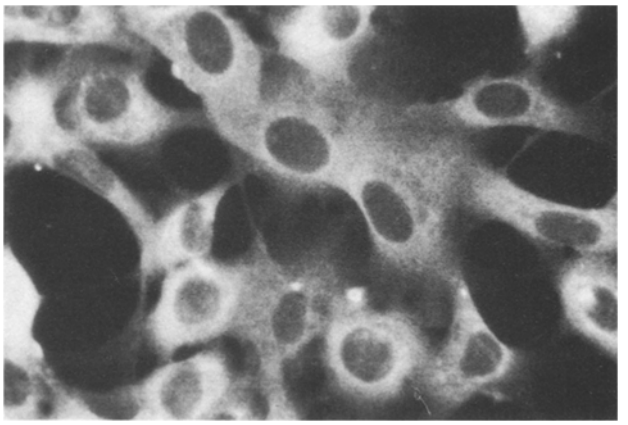
normal adult rat lung cells. On the basis of their morphological and growth characteristics and their ability to synthesize angiotensin converting enzyme the cells have been identified as endothelial cells. We have also shown that they synthesize glycosaminoglycans[7] and more recently have demonstrated that they produce crosslinked mature elastin[8]. In addition, we have detected surface antigens which appear to be specific for endothelial cells amongst the antigenic determinants of this cell line[9]. Further support for its identification as an endothelial cell became therefore of considerable interest. Blood coagulation factor VIII synthesis has been considered one of the most important criteria for the identification of endothelial cells in vivo and in culture. Monospecific antibodies to antihemophilic factor VIII derived from human plasma and bovine plasma have been used to identify human and bovine endothelial cells, respectively[6,10]. Previous attempts in our laboratory to produce anti-rat factor VIII were unsuccessful because adequate volumes of rat blood were not available for isolation of the antigen. Recently Schwartz[11] has shown that anti-human factor VIII crossreacts with bovine endothelial cells indicating that species specificity if it exists at all is not absolute. The present study was undertaken to determine whether anti-human factor VIII would also crossreact with rat lung cells and could be applied to support their identification as endothelial cells.

Coverslip cultures of the rat lung cells were incubated with rabbit antibodies to human factor VIII and associated proteins (Calbiochem-Behring, San Diego, CA) and fluorescein-conjugated goat anti-gamma globulin. They were then examined by indirect immunofluorescence microscopy. The figure shows a granular yellowish green fluorescence localized around the nucleus in the cytoplasm with little or no fluorescence in the nucleus. Fibroblast and epithelial cells derived from normal rat kidney (American Type Culture Collection No.CRL1570 and 1571) were tested as controls and showed no or little fluorescence indicative of factor VIII synthesis. Similarly rat lung cells incubated with normal rabbit serum produced no fluorescence beyond the very low level nonspecific background exhibited by fluorescein conjugated goat anti-gamma globulin. Results obtained confirm our previous identification of the rat lung cells. In addition, they demonstrate that although the clone of endothelial cells has been in culture for numerous passages, it has retained not only its morphological characteristics but also its capacity to synthesize factor VIII antigen.



Fluorescence staining of rat lung endothelial cells by rabbit anti-human factor VIII serum (dilution 1:8). × 125.

1 The data form part of the Ph.D. thesis submitted by A.T. Darnule to the Department of Biology, New York University. Supported in part by NIH USPHS Program Project grant HL15832.
2 Parshley, M.S., Cerreta, J.M., Mandl, I., Fierer, J.A., and Turino, G.M., In Vitro *15* (1979) 709.
3 Ryan, J.W., Chung, A., Martin, L.C., and Ryan, U.S., Tissue Cell *10* (1978) 555.
4 Caldwell, P.R.B., Seegal, B.C., Hsu, K.C., Das, M., and Soffer, R.L., Science *191* (1976) 150.
5 Jaffe, E.A., Hoyer, L.W., and Nachman, R.L., J. clin. Invest. *52* (1973) 2757.
6 Hoyer, L.W., de los Santos, R.P., and Hoyer, J.R., J. clin. Invest. *52* (1973) 2737.
7 Sampson, P., Parshley, M.S., Mandl, I., and Turino, G.M., Connect. Tissue Res. *4* (1975) 41.
8 Cantor, J.O., Keller, S., Parshley, M.S., Darnule, T.V., Darnule, A.T., Cerreta, J.M., Turino, G.M., and Mandl, I., Biochem. biophys. Res. Commun. *95* (1980) 1381.
9 Darnule, A.T., Stotzky, G., Darnule, T.V., Turino, G.M., and Mandl, I., Immun. Commun. (1983) in press.
10 Macarak, E.J., Howard, B.V., and Kefalides, N.A., Lab. Invest. *36* (1977) 62.
11 Schwartz, S.M., In Vitro *14* (1978) 966.

