

# Human T-cell leukaemia/lymphoma virus type 1 syncytium formation is regulated in a cell-specific manner by ICAM-1, ICAM-3 and VCAM-1 and can be inhibited by antibodies to integrin $\beta_2$ or $\beta_7$

Susan Daenke, Sharon A. McCracken and Sarah Booth

Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK

Human T-cell leukaemia/lymphoma virus type 1 (HTLV-1) is a pathogenic retrovirus responsible for a number of inflammatory pathologies and adult T-cell leukaemia. Although T-cell tropic *in vivo*, HTLV-1 can infect a wide variety of cell types *in vitro*. Cell-to-cell spread of HTLV-1 may require specific binding of envelope to its cellular receptor, with other cell-surface molecules facilitating fusion. Here it is shown that intercellular adhesion molecule-1 or -3 (ICAM-1, ICAM-3) or vascular cell adhesion molecule-1 (VCAM-1) are required for syncytium formation of K562 with HTLV-1-infected MT2 cells but not C91-PL cells. The effect of ICAMs and VCAM-1 on MT2-induced fusion can be blocked by antibodies that bind  $\beta$ -integrins. These fusion co-factor molecules are effective only when present in combination with HTLV-1 receptor-bearing cells and are not sufficient to mediate syncytium formation alone. The results suggest that engagement of HTLV-1-infected cells with susceptible target cells requires the simultaneous binding of viral envelope glycoprotein to the cellular receptor and co-factor molecules to  $\beta$ -integrins. The tissue-specific expression of adhesion molecules might therefore influence HTLV-1 virus tropism and pathogenic changes associated with syncytium formation.

## Introduction

Human T-cell leukaemia/lymphoma virus type 1 (HTLV-1) is spread most efficiently via cell-to-cell contact and free virions are poorly infectious. Intercellular infection occurs via fusion of infected cells with permissive target cells expressing the cellular receptor. The receptor for HTLV-1 has been mapped to the region q23.2–q25.3 of chromosome 17 in the human by using pseudotyping techniques (Sommerfelt & Weiss, 1990), and purified virions bound to mouse/human somatic cell hybrids (Gavalchin *et al.*, 1995). Studies also suggest that the HTLV-1 cellular receptor is shared by the related retroviruses HTLV-2, simian T-cell leukaemia/lymphoma virus type 1 and chimpanzee T-cell leukaemia virus, and has a well-defined host range (Sommerfelt & Weiss, 1990). Monoclonal antibody 34-23 blocks binding of HTLV-1 to activated cells and identifies a number of proteins of 31–70 kDa, one or all of which may constitute the cellular

receptor (Gavalchin *et al.*, 1993, 1995), although the exact nature of the receptor has yet to be characterized. Collectively, this evidence and the failure to date to identify any of the presumed receptor components may indicate that more than one cell-surface molecule confers susceptibility to infection (Dezzutti *et al.*, 1995; Ida *et al.*, 1995; Hildreth *et al.*, 1997).

Fusion co-factor proteins play a prominent role in retrovirus infections. Membrane proteins of the CCR and CXCR cytokine receptor family regulate the specific interaction of human immunodeficiency virus type 1 (HIV-1) with target cells (Fauci, 1996). Fusion assays in CHO cells with amphotropic and ecotropic type C retrovirus murine leukaemia virus also suggest that fusion may require the assembly, following transfection of cells with the specific receptor, of a polyvalent, heterotypic receptor complex (Siess *et al.*, 1996), which is achieved by a multistep pathway involving a number of cellular proteins.

Vascular cell adhesion molecule-1 (VCAM-1) has been identified as a putative co-receptor for HTLV-1 fusion between MT2 cells and K562 cells (Hildreth *et al.*, 1997), although the exact role of this or other molecules in inducing fusion has not

**Author for correspondence:** Susan Daenke.  
Fax +44 1865 220993. e-mail sdaenke@molbiol.ox.ac.uk

been investigated. Modification of the HTLV-1 envelope cytoplasmic domain has also been shown to modulate syncytium formation, suggesting that an interaction of the envelope protein cytoplasmic domain with some cellular factor may regulate fusion (Pique *et al.*, 1993). We have investigated the contributions of intercellular adhesion molecules (ICAM-1, ICAM-2, ICAM-3) and VCAM-1 in fusion of chronically HTLV-1-infected cell lines MT2 and C91-PL with human and murine target cells. We show that fusion between MT2 cells and target cells can be regulated by the interaction of addressins ICAM-1, ICAM-3 and VCAM-1 on the target cells with a ligand, presumably on infected cells. C91-PL cells, however, are constitutively fusion-competent on target cells that do not express these molecules. We suggest that co-factors ICAM-1, ICAM-3 and VCAM-1 regulate fusion in a cell-specific way and that this effect is mediated through integrin  $\beta$ -chains most likely to be expressed on the infected cell membrane.

## Methods

■ **Cells.** K562, COS7 (SV40-transformed monkey kidney) and MOP8 (polyomavirus-transformed mouse fibroblast) cell lines were obtained from the European Collection of Cell Cultures and maintained in Dulbecco's modified MEM (DMEM) supplemented with 2 mM L-glutamine and 5–10% foetal calf serum (FCS; Globe Farm). HTLV-1-expressing MT2 and C91-PL cell lines were maintained in RPMI 1640 medium, 2 mM L-glutamine with 10% FCS. All cell culture was done in the absence of antibiotics. All cultures tested negative for mycoplasma.

