Antibodies to sulfatide in leprosy and leprosy reactions

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Abstract. Antibodies to sulfatide have been reported in various demyelinating peripheral polyneuropathies. We have investigated the diagnostic value of these antibodies in leprosy. Anti-sulfatide IgM in leprosy patients was not significantly elevated. High anti-sulfatide IgG titers were observed in individuals from endemic areas, irrespective of their leprosy status, while western European controls were negative. No significant correlation was found between IgM or IgG antibody titers and leprosy classification, although multibacillary patients had higher anti-sulfatide IgM titers than paucibacillary patients. In addition, 23 patients developing leprosy reactions were followed longitudinally. Antibody titers in these patients fluctuated slightly during the follow-up period. There was no association with the occurrence of leprosy reactions or treatment. Thus, IgG titers against sulfatides are high in both leprosy patients and healthy controls in endemic areas, whereas such antibodies are not found in western European controls, suggesting that these antibodies are induced by environmental factors, such as microorganisms.

Leprosy is a chronic infectious disease that is caused by Mycobacterium leprae. Peripheral nerve damage is a major complication in leprosy and occurs across the entire leprosy spectrum, particularly in patients with acute inflammatory leprosy reactions. Reactional episodes in leprosy are accompanied by increased immune activity. A role for the immune system in the pathology of leprous neuritis has therefore long been suspected. A striking feature of M. leprae is its predilection for Schwann cells. Nerve damage could thus result from cellular or humoral immune reactivity towards M. leprae located in nerve tissue. Since nerve damage can also occur in uninfected tissue, nonspecific inflammatory or perhaps even autoimmune mechanisms may also play a role. To prevent nerve damage in leprosy, prediction or early detection of neuritis episodes will be crucial.

Loss of nerve function is often associated with demyelination. Antibodies might play an active role in this process. Anti-galactocerebroside antibodies are thought to induce myelin alterations, leading to inhibition of sulfatide synthesis and demyelination. These effects can be mimicked by injecting leprosy patients’ serum into Swiss white mice, suggesting that anti-glycolipid antibodies play an active role in the pathogenesis of leprosy neuritis. In sooty mangabey monkeys with experimental leprosy, antibodies towards ceramide, galactocerebroside or asialo-GM1 were reported to have potential diagnostic value in predicting leprous nerve damage. Antibodies directed to neural glycolipids are also related to leprosy: antibodies to ceramide and galactocerebroside were detected in the majority of leprosy patients, but titers did not differ significantly between patients with and without nerve damage.

Sulfatide is a glycosphingolipid that is expressed as surface determinant of myelin in the central and peripheral nervous system. Sulfatide and galactocerebroside play an important role in myelin function and stability. Antibodies to sulfatides have been detected in several neuropathies: 88% of patients with insulin-dependent diabetes mellitus had detectable antibodies to sulfatides, and these antibodies have also been found in patients with the Guillain Barré syndrome, Miller Fisher syndrome, and multiple sclerosis. For serologic detection of infection with M. leprae, various antigens can be used, including phenolic glycolipid-I, lipoolarabinomannan, and a number of M. leprae-specific proteins. However, no serologic assay is available for identification of leprosy reaction or nerve damage. Increased titers of antibodies to sulfatides and related neural components may be associated with nerve damage. In this study, we have therefore measured titers of antibodies to sulfatide in leprosy patients, including patients with type 1 or type 2 leprosy reactions, to investigate whether such antibodies might have prognostic or diagnostic value for the detection of leprosy neuritis.

MATERIALS AND METHODS

Patients. The population studied included 10 multibacillary and 10 paucibacillary patients, 10 household contacts, and 10 controls from the Philippines. Multibacillary leprosy patients included all borderline and lepromatous patients with a bacterial index (BI) of at least 2+ on the scale of Ridley and Jopling at any one site. Paucibacillary leprosy patients included indeterminate, tuberculoid, and borderline tuberculoid with BIs < 2+ at any one site. These parameters followed the World Health Organization recommendation at the time of collection. The contacts were persons living in the same household as the multibacillary or paucibacillary cases in the last 3 years. The normal population was composed of persons living in the same community as the patients who presented with other forms of skin diseases but who were free of clinical signs of leprosy and with no case of leprosy in their households. Five healthy west European controls were included as negative controls, and a group of 10 Guillian Barré Syndrome patients and 5 diabetic patients served as positive controls.

