Quantitation of Soluble E-Receptor of T Lymphocytes in Serum From HIV-1 Positive Patients

Guillermo Nuñez,¹ Nayla C. Moura,¹ Hector J. Hernandez,¹ Zulma F. Peixinho,¹ Roberto S. Zeballos,¹ Nilton Cavalcante,¹ Pedro Flores,¹ Ieda M. Longo,¹ and Nelson F. Mendes^{1*}

¹*Departamento de Microbiologia, Imunologia e Parasitologia-Disciplina de Imunologia Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brasil*

Human T lymphocytes carry a membrane receptor for sheep erythrocytes (E) related to the CD2 molecule. The E-receptor is found in a soluble form (Rs) in serum and can be quantitated by "rocket electrophoresis" using an anti-Rs serum obtained by immunizing sheep with autologous erythrocytes coated with Rs. Increased serum levels of Rs are found in patients with diseases associated with immunodepression. In the present study, 14 asymptomatic HIV-1 seropositive individuals were investigated regarding their Rs levels and delayed hypersensitivity skin tests every 3 months for a period of 35 months. All these patients progressed to AIDS in this period. Rs serum lev-

els have also been quantitated in 14 normal individuals. The mean Rs values in normal individuals, asymptomatic, and AIDS patients were, respectively: 4.8 ± 1.5 mm (SD), 9.6 ± 1.9 mm (SD) and 11.3 ± 2.4 mm (SD). An increase of Rs serum levels was observed when we compared normal individuals with CDC-II and CDC-IV clinical stage patients ($P < 0.05$, Mann-Whitney test) and CDC-II and CDC-IV patients, ($P < 0.05$, Wilcoxon test). We have observed a depressed delayed hypersensitivity response to ubiquitous antigens in CDC-IV patients. Our results indicate that Rs serum levels can be used as a progression marker in HIV infected patients. *J. Clin. Lab. Anal.* 11:69–72. © 1997 Wiley-Liss, Inc.

Key words: CD2; AIDS; retrovirus

INTRODUCTION

Human T lymphocytes carry on their membrane a receptor that is responsible for the phenomenon of sheep erythrocyte rosette formation (1–3). The molecules involved in this phenomenon are CD2 on the lymphocytes and T11TS on the sheep erythrocytes (E). The majority of anti-CD2 monoclonal antibodies inhibit T-cell functions, including mitogenesis (4–7), E-rosette formation (7,8), and mixed lymphocyte reaction (7). The CD2 molecule is involved in an alternative pathway of T lymphocyte activation (5,9–11). The E-receptor in a soluble form (Rs) is found in normal human serum and can be recovered from supernatant of heated lymphocytes (2,12).

We have obtained a specific anti-E-receptor serum (anti-Rs) by immunizing sheep with autologous erythrocytes coated with Rs. This antiserum is cytotoxic for T cells, inhibits E-rosette formation, agglutinates ERs complexes, identifies T lymphocytes by immunofluorescence, and shows a single precipitation line in gel diffusion with preparations containing Rs (13,14).

We have developed several methods for quantitation of Rs in our laboratory (13,14) and the most practical is "rocket electrophoresis" (electroimmunodiffusion). With this method we have found abnormally high levels of Rs in the serum

from patients with diseases associated with depression of cell-mediated immunity such as leukemia, lymphoma, carcinoma, uremia, leprosy, and bone marrow aplasia (15–17). Increased Rs levels also have been detected by other authors in sera from patients with cancer using anti-CD2 monoclonal antibodies (18).

We have identified and purified from the supernatant of heated peripheral lymphocytes (SHPL), by chromatography and SDS-PAGE, using anti-Rs, two molecules with molecular weights of ~ 58,000 and 150,000. These molecules also can be detected in serum from patients with cancer and uremia (19,20).

Several parameters are being used as progression markers in patients infected with HIV-1, such as p24, anti-p24, absolute number of CD4⁺ and CD8⁺ cells, ratio of CD4/CD8 (21–23), β 2 microglobulin, neopterin, soluble CD8, soluble IL-2 receptor, α interferon, 90-KDa serum protein (24–26), and delayed hypersensitivity skin tests (27).

*Correspondence to: Nelson F. Mendes, M.D., Caixa Postal 20300, 04038-990-São Paulo, SP, Brasil

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In the present study, HIV-1 seropositive individuals in different clinical stages were investigated regarding their Rs serum levels and delayed hypersensitivity skin tests with ubiquitous antigens in order to assess the potential use of Rs as a progression marker.

MATERIALS AND METHODS

Human Sera

Serum samples were obtained from 14 individuals (12 males and 2 females) with HIV-1 infection, followed clinically and laboratorially for a period of 35 months. Their ages ranged from 21 to 55 years (mean = 39.0). All patients were classified as group II CDC, 1986 [28] and none received antiviral drugs. The immunological evaluation was made every 3 months. All patients have progressed to AIDS (group IV, CDC).

The normal serum samples ($n = 14$) were obtained from our laboratory personnel, their ages varying from 28 to 32 years (mean = 30.7 years).