■ **Antibodies.** Antibodies to the following antigens were purchased from Serotec: ICAM-1 (MCA532); ICAM-2 (MCA1140); ICAM-3 (MCA 1337); VCAM-1 (MCA907);  $\beta_1$ -integrin (MCA1188);  $\beta_2$ -integrin (MCA503); VLA-4  $\alpha_4$  subunit (MCA697); LFA-1  $\alpha_L$  subunit (MCA1149) and CD98 (MCA1105). Mouse monoclonal antibody to  $\beta_7$ -integrin (BP6) was obtained from M. Jones, University of Oxford, UK. FITC-conjugated, rat monoclonal anti-mouse transferrin receptor (clone RI7217) and isotype-matched mouse and rat IgG antibodies were obtained from Sigma. Antiserum from a tropical spastic paraparesis (TSP) patient (TE) with high anti-HTLV-1 antibody titre was obtained from S. Nightingale, Midland Centre for Neurosurgery and Neurology, Birmingham, UK. All antibodies were dialysed against PBS to remove traces of azide or were purchased azide-free.

■ **Plasmid vectors for transfection.** pCDM8 expression vectors containing sequences encoding ICAM-1, ICAM-3 and VCAM-1 were obtained from D. Simmonds, University of Oxford, UK. HTLV-1 Tax expression vector pJFE-Tax and the reporter gene construct pU3R-CAT have been described (Niewiesk *et al.*, 1995). For stable transfections,  $5 \times 10^6$  cells were co-transfected with 5  $\mu$ g pCDNA3(neo), 5  $\mu$ g pCDM8 expression vector and 5  $\mu$ g pU3R-CAT by using Easyfactor reagent (EquiBio). Expression was determined by FACS after selection of clones in DMEM/10% FCS with 1 mg/ml G418.

■ **FACS analysis of membrane antigen expression.** Cells were fixed in fresh 1% paraformaldehyde in PBS and then labelled with 10  $\mu$ g/ml monoclonal antibody in PGS (PBS, 20 mM glucose, 5% human serum). After washing, FITC-conjugated anti-mouse Ig (Sigma) was added at the manufacturer's recommended concentration in PGS. Cells were washed, resuspended in fixative, held at 4 °C in the dark and analysed within 2 days. Analysis was on a Becton Dickinson FACSort.

■ **CAT assay for fusion.** Fusion of HTLV-1-infected cells with target cells was quantified by using an assay for CAT activity. Transfer of HTLV-1 Tax protein from infected cells to target cells (containing the CAT reporter gene driven by the HTLV-1 LTR promoter) was used as a measure of syncytium formation. CAT-reporter K562 cells expressing various adhesion molecules were seeded into 24- or 48-well plates at a density of  $2 \times 10^5$  cells/ml and allowed to settle overnight. Where appropriate, blocking antibodies were added to wells to achieve a final concentration of 2–10  $\mu$ g/ml immediately prior to addition of HTLV-1-infected cells. Antibody was left in the wells for the duration of the assay to maintain any effect on newly expressed membrane molecules. C91-PL or MT2 cells were added to the target cells at a ratio of 1 : 1 and duplicate wells of mixed cell cultures were incubated at 37 °C for 16 h. Cells were harvested from the wells into 30–50  $\mu$ l 100 mM Tris-HCl pH 8, 1% glycerol and lysed by freeze-thaw. The insoluble material was pelleted at 14 000 r.p.m. for 5 min. Supernatants were heated at 65 °C for 30 min to inactivate cellular deacetylases. Lysate (7  $\mu$ l) was added to 2  $\mu$ l chloramphenicol (1.6 mg/ml in 100 mM Tris-HCl pH 7.8) and 2  $\mu$ l [<sup>3</sup>H]acetyl coenzyme A (Amersham; 180–220 mCi/mmol, 50  $\mu$ Ci/ml) in a Wallac 96-well Betaplate. This was overlaid with 100  $\mu$ l lipophilic Betaplatescint, covered with a mylar film and incubated at 37 °C for 60 min. The amount of acetylated chloramphenicol that had diffused into the Betaplatescint was measured in a Wallac 1450 microbeta scintillation counter for 2 min per well.

■ **Antibody cross-linking assay.** Forty-eight-well flat-bottom tissue culture plates were coated overnight at 4 °C with 100  $\mu$ g/ml goat anti-mouse IgG in 50 mM Tris-HCl pH 9 (coating buffer). Plates were washed with 5% BSA in coating buffer and monoclonal antibodies to  $\beta_2$ - or  $\beta_7$ -integrins were added at 20  $\mu$ g/ml in 5% BSA in coating buffer for 1 h at 4 °C. Excess antibody was removed by washing twice with 2% BSA in coating buffer; twice with 1% BSA in PBS and once with RPMI 1640 medium with 10% FCS. MT2 cells ( $10^5$ ) were added to each well in duplicates and allowed to adhere at 37 °C for 2 h. K562-CAT cells ( $10^5$ ) were then added to all wells and mixed cultures were incubated overnight at 37 °C. Cells were harvested for CAT assay as described. Wells containing K562 cells alone served as controls for direct activation of CAT by antibody cross-linking.

