NOTES

Levels of Circulating Fibronectin Receptor in Adult and Pediatric Patients with Human Immunodeficiency Virus Type 1 Infection

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Received 27 September 1993/Returned for modification 15 October 1993/Accepted 13 January 1994

We found a significant increase in fibronectin receptor (FNR) levels in the sera of adult human immunodeficiency virus type 1 (HIV-1)-infected patients, especially in those with AIDS (1,026.9 ± 583.9 ng/ml; P < 0.0001). In contrast, AIDS patients with neurologic disorders and HIV-1-seropositive patients showed normal levels of FNR in serum. In addition, HIV-1-infected children showed increased levels of FNR in serum (824.4 ± 333.5 ng/ml; P = 0.03). We suggest that an increase of FNR levels in AIDS patients is related to enhanced expression of FNR on HIV-1-infected cells.

The fibronectin receptor (FNR) is a member of the integrin family of cell adhesion molecule receptors (2, 9). The integrin family includes the receptors for vitronectin (8) and fibrinogen (6) and some cell-cell adhesion receptors (4). Specific adhesive interaction between cells and substrata is crucial to a wide range of biological processes, including organ development, phagocytosis, cell motility, and immune response. FNR is considered a serum integrin, as it is involved in specific adhesive interaction between mononuclear cells and the extracellular matrix (10). This interaction is a prerequisite for accumulation of these cells at inflammatory sites and for subsequent phagocytosis and killing of organisms. FNR is implicated in facilitating fibroproliferative responses to tissue injuries. The presence of high levels of FNR in the sera of patients with chronic liver diseases selectively reflects hepatic fibrogenesis (15). We have previously demonstrated that levels of fibronectin in serum are significantly decreased in patients with human immunodeficiency virus type 1 (HIV-1) infection, especially in those with AIDS and with opportunistic infections (12). Decreased levels of circulating fibronectin may well correlate with its ability to interact directly with opportunistic pathogens, including bacteria (5), fungi (11), and protozoa (14), and with viral proteins or with viruses themselves (influenza A, parainfluenza 1, and mumps viruses) (3). More recently, we have demonstrated that fibronectin, in vitro, has the ability to bind to HIV-1-infected cells as well as to the HIV-1 glycoproteins gp41 and gp120 (7).

This study was designed to determine the levels of circulating FNR in the sera of adult and pediatric patients with HIV-1 infection in order to evaluate its role in the immune mechanisms and in the progress of the disease.

We studied 58 adult patients with HIV-1 infection and 11 children born to mothers with HIV-1 infection. Of the adult patients, 30 were asymptomatic and 28 had full-blown disease. Among the AIDS patients, 9 suffered from neurologic disorders and 19 had localized or disseminated opportunistic infections without involvement of the central nervous system. Among children born to HIV-1-infected mothers, six became seronegative for HIV-1 (confirmed by PCR) while five were seropositive for HIV-1, with symptomatic infections (class P-2).

Blood samples were collected and immediately centrifuged, and serum samples were stored at −20°C until assayed for FNR.

FNR levels were determined in the sera of our patients by a solid-phase enzyme immunoassay based on a sandwich method that utilizes two mouse monoclonal anti-FNR antibodies (Takara Shuzo, Kyoto, Japan). The amount of FNR was quantitated by measuring the A490-492 in a microtitrator plate reader. The enzyme immunoassay was sensitive over a range of 40 to 640 ng/ml.

An indirect immunofluorescent assay was used to determine FNR expression on human leukemic T cells chronically infected with HIV-1 (H9-V). This cell line was grown in RPMI 1640 medium supplemented with 10% fetal calf serum at 37°C in an atmosphere of 5% CO2. Twenty microliters of H9 (uninfected) and H9-V cellular suspensions (5 × 10^5 cells per ml) were added to wells of multitest slides to perform the indirect immunofluorescent assay. The slides were fixed with acetone solution and then challenged in duplicate with a murine monoclonal antibody against FNR (Takara Shuzo), diluted 1:5 in phosphate-buffered saline (PBS). Control wells were treated only with PBS. After a 30-min incubation at 37°C, the slides were washed and stained with fluoresceinated antibody against murine immunoglobulins (Technogenetics, Milan, Italy), diluted 1:10. After three washings, a drop of Evans blue was added to each well. The preparations were observed with an epifluorescent microscope.

Data are expressed below as means and standard deviations.

