chapter 6
sulfatide antibodies in leprosy and leprosy reactions

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abstract

Anti-sulfatide antibodies have been reported in various demyelinating peripheral polyneuropathies. We have investigated the diagnostic value of anti-sulfatide 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, twenty-three 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 anti-sulfatide antibodies are induced by environmental factors, such as microorganisms.

introduction

Leprosy is a chronic infectious disease that is caused by *Mycobacterium leprae* (Bloom and Godal 1983). 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 (Roth et al. 1985), leading to inhibition of sulfatide synthesis (Fry et al. 1974) and demyelination (Saida et al. 1979; Sergott et al. 1984). These effects can be mimicked by injecting leprosy patients’ serum into Swiss white mice (Shetty et al. 1985), 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-GM, were reported to have potential diagnostic value in predicting leprous nerve damage (Cho et al. 1993). Antibodies directed to neural glycolipids are also related to leprosy: anti-ceramide (Vemuri et al. 1996) and galactocerebroside antibodies (Vemuri and Mukherjee 1991; Vemuri et al. 1996) 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 (Dupouey et al. 1979). Sulfatide and galactocerebroside play an important role in myelin function and stability (Coetzee et al. 1996). Antibodies towards sulfatides have been detected in several neuropathies: eighty-eight percent of insulin dependent diabetes mellitus patients had detectable antibodies to sulfatides (Buschard et al. 1993), and anti-sulfatide antibodies have also been found in patients with the Guillain Barré syndrome (Ilyas et al. 1991; van den Berg et al. 1993) Miller Fisher syndrome (Willison and Veitch 1994) and multiple sclerosis (Ryberg 1978). For serological detection of infection with *M. leprae* various antigens can be used, including phenolic glycolipid-I, lipoarabinomannam, and a number of *M. leprae* specific proteins.
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(Buchanan 1995). However, no serological assay is available for identification of leprosy reaction or nerve damage. Raised titers of antibodies towards sulfatides and related neural components may be associated with nerve damage. In this study we have therefore measured anti-sulfatide antibody titers in leprosy patients, including patients with type 1 or type 2 leprosy reactions, in order 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 Ridley scale (Ridley and Jopling 1966) at any one site. Paucibacillary leprosy patients included indeterminate, tuberculoid (TT) and borderline tuberculoid (BT) with BIs < 2+ at any one site. These parameters follow the World Health Organization (WHO) recommendation at the time of collection (World Health Organization Expert Committee on Leprosy 1989). 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, presenting other forms of skin diseases but 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 Guillain 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 encountered a type 2 reaction and 9 patients 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 Leonard Wood Memorial Institutional Review Board (Human Rights Committee) (Cebu, The Philippines) and the Ministry of Health Ethical Committee (Manila, The Philippines). 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 immunoassay (ELISA)

Ninety-six wells flat bottom plates (Greiner GmbH, Solingen, Germany) were coated with sulfatide derived from bovine brain (Sigma Aldrich, St. Louis, MO) dissolved in methanol, overnight at 4°C. The plates were blocked with PBS/1% BSA (Sigma) for 90 minutes at room temperature and washed 5 times with PBS. Sera were titrated twofold ranging from 1/50 to 1/6400 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 1/1000 in PBS/1% BSA and added to the wells. After incubating 2 hours 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 Na$_2$HPO$_4$ (Merck) pH 5.0, containing 1mg/ml Phenylenediamine Dichloride (Sigma), 1 µl/ml 30% H$_2$O$_2$ (Sigma). The reaction was stopped by adding 10% sodium dodecyl sulfate (Sigma). After 20 minutes the optical density was read at 450 nm.
sulfatide antibodies in leprosy

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 bacillary index, regression analysis was executed.

results

IgM anti-sulfatide antibodies in untreated leprosy patients

Anti-sulfatide IgM titers in 10 multibacillary and 10 paucibacillary leprosy patients were determined and compared to those in endemic controls with non-leprosy related 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: 1600) than paucibacillary patients (median: 600), patients with skin dis-

statistical analysis

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IgM anti-sulfatide antibodies in untreated leprosy patients

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...and healthy contacts from the same endemic area (median: 400). Antibody titers in the latter two groups were in the similar range as 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 to the lepromatous group (figure 1c, p=0.02).

**IgG anti-sulfatide antibodies in untreated leprosy patients**

Regarding anti-sulfatide IgG titers (figure 1b), all 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 statistically not significant (p=0.09). The differences in IgG titers between the different groups of the leprosy spectrum were not significant either (figure 1d).

**Correlation between antibody titers and the bacillary index**

To examine whether anti-sulfatide antibody titers correlate with the bacterial load of the leprosy patients, anti-sulfatide IgM and IgG titers were plotted against the bacillary index (figure 2). Interestingly, a positive trend was found for IgM but a negative trend for IgG. Both trends failed to read statistical significance (p=0.18 for IgM, p=0.30 for IgG).

**Anti-sulfatide antibodies and leprosy reactions**

Twenty-three patients without any previous history of leprosy reactions were followed longitudinally. In 9 of them a type 1 reversal reaction occurred, 10 patients developed ENL and 4 patients 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).

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**figure 2: Correlation between bacillary index and anti-sulfatide IgM (a) and IgG (b). Regression coefficients and p-values are indicated in each graph.**
In this study, we have investigated the presence of anti-sulfatide antibodies 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 multi- 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-b). In contrast, anti-sulfatide IgG were absent in European controls and high in diabetic patients and patients with the Guillain Barré syndrome, as expected (data not shown). 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 anti sulfatide antibodies (Luna-Herrera et al. 1996). This suggests that the presence of such antibodies is not necessarily associated with nerve inflammation. The results also imply that measurement of anti-sulfatide antibodies 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 intracutaneously with *M. leprae* develop anti-ceramide, anti-asialo-GM₁, and anti-galactocerebroside antibodies, 1 to 2 years prior to developing nerve damage (Cho *et al.* 1993). In leprosy patients, anti-sulfatide IgM and IgG titers remained continuously stable over the time period studied (12 to 42 months). The fact that no increase in anti-sulfatide antibody titers could be detected prior to reactional episodes may be attributed to various causes. At the time of diagnosis, 40 to 70% of the leprosy patients already have nerve involvement. Therefore, in these patients raised anti sulfatide Ig titers might already be present before the onset of reactions and thus occur earlier during natural human infection compared to 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, like GFAP and S-100 (Thomas and Mukherjee 1990), remain to be studied in order to distinguish between leprosy nerve damage and healthy exposed individuals, as early detection of neural involvement will contribute to prevention of irreversible loss of nerve function.