Additionally, sera from 23 leprosy patients were collected longitudinally. The mean follow-up period was 28.3 months. During the follow-up period, 10 of them had a type 2 reaction and 9 patients had a type 1 reaction. The remaining 4 patients had no reaction during the follow-up period.

The collection of blood for the study was approved by the Leonord Wood Memorial Institutional Review Board (Human Rights Committee) (Cebu, the Philippines) and the Ministry of Health (Manila) Ethical Committee. Written informed consent was obtained from all subjects. For collection of sera from the Netherlands, approval was obtained.
from the Medical Ethical Committee of the Leiden University Medical Center (Leiden, The Netherlands).

**Enzyme-linked immunosorbent assay.** Ninety-six well, flat-bottom plates (Greiner GmbH, Solingen, Germany) were coated with sulfatide derived from bovine brain (Sigma, St. Louis, MO) dissolved in methanol overnight at 4°C. The plates were blocked with phosphate-buffered saline (PBS)/1% bovine serum albumin (BSA) (Sigma) for 90 min at room temperature and washed 5 times with PBS. Sera were titrated two-fold ranging from 1/50 to 1/6,400 and incubated overnight at 4°C. The wells were washed again 5 times with PBS. Peroxidase-conjugated rat anti-human IgG or IgM was diluted 1/1,000 in PBS/1% BSA and added to the wells. After incubating for 2 hr at room temperature, the wells were washed 3 times with PBS/0.05% Tween and 2 times with PBS. Enzymatic activity was determined in 50 mM citric acid (Merck, Darmstadt, Germany), 100 mM NaHPO4 (Merck) pH 5.0, containing 1 mg/ml of phenylenediamine Dichloride (Sigma), 1 μl/ml of 30% H2O2 (Sigma-Aldrich, Steinheim, Germany). The reaction was stopped by adding 10% sodium dodecyl sulfate (Sigma). After 20 min, the optical density was read at 450 nm.

**Statistical analysis.** The results of the sulfatide ELISA were scored as the highest dilution giving a positive reaction. The titers were transformed logarithmically. Analysis of variance was performed on the results of the different groups. Differences between groups were analyzed with an unpaired two-tailed t-test. To analyze the correlation between anti-sulfatide IgM titers and the BI, regression analysis was done.

**RESULTS**

**Anti-sulfatide IgM antibodies in untreated leprosy patients.** Anti-sulfatide IgM titers in 10 multibacillary and 10 paucibacillary leprosy patients were determined and compared with those in endemic controls with non-leprosy-related skin diseases (Skin) endemic healthy contacts (EnC), and west European controls (WeC). Median values are plotted for each group. Anti-sulfatide IgM (C) and IgG (D) were plotted against leprosy classification. IgM titers only differed significantly between lepromatous and tuberculoid patients (P = 0.02). Other differences were not significant.
lated skin diseases, healthy contacts from the same endemic area, and western European controls (Figure 1A). Untreated multibacillary patients appeared to have higher anti-sulfatide IgM titers (median = 1,600) than paucibacillary patients (median = 600), patients with skin diseases (median = 200), and healthy contacts from the same endemic area (median = 400). Antibody titers in the latter two groups were in the similar range as those in European healthy controls (median = 400). The differences between the four groups, however, were not statistically significant ($P = 0.11$). However, difference in IgM titers between the different groups of the leprosy spectrum showed significantly lower titers in the tuberculoid group compared with the lepromatous group (Figure 1C; $P = 0.02$).

Anti-sulfatide IgG antibodies in untreated leprosy patients. Regarding anti-sulfatide IgG titers (Figure 1B), all

Figure 2. Correlation between bacillary index and anti-sulfatide IgM (A) and IgG (B). Regression coefficients and $P$ values are indicated in each graph.