## Results

### Membrane expression of adhesion molecules on C91-PL, MT2 and K562 cells

K562 cells have been shown to be resistant to fusion with MT2 cells in the absence of high levels of expression of VCAM-1 (Hildreth *et al.*, 1997), although other target cell types fuse efficiently with MT2 cells (Nagy *et al.*, 1983). K562, MT2 and C91-PL cells were analysed for constitutive surface expression of a number of adhesion molecules (Table 1). K562 cells expressed low levels of ICAM-1 and ICAM-2 but little ICAM-3 or VCAM-1 on their surface. ICAM-1, -2 and -3 bind to the receptor LFA-1, a heterodimer composed of the integrin chains  $\alpha_L$  and  $\beta_2$ , and these interactions are involved in T-cell activation. VCAM-1 has been shown to bind to two receptors, VLA-4 (composed of integrin chains  $\alpha_4$  and  $\beta_1$ ) and LPAM-1 ( $\alpha_4$ ,  $\beta_7$ ). FACS analysis of MT2 and C91-PL cells showed that these cells differed in their expression of LFA-1 $\alpha$  and VLA-4 $\alpha$  but that expression of other integrin molecules was similar on both (Table 1). Notably, both HTLV-1-infected cell lines expressed low levels of  $\beta_2$ -integrin.

**Table 1.** FACS analysis of cell membrane phenotypes

Cells ( $10^4$ ) were examined by flow cytometry for cell surface staining with antibodies to the antigens shown. The intensity of cell staining is represented by the mean fluorescent emission (channel position) for the cell population. ND, Not done.

Antigen expressed	C91-PL	MT2	K562	MOP8 (T)*	COS7
Negative control†	< 5.6	< 3.8	< 2.6	< 4.0	< 8.8
CD98†	157.6	100.6	106.4	ND	ND
ICAM-1	281.3	437.7	25.5	4.3 (143.7)	6.4
ICAM-2	55.6	43.9	41.3	3.5	8.4
ICAM-3	138.5	31.1	2.6	5.1 (117.4)	7.3
VCAM-1	42.8	47.0	6.4	3.8 (103.6)	9.8
LFA-1 $\alpha$	58.7	4.3	2.5	3.4	9.8
VLA-4 $\alpha$	8.8	27.9	2.8	3.9	7.1
$\beta_1$ -integrin	11.7	19.9	47.8	3.3	327.1
$\beta_2$ -integrin	9.2	6.0	51.4	3.5	11.5
$\beta_7$ -integrin‡	+	+	—	—	—
Transferrin R†	ND	ND	ND	23.2	ND
W6/32†	354.6	267.5	3.7	4.3	694.5

\* Values show mean channel fluorescence in untransfected MOP8 cells, except for ICAM-1, ICAM-3 and VCAM-1 transfectants (T), where values are shown in parentheses.

† Control antibodies: mouse isotype-matched IgG control for each specific antibody. The highest negative control value is shown. Positive controls were anti-CD98 for leukocyte-derived cells, anti-mouse transferrin receptor (CD71) for MOP8 and W6/32 for HLA class I.

‡ Presence (+) of  $\beta_7$ -integrin was assayed by Western blot with monoclonal antibody BP6.

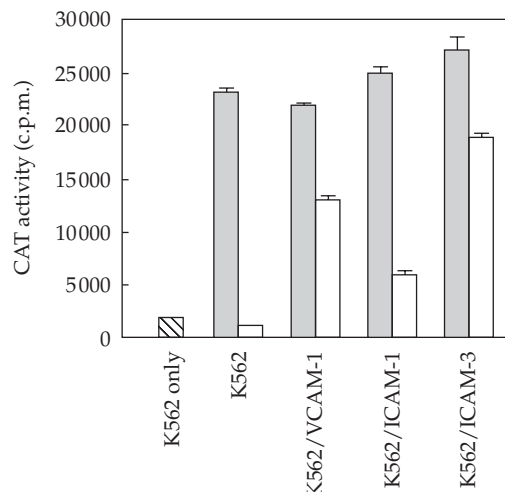
The sequences of the envelope coding region of proviruses integrated in MT2 (Gray *et al.*, 1990) and C91-PL are typical of the consensus sequence of a large collection of patient isolates (Daenke *et al.*, 1990; Paine *et al.*, 1991) and these cell lines have been shown to form syncytia with a number of different cell types (Nagy *et al.*, 1983). Furthermore, HeLa cells expressing MT2-derived envelope protein from plasmid p1118/14 (Carrington *et al.*, 1994) form syncytia *in vitro*. Therefore, there is no indication that the MT2 envelope protein is defective. C91-PL and MT2 cells were shown to express similar levels of envelope protein on their membranes (data not shown).

