Vascular cell adhesion molecule-1 induced by human T-cell leukaemia virus type 1 Tax protein in T-cells stimulates proliferation of human T-lymphocytes

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Among individuals infected with human T-cell leukaemia/lymphotropic virus type 1 (HTLV-1), a small percentage develop a lymphproliferative disease, adult T-cell leukaemia (ATL) or a chronic inflammatory disease, such as tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM) (reviewed by Uchiyama, 1997). HTLV-1 is considered to be a retrovirus that alters immunoregulatory pathways. The viral regulatory Tax protein has been shown previously to induce the expression of vascular cell adhesion molecule-1 (VCAM-1) by T-cells. To determine the functional role of this adhesion molecule, Jurkat T-cells stably expressing either Tax or both Tax and Rex (another viral regulatory protein) were used in binding and coculture assays performed with either control Jurkat cells or primary human T-lymphocytes. Evidence was provided that VCAM-1 acting in synergy with leucocyte function-associated antigen-3 (LFA-3) promotes T-cell–T-cell interactions and increases T-cell proliferation. Interestingly, Rex was found to modulate these events. These data establish that VCAM-1 induced by Tax on T-cells thus contributes to the immunopathological process triggered by HTLV-1 infection.

Human T-cell leukaemia/lymphotropic virus type 1 (HTLV-1), aetiologically linked to lymphproliferative as well as inflammatory diseases, infects and activates CD4+ helper T-cells and thus alters immunoregulatory pathways. The viral regulatory Tax protein has been shown previously to induce the expression of vascular cell adhesion molecule-1 (VCAM-1) by T-cells. To determine the functional role of this adhesion molecule, Jurkat T-cells stably expressing either Tax or both Tax and Rex (another viral regulatory protein) were used in binding and coculture assays performed with either control Jurkat cells or primary human T-lymphocytes. Evidence was provided that VCAM-1 acting in synergy with leucocyte function-associated antigen-3 (LFA-3) promotes T-cell–T-cell interactions and increases T-cell proliferation. Interestingly, Rex was found to modulate these events. These data establish that VCAM-1 induced by Tax on T-cells thus contributes to the immunopathological process triggered by HTLV-1 infection.

The viral regulatory protein Tax, which is involved in the transcriptional activation of the provirus and expression of many cellular genes implicated in T-cell activation and proliferation. More specifically, Tax has been shown to intervene in T-cell–T-cell interactions by enhancing the expression of an adhesion molecule, leukocyte function-associated antigen-3 (LFA-3 or CD58), which is the ligand for CD2 (Tanaka et al., 1995). The interaction of LFA-3 expressed by HTLV-1-infected T-cells with CD2 on resting T-cells defines an important costimulatory pathway in triggering the proliferation of resting T-cells (Wucherpfennig et al., 1992). In addition, other adhesion molecule interactions, such as intercellular adhesion molecule-1 (ICAM-1 or CD54) with LFA-1 (CD11a/CD18), have been suggested to participate, first, in HTLV-1-mediated activation of uninfected T-cells, facilitating lymphproliferation, and second, in increased adhesion to endothelial cells, resulting in tissue or organ infiltration (Ichinose et al., 1992, 1994).

We have reported previously the expression of vascular cell adhesion molecule-1 (VCAM-1 or CD106) by Jurkat cells stably expressing the lax gene and shown that Tax was trans-activating the VCAM-1 gene via two NF-xB sites present in the gene promoter (Valentin et al., 1997). Moreover, this VCAM-1 up-regulation has been confirmed in freshly isolated T-cells from TSP/HAM patients (Valentin et al., 1997). VCAM-1 may serve as either an accessory molecule or a potential coreceptor for HTLV-1-induced cell fusion (Daenke et al., 1999; Hildreth et al., 1997). VCAM-1 is normally expressed by stimulated endothelial cells, therefore favouring the adhesion of these cells to T-lymphocytes via VLA-4, which is the VCAM-1 ligand that is constitutively expressed by T-cells. Thus, this adhesion pathway may be important in mediating emigration of leukaemic T-cells into the organs of ATL patients and entry of activated T-cells into the central nervous system of individuals with TSP/HAM (Ishikawa et al., 1993; Uchiyama, 1997). Furthermore, a recent report has underlined the role of VCAM-1 in the induction of matrix metalloproteinase-2, which is an important mediator for the extravagation of T-cells and the degradation of the subendo-
Fig. 1. Comparative analysis by flow cytometry of cell surface (A) LFA-3 (CD58), (B) VCAM-1 (CD106) and (C) VLA-4 (CD29/CD49d) expression by parental Jurkat cells, by control neomycin-resistant Jurkat cells (C9), and by Jurkat cells stably expressing either Tax (clone 50) or both Tax and Rex (C11 and E12). Bars represent the mean of fluorescence intensity. Data represent the mean ± SD from 3 to 10 experiments. The asterisks (‡) indicate a statistically significant difference compared with control, as calculated by Student’s t-test (‡P < 0.05).

