

Expression of gp120 as a surrogate marker in HIV infection

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SUMMARY

Ninety seven patients infected with HIV -1 were studied to evaluate the expression of gp120 in peripheral blood CD4⁺ T lymphocytes as a surrogate marker of disease progression, analyzing correlation of it with viral load, T cell subsets, expression of activation markers, HAAR T and clinical signs and symptoms. In patients who had not received any antiretroviral therapy (naïve), expression of gp120 had a positive correlation with expression of CD95 in CD4⁺ T lymphocytes ($p=0.0409$) and a negative correlation with CD4⁺ T lymphocytes percentage ($p=0.0183$) and absolute values ($p=0.0165$), and with percentage of naïve CD4⁺ T cells ($p=0.0126$). In patients under highly active antiretroviral therapy (HAAR T) those correlations were not present. Expression of gp 120 had good correlations with some known surrogate markers of disease progression in naïve patients, but its low level of expression and the fact that those correlations disappear when HAAR T is initiated, limit the use of it as an independent surrogate marker.

Key words: gp 120 in HIV, T cell CD4⁺, surrogate marker.

Expresión de gp120 como marcador de progresión en la infección por VIH

RESUMEN

Se estudiaron 97 pacientes infectados con VIH-1 para determinar gp120 en células TCD4⁺ de sangre periférica como un marcador de progresión de enfermedad y su correlación con carga viral, subpoblaciones de células T, marcadores de activación, terapia antiretroviral, síntomas y signos clínicos. En los pacientes quienes no habían recibido terapia antiretroviral (vírgenes), la expresión de gp120 tenía una correlación positiva con la expresión de CD95 en los linfocitos TCD4⁺: ($p=0,0409$) y una correlación negativa con el porcentaje de linfocitos TCD4⁺ ($p=0,0183$) con los valores absolutos ($p=0.0165$) y con el porcentaje de células TCD4⁺ vírgenes ($p=0.0126$). En los pacientes con alta terapia antiretroviral estas correlaciones no estaban presentes. La expresión de gp120 tuvo buena correlación con algunos marcadores de progresión de enfermedad en pacientes vírgenes, pero su bajo nivel de expresión y el hecho de que esta correlación desaparece cuando la terapia antiretroviral es iniciada, limita su uso como marcador independiente de progresión.

Palabras clave: Glicoproteína 120, VIH, marcadores de progresión.

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Introduction

Close surveillance of the patient infected with HIV is needed to detect any change indicative of progression of the infection, and, at the right moment, begin or modify clinical interventions, as could be HAART. As HIV infection have a period of time when the patient has not any clinical symptom, some other indicators, known as surrogate markers, are used to know the stage of the infection at any given moment. Viral load and T cell subsets are two parameters widely used to fulfill this purpose (1). Expression of molecules associated with T cell activation (CD38, HLA-Dr, CD95) are used too, and so, level of apoptosis of these T cells (2,3).

Glycoprotein 120 (gp 120) is a structural molecule of HIV's envelope. It have a very important function in HIV infection, attaching virus to CD4 and CXCR4 molecules in the membrane of CD4⁺ T cells, so virus could invade cell. Cells infected with HIV can produce and release soluble gp 120 (4), and it had been detected in serum of AIDS patients (5). During infection patients produce anti-gp120 antibodies. Several studies had shown that interaction of gp120, independent of viral particle, with CD4 and CXCR4 receptors in the membrane of T cells mediate a rapid cell death, with apoptotic characteristics (6,7). A slower apoptotic effect could be seen too: crosslinking CD4 with gp120 and anti-gp120 antibodies cause characteristic DNA fragmentation after CD3 stimulation (8). In lymph nodes of infected patients, gp120 was present in the plasma membrane of apoptotic CD4⁺ T cells (9). All these findings interested us in study expression of gp 120 in peripheral blood CD4⁺ T cells, as a marker of susceptibility of these cells to suffer the deleterious effects observed in vitro, and, eventually, as a surrogate marker of disease progression. To do the test in peripheral blood had the advantage of the easy obtaining of the sample, so the test could be do as a routine procedure, and to use flow cytometry as the method, let us save time and expenses, facts specially important in a limited resources environment.