#### Fusion of K562 cells with HTLV-1-infected cell lines

To investigate the role of adhesion molecules in HTLV-1-induced fusion, K562 cells were stably transfected with a reporter plasmid, pU3R-CAT, with or without a pCDM8 plasmid encoding VCAM-1, ICAM-1 or ICAM-3. K562 clones with high cell-surface expression were selected by FACS analysis and tested for CAT activity by transient transfection with a plasmid expressing HTLV-1 Tax protein (Niewiesk *et al.*, 1995). Two stable clones with similar levels of adhesion molecule expression from each transfection were routinely used in experiments with indistinguishable results. Mean channel fluorescence of K562-CAT transfectant clones used in syncytium assays were typically 143.6 for ICAM-1, 179.6 for ICAM-3 and 250.4 for VCAM-1. Weakly expressing clones were less effective in syncytium formation with MT2 cells

(data not shown). K562-CAT cells were incubated with equal numbers of MT2 or C91-PL cells and allowed to fuse overnight at 37 °C, after which CAT activity was measured. In this system, expression of the CAT reporter gene is driven via the HTLV-1 LTR promoter by the fusion-dependent transfer of HTLV-1 Tax protein from MT2 or C91-PL cells into the target cells. We found that a quantitative CAT assay for syncytium formation had advantages over a visual assay when using K562 cells because of their morphological heterogeneity, particularly when overexpressing adhesion molecules. Large heterokaryons are common in the cultures and these are difficult to distinguish from syncytia in mixed cell populations. MT2 and C91-PL cells cultured under normal conditions *in vitro* aggregate but do not fuse with each other.

Addition of C91-PL cells to K562-CAT cells resulted in increased CAT activity in these cultures (Fig. 1). High-level expression on K562 cells of VCAM-1, ICAM-1 or ICAM-3 had no significant effect on the amount of fusion seen with C91-PL cells *in vitro*. In contrast, low CAT activity was seen in mixed cultures of MT2 with K562-CAT cells. Expression on K562-CAT cells of VCAM-1 increased CAT activity 7-fold after incubation with MT2 cells; expression of ICAM-1 and ICAM-3 increased CAT activity 3-fold and 9-fold, respectively (Fig. 1). Expression of ICAM-1, ICAM-3 or VCAM-1 on K562 cells did not activate the CAT reporter gene in the absence of a source of HTLV-1 Tax (data not shown). These data show that VCAM-1, ICAM-3 and to a lesser extent ICAM-1, when



**Fig. 1.** Fusion of C91-PL or MT2 cells with K562 transfectants. Cell fusion was measured by the CAT activity in K562-CAT cells transfected as shown, and after incubation with C91-PL (shaded bars) or MT2 cells (open bars) for 16 h. Bars show the mean values of duplicate samples and error bars represent the standard deviation of the mean. The hatched bar shows background CAT activity in K562-CAT cells in the absence of a source of HTLV-1 Tax protein.

expressed on target K562 cells, can increase HTLV-1-mediated fusion with MT2 cells, but that C91-PL cells can fuse in the absence of these co-factors.

### Inhibition of fusion by antibodies

MT2 cells express membrane VLA-4 $\alpha$  but not LFA-1 $\alpha$ , whereas C91-PL are positive for LFA-1 $\alpha$  but express VLA-4 $\alpha$  weakly (Table 1). It was unclear, therefore, whether the effects of ICAM-1, ICAM-3 and VCAM-1 on HTLV-1-specific fusion

were mediated directly via the integrin receptors described as ligands for these molecules, or via some other mechanism. No significant changes in expression of other membrane antigens were seen in ICAM-1-, ICAM-3- or VCAM-1-transfected K562-CAT cells with the panel of antibodies shown in Table 1.

To examine the interactions required of ICAM-1, ICAM-3 and VCAM-1 expressed on target cells in order to enhance syncytium formation with MT2 cells, we used antibodies to block these effects. Well-characterized antibodies were chosen that specifically inhibit adhesive or aggregation functions mediated by the appropriate molecules (see Methods for clone references). HTLV-1-specific fusion in MT2 and C91-PL was completely inhibited by addition of serum, obtained from an HTLV-1-seropositive, HTLV-1-associated myelopathy/TSP patient, at a 1 : 100 final dilution, regardless of the target cells (Table 2). This showed that, with or without expression of co-factor molecules, fusion was dependent on interaction of the target cell with the viral envelope protein. This antiserum bound similar levels of HTLV-1 envelope protein on the surface of MT2 and C91-PL cells and HTLV-1 envelope expressed from a recombinant vaccinia virus, WRproEnv-1 (data not shown).

A functional blocking antibody to VCAM-1 partially inhibited fusion of MT2 cells mixed with K562-CAT transfected with VCAM-1 (43.2% inhibition) but complete inhibition could not be achieved. An antibody specific for the  $\alpha_4$ -integrin subunit of VLA-4 was not inhibitory. Likewise, a  $\beta_1$ -integrin-specific antibody was also ineffective. Isotype-matched mouse IgG control antibodies had no effect on HTLV-1-induced fusion. This suggested that the fusion-regulatory effect of VCAM-1 on target cells was not via VLA-

**Table 2.** Fusion inhibition by antibodies

Percentage inhibition was calculated as the proportion of CAT activity in the absence of antibodies for each culture. Antibody concentrations were as stated in the text. mIgG, Negative-control mouse IgG antibody; a/s, antiserum; ND, not done.

