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QUANTITATION OF THE SOLUBLE RECEPTOR OF HUMAN T LYMPHOCYTES FOR SHEEP ERYTHROCYTES BY ELECTROIMMUNODIFFUSION IN THE SERUM OF PATIENTS WITH LEPROMATOUS AND TUBERCULOID LEPROSY

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ABSTRACT

Human T lymphocytes carry a membrane receptor for sheep erythrocytes (E) which is present in a soluble form in normal serum and that may play an immunoregulatory role. We have quantitated the soluble E-receptor by *rocket electrophoresis* in serum semples obtained from 43 normal controls, 32 patients with tuberculoid leprosy and 53 patients with lepromatous leprosy. The means of the *rockets* obtained in these 3 groups were respectively 5.0 mm, 7.5 mm and 10.9 mm. These differences were statistically significant ($p < 0.001$, Kruskal-Wallis test). Increased serum levels of Rs (E-receptor in soluble form) may be one of the mechanisms responsible by depression of cell-mediated immunity in leprosy.

INTRODUCTION

Human T lymphocytes carry in their membrane the so-called E-receptor which is responsible for the well known phenomenon of rosette formation with sheep erythrocytes (Lay *et al.*, 1971, reviewed by Mendes, 1977).

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The E-receptor in a soluble form (Rs) is found in normal human serum (Bernd *et al.*, 1983) and can be recovered from the supernatant of heated peripheral lymphocytes (Mendes *et al.*, 1975; Mendes *et al.*, 1982).

Several methods for demonstration and quantitation of Rs have been developed in our laboratory since we have obtained a specific anti-E receptor serum (anti-Rs) by immunizing sheep with autologous erythrocytes (E) coated with Rs. This antiserum is cytotoxic for T cells, inhibits E-rosette formation, agglutinates ERs complexes, identifies T lymphocytes by immunofluorescence and gives a single precipitation line in gel diffusion with preparations containing Rs (Mendes *et al.*, 1982; Bernd *et al.*, 1983).

The most practical method to quantitate Rs in human serum is "Rocket electrophoresis" or electroimmunodiffusion. By this method, we have found abnormally high serum levels of Rs in diseases associated with a depression of cell mediated immunity, such as carcinoma, leukemia, lymphoma, uremia, lepromatous leprosy and bone-marrow aplasia (Moura *et al.*, 1983; Falcão *et al.*, 1984).

The most extensively studied aspect of leprosy immunology has been the failure of lepromatous patients to control the multiplication of *M. leprae*. However, the exact nature of the immunologic deficiency in leprosy remains to be clarified, but there is evidence of a T cell deficiency, a macrophage disfunction and participation of serum factors which lead to a varying degree of nonspecific depression of cell-mediated immunity superimposed on the basic defect (reviewed by Godal, 1981 and Mendes, 1981).

In the present work we have quantitated Rs in the serum of normal individuals and in patients with lepromatous (LL) and tuberculoid leprosy (TT).

Materials and Methods

Serum samples: were obtained from 43 adult normal individuals, 32 adult patients with tuberculoid leprosy and 53 adult patients with lepromatous leprosy, undergoing treatment. The clinical form of the disease has been well characterized by clinical, histopathological and immunological parameters.

Anti-E receptor serum (anti-Rs): was obtained immunizing an adult sheep with autologous E sensitized with Rs. The soluble receptor was obtained from the supernatant of human peripheral lymphocytes

(SHPL) (Mendes *et al.*, 1982). The packed autologous E were incubated with an equal volume of SHPL at 4°C for 18 h under agitation. Sensitized E (ERs) were washed 3 times in cold Hanks' balanced salt solution (HBSS) at pH 7.2. The sheep received a 1 ml sc injection of packed ERs in Freund's complete adjuvant and the dose was repeated twice, 3 weeks apart. Then, weekly ERs injections were given, without adjuvant. Three months after the onset of immunization, the resulting antiserum was capable of blocking E-rosette formation, of agglutinating ERs complexes, it was cytotoxic to T lymphocytes and gave a precipitation line in gel with human serum containing increased amounts of Rs, showing total identity with SHPL.

Quantitation of Rs in serum samples by electroimmunodiffusion "Rocket Electrophoresis": rectangular glass plates (75 × 50 mm) were covered with 7 ml of the following mixture at 56°C: 0.2 ml of anti-Rs, 1.8 ml of saline and 5.0 ml of 1.5% agarose diluted in 3 parts of electrophoresis veronal buffer and 2 parts of distilled water. Seven wells of 3 mm diameter were made at 1 cm from one of the edges of the plate. Each well received 10 μl of serum to be tested and 1 well per plate was filled with 10 μl of control serum. The plates were subjected to 250 V for 3 h in an electrophoresis chamber (Gelman Instruments Company, Ann Arbor, Michigan, USA) containing 1 l 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 h at ambient temperature, then were 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.