Figure 3. Anti-sulfatide antibody titers in reaction-free patients (A–C), ENL (D–F) and reversal reactive (G–I) patients (3 representative individuals for each group). Reational episodes are marked in gray. IgM titers are indicated by open squares and IgG titers are indicated by solid squares.
individuals from the Philippines scored significantly higher than the European controls, which were all negative ($P < 0.0001$). Small differences were found between the four groups from the Philippines. Medians were 300 for the paucibacillary patients, 600 for the multibacillary patients, and 150 for the group with skin diseases and the control group from the same endemic area. These differences were not statistically significant ($P = 0.09$). The differences in IgG titers between the different groups of the leprosy spectrum were also not significant (Figure 1D).

**Correlation between antibody titers and the BI.** To examine whether titers of antibodies to sulfatide correlate with the bacterial load of the leprosy patients, anti-sulfatide IgM and IgG titers were plotted against the BI (Figure 2). Interestingly, a positive trend was found for IgM but there was a negative trend for IgG. Both trends failed to reach statistical significance ($P = 0.18$ for IgM and $P = 0.30$ for IgG).

**Antibodies to sulfatide and leprosy reactions.** Twenty-three patients without any history of leprosy reactions were followed longitudinally. Nine of them showed a type 1 reversal reaction, 10 developed erythema nodosum leprosum, and 4 remained free of leprosy reactions during the follow-up period. Anti-sulfatide antibody IgM and IgG titers fluctuated over time (Figure 3) in all three groups but did not correlate with either the onset or the occurrence of leprosy reactions. Neither IgM nor IgG titers were affected by therapy (Figure 3).

**DISCUSSION**

In this study, we have investigated the presence of antibodies to sulfatide in leprosy, with particular emphasis on their association with the occurrence of nerve damage and type 1 or type 2 leprosy reactions. Antibody titers in untreated multibacillary and paucibacillary patients, healthy contacts, patients with non-leprosy-related skin diseases, and European controls showed no statistically significant differences in IgG or IgM titers (Figure 1A and B). In contrast, anti-sulfatide IgG was absent in European controls and high in diabetic patients and patients with the Guillain Barré syndrome, as expected. The differences between western controls and controls from endemic areas might be attributed to the fact that the latter could have had (sub)clinical infections with mycobacteria or other environmental microbes. Such infections might have induced high levels of antibodies to sulfatide. This suggests that the presence of such antibodies is not necessarily associated with nerve inflammation. The results also imply that measurement of antibodies to sulfatide is not applicable for early diagnosis, prediction, or monitoring of leprosy reactions in endemic areas. Even though there was a trend towards a correlation between anti-sulfatide IgM titers and bacillary indices, this correlation was not significant.

In a longitudinal analysis, individual anti-sulfatide IgM and IgG titers fluctuated over time. There was no clear association with the onset of leprosy reactions. Anti-sulfatide Ig titers were unaffected by treatment. It has been reported that sooty mangabey monkeys inoculated intravenously and intracerebrally with *M. leprae* develop anti-ceramide, anti-asialo-GM$_{1}$, and anti-galactocerebroside antibodies 1–2 years prior to developing nerve damage. In leprosy patients, anti-sulfatide IgM and IgG titers remained continuously stable over the time period studied (12–42 months). The fact that no increase in antibody titers to sulfatide could be detected prior to reactional episodes may be attributed to various causes. At the time of diagnosis, 40–70% of the leprosy patients already have nerve involvement. Therefore, in these patients increased anti-sulfatide immunoglobulin titers might already be present before the onset of reactions and thus occur earlier during natural human infection compared with experimental infection of non-human primates. Indeed, experimental infection in the monkey model may not entirely reflect natural infection of the human host and this may strongly influence the titer and type of antibodies. In addition, genetic host factors in leprosy susceptible human hosts may also contribute to these differences. Other neural candidate glycolipids and proteins, such as glial fibrillary acidic protein and S-100, remain to be studied to distinguish between leprosy nerve damage and healthy exposed individuals, since early detection of neural involvement will contribute to prevention of irreversible loss of nerve function.

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