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The role of antiperipheral nerve antibodies in nerve damage in leprosy¶

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Summary The objective of this study was to determine the role of antineural antibodies in leprosy. Indirect ELISA using antigen prepared from normal human peripheral nerves was carried out on the sera from 100 leprosy patients and 18 normal controls. In total, 9% of the patients had demonstrable levels of IgG antineural antibodies and 11% had demonstrable levels of IgM antibodies. There was no correlation with the type of leprosy, bacteriological index, treatment taken, the presence of a reactional state, the presence of enlarged nerves or active neuritis.

Introduction

The role of antineural antibodies in leprosy directed against peripheral nervous system components is, to date, inconclusive and controversial. There is always some degree of nerve damage throughout the whole spectrum of the disease. However, it has not always been possible to elucidate the exact pathogenesis of nerve damage in cases with neuropathies, keeping in perspective the available information on the subject.[2,4,7,8,14] Hence, it is postulated that an autoimmune mechanism may be responsible in leprosy either as a primary process leading to nerve damage, or as a secondary process perpetuating an already existing nerve damage.[17]

Materials and methods

STUDY SUBJECTS

Keeping in perspective the results obtained from previous studies, which ranged from 0

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Table 1. Duration of the disease in all leprosy patients tested

Type of leprosy	Total no. of cases	Cases with disease duration < 1 yr	Cases with disease duration 1–4 yr	Cases with disease duration > 4 yr
LL	43	5	25	13
BL	18	1	12	5
BB	3	2	0	1
BT	34	10	20	4
TT	2	1	1	0
Total	100	19	58	23

through 4·1, 13, 22, 23·86, 25, 25·6, 40, 47 to 100%, the average (31%) was taken as the anticipated population proportion having antineural antibodies. The sample size required for this anticipated population proportion was calculated using a 95% confidence interval and a precision of 9 percentage points. The minimum sample size was found to be 100, when calculated according to the formula:

$$\frac{n = Z_{\alpha}^{z} \times p \times (1 - p)}{d^2}$$

where n is the sample size, z is the standard normal curve value at $1 - \alpha/2$, $100(1 - \alpha)$ is the confidence interval, p is the anticipated population proportion and d is the percentage point. Thus serum from 100 leprosy patients were studied. Representative samples from the entire spectrum of the disease were collected and tested. The patients were classified according to the Ridley–Jopling classification of leprosy.[15]

There were 43 patients with lepromatous leprosy (LL), 18 with borderline lepromatous (BL) leprosy, 3 with borderline (BB) leprosy, 34 with borderline tuberculoid (BT) leprosy and 2 with polar tuberculoid (TT) leprosy. Out of the total 100 patients, 35 were cases in reaction, 20 with Type I and 15 with Type II reaction. The duration of the disease in the patients ranged from 4 months to 11 years. Duration of the disease in all cases at the time of collection of serum samples for the study is shown in Table 1. In all, 52 of the patients had enlarged peripheral nerves, of whom 19 had signs of active neuritis, as detailed in Table 2. The patients were aged between 14 and 60 years and 61 patients had bacteriological indices (BI) ranging between 0 and 2+, 30 had BI > 2+; the BI of 9 patients were not known, and 67 patients were on multidrug therapy for leprosy.

Table 2. Leprosy patients with clinical evidence of nerve involvement

Type of leprosy	Total no. of cases	Cases with nerve enlargement	Cases with active neuritis
LL	43	17	6
BL	18	14	5
BB	3	1	0
BT	34	18	7
TT	2	2	1
Total	100	52	19

Table 3. Control sera used in the study

Type of sera	No. of sera
I Normal healthy controls	18
II Controls with neurological deficiencies due to causes other than leprosy:	
a Gullian–Barré syndrome	2
b Abortive Gullian–Barré syndrome	1
c Diabetic neuropathy	4
d Motor neuron disease	3
e Paraparesis	2
f Polio	1
g Anterior horn cell disease (other than polio)	1
h Crush injury	1
Alcohol induced sensory motor neuropathy	1
j Neurofibromatosis	1
k Viral encephalitis with sensory deficiency	1
l Post-diphtheritic bilateral VI nerve palsy with left VII nerve palsy	1
m Neuropathy due to unknown aetiology	4
Total	41

The remaining patients had not received any treatment for leprosy. Control sera were included in the study. There were normal healthy controls as well as controls with neurological deficits due to causes other than leprosy, as detailed in Table 3.

ANTIGEN PREPARATION

Normal human nerves were taken at autopsy within 1 hr of death and teased. They were treated with warm acetone (37°C) and petroleum ether and then homogenized and sonicated. The protein content was 1·2 mg/ml, calculated by Lowry's method.[9] The major bands migrated at 15–22 kDa and 25–27 kDa.