Indeed such interactions may lead to cell fusion and/or transmission of HTLV-1.

In the present study, we have investigated the functional consequences of Tax-induced VCAM-1 expression by T-cells in the activation and proliferation of normal resting T-cells. To that aim, we have used, in addition to parental Jurkat and Tax-negative neomycin-resistant Jurkat cells, three clones of Jurkat cells stably expressing either Tax (clone 50) or Tax and Rex, another viral regulatory protein, but acting at a post-transcriptional level (clones C11 and E12) (Lemasson et al., 1997). We first performed a flow cytometry analysis to compare the expression of VCAM-1 to that of LFA-3 by the different Jurkat cell lines. We confirmed that VCAM-1 was significantly expressed by Tax-expressing Jurkat cells (C11, E12 and 50) compared with the Tax-negative C9 and parental T-cells (Fig. 1B) (Valentin et al., 1997). Furthermore, the expression of LFA-3 was found to be up-regulated in the three Tax-positive clones when compared to the Tax-negative cells (Fig. 1A). We then analysed the profile of VLA-4 (CD29/CD49d) expression. In contrast to CD29 (β1 integrin) expression, CD49d (α4 integrin) down-regulation was statistically correlated, by the Student t-test, to Tax expression in T-cells (Fig. 1C). These data propose that Tax expression might induce a switch of VLA-4 expression to its VCAM-1 ligand on T-cells. As the level of CD49d expression has been reported to vary among ATL and HTLV-1-infected cases (Al-Fahim et al., 1999; Dhawan et al., 1993; Ishikawa et al., 1993; Uchiyama et al., 1996; Wake et al., 1995), it cannot be excluded that the expression of this integrin may be dependent on the level of Tax or of any viral or cellular protein.

Next, we investigated the functional consequences of VCAM-1 and/or LFA-3 expressed by Jurkat cells stably expressing Tax. We first evaluated the ability of Jurkat Tax-positive cells to adhere to Tax-negative T-cells by rosette assay analysis. Briefly, 1 x 10^6 Tax-negative T-cells were labelled with Hoechst H33342 fluorochrome (Sigma), as described previously (Weston & Parish, 1990), and then mixed with either Tax-negative or Tax-expressing T-cells for 15 min at 37 °C. No rosette formation was observed between parental Jurkat cells, between parental Jurkat and Tax-negative neomycin-resistant Jurkat cells (C9), and by Jurkat cells stably expressing either Tax (50) or both Tax and Rex (C11 and E12). From 15 to 48% of Tax-negative T-cells were found to bind to C11, E12 and 50 Tax-expressing T-cells (Table 1 and data not shown). These results underline the fact that Tax is mediating T-cell–T-cell interactions. To determine the involvement of adhesion molecules, Tax-expressing cells were first incubated with specific MAbs (10 µg/ml) for 40 min at 4 °C and then with labelled Tax-negative T-cells. Contrary to an incubation with an irrelevant MAb (to CD46) which was unable to interfere with rosette formation, inhibitions of 38 and 48% were observed when Tax-expressing T-cells were pre-incubated with blocking MAbs to LFA-3 or VCAM-1, respectively (Table 1). This inhibition increased up to 75% when Tax-expressing cells were treated with both MAbs.
Table 1. Effect of blocking VCAM-1 and LFA-3 MAbs on binding to parental Jurkat cells

Control parental Jurkat cells were labelled with Hoechst H33342 fluorochrome and incubated with Jurkat E12 cells stably expressing Tax and Rex, at a ratio of 10:1, respectively, for 30 min at 4 °C. E12 cells were pre-incubated with medium (none), an irrelevant MAb to CD46 (MCI20.6) or with blocking MAbs to LFA-3 (AICD58) or VCAM-1 (1G11) at 10 µg/ml. Cells were then observed with a fluorescence microscope (Nikon-Diaphot ELW D 0.3). A rosette was scored when at least three labelled cells were binding one unlabelled cell. Data represent the mean of three separate experiments (SD < 10%). Results are expressed as percentage rosette formation (labelled parental Jurkat cells bound to E12 cells) or percentage inhibition of rosette formation observed with MAb-treated E12 cells to that observed with untreated E12 cells.

