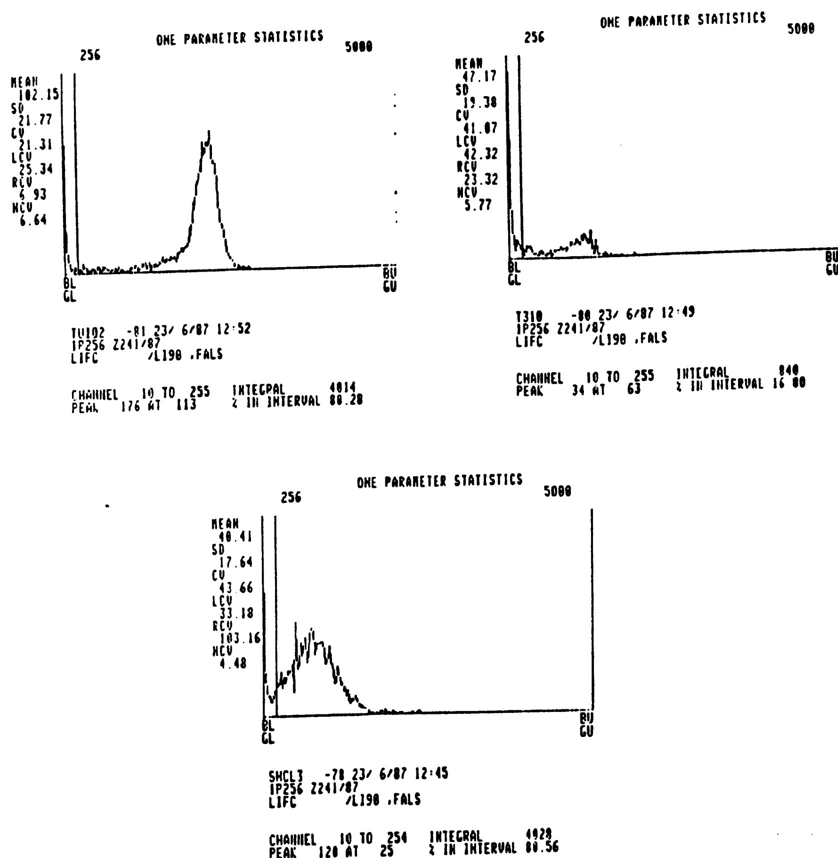

Fig. 3. Flow cytometry analysis of a case of CD8$^+$ T gamma-lymphoproliferative disorder with S-HCL3 (α-Leu-M5) mAb. Note that approximately 80 per cent of the gated lymphocyte population is positive for both CD8 and CD11c mAb.

malignant non-Hodgkin's lymphomas may be a good marker for late differentiation stages in both the B- and T-cell lineage.

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N1.8 The subunit specificity of the CD11a/CD18, CD11b, and CD11c panels of antibodies

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The integrin membrane glycoproteins participate in a wide range of cell–cell and cell–extracellular matrix (ECM) interactions. The leucocyte integrins, leucocyte function associated-1 molecule (LFA-1), Mac-1, and p150,95, are heterodimers consisting of distinct α-subunits (CD11a, b, c respectively) (M_r = 180 000, 170 000, 150 000) and a shared β-subunit (CD18) (M_r = 95 000). LFA-1 is expressed on virtually all leucocytes and functions in CTL–target cell conjugation, leucocyte-endothelial interaction, NK-killing, and ADCC of granulocytes and monocytes [1]. Two cell-surface ligands for LFA-1, intercellular adhesion molecules ICAM-1 [2–4],

and ICAM-2 [5], have been identified and cloned. Mac-1 and p150,95 are expressed primarily on myeloid cells [1]. Both Mac-1 and p150,95 have been reported to be complement receptors specific for iC3b. Mac-1 plays a role in myeloid cell–endothelial cell interaction [1]. The cDNA clones encoding these four subunits have recently been isolated [6–11]. Expression of these cDNA clones in COS cells was used to define the subunit specificity of antibodies in the CD11a/CD18, CD11b, and CD11c panels.