ELISA

Indirect ELISA using 60 ng of antigen per well (after checker board titration) was carried out using U-bottomed polystyrene Nunc Immunoplates. Briefly, the coated plates were kept overnight at 37°C in a moist chamber and washed next morning 3 times with PBS-T. Blocking was done with 3% BSA in PBS-T. The sera was diluted at 1 : 400 and the plates were incubated at 37°C for 1 hr in a moist chamber and washed with PBS-T. To test for IgG type of antibodies, 50 μl of antihuman IgG conjugated with HRPO (Lupin) at a dilution of 1 : 1000 in PBS-T per well was used. In order to test for IgM antibodies, 50 μl of antihuman IgM conjugated with HRPO at a dilution of 1 : 1000 in PBS-T per well was used. Plates were incubated at 37°C in a moist chamber for 1 hr and washed with PBS-T. Orthophenylene diamine (Sigma) with 30% hydrogen peroxide was used as a substrate. The reaction was stopped using 7% sulphuric acid. The optical density was read after 10–20 min using 492 nm filter in a Titertek Multiscan plus ELISA reader from

Table 4. Details of cases with demonstrable levels of IgG antibody

Type of leprosy	A/S	Duration of disease	BI	Nerve enlargement	Active neuritis	Whether on treatment
LL	33/M	2 yr	3+	+	+	–
LL	45/M	10 yr	2+	–	–	+
LL	40/M	8 months	3+	–	–	+
LL	28/M	1 yr	5+	–	–	+
BL	22/F	Relapsed 1 yr ago	Not known	+	+	+
BT	30/F	3 yr	–ve	+	+	+
BT	65/M	1 yr	–ve	+	–	+
BT	25/M	2 yr	–ve	–	–	+
BT	25/F	1 yr	–ve	+	–	–

Flow Laboratories. All assays were done in triplicate. The cut-off values for sero reactivity were determined by adding 2 SD to the mean absorbance of the healthy controls who had no apparent neurological damage.

Results

Out of 18 normal controls, one (5·6%) had significant levels of antineural antibodies of the IgM type and none had significant levels of antineural antibodies of the IgG type. Out of the neurological diseases other than leprosy, the sera obtained from the 2 Guillian–Barré syndrome sufferers had significant levels of antineural antibodies of the IgG type, whereas none of sera from the other diseases had any significant levels of these antibodies.

In total, 9 leprosy patients (9%) had significant levels of IgG antibodies and 11 patients (11%) had significant levels of IgM antineural antibodies. They formed 2 largely nonoverlapping groups with only 2 patients positive for both isotypes. The clinical and bacteriological details of these 2 groups are given in Tables 4 and 5. The optical density values obtained with ELISA for the sera of leprosy patients and normal controls are

Table 5. Details of cases with demonstrable levels of IgM antibody

Type of leprosy	A/S	Duration of disease	BI	Nerve enlargement	Active neuritis	Whether on treatment
LL	60/M	Relapsed 5 yr ago	1+	+	–	+
LL	35/M	Relapsed 2 yr ago	1+	+	–	+
LL	33/M	2 yr	3+	+	+	+
LL	45/M	6 yr	4+	–	–	+
LL	45/M	10 yr	2+	–	–	+
LL	45/M	11 yr	3+	+	–	+
BT	35/M	13 yr	–ve	+	–	–
BT	45/M	4 month	–ve	+	–	+
BT	30/M	6 yr	–ve	+	–	+
BT	40/M	2 yr	–ve	–	–	–
BT	50/M	4 month	–ve	–	–	–

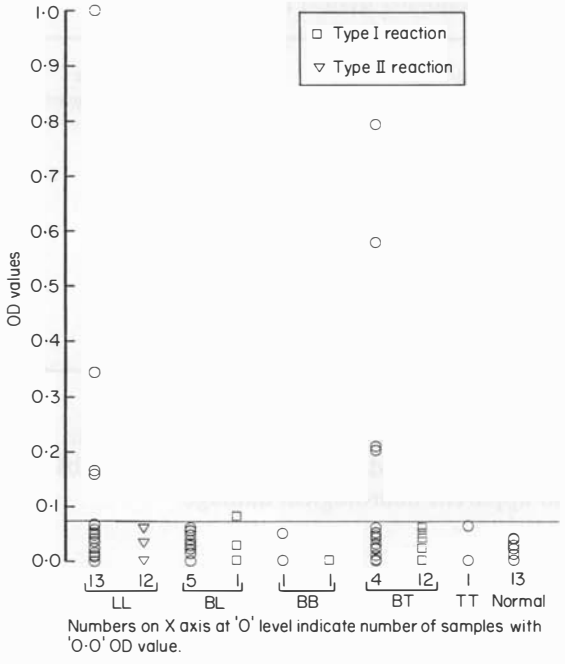

Figure 1. Optical density values with antigen derived from normal nerve using antiIgG.