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TABLE 1. Clinical characteristics of adult patients with HIV-1 infection

<table>
<thead>
<tr>
<th>Patient category</th>
<th>No. of males/no. of females</th>
<th>Age (yr)*</th>
</tr>
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<tbody>
<tr>
<td>Control (n = 15)</td>
<td>15/0</td>
<td>38.4 ± 10.8</td>
</tr>
<tr>
<td>Seropositive (n = 30)</td>
<td>24/6</td>
<td>26.1 ± 5.1</td>
</tr>
<tr>
<td>AIDS (n = 28)</td>
<td>22/6</td>
<td>33.3 ± 5.0</td>
</tr>
<tr>
<td>With opportunistic infections (n = 19)</td>
<td>13/6</td>
<td>34.5 ± 5.2</td>
</tr>
<tr>
<td>With neurologic disorders (n = 9)</td>
<td>9/0</td>
<td>30.6 ± 3.6</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation.

The Student's t test and Fisher's exact test, when appropriate, were used to assess differences between continuous variables. When samples were small, the Mann-Whitney nonparametric U test was used to assess differences between the two groups. A P value of <0.05 was considered significant. Tables 1 and 2 illustrate clinical characteristics of adult and pediatric patients with HIV-1 infection, respectively. Adult AIDS patients with neurologic disorders did not show localized or disseminated manifestations of opportunistic infections. HIV-1-infected children had asymptomatic infection (class P-2).

Figure 1 shows levels of FNR in the sera of adult patients with HIV-1 infection. As can be seen from the figure, increased and significant levels of FNR were noted in the sera of AIDS patients with opportunistic infections (1,026.9 ± 583.9 ng/ml; P < 0.0001). It is interesting that AIDS patients with neurologic disorders had normal levels of FNR in serum (255.6 ± 107.5 ng/ml), as did HIV-1-seropositive patients (234.1 ± 145.0 ng/ml). Figure 2 shows levels of FNR in the sera of HIV-1-infected children and in children seronegative to HIV-1. Increased levels of FNR in serum were detected in children seronegative for HIV-1 (349.3 ± 106.5 ng/ml; P = 0.0007). A more sustained increase of levels of FNR in serum was noted in HIV-1-infected children (824.4 ± 333.5 ng/ml; P = 0.03).

We also examined FNR expression on HIV-1-infected cells (H9-V) in vitro. There were significantly more positive fluorescent HIV-1-infected (H9-V) cells than positive fluorescent uninfected (H9) cells (71.6% ± 6.8% versus 26.8% ± 3.4%; P < 0.0001). No fluorescence was observed in H9 cells treated with PBS and stained with fluorescein-conjugated antibodies against murine immunoglobulins.

The results of our study clearly demonstrate increased levels of FNR in the sera of adult and pediatric patients with AIDS. In addition, we observed an increased in vitro expression of FNR on HIV-1-infected cells. In contrast, adult AIDS patients with neurologic disorders and HIV-1-seropositive patients had normal levels of FNR. In a previous study, we demonstrated decreased levels of plasma fibronectin in patients with AIDS-related complex and AIDS (12). Diminution of circulating fibronectin in these patients may be related to binding to various opportunistic pathogens and/or to a direct binding to HIV-1 itself or to viral glycoproteins.

FNR belongs to an integrin "superfamily," and as a serum integrin, it is involved in specific adhesive interaction between mononuclear cells and the extracellular matrix. This interaction is a prerequisite for the accumulation of these cells at inflammatory sites and for consequent phagocytic activity. FNR has also been identified as very late antigen 5 of the very late antigen integrin family (10). Very late antigen 5 integrins are expressed in activated human T cells as well as in many other cell types, including monocytes/macrophages. The in vivo cellular source of FNR is not known. Receptors of fibronectin have been detected on T lymphocytes (13), monocytes, and polymorphonuclear leukocytes (14). The increased levels of FNR in the sera of adult and pediatric patients with AIDS are related to enhanced expression, and consequently to release, of FNR from HIV-1-infected lymphocytes and monocytes. In addition, it could be postulated that HIV-1 itself may be able to stimulate expression and/or release of FNR. The increased levels of FNR in children seronegative for HIV-1 are in contrast with the normal levels of FNR noted in asymptomatic seropositive adults. Levels of fibronectin in the sera of children seronegative for HIV-1 were normal, but levels were signifi-

![FIG. 1. FNR levels in the sera of adult patients seropositive for HIV-1, with neurologic disorders, and with AIDS. Horizontal bars indicate mean values.](image1)

![FIG. 2. FNR levels in the sera of pediatric patients with HIV-1 infection. Horizontal bars indicate mean values.](image2)
cantly decreased in children with HIV-1 infection (data not shown).

In conclusion, increased expression of FNR on HIV-1-infected cells, together with increased levels of FNR in the sera of AIDS patients, probably reflects an ability of fibronectin to interact with HIV-1 itself and a role in counteracting and limiting HIV-1 infection.

REFERENCES