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Quantitation of the soluble receptor of human T lymphocytes for sheep erythrocytes by electroimmunodiffusion in the serum of patients with cancer, uremia and leprosy[1]

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Summary. Abnormally high serum levels of the soluble receptor of human T lymphocytes for sheep erythrocytes were found, by electroimmunodiffusion, in patients with carcinoma or other solid tumors, leukemia, lymphoma, uremia and lepromatous leprosy.

Human T lymphocytes carry a membrane receptor for sheep erythrocytes[2-5] which can be recovered in a soluble form (R_s) from the supernatant of heated (45 °C) peripheral lymphocytes[6]. We have obtained a specific anti-receptor serum (anti-R_s) by immunizing sheep with autologous erythrocytes (E) sensitized by R_s. This antiserum is cytotoxic for T cells, inhibits E-rosette formation, agglutinates ER_s complexes and can be used to identify T lymphocytes by immunofluorescence[7]. In this study, we have investigated the ability of the anti-R_s serum to detect R_s in human

serum by double diffusion in a gel, and to quantitate it by electroimmunodiffusion in normal individuals and in patients with cancer, leprosy and uremia.

Materials and methods. The anti-R_s serum was obtained by immunizing an adult sheep with autologous E sensitized with R_s. The soluble receptor was obtained from the supernatant of human peripheral lymphocytes (SHPL)[6,7]. The packed autologous E were incubated with an equal volume of SHPL at 4 °C for 18 h under agitation. Sensitized E (ER_s) were washed 3 times in cold Hanks'balanced salt solution (HBSS) at pH 7.2. The sheep received a 1-ml s.c. injection of packed ER_s in Freund's complete adjuvant and the dose was repeated twice, 3 weeks apart. Then, weekly ER_s injections were given, without adjuvant. 3 months after the onset of immunization, the resulting antiserum was capable of blocking E-rosette formation and of agglutinating ER_s complexes, and it was cytotoxic to T lymphocytes.

Ouchterlony's double diffusion was carried out on rectangular glass plates covered with 1% agarose in saline. The central well was filled with anti-R_s, while the outer wells were filled with the serum samples to be tested and with supernatant of heated peripheral lymphocytes prepared as described elsewhere[6,7]. For electroimmunodiffusion ('Rocket immunoelectrophoresis)[8], rectangular glass plates (75 × 50 mm) were covered with 7 ml of the following mixture at 56 °C: 0.2 ml of anti-R_s, 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. 7 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 R_s 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 R_s in the serum samples tested is proportional to the height of the 'rockets' obtained. All sera were obtained from adult individuals, male and female; 23 normal individuals, 64 patients with carcinomas or other solid tumors, 11 patients with leukemia or lymphoma, 20 patients with lepromatous leprosy and 34 patients with uremia.

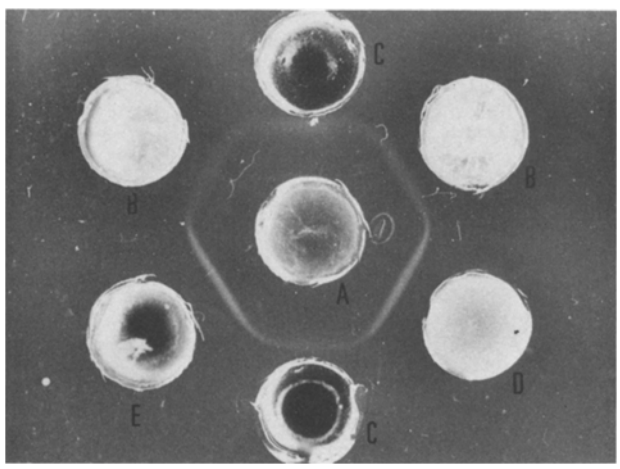

Figure 1. Detection of R_S in human serum by double diffusion in gel. A, Anti-R_s serum; B, supernatant of heated lymphocytes; C, concentrated normal human serum; D, serum from patient with melanoma; E, serum from patient with fibrosarcoma.

