

CORRELATION BETWEEN RUBINO'S REACTION AND SOLUBLE SHEEP ERYTHROCYTE RECEPTORS IN HANSEN'S PATIENTS¹

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ABSTRACT: *This study investigated the correlation between either factors or molecules that interact with formolate sheep's erythrocytes (Rubino's reaction) and T lymphocytes soluble receptors for sheep erythrocytes (Rs2) present at a high level in the serum of Hansen's patients. The analysis of fractions obtained by chromatography on Sephadex G-200 showed positive Rubino's reaction in the fraction that correspond to Rs2 with more than 150 kDa, detected by a specific anti-soluble receptor. Additionally, the level of the soluble receptor present in the serum of Hansen's patients, showing positive or negative Rubino's reaction, was determined by immunodiffusion (Mancini). The result demonstrated no statistical difference between serum soluble receptor level in positive (7.3 mm) or negative (7.8 mm) Rubino's reactions, giving evidence that the high molecular weight E-soluble receptor is not responsible for Rubino's reactions.*

KEY-WORDS: *Rubino's reaction, Leprosy, E-receptors, CD2*

INTRODUCTION

Leprosy is a chronic infectious disease caused by the acid-fast *Mycobacterium leprae* and exhibiting a spectrum of clinical manifestations between extreme lepromatous leprae the anergic form and tuberculoid leprae with cell-mediated immunity form (BOOTH et al., 1988).

The property of the leprosy patient's serum to cause rapid agglutino-sedimentation of the formolate sheep erythrocytes has been described by Rubino (1926) and the nature of the element responsible for these Rubino's reaction is not characterized. An increased IgG, IgA and IgM level has been shown in the leprosy patient's serum from lepromatous leprosy with positive Rubino's reaction (SILVA et al., 1976) and the higher frequency of the Rubino's reaction positivity in multibacilíferos patients (CURBAN, 1962). Also it has been shown no correlation between the presence of circulating immune complex and positive Rubino's reaction (WAGNER et al., 1978).

MENDES et al. (1982) after immunizing sheep with autologous erythrocytes sensitized with solubilized receptor from human lymphocytes, obtained polyclonal antibodies specific to the E receptor. When these anti-soluble receptor (anti-Rs) sera were used in electro-immunodiffusion, an increase in Rs levels was detected in the serum of patients with cancer, uremia and leprosy (MOURA et al., 1983). The T lymphocyte

membrane receptor for sheep erythrocyte detected by monoclonal antibodies (CD2), has been biochemically defined as polypeptides of molecular weights from 40 to 58 kDa (KAMOUN et al., 1981; VERBI et al., 1982; BROWN et al., 1987). This heterogeneity in molecular weight is due to variations in the side branches of carbohydrates (BROWN et al., 1987). The anti-Rs serum recognizes molecules of approximately 58 kDa (Rs1), therefore similar to CD2 and molecules higher than 150 kDa (Rs2), the latter being detected in high levels in the serum of patients with cancer and uremia (ITANO et al., 1991). The Rs2 showed electrophoretic migration similar to alpha 2 globulin, is not related to IgM and is not an immune complex with IgG (ITANO et al., 1991).

Since that soluble receptor also exhibits the ability to interact with formolated sheep erythrocytes (MENDES et al., 1982), it is important to study whether or not that receptor shows any relationship between both soluble receptor (Rs2) increased in hansen's patients and factors responsible for Rubino's reaction.

MATERIALS AND METHODS

HUMAN SERUM

Human serum was obtained from peripheral blood of hansen's patients from Centro de Saúde de Londrina, classified by Dr. Roberto Schnitzler in: Lepromatous leprosy (LL), Tuberculoid leprosy (TT), Bordeline leprosy

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(BL) and Indeterminate leprosy (IL). The normal human serum was obtained from teacher and public official from Universidade Estadual de Londrina.

RUBINO'S REACTION

The Rubino's reaction was carried out in two series of serum dilution (1/2), one with formulated sheep erythrocyte and another with natural sheep erythrocyte (1.8%). The reaction was considered positive only when a series of formulated erythrocytes agglutino-sedimentated. For Sephadex fraction's analysis, a mixture of samples of fraction and normal human serum (v/v) was used.

