Evaluation of Circulating Immune Complexes and Antinuclear Antibodies in Japanese Patients with Leprosy*

F. Furukawa 1, K. Sekita 1, Y. Hamashima 1, M. Ozaki 2, and S. Imamura 2

1 Department of Pathology (Head: Prof. Y. Hamashima, MD), Faculty of Medicine, Kyoto University, Sakyo, Kyoto 606, Japan
2 Department of Dermatology (Head: Prof. S. Imamura, MD), Faculty of Medicine, Kyoto University, Sakyo, Kyoto 606, Japan

Summary. In 79 patients with leprosy a significant increase of anti-extractable nuclear antigen (ENA) antibodies and circulating immune complexes (CIC) was found. No correlation between CIC and anti-ENA antibodies was demonstrable. Since such a correlation is known from antinuclear antibodies and CIC in patients with systemic lupus erythematosus, it appears likely that anti-ENA antibodies do not play a causative role in CIC-mediated pathogenesis of leprosy.

Key words: Circulating immune complexes — Anti-dsDNA antibodies — Anti-ssDNA antibodies — Anti-ENA antibodies — Leprosy

Introduction

It is well known that autoantibodies may be demonstrated in sera of patients with leprosy [1]. Both antinuclear antibodies (ANA) and antithyroglobulin antibodies were frequently demonstrated [1]. Good correlation between the occurrence of autoantibodies and circulating immune complexes (CIC) has been reported [2]. This investigation was designed to evaluate CIC and ANA in patients with leprosy.

Material and Methods

Patients

Seventy-nine patients with leprosy in Nagashima Aiseien, Okayama, Japan, were studied. All patients were classified according to clinical and pathological criteria [5]. There were 57 lepromatous patients, whose ages ranged from 20 to 81 years. Lepromatous patients were divided into two groups, i.e. in the active (pa) and inactive (pa) stage according to the criteria of the Japanese leprosy committee [4, 6]. The number of patients in each group was 21 and 36, respectively. There were 22 tuberculoid patients, whose ages ranged from 52 to 84 years and whose clinical stage was inactive. All patients were under treatment with dapsone, rifampicin, and others. Patients with systemic infectious diseases, cancer, liver diseases, and autoimmune diseases were excluded from this study. Twenty-five serum samples were obtained as controls from healthy individuals whose ages ranged from 25 to 79 years. All sera were stored at −80°C until needed.

Clq Solid Phase Assay

This assay was performed as a modification of the method of Hay et al. [7]. The procedure has been described in detail previously [4].

Measurement of Anti-ssDNA Antibodies

Anti-ssDNA antibodies were assayed by the Farr assay as a modification of the method of Yoshida et al. [8]. The reaction mixture (200 µl) contained 5 µl heat-inactivated test serum and 5 ng heat-denatured 125I-ssDNA (calf thymus) in borate buffer, pH 8.4, ionic strength 0.1. The mixture was incubated at 37°C for 1 h and then at 4°C for 16 h. After 1 ml 60% cold saturated ammonium sulfate was added, the mixtures were incubated at 4°C for 30 min, and centrifuged at 1500 x g for 30 min at 4°C and the precipitates were washed once with 2 ml 50% saturated ammonium sulfate. The amount of anti-ssDNA antibodies was expressed as a percentage of 125I-ssDNA precipitated. Anti-ssDNA titers higher than 2 s.d. values above the mean level of healthy subjects were regarded as positive (15%).

Measurement of Anti-dsDNA Antibodies

Anti-dsDNA antibodies were assayed by the Farr assay as a modification of the method of Yoshida et al. [8]. The reaction mixture (200 µl) contained 5 µl heat-inactivated test serum and 5 ng heat-denatured 125I-dsDNA (E. coli 4C) was prepared by S 1 nuclease digestion and methylated-albumin kieselgur column elution [9]. The S 1 nuclease analysis [10] revealed ssDNA contamination of less than 5%. The reaction mixture (200 µl) contained 20 ng 14C-dsDNA and 10 µl heat-inactivated test serum. After incubating these mixtures with 1 ml 60% saturated ammonium sulfate, the resulting precipitates were washed once with 50% saturated ammonium sulfate and dissolved in 0.1 N HCl. The amount of radioactivity in the precipitate was determined in a liquid scintillation counter. The results are expressed as a percentage of 14C-dsDNA precipitated. Titters of anti-dsDNA antibodies higher than 2 s.d. values above the mean level of healthy subjects were regarded as positive (5%).
Measurement of Anti-Extractable Nuclear Antigen (ENA) Antibodies

A lyophilized preparation of a saline-soluble acetone extract of rabbit thymus (Pel-Freeze Biologicals Inc., Rogers, AR, USA) was used as a source of ENA [11]. A modified radioimmunoassay was designed according to the method of Billings et al. [12]. Briefly, each polyvinyl chloride well was first coated with 100 μl rabbit thymus extract solution (30 μg/ml) in PBS and then with 1% BSA PBS. After washing the wells, 100 μl of the serum sample, which was diluted 20 times with PBS-BSA-Tween, was transferred to duplicate rabbit-thymus-extract-coated wells and incubated at 37°C for 1 h. After washing three times, the wells were incubated with 100 μl 125I-protein A solution labelled by the chloramine T method [13]. After washing, radioactivity was counted for 1 min. Anti-ENA titers higher than 2 s.d. above the mean level of healthy subjects were regarded as positive (1284 cpm).

Statistical Analysis

The association of each phenotype was evaluated using the χ² test. Probability values of over 5% were considered insignificant.

Results

The incidence of the appearance of ANA is shown in Fig. 1. Anti-dsDNA antibodies were found in three patients (8.3%) with inactive lepromatous leprosy and one patient (4.5%) with tuberculoid leprosy. Anti-dsDNA antibodies were not found in patients with active lepromatous leprosy or in controls. Anti-dsDNA antibodies were found in four patients (19.1%) with active lepromatous leprosy, nine (25%) with inactive lepromatous leprosy, two (9.1%) with tuberculoid leprosy, and four in controls (16%). Anti-extractable nuclear antigen antibodies were found in five (23.8%) patients with active lepromatous leprosy, six (16.7%) with tuberculoid leprosy, seven (31.8%) with lepromatous leprosy, and in two controls (8%). There were no patients in whom all three kinds of ANA described above could be detected, but five out of 79 patients had two types of ANA. The presence of both anti-ssDNA antibodies and anti-ENA antibodies were demonstrated in three patients, anti-dsDNA antibodies and anti-ENA antibodies in one patient, and anti-dsDNA antibodies and ssDNA antibodies in one patient.

Circulating immune complexes (CIC) were demonstrated in 14 (66.6%) patients with active lepromatous leprosy, 17 (47.2%) with inactive lepromatous leprosy, 12 (54.5%) with tuberculoid leprosy and in two controls (8%).

Correlation studies between CIC and ANA were performed in order to know the possibility that ANA were one component of CIC detected in patients with lepromatous leprosy. As shown in Table 1 and 2, there were no significant associations among CIC, anti-ssDNA antibodies and anti-ENA antibodies. Needless to mention, no association was present between CIC and anti-dsDNA antibodies.

Discussion

Anti-nuclear antibodies and other autoantibodies are frequently found in patients with leprosy. The reported incidence of ANA in leprosy, especially in patients with lepromatous leprosy, varies from 0—54% [1]. The specificity of these ANA has not been fully investigated. Our study shows a marked and significantly high incidence of anti-ENA antibodies in patients with leprosy. ENA is considered to contain two major antigens, one a ribonucleoprotein, RNP, and the other an acidic pro-