Results

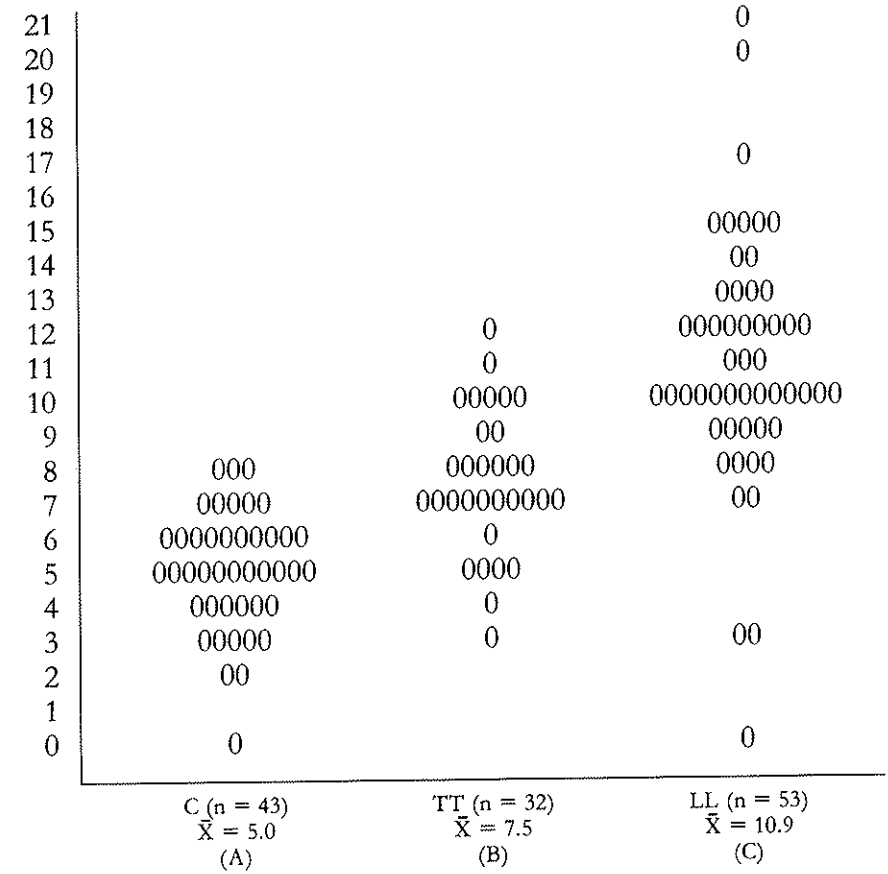
The results are illustrated in Figure 1 and the statistical analysis using the Kruskal-Wallis test showed a significant increase of Rs in the serum of patients with lepromatous and tuberculoid leprosy as compared with normal individuals ($p < 0.001$). Patients with tuberculoid leprosy showed Rs serum levels in an intermediate range, between normal controls and lepromatous leprosy. The means of the "rockets" obtained in each group were as follows: lepromatous leprosy: 10.9 mm; tuberculoid leprosy: 7.5 mm; normal controls: 5.0 mm.

Discussion

Our results have shown increased serum levels of Rs in lepromatous and tuberculoid leprosy patients as compared with normal individuals.

Studies from our institution (Musatti *et al,* 1979; Musatti *et al.,* 1980; Moura *et al.,* 1983) and others (Owen *et al,* 1976; Oh *et al.,* 1981) have indicated that Rs may be a regulatory molecule, released from the surface of T cells, that serves as a negative feedback control of their multiplication and function. Recently, our laboratory has demonstrated that Rs is present in preparations of the so-called immunoregulatory α--globulin (IRA), a substance that suppresses T cell-dependent immune responses and inhibits E rosette formation (Musatti *et al.,* 1983). The increase of Rs serum levels in pathological conditions may be explained by one of the following mechanisms: lymphocyte destruction, increased synthesis, decreased catabolism or diminished elimination. In lepromatous leprosy, the affected lymph nodes show a depletion of T cells as revealed by the adherence to sheep erythrocytes in cryostat tissue sections, probably indicating local lymphocyte destruction (Mendes *et al.,* 1974).

The observation that augmentation of Rs indicates depressed cell-mediated immunity, elucidates our results, with higher Rs values in lepromatous than in tuberculoid patients. This latter group of patients also showed abnormally high levels of Rs as compared with normal individuals, supporting other studies that demonstrated that even in the tuberculoid form there is a relative immunodeficiency (Bullock *et al.,* 1968; Han *et al.,* 1971),



C normal controls
TT tuberculoid leprosy patients
LL lepromatous leprosy patients
$\bar{X}$ mean
n number of individuals

Kruskal-Wallis between A,B,C: H' = 69.62; $p < 0.001$
Multiple comparisons:

Groups	$\lvert\bar{R}_i - \bar{R}_j\rvert$	msd	msl
A × B	30.34	22.47	$\simeq 0.001$
A × C	63.26	20.05	< 0.001
B × C	32.91	21.88	< 0.001

$\lvert\bar{R}_i - \bar{R}_j\rvert$ = difference between means of the ranks in the two groups compared
msd = minimal significant difference
msl = minimal significant level

FIGURE 1. Quantitation of Rs in the serum of patients with lepromatous and tuberculoid leprosy.

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