Antibody	C91-PL		MT2		
	COS7	K562	K562 + ICAM-1	K562 + ICAM-3	K562 + VCAM-1
mIgG	1.3	1.9	0.6	2.1	0.5
TSP a/s	98.6	94.7	100.0	93.2	91.4
ICAM-1	0.0	3.3	9.6	4.0	ND
ICAM-2	ND	4.6	4.3	6.5	5.1
ICAM-3	2.4	13.2	ND	11.5	ND
VCAM-1	4.8	7.1	ND	15.6	43.2
LFA-1 $\alpha$	ND	2.7	4.2	3.4	ND
VLA-4 $\alpha$	ND	1.3	ND	ND	8.0
$\beta_1$ -integrin	3.6	— 1.5	ND	2.8	9.9
$\beta_2$ -integrin	4.4	70.4	53.9	61.1	18.4
$\beta_7$ -integrin	0.3	64.5	ND	31.9	82.6

4 ( $\alpha_4$ ,  $\beta_1$ ) expressed on MT2 cells. We therefore tested the  $\beta_7$ -integrin-specific antibody BP6 for the ability to block MT2-induced fusion with target cells expressing VCAM-1. Fusion was significantly reduced in these assays (82.6% inhibition). Fusion between C91-PL and K562 cells was not inhibited by antibodies specific for VLA-4 $\alpha$  or VCAM-1, although BP6 inhibited fusion by 64.5%. Therefore fusion mediated by MT2 or C91-PL cells may require engagement of integrin  $\beta_7$  during interaction and subsequent fusion with their target cells. The BP6 antibody did not allow us to quantify the level of  $\beta_7$ -integrin expression on MT2 and C91-PL cells, as this reagent performed poorly in FACS analysis, even on phytohaemagglutinin-stimulated peripheral lymphocytes. We could not, therefore, correlate levels of fusion inhibition with expression.

Fusion between MT2 cells and K562-CAT expressing ICAM-1 or ICAM-3 was inhibited by anti- $\beta_2$  but not anti-LFA-1 $\alpha$  antibodies. Anti- $\beta_2$  inhibited C91-PL fusion with K562 cells by 70.4% and anti-ICAM-3 inhibited by 13.2%. Anti-LFA-1 $\alpha$ , anti-ICAM-1 and anti-ICAM-2 antibodies were ineffective. Collectively, these data suggest that in both MT2 and C91-PL cells, integrins  $\beta_2$  and  $\beta_7$  are involved in an HTLV-1 envelope-dependent process that leads to fusion with susceptible target cells. The fact that complete inhibition was not seen even when high concentrations of blocking antibody were used suggests that several integrins might contribute simultaneously to this effect. Blocking experiments with C91-PL cells using a combination of anti- $\beta_2$  and anti- $\beta_7$  antibodies showed some additive effect but not complete inhibition (data not shown). To ensure that the effects of these antibodies on blocking of syncytium formation was specific to a  $\beta_2$ - or  $\beta_7$ -integrin interaction, we tested them in syncytium assays using COS7 target cells transiently transfected with pU3R-CAT. COS7 cells do not express ICAM-1, ICAM-2, ICAM-3, VCAM-1 or  $\beta_2$ - or  $\beta_7$ -integrin but are strongly positive for  $\beta_1$ -integrin (Table 1). With the exception of TSP antisera, all the antibodies added failed to block syncytium formation in these cultures (Table 2).

#### Co-factor molecules must be present in combination with the HTLV-1 receptor on target cells

The antibody blocking data suggested that the effects of ICAM-1, ICAM-3 or VCAM-1 in inducing fusion between MT2 and K562-CAT cells were strongly dependent on interactions involving the integrin molecules  $\beta_2$  or  $\beta_7$ . The mechanism by which addressin-integrin interactions induce syncytium formation in mixed cultures of K562 and MT2 cells is not clear. This could be a consequence of a specific interaction between the HTLV-1 envelope glycoprotein and a receptor/co-receptor on the target cells. Alternatively, the fusion co-factor may render the target cell susceptible to fusion via some mechanism (such as activation) which itself is not directly related to viral envelope protein binding. We therefore tested if these fusion co-factor molecules were effective if presented *trans* by a third-party cell that itself is not susceptible to HTLV-

**Table 3. Co-receptor effect is not provided in *trans* or by antibody cross-linking**

Fusion in mixed cell cultures was measured by CAT assay as described. Values represent the means of duplicate samples  $\pm$  standard deviation.

(a) Co-factor presented on MOP8	CAT activity in K562 + MT2 (c.p.m.)
None	843 $\pm$ 34
ICAM-1	1065 $\pm$ 22
ICAM-3	1346 $\pm$ 46
VCAM-1	962 $\pm$ 17
(b) Cross-linking antibody	CAT activity in K562 + MT2 (c.p.m.)
GAM*	1024 $\pm$ 91
$\beta_2$ -integrin	986 $\pm$ 32
$\beta_7$ -integrin	1001 $\pm$ 30

\* Plates coated with goat anti-mouse IgG capture antibody alone were used.

1-induced fusion. MOP8 cells were transiently transfected and analysed for expression of human adhesion molecules as described for K562 cells (see Methods). Untransfected MOP8 cells were uniformly negative when stained with the panel of antibodies to human membrane antigens (Table 1). Forty-eight hours after transient transfection of MOP8 cells with ICAM-1, ICAM-3 or VCAM-1, expression levels represented by mean channel fluorescence levels of 143.7, 117.4 and 103.6, respectively, were found in approximately 25–30% of the cell population, reflecting the efficiency of the transient transfection procedure. MOP8 mouse cells expressing ICAM-1, ICAM-3 or VCAM-1 were co-incubated with MT2 cells for 4 h. MT2 cells were then either washed from the MOP8 monolayer and added to K562-CAT cells or K562-CAT cells were added directly to the wells containing MOP8 and MT2. After 16 h, the results showed that the co-factor molecules presented to MT2 cells on MOP8 were ineffective in rendering the MT2 cells fusion-competent with K562 cells (Table 3). This was in contrast to their effects when presented on K562 target cells. It has been suggested that VCAM-1 may interact directly with a viral protein, triggering fusion (Hildreth *et al.*, 1997). However, MOP8 cells expressing ICAM-1, ICAM-3 or VCAM-1 did not form visible syncytia with either MT2 or C91-PL HTLV-1-infected cells, despite high levels of expression on their surface and long incubation times (data not shown).

