Expression of the $\alpha 5 \beta 1$ fibronectin receptor on T lymphocytes of patients with HIV-1 infection

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Abstract

Aims—To evaluate the expression of the $\alpha 5 \beta 1$ integrin fibronectin receptor (FNR), 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 FNR and then with monoclonal antibodies, conjugated with phycoerythrin, directed against CD3, CD4 and CD8 positive cells. Expression of FNR on CD3, CD4 and CD8 positive cells was analysed using flow cytometry.

Results—Normal expression of FNR was observed on CD3 positive cells from asymptomatic HIV positive patients and those with AIDS. Increased expression of FNR was observed on CD8 positive cells from asymptomatic HIV positive patients and on CD4 positive cells from patients with AIDS. Increased FNR 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 FNR 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 FNR expression has a direct role in the pathogenesis of AIDS.

(J Clin Pathol 1996;49:733-736)

Keywords: fibronectin receptor, T lymphocytes, AIDS, HIV-1 infection.

The $\alpha 5 \beta 1$ fibronectin receptor (FNR) belongs to the integrin family of cell adhesion molecule receptors.^{1,2} FNR has also been identified as very late antigen (VLA)-5.³ VLAs are expressed in phytohaemagglutinin activated human T cells. FNR is regarded as a serum integrin, as it is involved in specific adhesive interaction between mononuclear cells and the extracellular matrix.⁴ In particular, lymphocytes interact with fibronectin via two main receptors: the $\alpha 5 \beta 1$ integrin, which is the receptor for the RGD sequence and synergistic sites,⁵ and the $\alpha 4 \beta 1$ integrin.⁶ The fibronectins are a family of high molecular weight glycopro-

teins found in plasma and tissue.^{7,8} Most cellular fibronectins are produced constitutively, one of which is synthesised and secreted by T lymphocytes in response to antigenic stimulation.⁹ During diverse biological processes, including inflammation, wound healing and tumour metastasis, cells must interact with components of the extracellular matrix.¹⁰ The interaction between fibronectin and cells expressing the $\alpha 5 \beta 1$ FNR results in augmented cell adhesion, migration and differentiation.¹¹

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.¹² More recently, increased serum concentrations of circulating FNR were observed in adult and paediatric patients with HIV-1 infection.¹³

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/ μ l). 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/ μ l). 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 by using a non-parametric

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Accepted for publication 7 May 1996

Table 1 Expression of FNR on T lymphocytes from patients with HIV-1 infection. Results are expressed as mean (SD)

Subjects	FNR (% positive cells)		
	CD3 positive	CD4 positive	CD8 positive
Controls (n = 32)	49.9 (16.3)	49.1 (11.6)	49.9 (10.2)
Asymptomatic HIV positive patients (n = 13)	54.5 (5.7)	47.9 (12.3)	57.1 (6.5)*
Patients with AIDS (n = 33)	58.7 (15.5)	76.8 (16.8)†	53.5 (13.4)

*p = 0.0235 and †p = 0.0001 v 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-

Figure 1 Dot plot illustration of expression of FNR on CD4 and CD8 positive peripheral blood lymphocytes from a healthy control subject (A and B), from a patient with AIDS (C), and an asymptomatic HIV positive patient (D). The contour plots were divided into four quadrants: bottom left, gated and unstained cells; bottom right, cells with FITC fluorescence only; top left, cells with PE fluorescence only; top right, cells with FITC and PE fluorescence.

Figure 2 FNR expression on CD4 positive T lymphocytes from 30 patients with AIDS. PCP = P carinii pneumonia; MI = mycobacterial infection; CT = cerebral toxoplasmosis; CM = cryptococcal meningitis; HIV-ENC = HIV-1 encephalopathy; RC = retinitis caused by infection with cytomegalovirus; KS = Kaposi sarcoma. $p = 0.0001$, PCP and MI versus controls; $p = 0.002$, CT versus controls; $p = 0.0003$, CM versus controls; $p = 0.025$, HIV-ENC versus controls.

Figure 3 FNR expression on CD8 positive T lymphocytes from 30 patients with AIDS. PCP = P carinii pneumonia; MI = mycobacterial infection; CT = cerebral toxoplasmosis; CM = cryptococcal meningitis; HIV-ENC = HIV-1 encephalopathy; RC = retinitis caused by infection with cytomegalovirus; KS = Kaposi sarcoma. Horizontal bar = mean value.

ebreal toxoplasmosis, cryptococcal meningitis and HIV-1 encephalopathy. By contrast, increased, but not significantly, FNR expression was observed on CD4 positive cells from patients with AIDS with retinitis caused by infection with cytomegalovirus and in those with Kaposi sarcoma. However, normal expression of FNR was noted on CD8 positive cells from all patients with AIDS with pulmonary involvement, cerebral disorders and other clinical manifestations (fig 3).

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 gp41.¹⁴ 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.¹³ Weeks *et al*¹⁵ have shown that acute HIV-1 infection of peripheral blood lymphocytes is associated with enhanced binding to fibronectin in vitro, which is mediated through $\alpha 5 \beta 1$ 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.¹² Godfrey¹⁶ 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 expressed 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,¹⁷ fungi,¹⁸ and bacteria.¹⁹

In conclusion, increased expression of FNR 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 up-regulation of FNR expression has a direct role in the pathogenesis of AIDS.

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