Materials and methods

Patients: Ninety seven patients (74 men and 23 women) infected with HIV-I, were received in our laboratory, Bio Cell, in Caracas, and studied as a part of their surveillance and treatment program Staging of infection was achieved according to CDC's classification system (10). The median age of patients was 34.9 ± 12.4 years. Thirty patients were attended before they received any antiretroviral therapy (Group of Naïve Patients) and sixty-seven were under HAART (Group of Treated Patients), following a protocol which include two nucleoside analogs inhibitors or reverse transcriptase (lamivudine and stavudine) and a combination of two protease inhibitors (lopinavir and ritonavir). These patients had received this antiretroviral treatment for 8.7 ± 4.4 months by the moment this study was made.

Controls: Twenty volunteer individuals served as controls. They were asymptomatic, with serology negative to HIV infection and with similar characteristics to patients in age and gender.

Samples: 10 ml. of whole blood was drawn for each patient and control, and it was mixed with EDTA.

Flow cytometry determinations: 50).11. of EDTA whole blood were mixed, in several combinations, with 5).11 of the respective monoclonal antibodies, and incubated for 15 minutes, at room temperature, protected from light. After incubation, 2 ml. of lysing solution was added to test tubes, mixed, and incubated for 15 minutes. Test tubes were centrifuged at 1.500 rpm, for 5 minutes. Supernatant was discarded and pellet was mixed with 200 01 of PBS. Then samples were acquired in a FACScan flow cytometer (Becton Dickinson), 10.000 events were saved and analyzed by Cell Quest and/or Pain-a-Gate Pro (Becton Dickinson). Isotypic controls were used to set cut-off, especially for molecules of weak expression, as gp120. In this work we considered as positive to expression of CD95 only cells with "high expression" (CD95 high). Expression of molecules was reported as percentage of positive cells in a given subset. Monoclonal antibodies used were antiFITC (ImmunoDiagnostics Inc., Wobum, MA); anti-CD95-PE, anti-FITC and anti-CD4-PC5 (Immunotech, France); anti-CD8-FITC, anti-CD38-RPE and anti-CD4-FITC/anti-CD45RA-RPE (Dako A/S, Denmark). Isotypic control monoclonal antibodies used were mouse IgG 11RDI/ mouse IgG1-FITC (Beckman-Coulter, Miami, FL), mouse IgG1IPC5 (Immunotech, France) and mouse IgG1-FITC (Dako A/S, Denmark).

Viral load determinations: It was determined using Amplicor-HIV-I-Monitor (Roche Diagnostic System), following instructions of the commercial kit.

Statistics: Data were analyzed using Graph Pad Prism (Graph Pad Software Inc., San Diego, CA). For determination of differences between groups Mann-Whitney U test was used, and for determination of correlation between parameters Spearman test was used.

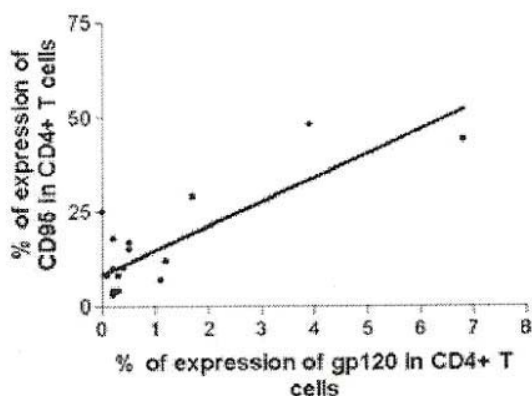
Results

Expression of gp 120 in CD4⁺ T cells was 1.14 ± 1.77 % in the group of naïve patients and 0.65 ± 0.57 % in the group of treated patients.

In the group of naïve patients we found that expression of gp 120 had a positive statistically significant correlation with expression of CD95 in CD4⁺ T lymphocytes ($p=0.0409$). The patients with higher percentages of expression of gp 120 had higher percentages of expression of CD95 in CD4⁺ T cells (Graph 1).