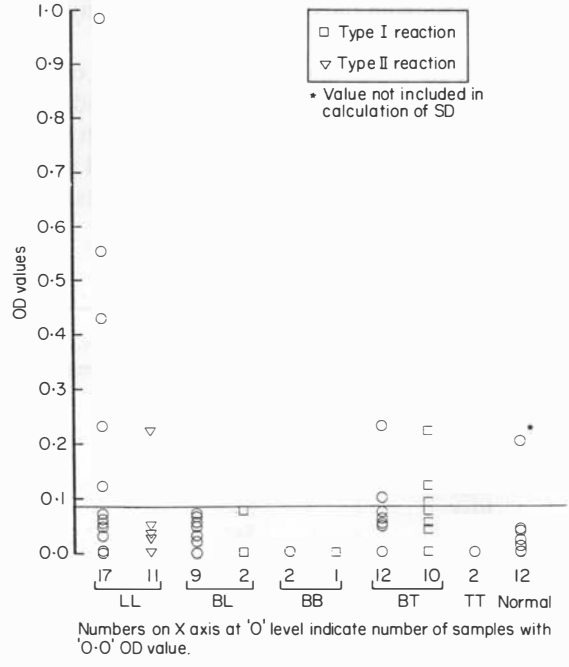

Figure 2. Optical density values with antigen derived from normal nerve using antiIgM.

Table 6. Comparison of the various clinical and laboratory attributes of leprosy with the prevalence of significant levels of antineural antibodies of the IgG type

	LL ($n = 43$)		BL ($n = 18$)		BB ($n = 3$)		BT ($n = 34$)		TT ($n = 2$)	
	+	−	+	−	+	−	+	−	+	−
	4	39	1	17	0	3	4	30	4	2
No. of cases with active neuritis	1	5	1	4	−	0	1	6	−	1
No. of cases with nerve enlargement	1	16	1	17	−	1	3	15	−	2
No. of cases with reaction	0	15	0	2	−	1	0	20	−	0
No. of cases on treatment	3	30	1	14	−	0	3	15	−	1
No. of cases with BI: 0 − 2+	1	22	0	6	−	2	4	25	−	1
No. of cases with BI: > 2+	3	17	0	10	−	0	0	0	−	0
No. of cases with BI: Not known	0	0	1	1	−	1	0	5	−	1
No. of cases with duration of dis.*: < 1 yr	1	4	0	1	−	2	0	10	−	1
No. of cases with duration of dis.*: 1 − 4 yr	2	23	1R	11	−	0	4	16	−	1
No. of cases with duration of dis.*: > 4 yr	1	12	0	5	−	1	0	4	−	0

+, No. of cases with significant levels of antineural antibodies; −, No. of cases without any significant levels of antineural antibodies; dis.*, disease; R, relapsed case.

shown in Figures 1 and 2. A comparison of the clinical and laboratory attributes of leprosy with the prevalence of significant levels of antineural antibodies of IgG and IgM was done. The details are given in Tables 6 and 7.

Using the χ^2-test, there was no significant association ($P > 0{\cdot}05$) between the type of leprosy, the BI and the presence of significant levels of antineural antibodies of the IgG or IgM type; nor was there any significant association ($P > 0{\cdot}05$) between the treatment taken and the presence of detectable levels of antineural antibodies. Using Fisher's exact test, there was no significant association between the presence of detectable levels of antineural antibodies and nerve enlargement, active neuritis and duration of the disease.

Table 7. Comparison of the various clinical and laboratory attributes of leprosy with the prevalence of significant levels of antineural antibodies of the IgM type

	LL ($n = 43$)		BL ($n = 18$)		BB ($n = 3$)		BT ($n = 34$)		TT ($n = 2$)	
	+	−	+	−	+	−	+	−	+	−
	6	37	0	18	0	3	5	29	0	2
No. of cases with active neuritis	1	5	−	5	−	0	0	7	−	1
No. of cases with nerve enlargement	4	13	−	14	−	1	3	15	−	2
No. of cases with reaction	1	14	−	0	−	0	3	2	−	0
No. of cases on treatment	6	27	−	15	−	0	2	16	−	1
No. of cases with BI: 0 − 2+	3	20	−	6	−	2	4	25	−	1
No. of cases with BI: > 2+	3	17	−	10	−	0	0	0	−	0
No. of cases with BI: Not known	0	0	−	2	−	1	0	5	−	1
No. of cases with duration of dis.*: < 1 yr	0	5	−	1	−	2	2	8	−	1
No. of cases with duration of dis.*: 1 − 4 yr	2 (1 + R)	23	−	12	−	0	1	19	−	1
No. of cases with duration of dis.*: > 4 yr	4 (3 + R)	9	−	15	−	1	2	2	−	0

+, No. of cases with significant levels of antineural antibodies; −, No. of cases without any significant levels of antineural antibodies; dis.*, disease; R, relapsed case.