The cDNAs encoding the LFA-1, Mac-1, and p150,95 α-subunits and the common β-subunit were subcloned into the expression vector pCDM8. COS cells were transfected [12] with a single α-subunit cDNA together with the β-subunit cDNA for expression of heterodimers; transfection with α-subunit cDNA alone or β-subunit cDNA alone was also tested. Cell-surface expression was examined by indirect immunofluorescence using a panel of previously characterized antibodies (Fig. 1). Using this expression system we typically achieve transient cell-surface expression of heterodimers on 25–75 per cent of the transfected cells. Many of the expressing cells are several log channels brighter than controls indicating an extremely high density of surface protein. Immunoprecipitation studies suggest that the α- and β-subunits are associated on the cell surface [unpublished data]. When α- or β-subunit cDNAs were transfected alone, expression of β was detected with some but not all anti-β mAb (TS1/18 in Fig. 1 and see below); expression of CD11a, CD11b, or CD11c α-subunits alone was not detected. Whether the leucocyte β-subunit can associate with ECM integrin α-subunits and thus be transported to the cell surface or whether it is expressed alone is unknown. It is likely that overexpression in COS cells of the leucocyte β-subunit drives lower-affinity interaction with an inappropriate α-subunit or drives expression through a system which is normally inefficient.

Using this expression system, we tested the CD11a/CD18, CD11b, and CD11c panels of Workshop antibodies for the ability to stain various transfectants (Table 1). In the CD11a/CD18 antibody panel, 18 mAb stained LFA-1 transfectants uniquely, defining them as CD11a, while eight antibodies stained the LFA-1, Mac-1, and p150,95 transfectants, defining them as CD18. No antibody clustered as CD11a/CD18 was found to react with a Mac-1 or p150,95 member unless it reacted with all three transfectants. Seven antibodies did not react with any of the transfectants.

We verified this pattern of antibody reactivity using Epstein–Barr virus (EBV)-transformed B-cell lines that lack or possess LFA-1 cell surface expression, KOS and CO3 respectively [13]. The staining patterns achieved with these cell types was similar to the staining patterns of the LFA-1 transfected cells with a few exceptions. Two antibodies, BU-49 and GRT22, react with both LFA-1^{+}

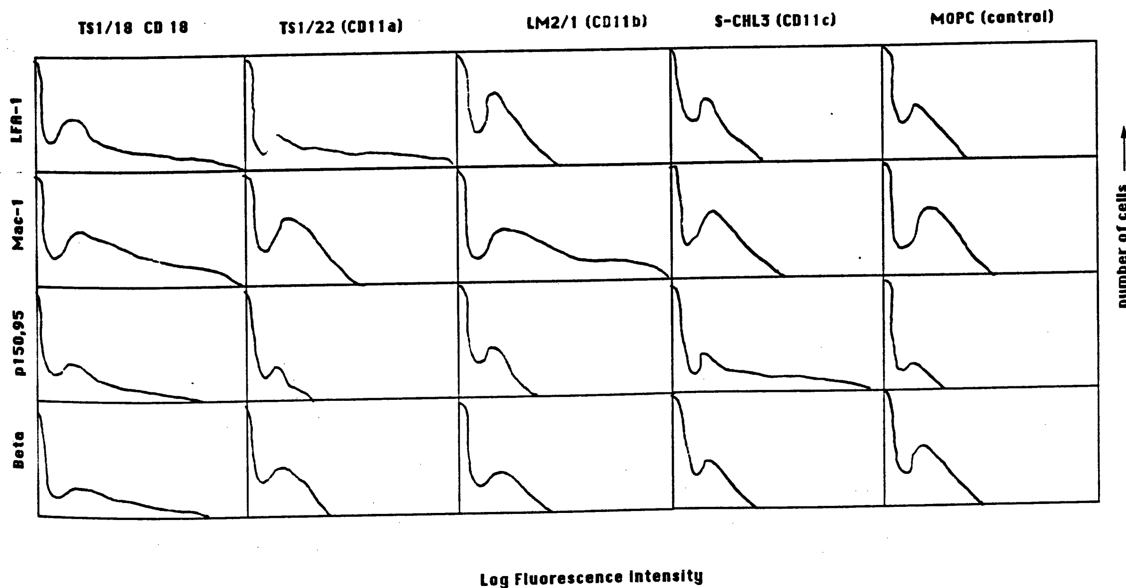

Fig. 1. Indirect immunofluorescence and FACS analysis of COS cells co-transfected with LFA-1, Mac-1, p150,95, and the common β-subunit cDNA. COS cells were transfected according to established procedures [12] using 400 μg/ml DEAE–dextran, 100–200 μM chloroquine, and 0.5–1.0 μg/ml of each plasmid. Transfectants were stained using indirect immunofluorescence according to the guidelines of the Conference.

and LFA-1⁻ cells but not LFA-1 transfectants, indicating that these antibodies recognize a non-CD11/18 molecule on the surface of B-cells. Interestingly, VIPIIIBI stained the LFA-1$^+$ cell line but not the LFA-1$^-$ patient cell line or the LFA-1 transfected COS cells. This observation has several explanations. A LFA-1 polymorphism exists in the mouse [14] and in humans [15]. This antibody could be recognizing a polymorphic difference. Alternatively, this antibody could recognize conformational, posttranslational processing or posttranscriptional differences. cDNA clones for human Mac-1 [7, 9] have a one amino-acid difference, the function of which is unknown. Finally, glycosylation differences of LFA-1 on different cell types [16] could be involved.