We tried inducing fusion in MT2 cells by direct antibody cross-linking of integrin  $\beta_2$  or  $\beta_7$  on the membrane. MT2 cells were added to tissue-culture plates that had been coated with

goat anti-mouse Ig followed by mouse monoclonal antibodies to  $\beta_2$ - or  $\beta_7$ -integrin. After 2 h exposure to the antibody-coated plates, MT2 cells adhered tightly to the surface. K562–CAT cells were added and cultures were incubated overnight. CAT activity in K562 cells cultured with antibody cross-linked MT2 was similar to background levels (Table 3). No direct effect of exposure to antibody-coated plates was seen on the K562–CAT cells.

These data show firstly that ICAM-1, ICAM-3 and VCAM-1 do not act as receptors for HTLV-1 envelope glycoprotein (e.g. on MOP8 cells) and are not effective in isolation, and secondly, that the effects of these co-factors on MT2-induced fusion cannot be mimicked by antibody cross-linking of integrins. Therefore, it is likely that simultaneous engagement of receptor–envelope and co-receptor–ligand is required for HTLV-1 fusion in MT2 cells.

## Discussion

Adhesion molecules mediate homotypic and heterotypic recognition and attachment functions between cells (Gahmberg *et al.*, 1997). ICAM-1, ICAM-2 and ICAM-3 are found on most haematopoietic cells and on a wide range of non-haematopoietic cells where, with the exception of ICAM-3, expression may be inducible. The normal interaction is through engagement with a natural ligand(s); for the ICAMs this is LFA-1; for VCAM-1 it is VLA-4, although there is some promiscuity in ligand recognition. Adhesion molecules have additionally been shown to be involved in a number of non-classical functions. For example, ICAM-1 is a receptor for human rhinoviruses (Staunton *et al.*, 1989) and *Plasmodium falciparum*-infected erythrocytes (Berendt *et al.*, 1992); VCAM-1 can act as a receptor for encephalomyocarditis virus (Huber, 1994) and the  $\beta_1$ -integrin VLA-2 is the cellular receptor for echovirus (King *et al.*, 1995).

A role for adhesion molecules in syncytium formation of retroviruses has been well documented. Host-derived ICAM-1 can be incorporated on HIV-1 virions and enhance virus infectivity by interaction with LFA-1 on target cells (Fortin *et al.*, 1997). Antibodies to ICAM-3 and  $\beta_2$ -integrin have also been reported to block HTLV-1-induced syncytium formation (Ida *et al.*, 1995). Most recently, VCAM-1 has been identified as a putative co-receptor for HTLV-1 (Hildreth *et al.*, 1997).

In this study, we have confirmed and extended observations by others that adhesion molecules play a role in HTLV-1 syncytium formation. We show that ICAM-1, ICAM-3 and VCAM-1 regulate HTLV-1-mediated fusion in a cell-specific manner, but that expression of these molecules alone on target cells is not sufficient to confer susceptibility to syncytium formation with HTLV-1-infected cells. Furthermore, the fact that syncytium formation in mixed cultures could not be inhibited completely by antibodies that block the functional association with their respective ligands shows that their effects are contributory but not mandatory for fusion.

Antibodies used to block the  $\alpha$ -integrin-binding domains of LFA-1 or VLA-4 were ineffective in inhibiting HTLV-1 fusion. The  $\alpha$ -domains recognized by these antibodies contain the major binding sites for each addressin and have been shown to disrupt ligation-dependent functional responses such as T-cell activation. These data suggest that ICAM-1, ICAM-3 and VCAM-1 expressed on K562 cells interact with MT2 cells in a way that differs from that classically described for cellular activation.

MT2-induced fusion with K562–CAT expressing ICAM-1 or ICAM-3 was significantly reduced by a  $\beta_2$ -specific antibody and fusion with K562–CAT expressing VCAM-1 by a  $\beta_7$ -specific antibody. Likewise, fusion of C91-PL and K562 was also inhibited by these antibodies. Using the assays described, we were unable to determine whether antibody-blocking of  $\beta_2$ -integrins was operating on the infected cell membrane or the target cell membrane, as both cell types express  $\beta_2$ -integrins constitutively and K562 cells have significantly higher levels of expression than MT2 or C91-PL cells. Furthermore, MT2 and C91-PL cells express ICAM-1 and ICAM-3 on their surfaces, raising the possibility that the fusion co-factor effect is mediated by ligand recognition of these proteins by the  $\beta_2$ -integrins expressed on the target cells. However, if this is the case, it is difficult to reconcile the MT2-specific requirement for ICAM-1 or ICAM-3 expression on K562 target cells for fusion to occur. With the exception of lower ICAM-3 expression on MT2 compared with C91-PL cells, levels of expression of the other co-factor molecules are similar. This suggests that it is the  $\beta_2$ -integrins on the infected cell membrane that promote syncytium formation when suitable ligands are presented on the target cells. The efficacy of anti- $\beta_7$ -integrin antibodies in blocking syncytium formation of both MT2 and C91-PL cells also suggests that blockage occurs at the HTLV-1-infected cell membrane, as we were unable to detect  $\beta_7$ -integrin expression in K562 cells by Western blot.

