INTERNATIONAL JOURNAL OF LEPROSY Volume 35, Number 3
Printed in U.S.A.

Rheumatoid-factor-like Substance and Antistreptolysin O Antibody in Leprosy Serum

Significance in Erythema Nodosum Leprosum[1]

Masahide Abe, Shiro Chinone and Tadashi Hirako[2]

The phenomenon of autoimmunity is well recognized in a great variety of disorders (12). In leprosy, however, little is known of this phenomenon, although a role is suggested in the reactional states in leprosy (21). Recent investigations of Cathcart *et al.* (3) and Bonomo *et al.* (1) have shown that autoantibodies, such as rheumatoid factors (RF) and thyroglobulin antibodies, are found in the serum of leprosy patients, without a demonstrable relation, however, between the presence of these antibodies and clinical findings in the disease. On the other hand, Matthews and Trautman (14) have emphasized the resemblance of reactive episodes in leprosy to manifestations of collagen diseases, on the basis of positive serologic findings, including RF, thyroglobulin antibodies, false-positive serologic tests for syphilis, and cold-precipitable proteins. Our own interest was directed toward RF or RF-like substances in leprosy serum, from the point of view of a possible role of such substances as evidence in the repeated occurrence of antigen-antibody reactions *in vivo* associated with the course of leprosy, especially with erythema nodosum leprosum (ENL). Antistreptolysin O antibody was also investigated, in order to throw light on the serologic resemblance of ENL to collagen diseases.

MATERIALS AND METHODS

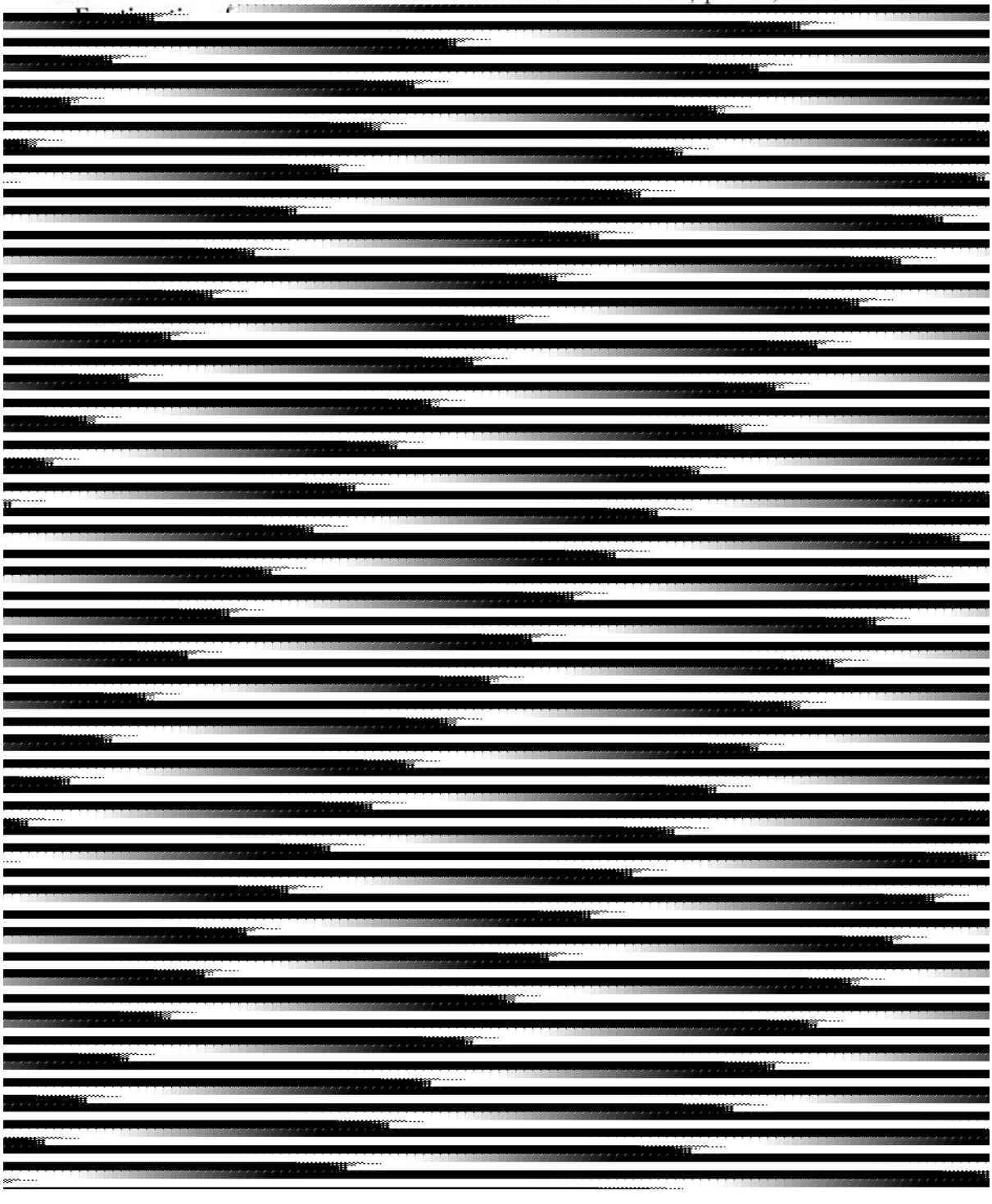
Sera. Leprous sera were collected from leprosy patients in the National Leprosarium Tama Zensho-en. Results of serologic tests on patients whose disease was found to be complicated with nonleprous illness were omitted. Sera from healthy persons were donated by the Blood Transfusion Service of the University Hospital in the University of Tokyo. All sera were stored at −20°C until use.