Results. The double diffusion experiments showed that normal human serum only produces a visible precipitation line with anti-R_s after at least 5-fold concentration. On the other hand, unconcentrated serum samples from some patients formed visible precipitation lines. The coalescent pattern of the lines indicated total identity between the antigen detected in each serum and the supernatant of heated lymphocytes which was the known source of R_s (fig. 1). The results of quantitation of R_s in serum samples by electroimmunodiffusion are illustrated in figure 2. The statistical analysis of the results by the Mann-Whitney test showed a significant increase of R_s levels in patients with carcinoma, leukemia or lymphoma, uremia and lepromatous leprosy as compared with normal individuals ($p < 0.01$).

Discussion. We have described the detection of R_s in human serum by double diffusion in gel and its quantitation by electroimmunodiffusion, using a specific anti-R_s serum. By gel diffusion, it was possible to demonstrate total identity between the single precipitation lines obtained with different patients' sera, concentrated normal serum and supernatant of heated lymphocytes. Only sera with increased levels of R_s or concentrated normal serum produced a visible precipitation line in the gel diffusion studies. The quantitation of R_s by electroimmunodiffusion showed a narrow range of R_s levels in normal individuals. The diseases studied showed a significant increase in the serum levels of R_s. Those diseases have a depression of cell-mediated immunity in common. The role played by R_s in vivo seems to be related to immunoregulation, as was demonstrated in the study of patients with uremia[9,10]. High levels of R_s inhibit the proliferative response of lymphocytes in the presence of mitogens or allogeneic cells[11]. The increase of the serum level in the diseased states may be explained by one of the following mechanisms; lymphocyte destruction, increased synthesis, decreased catabolism or diminished elimination. A monoclonal antibody which

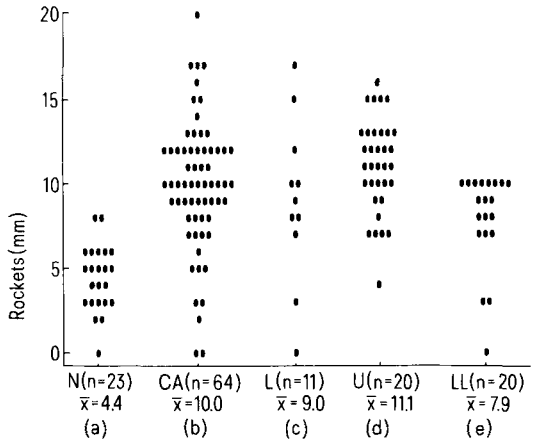

Figure 2. Quantitation of R_S in human serum by electroimmunodiffusion. N, Normal individuals; CA, patients with carcinoma (22 breast, 9 uterus, 8 stomach, 4 rectum, 3 skin, 3 lung, 2 prostate, 2 ovary, 2 colon, 1 palate, 1 kidney and 1 esophagus) or others solid tumors (4 melanoma, 1 rhabdomiosarcoma and 1 fibrosarcoma); L, patients with leukemia or lymphoma (2 acute myeloid leukemia, 2 acute lymphocytic leukemia, 2 chronic lymphocytic leukemia, 2 lymphocytic lymphoma, 1 hystiocytic lymphoma, 1 Hodgkin's and 1 thymoma); U, patients with uremia; LL, patients with lepromatous leprosy; $\bar{X}$, mean; n, number of individuals.
Mann-Whitney test (one-tailed)
(a) × (b): $z = 5.67$; $p < 0.01$
(a) × (c): $z = 3.04$; $p < 0.01$
(a) × (d): $z = 5.99$; $p < 0.01$
(a) × (e): $z = 2.37$; $p < 0.01$

probably recognizes the E receptor has recently been obtained[12]. However, these antibodies cannot be used in gel diffusion or other methods involving precipitation, such as electroimmunodiffusion. The identification of R_s as a possible immunoregulatory substance and the demonstration of increased serum levels of R_s in diseases associated with depression of cell-mediated immunity opens a new line of investigation in clinical immunology.

1 Supported by FINEP, CNPq, CAPES and FAPESP.

2 Lay, W.H., Mendes, N.F., Bianco, C., and Nussenzweig, V., Nature *230* (1971) 531.
3 Silveira, N.P.A., Mendes, N.F., and Tolnai, M.E.A., J. Immun. *108* (1972) 1456.
4 Mendes, N.F., Tolnai, M.E.A., Silveira, N.P.A., Metzgar, R.S., and Gilbertsen, J., J. Immun. *11* (1973) 860.
5 Mendes, N.F., Miki, S.S., and Peixinho, Z.F., J. Immun. *113* (1974) 531.
6 Mendes, N.F., Saraiva, P.J., and Santos, O.B.O., Cell, Immun. *17* (1975) 560.
7 Mendes, N.F., Lymphology *10* (1977) 85.
8 Laurell, C.B., Analyt. Biochem. *15* (1966) 45.
9 Musatti, C.C., Soares, V.A., Santos, L.M.B., DeLima, J.J.G., and Mendes, N.F., Clin. Immun. Immunopath. *14* (1979) 403.
10 Owen, F.L., Bernier, G.M., and Fanger, M.W., Immunochemistry *13* (1976) 129.
11 Musatti, C.C., Santos, L.M.B., and Mendes, N.F., Clin. Immun. Immunopath. *17* (1980) 323.
12 Kamoun, M., Martin, P.J., Jansen, J.A., Siadak, A.W., and Novinsky, R.X., J. exp. Med. *153* (1981) 207.