<table>
<thead>
<tr>
<th>Pre-treatment of E12 cells</th>
<th>Rosette formation (%)</th>
<th>Inhibition of rosette formation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>36.5</td>
<td>-</td>
</tr>
<tr>
<td>Anti-CD46</td>
<td>34.6</td>
<td>5.2</td>
</tr>
<tr>
<td>Anti-LFA-3</td>
<td>22.5</td>
<td>38.3</td>
</tr>
<tr>
<td>Anti-VCAM-1</td>
<td>19.0</td>
<td>47.9</td>
</tr>
<tr>
<td>Anti-LFA-3 + anti-VCAM-1</td>
<td>9.0</td>
<td>75.3</td>
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These data clearly provide evidence that VCAM-1 induced in Tax-positive T-cells is involved in T-cell–T-cell interactions and that both VCAM-1 and LFA-3 are acting synergistically in this adhesion process.

These data suggest that these adhesion molecules may increase T-cell proliferation by enhancing binding of Tax-expressing T-cells to normal T-cells. To verify this hypothesis, human T-lymphocytes were purified and cultured, as described elsewhere (Huet et al., 1986). Briefly, mononuclear cells, obtained by Ficoll–hypaque centrifugation of peripheral blood from normal healthy donors (Établissement de Transfusion Sanguine de Lyon), were incubated for 1 h at 37 °C in plastic culture dishes. Nonadherent cells were then depleted of phagocytic cells by carbonyl iron ingestion and passage over a magnet. Accessory cells were lysed after incubation with 3H-leucine methyl ester. After eliminating residual HLA-DR cells, this preparation was analysed by flow cytometry and found to contain more than 95% CD2−CD3+ cells (data not shown). Under these conditions, these T-lymphocytes were unable to proliferate when stimulated via the CD3/TCR pathway unless they were incubated with accessory cells. We therefore investigated whether the Tax-expressing Jurkat cells were endowed with such a property. To investigate this, Tax-negative or -positive cells were fixed with 1% paraformaldehyde. No significant [3H]thymidine incorporation by these cells could be detected (less than 300 c.p.m.). Then, 2 × 10⁴ fixed cells were incubated with 5 × 10⁴ T-lymphocytes in the presence of 5 µg/ml CD3 MAb pre-adsorbed onto a 96-well plate. The proliferation of these T-lymphocytes was assessed following the addition of [3H]thymidine. As shown in Fig.
2(A). Tax-negative Jurkat cells were unable to induce DNA synthesis whereas Tax-positive cells induced a significant level of $[^{3}H]$thymidine incorporation. These observations indicate that a costimulatory signal is delivered by Tax-positive Jurkat cells. To determine whether VCAM-1 induced by Tax is involved in the proliferation of CD3-stimulated T-lymphocytes, we performed blocking experiments in which Tax-expressing Jurkat cells were pre-treated with 10 µg/ml VCAM-1-blocking MAb for 30 min at 4°C, fixed and then cocultivated with CD3-stimulated T-lymphocytes. A 15 to 20% inhibition of the proliferation of CD3-stimulated T-lymphocytes cocultivated with C11 and E12 cells (which express both Tax and Rex) was observed, whereas a 90% inhibition was reached in the presence of cells from clone 50 (which expresses only Tax) (Fig. 2B). Likewise, $[^{3}H]$thymidine incorporation by CD3-stimulated T-lymphocytes was reduced by about 70% only with fixed cells of clone 50 which had been pre-treated with the LFA-3-blocking MAb (Fig. 2B). The discrepancy in the inhibition of T-cell proliferation between Jurkat cells expressing Tax and Rex and those expressing only Tax proposes that the stimulatory pathway induced by VCAM-1 and LFA-3 is the only one operating in cells expressing Tax alone. Consequently, other molecules in cells expressing both Tax and Rex might be required to fully activate resting T-cells (reviewed by Hölsberg, 1999). Collectively, these data indicate that Rex may be involved in the expression of these molecules and/or in the down-regulation of the stimulatory potential linked to VCAM-1 and LFA-3. This former hypothesis is supported by previous observations indicating that Rex has the capability to increase cellular gene expression. Indeed, it has been reported that Rex may contribute to human interleukin-2 gene expression regulation and in stabilizing the interleukin-2 receptor α-chain mRNA in T-cells (Kanamori et al., 1990; McGuire et al., 1993). More interestingly, a recent study has underlined that the concomitant expression of Tax and Rex in Jurkat cells resulted in the overexpression of a protein tyrosine kinase, FynB, probably through a splicing mechanism controlled by Rex (Weil et al., 1999).

In conclusion, VCAM-1 induced by HTLV-1 Tax protein in infected T-cells is able to promote T-cell–T-cell adhesion, thus contributing to the proliferation of uninfected T-lymphocytes. Such events may have particular relevance to an understanding of the immunopathogenesis of HTLV-1 and of the spontaneous proliferation of T-cells of HTLV-1-infected individuals, which contributes to an amplification of immune system activation in an antigen-independent manner, thus favouring HTLV-1 dissemination and maintenance of the disease.

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