Serologic tests. The hemagglutination of sensitized sheep blood cells (Waaler-Rose test) was performed by the original method of Rose (7) with slight modification. Differential agglutination ratios of 1:16 or more were regarded as positive. The modified method of Heller *et al.* (6) also was used, for the test with serum protein fractions. The RA test (a rapid slide screening test for rheumatoid arthritis) was performed with the commercial human gamma globulin-coated latex particles (Hyland). The agglutination titer was measured on a glass slide with this latex-globulin reagent, the sera or isolated gamma globulin fractions being serially diluted with glycine-NaOH-buffered saline (pH 8.2). Human FII-tanned (i.e., Cohn's Fraction II) red blood-cell agglutination (FII-S.C.A. (sensitized cell agglutination)) was tested with the sheep erythrocytes, which were treated with tannic acid (2) and sensitized with Cohn's blood Fraction II solution heated at 63°C for 10 minutes. Agglutination titers of 1:20 or more were regarded as positive. The antistreptolysin-O (ASLO) test was carried out with the use of commercial reagent (Difco). The antibody titer was expressed in terms of Todd's unit, i.e., the reciprocal of the highest dilution of serum showing no hemolysis (16).

Mercapto-ethanol treatment of serum. One volume of 1M 2-mercapto-ethanol (ME) was mixed with nine volumes of serum, and the mixture was incubated at

[1]Received for publication 30 January 1967.

[2]Masahide Abe, M.D., Chief, Laboratory of Serology, National Institute for Leprosy Research, Tokyo, Japan; Shiro Chinone, Laboratory of Serology, National Institute for Leprosy Research; *Present address:* Department of Parasitology, Azabu Veterinary College, Fuchinobe, Kanagawa, Japan; Tadashi Hirako, National Leprosarium, Tama Zensho-en, Tokyo, Japan.

room temperature for two hours. It was then dialyzed overnight against large volumes of saline containing 0.02 M iodoacetamide. As a control, the same serum, mixed in the proportion of 9 to 1 with saline, instead of ME, was treated in the same manner.

according to the usual technic. A special column, measuring 25 x 450 mm. (Pharmacia), was used for good separation of protein. Three to 5 ml. of the sample, mixed with sucrose (200 mgm./ml.), were applied to the column, and then the buffer solution of 0.1 M Tris-HCl, pH 8.0, in 1.0 M NaCl