Expression of the α5β1 fibronectin receptor on T lymphocytes of patients with HIV-1 infection

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Abstract
Aims—To evaluate the expression of the α5β1 integrin fibronectin receptor (FNRI) which mediates several processes, including phagocytosis, cell motility and the immune response, on T lymphocytes of patients with HIV-1 infection.

Methods—T lymphocytes were incubated with monoclonal antibody directed against FNRI and then with monoclonal antibodies, conjugated with phycoerythrin, directed against CD3, CD4 and CD8 positive cells. Expression of FNRI on CD3, CD4 and CD8 positive cells was analysed using flow cytometry.

Results—Normal expression of FNRI was observed on CD3 positive cells from asymptomatic HIV positive patients and those with AIDS. Increased expression of FNRI was observed on CD8 positive cells from asymptomatic HIV positive patients and on CD4 positive cells from patients with AIDS. Increased FNRI expression was observed on CD4 positive cells from patients with AIDS, particularly those with opportunistic infections caused by Pneumocystis carinii, Mycobacterium sp, Toxoplasma gondii, and Cryptococcus neoformans.

Conclusion—Increased expression of FNRI on CD8 and CD4 positive cells in asymptomatic HIV positive patients and those with AIDS, respectively, may be an epiphenomenon correlated with lymphocyte activation by HIV-1 or opportunistic infection. Further study is required to determine whether upregulation of FNRI expression has a direct role in the pathogenesis of AIDS.

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The α5β1 fibronectin receptor (FNRI) belongs to the integrin family of cell adhesion molecule receptors. FNRI has also been identified as very late antigen (VLA)-5. VLAs are expressed in phagocytic cells, and FNRI is regarded as a serum integrin, as it is involved in specific adhesive interaction between mononuclear cells and the extracellular matrix. In particular, lymphocytes interact with fibronectin via two main receptors: the α5β1 integrin, which is the receptor for the RGD sequence and synergistic sites, and the α4β1 integrin. The fibronectins are a family of high molecular weight glycoproteins found in plasma and tissue. Most cellular fibronectins are produced constitutively, one of which is synthesised and secreted by T lymphocytes in response to antigenic stimulation. During diverse biological processes, including inflammation, wound healing and tumour metastasis, cells must interact with components of the extracellular matrix. The interaction between fibronectin and cells expressing the α5β1 FNRI results in augmented cell adhesion, migration and differentiation. Previously, we have shown that serum concentrations of fibronectin are significantly decreased in patients infected with HIV-1, particularly in patients with AIDS with concomitant opportunistic infections. More recently, increased serum concentrations of circulating FNRI were observed in adult and paediatric patients with HIV-1 infection.

Methods

The study population comprised 46 patients with HIV-1 infection, 13 of whom (eight men and five women; mean (SD) age 34.1 (7.8) years) were asymptomatic (CD4 cell counts > 400 cells/μl). The remaining 33 patients (20 men and 13 women; mean age 32.8 (8.4) years) had frank AIDS (CDC stage IV; CD4 cell counts < 100/μl). Thirty two healthy subjects (19 men and 13 women; mean age 36.3 (4.0) years) without HIV-1 infection served as controls.

ANALYSIS OF RECEPTOR EXPRESSION BY FLOW CYTOMETRY

Blood from all patients and controls was collected into tubes containing heparin. After lysis of red blood cells, white blood cells were washed and pelleted in phosphate buffered saline (PBS). Pellets were resuspended in PBS containing mouse anti-human fibronectin receptor IgG monoclonal antibody (diluted 1 in 100; Takara Shuzo, Kyoto, Japan) and incubated at 4°C for 25 minutes. After being washed twice in PBS, samples were incubated in PBS containing goat anti-mouse IgG fluorescein isothiocyanate (FITC) conjugated antibodies (diluted 1 in 50; Technogenetics, Milan, Italy) at 4°C for 25 minutes. After two further washings, pellets were resuspended in PBS and lymphocytes were incubated with phycoerythrin (PE) conjugated anti-CD3, anti-CD4, and anti-CD8 (Ortho Pharmaceuticals, Raritan, New Jersey, USA), and analysed using flow cytometry.