PREPARATION OF ANTI-Rs SHEEP SERUM

The procedure was that recommended by MENDES et al., (1982) with some modifications. Briefly, sensitized autologous E with Rs present in supernatant of heated peripheral lymphocytes (E receptors) were inoculated subcutaneously into a sheep with complete Freund's adjuvant and another two inoculation with incomplete Freund's adjuvant, with a 30 day interval between inoculation.

CHROMATOGRAPHY ON SEPHADEX G-200

Three-milliliter samples of the Rubino's positive serum were applied to Sephadex G-200. The column (2 X 60cm) was equilibrated with 0.15 M Tris-NaCl buffer, pH 8.0.

DOUBLE IMMUNODIFFUSION (OUCHTERLONY)

The procedure was carried out on glass slides (2.5 X 7.5 cm) covered with a 3.0 mm high layer of 1% agar in 0.85% NaCl containing 0.02% sodium azide.

SIMPLE IMMUNODIFFUSION (MANCINI)

The procedure was carried out on glass slides (5.0 X 7.5 cm) covered with a 3.0 mm high layer of anti-Rs serum and agar. The samples (10 ul) were applied to orifices in the gel and maintained in a humid chamber for 48 hs. The gel was washed, dried and stained and readings were taken (mm).

STATISTICAL ANALYSIS

Statistical analysis was done using Student t-test.

RESULTS

FREQUENCY OF THE RUBINO'S REACTION

The frequency of the Rubino's reaction detected was: 45.6% in 92 serum of the LL patients, 0% in 8 serum of BL patients, 0% in 25 serum of TT patients, 0% in 12 serum of IL patients and 0% in 36 serum of normal human.

CHROMATOGRAPHY ON SEPHADEX G-200

Analysis of the chromatographic eluates of positive Rubino's reaction serum showed the presence of one fraction with positive Rubino's reaction, at the end of the

exclusion volume, correspondent to fraction capable of interacting with anti-Rs serum in double immunodiffusion (Figure 1).

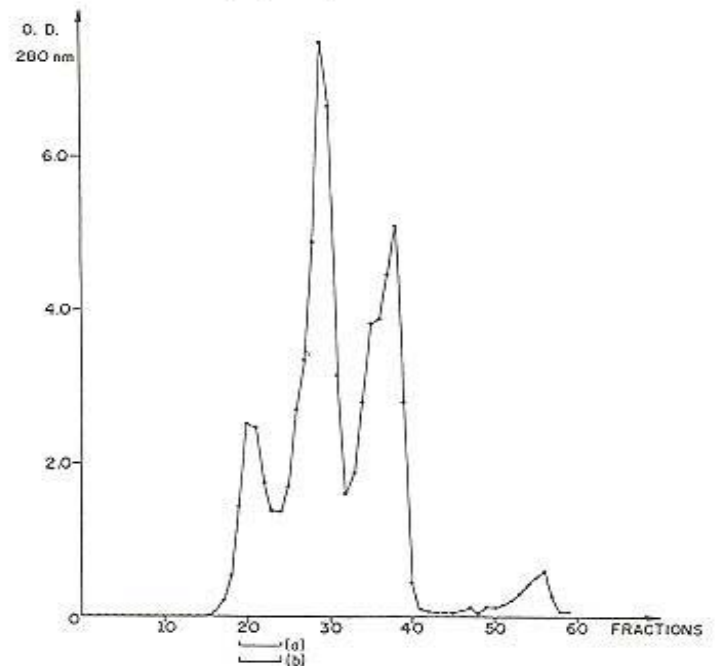


FIG. 1. Spectrophotometric profile at 280 nm of Sephadex G-200 chromatography of sera (3.0 ml) from Hansen's patients. Fractions (2.5 ml) were collected and analyzed by immunodiffusion (Ouchterlony) using sheep anti-E receptor serum (a) and by Rubino's reaction (b) and the positive fractions are indicated as ———.

SIMPLE IMMUNODIFFUSION (MANCINI)

The study of level of soluble receptors in the serum by simple immunodiffusion (Mancini) showed elevated Rs levels in the serum of Hansen's patients (7.3 mm) in relation to normal human serum (6.1 mm) ($p < 0.02$), but was not different between serum of the Hansen's patients with positive Rubino's reaction (7.3 mm) and negative Rubino's reaction (7.8 mm) ($p > 0.1$). Table 1.