In the CD11b panel, six of eight antibodies reacted uniquely with COS cells transfected with Mac-1. All four CD11c panel mAb reacted exclusively with p150,95 transfectants. The antibody, MJ5/1, in the CD11b antibody panel reacted with the LFA-1, Mac-1, and p150,95 transfectants and was classified a CD18 mAb.

Although the nine antibodies in the CD11a/CD18 and CD11b antibody panels reacting with all three transfectants could be recognizing cross-reactive epitopes, we believe that it is likely that they are recognizing CD18. Consistent with this explanation, no antibodies were found that interacted with just two transfectants, a phenomenon which could be postulated since Mac-1 and p150,95 are more structurally related than with LFA-1 [6–8].

COS cells transfected with the β cDNA alone were stained by only three of nine CD18 mAb suggesting that, in the absence of the CD11a, b, or c subunits, the β-subunit does not maintain antigenic and structural integrity.

We have expressed the LFA-1, Mac-1, and p150,95 heterodimers on the surface of COS cells and defined the subunit specificity of the CD11a/CD18, CD11b, and CD11c antibody panels. With one exception, the results with LFA-1$^+$ and LFA-1$^-$ cell lines were concordant with the results using COS cells expressing LFA-1. The reactivity of the panel of antibodies suggests that the antigenic and structural integrity of the molecules

Table 1. Staining of COS cells transfected with LFA-1, Mac-1, p150,95 $\alpha\beta$-complexes or the β-subunit alone and of LFA-1$^+$ and LFA-1$^-$ B-cell lines

Antibody		Transfectants				B-cell lines		Conclusion
Workshop no.	Name	LFA-1	Mac-1	p150,95	β	LFA-1$^+$	LFA-1$^-$	
N201	11H6	++	±	+	−	+	−	CD18
N202	CRIS-3	++	−	−	−	+	−	CD11a
N203	122-2A5	++	−	−	−	+	−	CD11a
N204	BU-17	−	−	−	−	−	−	−
N205	BU-49	−	−	−	−	+++	+++	−
N206	GRT22	−	−	−	−	+++	+++	−
N207	M232	++	++	++	−	+	−	CD18
N208	0501	−	−	−	−	−	−	−
N209	MEM-25	++	−	−	−	+	−	CD11a
N210	MEM-30	++	−	−	−	+	−	CD11a
N211	MEM-83	++	−	−	−	+	−	CD11a
N212	MEM-95	++	−	−	−	++	−	CD11a
N213	25.3.1	++	−	−	−	++	−	CD11a
N214	IC11	++	++	++	++	+	−	CD18
N215	459	++	−	−	−	++	−	CD11a
N216	MHM23	++	++	++	−	++	−	CD18
N217	MHM24	++	−	−	−	++	−	CD11a
N218	CLB-54	++	++	++	++	++	−	CD18
N219	YFC51.1	++	+	++	−	+	−	CD18
N220	YTH 81.5	++	−	−	−	+	−	CD11a
N221	YFC118.3	+	+	±	−	+	−	CD18

Table 1—*Continued*

Antibody		Transfectants				B-cell lines		Conclusion
Workshop no.	Name	LFA-1	Mac-1	p150,95	β	LFA-1$^+$	LFA-1$^-$	
N222	1524	++	–	–	–	+	–	CD11a
N223	2F12	++	–	–	–	++	–	CD11a
N224	F110.22	++	–	–	–	±	–	CD11a
N225	TMD3-1	++	–	–	–	++	–	CD11a
N226	ITM3-2	–	–	–	–	–	–	–
N227	CC5 1D7	++	–	–	–	++	–	CD11a
N228	VIPIIIB1	–	–	–	–	++	–	CD11a or CD18
N229	GRF1	++	++	++	–	+	–	CD18
N230	GRS3	+	–	–	–	±	–	CD11a
N231	HI111	++	–	–	–	++	–	CD11a
N232	M10	++	–	–	–	++	–	CD11a
N301	LPM19C	–	++	–	–	–	–	CD11b
N302	14B6.E2	–	++	–	–			CD11b
N303	5A4.C5	–	++	–	–			CD11b
N304	MO1	–	–	–	–			–
N305	MN41	–	++	–	–			CD11b
N306	MJ5/1	+	++	+	+	±	–	CD18
N307	TMG6-5	–	++	–	–			CD11b
N308	VIM12	–	++	–	–			CD11b
N351	F9083	–	–	++	–			CD11c
N352	S-HCL3	–	–	++	–			CD11c
N353	L29	–	–	++	–	–	–	CD11c
N354	B-ly6	–	–	+	–			CD11c