DATA ANALYSIS

Data are expressed as mean (SD). Statistical analysis was done using a non-parametric
Table 1  Expression of FNR on T lymphocytes from patients with HIV-1 infection. Results are expressed as mean (SD)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>FNR (% positive cells)</th>
<th>CD3 positive</th>
<th>CD4 positive</th>
<th>CD8 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 32)</td>
<td>49.9 (16.3)</td>
<td>49.1 (11.6)</td>
<td>49.9 (10.2)</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic HIV positive</td>
<td>54.5 (5.7)</td>
<td>47.9 (12.3)</td>
<td>57.1 (6.5)*</td>
<td></td>
</tr>
<tr>
<td>patients (n = 13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with AIDS (n = 33)</td>
<td>58.7 (15.5)</td>
<td>76.8 (16.8)t</td>
<td>53.5 (13.4)</td>
<td></td>
</tr>
</tbody>
</table>

*p = 0.0235 and tP = 0.0001 vs controls.

Mann-Whitney U test. All p values are two-tailed. Statistical significance was set at p < 0.05 for all tests.

Results
Table 1 shows expression of FNR on T lymphocytes from HIV-1 infected patients. As can be seen, normal expression of FNR was observed on CD3 positive cells from asymptomatic seropositive patients and those with AIDS. However, on analysis of CD3 subpopulations a significant increase in FNR expression on CD4 positive cells was observed only in patients with AIDS. Increased FNR expression was observed on CD8 positive cells from asymptomatic seropositive patients, but not from those with AIDS. Figure 1 shows representative cytofluorographs of a patient with AIDS and a healthy control subject, analysed using a dual immunofluorescence technique. The FNR positive cell population can be seen in the top right quadrants.

Figure 2 illustrates FNR expression on CD4 positive cells from 30 patients with AIDS with pulmonary infection, cerebral disorders and other clinical manifestations. As shown, increased expression of FNR was observed on CD4 positive cells from patients with AIDS with Pneumocystis carinii pneumonia, disseminated mycobacterial infection, and from those patients with cerebral disorders, including cer-
Discussion

The results of the present study show clearly that FNR expression is raised significantly on CD4 positive cells from patients with AIDS and on CD8 positive cells from asymptomatic HIV positive patients. An increase in the number of CD8 positive cytotoxic T lymphocytes has been observed in the peripheral blood of HIV-1 infected patients, predominantly in the early stages of the infection, and such an increase may directly or indirectly stimulate FNR expression on these CD8 positive cells. As the disease progresses, HIV-1, infecting a larger number of CD4 positive cells, may upregulate expression of FNR on these cells.

Recently, we have demonstrated that fibronectin binds to HIV-1-infected cells and to HIV-1 glycoproteins, such as gp120 and gp41. A significant increase in FNR expression has been observed in the serum of adult and paediatric patients with AIDS; increased expression of FNR was also noted in vitro on HIV-1 infected cells. Weeks et al have shown that acute HIV-1 infection of peripheral blood lymphocytes is associated with enhanced binding to fibronectin in vitro, which is mediated through α5β1 FNR. We have reported previously that serum concentrations of fibronectin are significantly decreased in patients with HIV-1 infection, particularly in those with AIDS with concomitant opportunistic infections. Godfrey demonstrated that T lymphocytes synthesise and secrete fibronectin in response to antigen or mitogen activation. Increased expression of FNR on CD4 positive cells in patients with AIDS and on CD8 positive cells in asymptomatic seropositive patients may result from direct or indirect stimulation by viral products of HIV-1 or by HIV-1 itself, or both, which by infecting these cells, provokes overexpression of FNR.

Circulating, soluble fibronectin binds to CD4 positive and CD8 positive cells, via FNR expression on their surfaces. This binding may lead to opsonisation or sequestration of HIV-1 infected cells. Alternatively, overexpression of FNR on CD4 or CD8 positive cells could stimulate the synthesis and secretion of fibronectin by these cells. In inflammatory states, fibronectin has been shown to bind and opsonise several opportunistic pathogens, including protozoa, fungi, and bacteria.

In conclusion, increased expression of FNR on CD4 and CD8 positive cells in a symptomatic HIV positive patients and those with AIDS, respectively, may be an epiphemogeneous correlated with lymphocyte activation by HIV-1 or opportunistic infection. Further study is required to determine whether upregulation of FNR expression has a direct role in the pathogenesis of AIDS.