Discussion

The results obtained after ELISA showed that 9% had significant levels of IgG antineural antibody and 11% had significant levels of IgM antibody. None of the normal controls had detectable levels of the IgG type of antineural antibodies, but 1 control (5·6%) had significant levels of IgM type of antineural antibody. There was no significant association with the type of leprosy, BI, treatment received, presence of nerve enlargement or active neuritis and the duration of disease.

Most of the patients with significant levels of antineural antibodies belonged to either the LL or BT group, but considering the fact that a large number of patients who were included in the study belonged to these 2 groups, in comparison with the other groups this was quite predictable.

Of the other neurological diseases tested for antineural antibodies, the sera from the 2 Gullian–Barré syndrome sufferers had significant levels of antineural antibodies of the IgG type and none of the others had significant levels. This was expected because the Gullian–Barré syndrome has a documented autoimmune pathogenesis. Thus these 2 sera have acted as positive controls for the ELISA test.

Similar to the results obtained in our study, Chujor *et al.*[3] have reported 4·1% of the leprosy patients and 5·6% of the normal sera positive for antineural IgG antibodies. Ghaswala *et al.*[6] were not able to demonstrate any antibodies in their series as the optical density values of patients' sera were within those of normal controls. Thomas & Mukherjee,[16] on the other hand, have shown 100% positivity in all of their 258 sera tested. None of their normal controls were positive. Such variation in results are difficult to explain. The 100% positivity shown by this group[16,11,12] has not been substantiated by any of the studies carried out so far.[3,5,6,10,13,18]

Benjamin *et al.*,[1] using immunoblot and antigen prepared from intermediate filament derived from human spinal cord, showed a high positivity of antineural antibodies (47%) in leprosy patients, but the positivity in normal controls was also high, ranging from 20% in American subjects to 41·6% in Ethiopians. Eustis Turf *et al.*[5] used indirect immunofluorescence and found 40% positivity in leprosy patients. Our study gave a 9% level of significant IgG antibodies in leprosy patients.

This variation in results is probably due to different kinds of antigens used. In the studies where human peripheral nerve antigen has been used,[3,11,12,16] the type of proteins predominating in the preparation were probably different. The major protein in experiments by Chujor *et al.*[3] was PNS myelin protein Po with 28 kDa mol. wt, whereas, in the antigen prepared by Thomas & Mukherjee[16] there was a major band in the 40–70 kDa mol. wt. The SDS–PAGE protein analysis of the antigen used in this study showed bands migrating at about 15–22 kDa and 25–27 kDa.

Other studies using animal nerve as antigen have found varying percentages of positivity. Wright & Hirst,[18] using rat sciatic nerve, found 25% positivity, but 22·2% of normal controls were also positive. Mshana *et al.*[10] used bovine sciatic nerve myelin basic protein and found 13% positive and Om Parkash *et al.*[13] used rabbit nerve and found 22% positive. However, we have used human peripheral nerve as the source of antigen. This might explain the differences in these results and the results obtained by us.

In our study the number of leprosy patients with significant levels of IgM antineural antibodies (11%) were more than those with detectable levels of IgG antibodies (9%).

Patients with significant levels of IgM antibodies belonged only to the LL and BT group. However, again considering the fact that a large number of patients who were included in the study belonged to these 2 groups in comparison with the other groups, this was quite predictable. Thomas & Mukherjee[16] tested only 35 patients with antihuman IgM conjugates. They obtained IgM antibody titres in the range of 0·14 to 0·67 OD. We have also obtained values in a similar range, with only 1 lepromatous leprosy patient having a high value of 0·984 OD. Chujor *et al.*[3] also characterized antineural antibodies with respect to immunoglobulin classes and found that the antibodies they detected belonged mainly to the IgG and IgM class. However, Benjamins *et al.*,[1] using intermediate filament fraction of the human spinal cord as an antigen, were not able to detect any IgM antibodies using the immunoblot technique. Others[5,6,13,18] did not study the sera for antineural antibodies of the IgM type. IgM antibodies are the earliest antibodies to appear. However, in our study, there was no significant association between the presence of IgM antibodies and a recent onset of the disease. We looked for such an association because IgM antibodies are usually associated with the early stages of any infection.

Conclusions

This study is not able to demonstrate any statistically significant levels of antineural antibodies associated with any type of leprosy, or any specific feature of the disease ($P > 0{\cdot}05$). It cannot be said with any certainty that an autoimmune pathogenesis exists for neuropathy in leprosy. However, further studies will be required to confirm this assumption.

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