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Natural killer cell activity in fawn-hooded rats[1]

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Summary. Fawn-hooded (FH) rats were shown to lack the genetically conditioned defect of natural killer (NK) activity hypothesized to be present by analogy with the Chediak-Higashi syndrome (CHS) in mice and human beings. In 4-h ^{51}Cr release assays, splenic NK cells from FH rats killed YAC-1, RL♂1 and G_1-TC tumor targets without deficiency based upon comparison with cells from BD-IV, BD-IX and NBR inbred rat strains. Progeny of BD × FH F_1 rats backcrossed to FH failed to reveal a correlation of reduced NK activity and dilute coat color. From these, and other data presented, it is concluded that despite similarities in coat color dilution and platelet storage pool deficiency, FH rats do not closely resemble CHS mice or human beings in having deficient NK activity and cannot be considered the rat homolog of the CHS.

Fawn-hooded (FH) rats are mutants which manifest a genetic condition consisting of a coat color dilution and a platelet storage pool deficiency[2,3]. In these regards they resemble animals and human beings with the Chediak-Higashi syndrome (CHS)[4,5]. In addition to the coat color dilution and platelet storage pool deficiency, CHS mice and human beings have a marked deficiency of natural killer (NK) cell activity[6–8]. NK cells are lymphocytes which without antibody or previous exposure can kill certain tumor and virus-infected cells[9]. CHS mice, because of their lack of NK cell activity, have been used extensively to elucidate the mechanisms of NK cell activity and have proven useful in the demonstration of the in vivo relevance of NK cell activity[10]. In spite of the similarities of the conditions in CHS mice and FH rats no information is available regarding the NK cell activity of FH rats. The purpose of this investigation was to determine the levels of NK cell activity in FH rats.

Materials and methods. Animals. 6–12-week-old pedigree BD-IX, BD-IV, NBR and FH rats were used in these studies[1]. BD-IX, BD-IV and NBR are completely inbred strains. The FH animals used in this study were highly but incompletely inbred animals. BD-IV × FH F_1 and BD-IX × FH F_1 rats were obtained from matings between BD females and FH males. F_1 females were backcrossed to FH males and the progeny were used in these studies.

Cell lines and culture conditions. Three tumor cell lines sensitive to NK cell mediated lysis were employed[1]: YAC-1, RL♂1 and G_1-TC. These tumor target cell lines were cultured in HEPES buffered RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (complete HRPMI) as previously described[11].

NK cell mediated tumor cell lysis. For the completely inbred strains, splenic effector cells were prepared from a pool of 2 rats whereas individual rats were the source of effector cells for FH and backcrossed rats. Rat nylon-wool non-adherent spleen cells were obtained as previously described for the mouse[11] except that red cell lysis was achieved using a 6 sec rather than a 4 sec exposure to sterile distilled water. Nylon wool fractionation was carried out after preincubation at 37 °C for 18 h to allow for increased expression of effector reactivity[12]. Assays for NK cell reactivity were performed as previously described[10] using ^{51}Cr prelabeled target cells for the 4 h and ^{125}iododeoxyuridine (^{125}IUDR) prelabeled target cells for the 16 h assay. The percent specific release of ^{51}Cr or ^{125}I was determined using the following formula:

$$\frac{\text{counts released from experimental well} - \text{spontaneous release}}{\text{counts released from SDS lysed well} - \text{spontaneous release}} \times 100$$

The in vitro augmentation of NK activity was attempted by incubation of non-fractionated spleen cells for 18 h at 37 °C in complete HRPMI medium containing 50 µg/ml poly I:C and 25 µg/ml DEAE-dextran.

An outbreak of *Mycoplasma pulmonis*-associated respiratory disease which occurred in the room housing all the rat strains, afforded an opportunity to examine natural in vivo augmentation of NK activity. The splenic NK activity of exposed but clinically healthy rats was assessed during the second week of the disease outbreak.