Amyloid-Related Serum Component (Protein ASC) in Leprosy Patients

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The presence of amyloid-related serum component, protein ASC, in serum samples from 63 leprosy patients was investigated. Protein ASC was detected in 38% of the patients. A correlation to the disease spectrum of leprosy was apparent: polar lepromatous cases, 64% positive; borderline lepromatous, 50%; borderline tuberculoid, 36%; subpolar tuberculoid, 17%; and polar tuberculoid, negative. Antibody activity against the antigen of Mycobacterium leprae was also determined, showing a similar correlation to the disease spectrum. Serum samples from 23 apparently healthy Ethiopians serving as controls showed a protein ASC incidence of 22%. This figure is significantly higher than the frequency found by others among healthy Norwegian blood donors. Immunoglobulin M levels among patients were elevated in the borderline lepromatous and polar lepromatous groups. The three tuberculoid groups did not differ in this respect from the control group but were all elevated as compared to a normal Caucasian serum pool. Although raised immunoglobulin M levels seemed to parallel increased frequencies of protein ASC in the patient groups as well as in controls, this correlation might be only secondary to a primary derangement in T-cell function.

Leprosy patients with the low-resistant form of the disease, so-called lepromatous leprosy, have a more or less complete lack of cell-mediated immunity against the leprosy bacillus (7, 10). At the same time the humoral immune system is hyperstimulated, with consequent hypergammaglobulinemia and the occurrence of various kinds of autoantibodies (6, 8, 18, 28, 30–33). These humoral changes are very similar to the immunological derangements found in autoimmune disorders, e.g., SLE and rheumatoid arthritis. The similarities in features also extend to a rather high frequency of amyloidosis in lepromatous leprosy as well as in rheumatoid arthritis. Studies in the United States showed that amyloidosis was the actual cause of death in as many as nine out of 20 leprosy cases (29). In another study of 101 patients at Carville more than 40% were clinically diagnosed as having secondary amyloidosis, with 31% positive gingival biopsies (35). Similar studies in South America give figures of about the same order (18, 20), whereas in India secondary amyloidosis seems to be rarer (16, 27). No studies have so far been reported from Africa.

Recent progress in studies of amyloid substance (4, 9, 13, 14, 17, 23) has revealed a serum protein (amyloid-related serum component [ASC]), present in most cases of amyloidosis (91%) and in high frequencies in disorders characterized by a tendency to develop secondary amyloidosis, for example, rheumatoid arthritis (63%) (11). In the present investigations serum samples from leprosy patients along the disease spectrum have been tested for the presence of protein ASC. The results show a high incidence of protein ASC not only in polar lepromatous cases but also in the borderline groups.

MATERIALS AND METHODS

Serum samples from patients and normals. Serum samples from 63 leprosy patients were included in the study. The patients were all registered at the All Africa Leprosy and Rehabilitation Training Center (ALERT), Addis Ababa, and were classified by histological examination of biopsies according to the Ridley and Jopling system (24–26). Forty-six of the patients were untreated. The mean age of the patients was 25.5 years, with a standard deviation of ±10.6 years. None of the patients had reversal reaction or erythema nodosum leprosum at the time of bleeding.

Serum samples from 23 apparently normal healthy adult Ethiopians served as controls. The sera were kindly supplied by Mehari Gebre-Medhin, Ethiopian
Nutrition Institute, Addis Ababa. There was no history of contact with leprosy among these normal people. The mean age was 30.2 years (standard deviation, ±5.0 years). All serum samples were stored at −20 C until use. Aliquots of the sera were shipped on dry ice to Oslo for protein ASC determinations.

**Protein ASC determination.** The presence of protein ASC in serum samples was determined in agarose diffusion experiments as described (11), using antiserum to amyloid protein AS as an antibody reagent.

**Determination of antibody activity against a mycobacterial antigen.** Thirty-eight of the serum samples were tested for antibody activity against the antigen of *Mycobacterium leprae*, an antigen common to all mycobacteria (submitted for publication). An extract of *M. duchovii* was used as antigen in crossed immunolectrophoresis with the patient's serum in the intermediate gel against a pool of rabbit anti-*M. duchovii* (1–3, 36–38). The amount of antibody present in the serum samples included in the intermediate gel was semiquantitated by comparison with dilutions of a high-titered antiserum run in parallel experiments.

**Immunoglobulin quantitation.** The serum concentrations of immunoglobulin M (IgM) was determined by radial immunodiffusion according to Mancini et al. (19). A monospecific antiserum was kindly provided by K. Lindqvist, University of Dar Es Salaam, Tanzania. Serum concentrations were expressed as percentage of a reference serum pool of Caucasian sera.