Table 1. LEVEL OF SOLUBLE T LYMPHOCYTE RECEPTOR FOR E IN THE SERUM OF HANSEN'S PATIENTS WITH POSITIVE (RP) AND NEGATIVE (RN) RUBINO'S REACTION AND NORMAL HUMAN SERUM (NHS).

SERUM	Precipitation Circle Diameter (mm) ^a
Positive Rubino's reaction (25) ^b	7.3
Negative Rubino's reaction (41)	7.8
Normal human serum (18)	6.1

a) Hansen's patients sera and sheep anti-E receptors serum.

b) Number of serum analysed.

$p < 0.02$ NHS X RP

$p < 0.001$ NHS X RN

$p > 0.1$ RP X RN

DISCUSSION

The high frequency of Rubino's reaction detected in lepromatous leprosy serum is in agreement with literature data that has shown greater frequency in multibacilliferous patients (CURBAN, 1962) usually belonging to lepromatous leprosy.

The molecules responsible for Rubino's reaction, have not been characterized yet, but these molecules should have some important biological role associated with immunosuppression, considering their presence only in polar lepromatous leprosy, an anergic form of cell-mediated immunity. In addition, an immunosuppressive effect of soluble receptors in blastogenic response has been shown (MUSATTI et al., 1980) and high levels have been found in serum of patients with cancer, uremia and lepromatous leprosy (MOURA et al., 1983), all of those associated with immunosuppression. MUTHUKKARUPPAN et al. (1988) demonstrated that the T cell activation pathways through the CD3 and CD2 receptors are impaired in lepromatous leprosy patients. The impairment appears to be due to the modulation of the CD2 receptor specifically by *M. leprae*. These data demonstrate the importance of studying the receptors for sheep erythrocytes and the molecules responsible for Rubino's reaction, present both in lepromatous leprosy patient's serum and exhibiting the ability to interact with formulated sheep erythrocytes. The fractions, Rubino positive, obtained by chromatography, were detected by Rubino's reaction only after the addition of the normal human serum. Therefore the proteins present in normal human serum should be necessary to neutralize either the electrical charge on the surface of erythrocytes or to react with a factor with distinct molecular weights necessary for Rubino's reaction to take place.

Since analysis of the fractions obtained by chromatography on Sephadex G-200 demonstrated the positive Rubino's reactions in the same fractions correspondent to Rs2 detected by anti-soluble receptor serum, the molecule responsible for Rubino's reaction should bear similar molecular weight and be higher than 150 kDa, with elution before IgG (second peak).

The study of soluble receptor's levels has shown a higher level of soluble receptors in Hansen's patients, but no statistical difference was detected between Hansen's patients serum showing positive or negative Rubino's reaction. These results give evidence that the soluble receptor is not responsible for Rubino's reaction. Even though the relationship was not demonstrated with the soluble receptor, the study of molecules responsible for Rubino's reaction should be important since these molecules have been detected only in lepromatous leprosy anergic form.

CONCLUSIONS

The molecule responsible for Rubino's reaction and the soluble human T lymphocyte receptor for sheep erythrocytes (Rs2) present in lepromatous leprosy patient's serum should bear similar molecular weight, higher than 150 kDa. The correlation between soluble receptors levels in positive and negative Rubino's reaction serum show that the soluble receptor is not responsible for Rubino's reaction.

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RESUMO: Neste trabalho correlaciona-se fatores, presentes em soros de pacientes hansenianos, que interagem com hemácias de carneiro formuladas (Reação de Rubino), com receptor solúvel de linfócitos T para eritrócitos de carneiro (Rs2) presente em nível elevado em soros de pacientes com hanseníase. A análise de frações obtidas por cromatografia em Sephadex G-200 demonstrou reação de Rubino na fração que corresponde ao Rs2 com peso molecular superior a 150 kDa, detectado por soro anti-receptor. Adicionalmente, analisou-se o nível de receptor solúvel em soros de pacientes com hanseníase, apresentando reação de Rubino positiva e negativa, por imunodifusão (Mancini). Os resultados obtidos não demonstram diferença significativa entre nível de receptor solúvel em reação de Rubino positiva (7.3 mm) ou negativa (7.8 mm) indicando que o receptor solúvel para E de alto peso molecular não é responsável pela reação de Rubino.