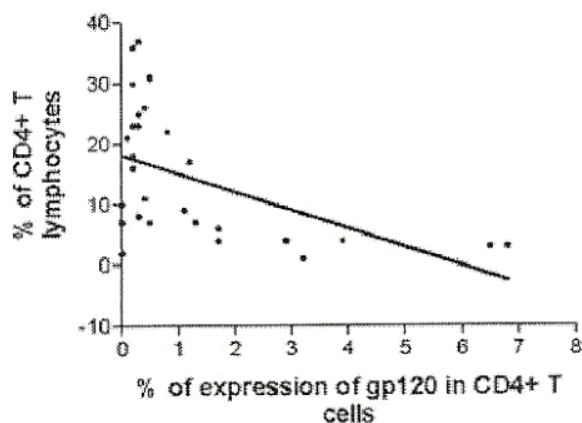
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Statistics (correlation:*)
Number of XY pairs = 18
Spearman $r = 0,4859$
P value (Two-tailed) = 0,0409

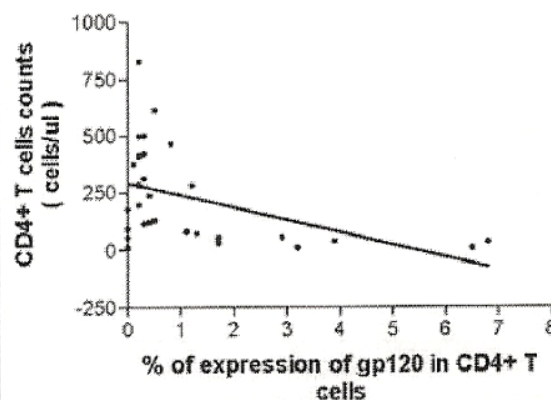
Graph 1. Expression of gp 120 and CD95 in CD4+T cells (Group of naïve patients).

In the group of naïve patients, individuals with percentages of expression of gp120 higher than 1.5 % had low percentages and absolute values of CD4+ T lymphocytes (Graphs 2 and 3), and there were negative statistically significant correlations between these parameters ($p=0.0183$ and $p=0.0165$, respectively). Nevertheless not all patients with low levels of CD4+ T cells had high percentages of expression of gp120. Also, there were negative statistically significant correlation ($p=0.0126$) between expression of gp 120 and percentage of expression of CD45RA (naïve cells) in CD4+ T cells (Graph 4).



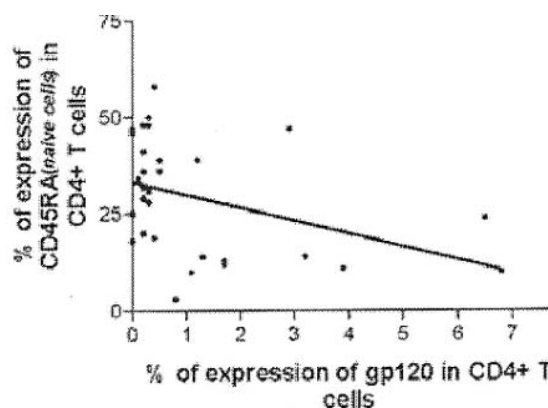
Statistics (correlation:*)
Number of XY pairs = 30
Spearman $r = -0,4280$
P value (Two-tailed) = 0,0183

Graph 2. Expression of gp 120 % of CD4 T cells (Group of naïve patients).



Statistics (correlation:*)
Number of XY pairs = 30
Spearman $r = -0,4341$
P value (Two-tailed) = 0,0165

Graph 3. Expression of gp 120 and CD4+T cells (/ul) (Group of naïve patients).



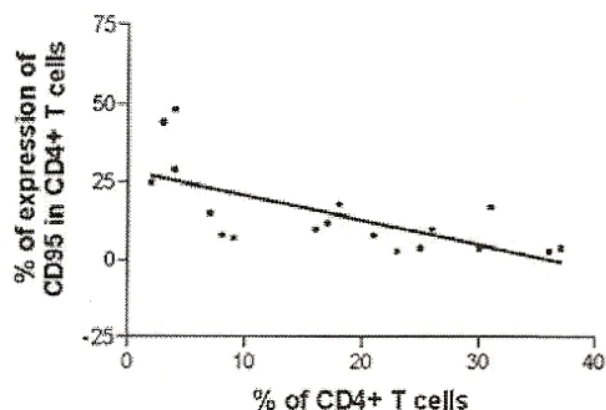
Statistics (correlation:*)
Number of XY pairs = 30
Spearman $r = -0,4459$
P value (Two-tailed) = 0,0126

Graph 4. Naïve CD4+ T cells and expression of gp 120 (Group of naïve patients).