MT2 cells do not express LFA-1  $\alpha$ -subunit and C91-PL have very low levels of VLA-4 $\alpha$ -integrin expression. The apparently low reactivity of C91-PL cells with  $\beta_2$ -integrin-specific antibodies contrasts with the high expression level of LFA-1 $\alpha$  chain on these cells (Table 1). Transport of integrin  $\alpha$ -chain subunits to the membrane is known to depend on their complexing intracellularly with  $\beta$ -chain subunits (Gahmberg *et al.*, 1997). Therefore, one would expect a close correlation between reactivity with antibodies to  $\alpha_L$  and  $\beta_2$  subunits. Our results with these antibodies, while reproducible, are difficult to explain unless a proportion of the  $\beta_2$ -integrin subunits are present at the cell surface in a form that is unreactive with the antibody used. Flexibility within the integrin system is achieved by the combinatorial pairing of  $\beta$ -chains with several  $\alpha$ -chains to form heterodimers of distinct specificities. It is possible that  $\beta_2$ - and  $\beta_7$ -integrins are presented on the membrane as novel complexes with other  $\alpha$ -integrin chains that constitute co-factor molecules, although there is no direct evidence for this. The efficiency of fusion may be dependent on

the net effect of a combination of co-factor interactions or glycoprotein receptor density, and C91-PL might be able to maintain a stable interaction with target cells more successfully than MT2 cells. Other fusion-susceptible cell types (e.g. COS7) may use a different array of co-factor molecules, or require none.

The cytoplasmic domains of  $\beta_2$ - and  $\beta_7$ -integrins are known to have a regulatory function in ligand interaction (Hibbs *et al.*, 1991). Deletion of the cytoplasmic domains of  $\beta_2$  and  $\beta_7$  subunits leads in some cases to constitutively active molecules (Crowe *et al.*, 1994; Lub *et al.*, 1997). We have shown in MT2 cells that co-factor engagement must occur together with the presumed binding of gp46 to the cellular receptor, although cross-linking of  $\beta_2$ - or  $\beta_7$ -integrin on the surface of MT2 cells by antibodies is not sufficient to induce fusion. Similar to other retroviruses, HTLV-1 envelope proteins with truncated or mutated cytoplasmic domains have been shown to affect syncytium formation (Pique *et al.*, 1993). Modification of the gp21 cytoplasmic tail may be a consequence of integrin engagement, although the antibody cross-linking experiments do not support this hypothesis.

This study suggests that HTLV-1-infected cells can use cell surface adhesion proteins as regulators of fusion with target cells. The pattern and level of expression of ICAMs and VCAM-1 vary widely in human cell types, and some are inducible. Notably, VCAM-1 is expressed mainly on endothelial cells whereas ICAM-1 and ICAM-3 tend to be leukocyte specific. Using these proteins for recognition in conjunction with the envelope-specific receptor interaction, HTLV-1 could target certain populations of cells according to their membrane phenotypes, which might themselves be influenced by factors such as chemokines. It is widely accepted that HTLV-1 infection is carried to sites of inflammation by the peripheral T-cell population. Membrane co-factor expression in cells at these sites may then be important for the subsequent establishment of a focus of infection or for pathological effects resulting from syncytium formation.

We thank Dr D. Simmonds, Institute of Molecular Medicine, University of Oxford, for the kind gift of the pCDM8 expression vectors. We also thank N. Rust, Department of Surgery, University of Oxford, for help in FACS analysis of samples and M. Jones, Department of Cellular Sciences, University of Oxford, for provision of the BP6 monoclonal antibody. This work was supported by a Career Development Grant (S.D.) from the Wellcome Trust.