PALAVRAS-CHAVE: Reação de Rubino, hanseníase, receptor para E, CD2

REFERENCES

BOOTH, J.R.; HARRIS, D.P.; LOVE, J.M.; WATSON, J.D. Antigenic proteins of *Mycobacterium leprae* - Complete sequence of the gene for the 18 kDa protein. *J. Immunol.*, v. 140, n. 2, p. 597-601, 1988.

BROWN, M.H.; KRISANSSEN, G.W.; TOTTY, N.F.; SEWELL, W.A.; CRUMPTON, M.J. Purification and N-terminal amino acid sequence of the human T lymphocyte CD2 (T11) surface antigen. *Eur. J. Immunol.*, v. 17, p. 15-20, 1987.

- CURBAN, G.H. Contribuição para o estudo da reação de Rubino. *Rev. Bras. Lepr.*, v. 30, p. 179-216, 1962.
- ITANO, E.N.; ONO, M.A.; SUMIGAWA, M.; LONGO, I.M.; MOURA, N.C.; MENDES, N.F. Molecular forms of soluble human T lymphocyte receptor for sheep erythrocytes in serum and saliva. *J. Clin. Lab. Anal.*, v. 5, p. 114-120, 1991.
- KAMOUN, M.; MARTIN, P.J.; HANSEN, J.A.; BROWN, M.A.; SIADAK, A.W.; NOWINSKI, R.C. Identification of a human T lymphocyte surface protein associated with the E-rosette receptor. *J. Exp. Med.*, v. 153, p. 207-212, 1981.
- MENDES, N.F. BERND, L.A.G.; CINTRA, H.H.A.; MENDES, C.M.F.; PRAÇA, C.L.; MOURA, N.C. Human T lymphocyte receptor for sheep erythrocytes: characterization of a specific antiserum and its application in the detectin and quantitation of receptor in a soluble form. *Cell. Immunol.*, v. 72, p. 143-150, 1982.
- MOURA, N.C.; LONGO, I.M.; BERND, L.A.G.; MENDES, N.F. Quantitation of the soluble receptor of human T lymphocytes serum of patients with cancer, uremia and leprosy. *Experientia*, v. 39, n. 3, p. 306-308, 1983.
- MUSATTI, C.C.; SANTOS, L.M.B.; DAHER, S.; MENDES, N.F. Immunoregulation of the proliferative response of lymphocytes by soluble E receptors. *Clin. Immunol. Immunopathol.*, v. 17, p. 323-329, 1980.
- MUTHUKKARUPPAN, V.; CHAKKALATH, H.R.; MALARKANNAN. The classical and alternative pathways of T cell activation are impaired in leprosy. *Immunol. Lett.*, v. 19, p. 55-58, 1988.
- RUBINO, M.C. Nuevas reacciones serológicas en la lepra. *Rev. Soc. Arg. Biol.*, v. 2, p. 407-416, 1926.
- SILVA, O.P.; FERRI, R.G.; MORAES, N.; MARQUES, A.L.V. Estudos imunológicos na hanseníase. II. Quantificação de imunoglobulinas séricas. Tentativa de associação com a reação de Rubino. *Hans. Intern.*, v. 1, n. 1, p. 42-52, 1976.
- VERBI, W.; GREAVES, M.F.; SCHNEIDER, C.; KOUBEK, K.; JANOSSY, G.; STEIN, H.; KUNG, P.; GOLDSTEIN, G. Monoclonal antibodies OKT11 and OKT11A have pan-T reactivity and block sheep erythrocyte "receptors". *Eur. J. Immunol.*, v. 12, p. 81-86, 1982.
- WAGNER, O.; PENTTINEN, K.; ALMEIDA, J.D.; OPROMOLLA, D.V.A.; GODAL, T.; KRONVALL, G. Circulating complexes in leprosy studied by the platelet aggregation test - The platelet aggregation test and its relation to the Rubino test and other sero-immunological parameters in 135 patients with leprosy. *Clin. Exp. Immunol.*, v. 34, p. 326-337, 1978.

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