**RESULTS**

**Protein ASC determination.** Protein ASC was detected in 24 of 63 sera from leprosy patients, giving an overall frequency of 38%. When the frequencies of protein ASC positivity in the various diagnostic groups along the leprosy spectrum were calculated, a striking correlation to the spectrum appeared (Fig. 1). The incidence of protein ASC was highest at the lepromatous end, with nine out of 14 being positive among the polar lepromatous (LL) cases (64%). The incidence decreased gradually towards the tuberculoid end. The overall incidence among the 42 LL, borderline lepromatous (BL), and borderline tuberculoid (BT) cases was 50%. This contrasts to the low incidence among polar tuberculoid (TT) and subpolar tuberculoid (TT/BT) cases with only three out of 21 being positive (14%).

Protein ASC was detected in five out of 23 serum samples (22%) from normal Ethiopians serving as controls. When compared statistically with the 21 positive out of the 42 BT, BL, and LL cases, the high incidence among the latter was shown to be significantly different from the control group ($\chi^2 = 4.95; P < 0.05$). The BT patients did not differ significantly from the BL and LL patients regarding the occurrence of protein ASC. Short treatment did not significantly influence the frequency of protein ASC positivity.

**Correlation to antibody and IgM levels.** The presence of antibodies against the antigen of mycobacteria, including *M. leprae*, was determined in 42 patients, and the results are shown in Fig. 2. All LL and BL cases were positive, whereas BT and TT/BT cases showed antibody activity in five out of eight and three out of nine cases, respectively. The overall picture is parallel to the findings regarding protein ASC. On an individual basis, however, there was not a strict correlation between the two parameters. Anti-a activity was not detected among normals.

IgM concentrations were also determined in the 63 leprosy sera, as well as in the 23 serum samples from adult Ethiopians. IgM levels in sera from LL and BL patients were significantly higher than in the control sera (Fig. 3). Concentrations in the control sera were similar to the three tuberculoid groups (BT, TT/BT, TT) but were all elevated as compared to the Caucasian serum pool (Fig. 3).

**DISCUSSION**

In the present investigations a high proportion of leprosy patients had detectable amounts
of amyloid-related serum component (protein ASC) in their serum. The figures correlated with the disease spectrum of leprosy, with the highest frequency among LL cases (64%) and decreasing towards the tuberculoid end of the spectrum (BL, 50%; BT, 35.7%; TT, negative). Our findings are in line with previous reports on the incidence of amyloidosis in leprosy (5, 20, 21, 29, 35). The tendency to develop amyloidosis and to give detectable amounts of protein ASC seems to parallel the general and specific activation of humoral immunity which is a characteristic of lepromatous leprosy. This is illustrated by our finding of an overall correlation of protein ASC positivity to antibody activity towards an M. leprae antigen (Fig. 2) as well as to IgM levels (Fig. 3). The same causes underlying the elevation of these two parameters might also be operative in creating the amyloid or preamyloid state (12). Since the causative agent is known in leprosy and since leprosy is a chronic but curable disease it might constitute a convenient model for studies of amyloidosis.

The correlation of clinical as well as laboratory findings in leprosy to the histological classification according to Ridley and Jopling and Ridley and Waters (24–26) has been well established (7, 10). In previous studies it became apparent that an intermediate group in the tuberculoid end of the spectrum could be delineated, the so-called TT/BT group (D. Samuel, unpublished data; 22). Our results of protein ASC testing, as well as of the presence of antibodies against the antigen of M. leprae, also support the delineation of an intermediate TT/BT group.

The number of protein ASC-positive serum samples from adult, apparently healthy Ethiopians was as high as five out of 23 (22%). The incidence is significantly higher than the frequency of protein ASC-positive sera among adult Norwegian blood donors (2.9%; \( \chi^2 = 8.661; P < 0.005 \) (11). The IgM determinations show that the humoral immune system in the Ethiopian controls is considerably more stimulated than the immunoglobulin system in Caucasians. This has been well documented by others previously (15, 34). The high protein ASC incidence among normal Ethiopians might therefore merely reflect an immunological disposition, giving a parallel increase in humoral antibody activity.

The basic characteristic of lepromatous leprosy is a defective cell-mediated immune response towards M. leprae (7, 10). The exact nature of this defect in the T-cell system has not yet been defined. Since a high frequency of protein ASC has been found in sera of patients with Hodgkin's disease (11), it also remains a

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**FIG. 2.** Antibody activity against the antigen of M. leprae in serum samples from leprosy patients along the disease spectrum. Bars indicate percentage of anti-a-positive serum samples, with actual numbers given below the bars.

**FIG. 3.** Percentage of protein ASC positivity plotted against mean IgM serum concentrations in five groups of leprosy patients histologically classified (TT, TT/BT, BT, BL, LL) and in a group of adult, apparently healthy Ethiopians. The IgM concentration is expressed as percentage of a pool of normal Caucasian blood donors (100%).
possibility that protein ASC production might primarily reflect a defective T-cell system and therefore only parallel a secondary humoral hyperactivity.

LITERATURE CITED


