

1. Springer, T. A. *Nature* **346**, 425 (1990).
2. Diamond, M. S., Garcia-Aguilar, J., Bickford, J. K., and Springer, T. A. *J. Cell Biol.* **120**, 1031 (1993).
3. Bilsland, C. A. G., Diamond, M. S., and Springer, T. A. *J. Immunol.* **152**, 4582 (1994).
4. Stacker, S. A. and Springer, T. A. *J. Immunol.* **146**, 648 (1991).

AS5.10 Identification of cation-dependent epitopes on β1 and β2 integrins

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We previously identified a monoclonal antibody (mAb) S140 (NKI-L16) that recognized a bivalent-cation-dependent epitope (Ca^{2+}). Here we screened the Adhesion Structure Panel (leucocyte integrins) for mAb with similar properties. We identified two other mAb directed against CD11a, S158 (TS2/14) and S159

Table 1 Antibodies that recognize cation-dependent epitopes

Workshop mAb			Expression on Peer T cells in the presence of*				
Code	Clone name	Antigen	PBS	EDTA	Ca^{2+}	Mg^{2+}	Mg^{2+}/Ca^{2+}
—	—	—	—	—	—	—	—
S160	TS2/4	CD11a	+	+	+	+	+
S140	NKI-L16	CD11a	−	−	+	−	+
S158	TS2/14	CD11a	±	−	−	+	+
S159	TS1/22	CD11a	±	−	−	+	+

Peer T cells were treated with EDTA or washed with PBS. Subsequently, 1 mM Ca^{2+}, Mg^{2+}, or Mg^{2+}/Ca^{2+} was added, and the cells were stained with mAb.