Other relevant strong negative statistically significant correlations observed, both in naïve and treated patients, were between CD95 expression in CD4+ T cells and percentage and absolute values of these CD4+ T cells, with p values of 0.0009 and 0.0054 in naïve patients, and 0.004 and 0.0003 in treated patients, respectively (Graphs 5 and 6, as respectatives).

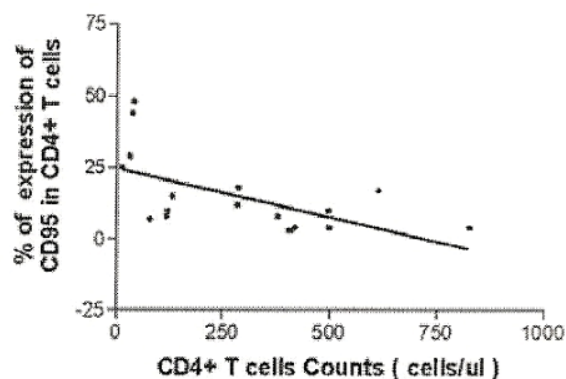
When the group of treated patients was analyzed, no correlations between expression of gp120 and other parameters were observed.

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Statistics (correlation:***)
Number of XY pairs = 18
Spearman $r = -0,7119$
P value (Two-tailed) = 0,0009

Graph 5. CD95 and % of CD4+ T cells (Group of naive patients).



Statistics (correlation:**)
Number of XY pairs = 18
Spearman $r = -0,6266$
P value (Two-tailed) = 0,0054

Graph 6. CD95 and CD4+ T cells Counts (Group of naive patients).

Conclusions

Taking account the deleterious effect that gp120 has in CD4+ T cells (6-9), it was not a surprise to found a negative correlation between expression of gp120 and level of CD4+ T cells in naïve patients. Nevertheless not all patients with low levels of CD4+ T cells had high expression of gp120. So, we can think in other causes of T cell destruction, as could be tat protein (11), or other deleterious effects of HIV infection. On the other hand, it is possible that gp120 attach a cell, induce an

apoptotic effect and leave this cell to join another. So, at any given moment not all cells affected by interaction with gp120 will display this molecule in its membrane. It is relevant that higher expression of gp120 is associated with lower levels of naïve CD4+ T cells (Graph 4), that could mean that this is a more susceptible subset to gp120.

Considering what high expression of CD95 means to susceptibility of a cell to suffer apoptosis (12), and the association observed between CD95 and HIV infection (13, 14), is so relevant the correlation observed between CD95 and gp120 expressions in CD4+ T cells in naïve patients.

In the group of patients studied, when HAART is initiated, we observed a rapid fall in viral load levels, but a slower recuperation of CD4+ T lymphocyte population. So, in this group level of CD4+ T cells.

Is not any more a good surrogate marker of progression to disease, at least in the first moment, after beginning HAART. This could explain why expression of gp120, which is correlated with CD4+ T lymphocyte population, has not correlation with any other surrogate marker in patients under HAART.

It is relevant that 92% of the patients (naïve and treated) with expression of gp120 > 1.5 % had one or more of the following features: viral load;³180.000 copies/ml; CD4+ T lymphocytes £157 /µl or specific AIDS symptoms. But only 12 % of all evaluated patients had expression of gp120 1.5 %. Other disadvantage is that those patients under HAART with viral load levels ³ 10.800 copies/ml (suggestive of any fail in antiretroviral therapy) had low levels of expression of gp120 (<1.5 %).

We conclude that, although expression of gp120 in peripheral blood CD4+ T lymphocytes had good correlations with known surrogate markers in naïve patients, its low level of expression, and the fact that no correlation was observed in patients under HAART, limits its use as an independent surrogate marker.

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