++, mean fluorescence of positive cells > 10 channels difference from control cells; +, 6–10 channels difference; ±, 4–6 channels difference or + on one occasion and – on another; –, <4 channels difference.
Staining of EBV-transformed LFA-1$^+$ (CO3) and genetically LFA-1$^-$ (KOS) B-cell lines: –, <20 per cent cells; +, 20–49 per cent positive; ++, 50–75 per cent positive; ±, > 15 per cent when a 10-fold lower dilution of antibody was used.

expressed on COS cells has been maintained. The obvious effectiveness of this expression system will allow for functional studies, production of mutant heterodimers, and hence more fine mapping of the antibody epitopes.

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N1.9 Differential effects on leucocyte functions of CD11a, CD11b, and CD18 mAb

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Mac-1 (CD11b/CD18), LFA-1 (CD11a/CD18), and p150,95 (CD11c/CD18) constitute a family of functionally important leucocyte integrins that share a common β-subunit (M_r, 95 000) and participate in cellular adhesion [1, 2]. Mac-1 (α-subunit, M_r 165 000) is expressed basally on granulocytes, monocytes, and NK-cells. A second intracellular, vesicular pool is mobilized to the surface in granulocytes and monocytes by inflammatory mediators (fMLP, C5a, phorbol esters). Mac-1 is known to bind soluble ligands (iC3b, clotting factor X, fibrinogen) [1, 3–5] and to promote a series of neutrophil functions including adherence to endothelial cells, margination through high endothelial venules, homotypic aggregation, and chemotaxis [6]. LFA-1 (α-subunit, M_r 170 000) is expressed on B- and T-lymphocytes, NK-cells, monocytes, and granulocytes. mAb to LFA-1 block cytolytic T-cell-mediated killing and NK-mediated killing by inhibiting adhesion to target cells, and block T helper cell responses by preventing cell–cell contact. Ligands for LFA-1 have recently been characterized and cloned and include ICAM-1 (CD54) [7, 8] and ICAM-2 [9]. p150,95 (α-subunit, M_r 150 000) is expressed on phagocytic cells, NK-cells, certain cytolytic T-cells, and CD5$^-$ B-cells [6]. The role of p150,95 is less clearly defined, although mAb inhibit conjugate formation between cytotoxic T-cells and target cells as well as some neutrophil and monocyte adherence functions [10–12]. Recently, both rosetting experiments [13] and affinity chromatography [14] have suggested iC3b as a ligand for p150,95.

In this study, we have characterized functionally the Workshop antibodies directed to the CD11a, CD11b, and CD18 antigens for their ability to inhibit homotypic cell aggregation, to block the binding of T-lymphoma or B-lymphoblastoid cells to immunoaffinity-purified, solid-phase Mac-1 and LFA-1, and to decrease the binding of ^{125}I-labelled Mac-1 to iC3b-coated sheep erythrocytes (iC3b-E). The specificity of Workshop antibodies for CD11a, CD11b, and CD18 was defined elsewhere by their reactivity with COS cells transfected with CD11a/CD18, CD11b/CD18, and CD11c/CD18 cDNA clones [Larson *et al.*, N1.8].

Since Mac-1 is known to bind to a multiplicity of soluble and cellular ligands, we hypothesized that, by testing the Workshop CD11b and CD18 panels, we might find particular antibodies that block restricted functions; such a trend might suggest the presence of multiple functional domains on Mac-1 that are critical for ligand specificity. A similar technique has been utilized with five other CD11b antibodies to distinguish two functional domains, one responsible for iC3b-E binding and a second for neutrophil spreading on plastic and neutrophil chemotaxis [15, 16]. For our studies three separate assays were selected: a binding assay between purified ^{125}I-Mac-1 and iC3b-E, a Mac-1-dependent neutrophil homotypic cell–cell aggregation assay, and a ^{51}Cr-SKW3 T-lymphoma cell binding assay to solid-phase purified Mac-1. In the latter two assays, Mac-1 interacts with undefined ligands on neutrophils and T-cells.

In the homotypic neutrophil aggregation assay (Table 1), the data demonstrate that, in general, the CD18 β-chain Workshop antibodies are better blockers of phorbol-ester-induced aggregation than the CD11b panel. Six of the eight CD18 antibodies tested inhibit neutrophil aggregation by at least 50 per cent and four of the eight inhibit it by more than 75 per cent; furthermore, other non-Workshop β-chain antibodies (TS1/18, 60.3) have been described as inhibitors of neutrophil aggregation. In contrast, only one of six α-chain antibodies, N301