## References

- Berendt, A. R., McDowall, A., Craig, A. G., Bates, P. A., Sternberg, M. J., Marsh, K., Newbold, C. I. & Hogg, N. (1992). The binding site on ICAM-1 for *Plasmodium falciparum*-infected erythrocytes overlaps, but is distinct from, the LFA-1-binding site. *Cell* **68**, 71–81.
- Carrington, C. V. F., Weiss, R. A. & Schulz, T. F. (1994). A truncated HTLV-1 envelope protein, lacking the hydrophobic membrane anchor domain, is associated with cellular membranes and virions. *Virology* **202**, 61–69.
- Crowe, D. T., Chiu, H., Fong, S. & Weissman, I. L. (1994). Regulation of the avidity of integrin alpha 4 beta 7 by the beta 7 cytoplasmic domain. *Journal of Biological Chemistry* **269**, 14411–14418.
- Daenke, S., Nightingale, S., Cruickshank, J. K. & Bangham, C. R. M. (1990). Sequence variants of human T-cell lymphotropic virus type I from patients with tropical spastic paraparesis and adult T-cell leukemia do not distinguish neurological from leukemic isolates. *Journal of Virology* **64**, 1278–1282.
- Dezzutti, C. S., Rudolph, D. L. & Lal, R. B. (1995). Infection with human T-lymphotropic viruses types I and II results in alterations of cellular receptors, including the up-modulation of T-cell counterreceptors CD40, CD54, and CD80 (B7-1). *Clinical and Diagnostic Laboratory Immunology* **2**, 349–355.
- Fauci, A. S. (1996). Host factors and the pathogenesis of HIV-induced disease. *Nature* **384**, 529–534.
- Fortin, J.-F., Cantin, R., Lamontagne, G. & Tremblay, M. (1997). Host-derived ICAM-1 glycoproteins incorporated on human immunodeficiency virus type 1 are biologically active and enhance viral infectivity. *Journal of Virology* **71**, 3588–3596.
- Gahmberg, C. G., Tolvanen, M. & Kotovuori, P. (1997). Leukocyte adhesion – structure and function of human leukocyte  $\beta_2$ -integrins and their cellular ligands. *European Journal of Biochemistry* **245**, 215–232.
- Gavalchin, J., Fan, N., Lane, M. J., Papsidero, L. & Poiesz, B. J. (1993). Identification of a putative cellular receptor for HTLV-1 by a monoclonal antibody, Mab 34-23. *Virology* **194**, 1–9.
- Gavalchin, J., Fan, N., Waterbury, P. G., Corbett, E., Faldasz, B. D., Peshick, S. M., Poiesz, B. J., Papsidero, L. & Lane, M. J. (1995). Regional localization of the putative cell surface receptor for HTLV-1 to human chromosome 17q32.2–17q25.3. *Virology* **212**, 196–203.
- Gray, G. S., White, M., Bartman, T. & Mann, D. (1990). Envelope gene sequence of HTLV-1 isolate MT-2 and its comparison with other HTLV-1 isolates. *Virology* **177**, 391–395.
- Hibbs, M. L., Jakes, S., Stacker, S. A., Wallace, R. W. & Springer, T. A. (1991). The cytoplasmic domain of the integrin lymphocyte function-associated antigen 1 beta subunit: sites required for binding to intercellular adhesion molecule 1 and the phorbol ester-stimulated phosphorylation site. *Journal of Experimental Medicine* **174**, 1227–1238.
- Hildreth, J. E. K., Subramaniam, A. & Hampton, R. A. (1997). Human T-cell lymphotropic virus type 1 (HTLV-1)-induced syncytium formation mediated by vascular cell adhesion molecule-1: evidence for involvement of cell adhesion molecules in HTLV-1 biology. *Journal of Virology* **71**, 1173–1180.
- Huber, S. A. (1994). VCAM-1 is a receptor for encephalomyocarditis virus on murine vascular endothelial cells. *Journal of Virology* **68**, 3453–3458.
- Ida, H., Eguchi, K., Mizokami, A., Yamashita, I., Origuchi, T., Takashima, H., Shimada, H., Kawabe, Y., Nakamura, T. & Nagataki, S. (1995). CD18 and CD50(ICAM-3) mAb block human T-cell lymphotropic virus type 1 (HTLV-1)-induced syncytium formation. In *Leukocyte Typing. V. White Cell Differentiation Antigens*, pp. 1598–1599. Edited by S. F. Schlossman, L. Boumsell & W. Gilks. Oxford: Oxford University Press.
- King, S. L., Cunningham, J. A., Finberg, R. W. & Bergelson, J. M. (1995). Echovirus 1 interaction with the isolated VLA-2 I domain. *Journal of Virology* **69**, 3237–3239.
- Lub, M., van Vliet, S. J., Oomen, S. P., Pieters, R. A., Robinson, M., Figdor, C. G. & van Kooyk, Y. (1997). Cytoplasmic tails of beta 1, beta 2, and beta 7 integrins differentially regulate LFA-1 function in K562 cells. *Molecular Biology of the Cell* **8**, 719–728.
- Nagy, K., Clapham, P., Cheingsong-Popov, R. & Weiss, R. A. (1983).

Human T-cell leukemia virus type I: induction of syncytia and inhibition by patients' sera. *International Journal of Cancer* **32**, 321–328.

**Niewiesk, S., Daenke, S., Parker, C. E., Taylor, G., Weber, J., Nightingale, S. & Bangham, C. R. M. (1995).** Naturally occurring variants of human T-cell leukemia virus type I Tax protein impair its recognition by cytotoxic T lymphocytes and the transactivation function of Tax. *Journal of Virology* **69**, 2649–2653.

**Paine, E., Garcia, J., Philpott, T. C., Shaw, G. & Ratner, L. (1991).** Limited sequence variation in human T-lymphotropic virus type I isolates from North American and African patients. *Virology* **182**, 111–123.

**Pique, C., Pham, D., Tursz, T. & Dokhelar, M.-C. (1993).** The cytoplasmic domain of the human T-cell leukemia virus type I envelope

can modulate envelope functions in a cell type-dependent manner. *Journal of Virology* **67**, 557–561.

**Siess, D. C., Kozak, S. L. & Kabat, D. (1996).** Exceptional fusogenicity of Chinese hamster ovary cells with murine retroviruses suggests roles for cellular factor(s) and receptor clusters in the membrane fusion process. *Journal of Virology* **70**, 3432–3439.

**Sommerfelt, M. A. & Weiss, R. A. (1990).** Receptor interference groups of 20 retroviruses plating on human cells. *Virology* **176**, 58–69.

**Staunton, D. E., Merluzzi, V. J., Rothlein, R., Barton, R., Marlin, S. D. & Springer, T. A. (1989).** A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. *Cell* **56**, 849–853.

---

Received 26 October 1998; Accepted 8 February 1999