Anti-E-Receptor Serum (Anti-Rs)

The anti-Rs were obtained by immunizing adult sheep with autologous E coated with Rs. The soluble receptor (Rs) was obtained from the supernatant of heated human peripheral lymphocytes (SHPL). Packed autologous E was incubated with equal volume of SHPL at 4°C for 18 hr, with constant agitation. "Sensitized" E (ERs) was washed three times in cold Hanks' balanced salt solution (HBSS) at pH 7.2. The immunization schedule has been described by us elsewhere (13).

Rocket Electrophoresis

Rectangular glass plates (75 × 50 mm) were covered with 7 ml of the following mixture at 56°C: 0.4 ml of anti-Rs, 1.6 ml of saline, and 5 ml of 1.5% agarose diluted in three parts of electrophoresis veronal buffer and two parts of distilled water. Five wells of 3 mm diameter were made at 1 cm of the edge of the plate. Each well received 10 µl of serum to be tested and 1 well per plate was filled with 10 µl of the control serum. The plates were subjected to 250V for 3 hr in an electrophoresis chamber (Gelman Instruments, Ann Arbor, MI), containing 1,000 ml of veronal buffer pH 8.6, and the migration of Rs was from the cathode to the anode. After migration, the plates were washed in saline for 24 hr at ambient temperature, then dried at 37°C, and stained with amido black. The resulting "rockets" were measured in mm. The concentration of Rs in the serum samples tested is proportional to the height of the "rockets" obtained.

Delayed Hypersensitivity Skin Tests

The antigens employed in this test were: Candidin, Tricophytin, PPD and Streptokinase-Streptodornase (Fundação Atauilpho de Paiva, Rio de Janeiro, Brazil). They

were injected intradermally (0.1 ml) on the ventral surface of the forearm. The results were expressed in millimeters as the mean of the two major and perpendicular diameters of the cutaneous induration 48 hr after injection.

RESULTS

The mean Rs values of normal individuals, asymptomatic (CDC-II), and AIDS patients (CDC-IV) were, respectively: 4.8 ± 1.5 mm (SD), 9.6 ± 1.9 mm (SD) and 11.3 ± 2.4 mm (SD) as expressed in Figure 1. A significant increase in the Rs serum levels was observed when we compared normal individuals and patients in the asymptomatic phase, $z = 4.5$, $P < 0.05$, (0.000007), Mann-Whitney test. The same was found when we compared normal individuals and patients in the AIDS phase, $z = 4.5$, $P < 0.05$, (0.000007), Mann-Whitney test. A significant Rs increase was also observed when we compared the patients in the two phases of the disease, $T = 10$, $P < 0.05$, Wilcoxon test (Fig. 1).

The results of the delayed hypersensitivity skin tests are depicted in Table 1. A depressed delayed hypersensitivity response to ubiquitous antigens was observed in group IV patients.

Fig. 1. Quantitation of the soluble E-receptor (Rs) in the serum of HIV-1 positive patients followed from CDC-II to CDC-IV and normal controls.

N = normal controls; $X = 4.8 \pm 1.5$ mm (SD)

II = CDC-II; $X = 9.6 \pm 1.9$ mm (SD)

IV = CDC-IV; $X = 11.3 \pm 2.4$ mm (SD)

n = number of individuals

Mann-Whitney test between: N x CDC-II; $z = 4.5$, $P < 0.05$.

N x CDC-IV, $z = 4.5$, $P < 0.05$.

Wilcoxon test between: -CDC-II x CDC-IV; $T = 10$, $P < 0.05$

TABLE 1. Delayed Hypersensitivity Skin Tests in the Follow-up of HIV-1 Positive Patients

Patients number	Clinical classification							
	CDC-II ^a				CDC-IV ^a			
	C	P	T	V	C	P	T	V
1	—	—	5 ^b	—	ND	ND	ND	ND
2	16	—	50	12	—	—	—	—
3	10	10	15	—	—	—	—	—
4	—	—	22	12	—	—	—	—
5	10	5	15	—	5	—	—	—
6	6	—	—	25	—	—	—	—
7	—	—	10	45	—	—	—	—
8	20	—	—	—	—	—	—	—
9	7	—	—	—	—	—	—	—
10	—	—	—	12	—	—	—	—
11	5	—	5	15	—	—	—	—
12	—	—	—	—	—	—	—	—
13	—	—	40	—	—	—	—	—
14	5	8	7	—	—	—	—	—

^aC = candidin; P = PPD, T = tricothylin, V = streptokinase-streptodornase.

^bNumber express the diameter of the cutaneous reaction in mm.

ND = not done.

— = negative reaction.

DISCUSSION

We have demonstrated that quantitation of the E-soluble receptor (Rs) is a valuable marker in the follow-up of HIV seropositive patients. We have detected a statistically significant increase of Rs from asymptomatic period to AIDS. A concomitant loss of in vivo cell-mediated immunity reactions to ubiquitous antigens also has been observed.

Rs serum levels are also increased in diseases associated with depression of cell-mediated immunity (15–17). In cancer patients, we also have observed that when the immune response is restored, Rs decreases to normal levels (15). The role played by Rs in vivo seems to be related to immunoregulation, as demonstrated by us in uremic patients (29). High levels of Rs inhibit proliferative response of lymphocytes in the presence of mitogens or allogeneic cells (30). Our results indicate that Rs serum levels can be used as a serum marker of disease progression in HIV.

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