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# Neural Auto-Antibodies in Leprosy

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A new axonal antibody confined to peripheral nerve has been found at a significant titre of 1/30 by indirect immunofluorescence. This antibody occurred in 23 out of 59 sera from patients with lepromatous leprosy, 2 out of 10 sera from patients with tuberculoid leprosy from Malaysia and 1 out of 12 sera from patients with leprous neuropathy from Ethiopia, 4 out of 6 sera from patients with post-infective polyneuropathy and 6 out of 271 control sera.

Failure to remove the axonal antibody by absorption with BCG in six sera from patients with lepromatous leprosy and four sera from patients with post-infective polyneuropathy suggested that this was not a cross-reaction with *Myco. leprae* or other mycobacteria, but probably occurred as an epiphenomenon following nerve injury. The finding of axonal antibodies at a titre of 1/10 in 43 out of 271 control sera (15.8%) also suggests that the antibody's presence is not related to nerve damage. There were no antimyelin antibodies found in any of the sera studied.

## Introduction

The presence of auto-antibodies in lepromatous leprosy has been widely investigated. Auto-antibodies have been found against testis (Wall and Wright, 1974), thyroid, nuclear material (Bonomo *et al.*, 1963) and cardiolipin (Ruge *et al.*, 1960), as well as rheumatoid factors and cryoglobulins (Matthews and Trautman, 1965). Antibody plays a part in the pathogenesis of erythema nodosum leprosum (ENL) and appears to cause tissue damage by immune complex formation (Wemambu *et al.*, 1969; Moran *et al.*, 1972).

Nerve involvement is found across the whole spectrum of leprosy (Ridley and Jopling, 1966; Pearson, 1972). In the tuberculoid form of the disease, nerve damage is associated with few leprosy bacilli and a granulomatous reaction

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(Pearson, 1972; Ridley, 1973) indicating an increase in cell mediated immunity. In contrast, in early lepromatous leprosy there is an insidious, continued loss of endoneurium associated with increasing numbers of *Mycobacterium leprae* in the surrounding Schwann cells (Ridley, 1973; Weddell *et al.*, 1964); at that stage little or no sensory loss may be detected, although at a later stage of the disease, progressive clinical neuropathy frequently occurs. In addition, especially in treated lepromatous patients, episodes of mononeuritis multiplex is a common feature of ENL. As it was considered that antibodies might play a role in producing lepromatous (but not tuberculoid) neuritis, the sera of lepromatous patients were investigated for the presence of antibodies capable of reacting with nerve tissue. For comparison, sera from patients with acute post-infective polyneuritis (Guillain-Barré syndrome) were also studied, as an immunological pathogenesis (Luijten *et al.*, 1972) has been suggested in this condition.

## Sources of Sera

### (1) LEPROSY

These studies were undertaken on leprosy patients from the Leprosy Research Unit at Sungei Buloh, Malaysia. Sera were examined from 59 adult patients, 50 males, aged from 14 to 71 (mean age 39 years, average age 35.5 years) and nine females, aged 21 to 48 (mean age 33 years, average age 32.7 years), suffering from bacilliferous leprosy; 56 were classified as lepromatous (LL) and three borderline lepromatous (BL) on the Ridley-Jopling spectrum (1966). Three patients were untreated, and six more also had active uncontrolled leprosy, three having relapsed through failure to continue on dapsone therapy and three having developed drug-resistant disease. The remaining 50 patients were all receiving effective treatment of varying duration. Forty-two had ENL at the time of entering the study, and three had had ENL in the past. None of the BL patients, and none of the untreated, or relapsed, off treatment LL patients had ENL. In 12 of the patients, a second sample of serum was obtained, 24 days to 15 months after the first. Some degree of peripheral nerve enlargement was detected in all patients, and evidence of motor and/or sensory nerve damage was found in the majority. Six patients were suffering from active ENL neuritis at the time of the taking of their blood specimen, and 21 more had either been diagnosed as suffering from ENL neuritis in the past or else developed the condition within 3 months of the time of study. Ten sera from patients with tuberculoid leprosy were also tested.

The patients were of Malay, Chinese, Indian or aboriginal origin, or a mixture of these races.

In addition, 12 sera were taken from patients with leprosy attending the MRC research unit, Addis Ababa, Ethiopia, in whom clinical nerve damage was present. The tuberculoid form was present in four of the patients, two were borderline and the remaining six patients had the lepromatous form, four with ENL.

### (2) NERVOUS SYSTEM DISEASE

Sera from 33 adult patients under the care of the Neurological Department, Guy's Hospital, with a variety of nervous diseases (see Table 1) were studied. Nineteen were males and 14 females (mean age 37 years). The diagnosis of post-infective polyneuropathy was accepted only when there was a predominantly motor peripheral neuropathy following a minor upper respiratory or gastro-

intestinal infection, combined with cyto-albuminuric dissociation in the cerebrospinal fluid (Hinman and Magee, 1967).

(3) AUTO-IMMUNE DISEASE

This group consisted of 59 sera with auto-antibodies against nuclear material (24), gastric parietal cells (10), thyroid microsomal cells (10), reticulin (5), mitochondria (10); in addition four sera with high agglutinin titres against A and B blood group substances and two rabbit sera with antibodies experimentally raised against rat myelin (Gregson *et al.*, 1971) were studied.

(4) CONTROLS

Thirty eight sera from male Malaysian Chinese patients suffering from minor non-neurological conditions, 40 lyophilised sera from Ethiopian patients with relapsing fever, 40 sera from general hospital in-patients (Guy's) and 90 sera from English blood donors were studied as controls.

## Methods

(1) IMMUNOFLUORESCENT TESTS

The unfixed and delipidated frozen rat tissue substrates used included transversely sectioned sciatic nerve with striated muscle, large intestine for myenteric nerve, and cerebellum. Unfixed guinea-pig peripheral nerve sections were also tested. Delipidation was carried out by soaking the 5 $\mu$m frozen section in warm acetone (28°C) for 2 min, followed by a series of "soaks" in warm ether (Lacey, 1972). In addition, frozen human testicular sections were treated with the lepromatous leprosy sera.

The indirect immunofluorescent test was carried out according to the WHO Manual of auto-immune serology (1969) except that it was not found necessary to inactivate sera at 56°C and the test was conducted at room temperature. Saline dilutions of positive sera at 1:10, 1:30 and doubling dilutions thereafter were tested to determine the antibody titre. A significant titre was taken as 1:30 (see Table 2). The following conjugates were used: sheep antipolyvalent human globulin conjugate (Wellcome Laboratories), specific sheep anti-human IgG, IgM and IgA conjugates (Wellcome Laboratories), rabbit anti-human $\beta$ iC/$\beta$ia conjugate (Behringwerke), anti-Fic conjugate (Hyland), and sheep anti-rabbit mixed immunoglobulin conjugate (Wellcome Laboratories). Results were recorded as negative, equivocal (±), positive or strongly positive. The microscope used was an "Ortholux" Leitz incident light microscope, with a quartz iodine light source for transmitted light and an HBO 50 lamp for incident light, a Turner interference filter 4950 AU (Gillett and Sibert) and an Ilford micro 4-blue gelatine secondary filter number 110. The incident light was used with a number three beam splitting dichroic mirror (TK 495) with two K 495 suppression filters in the turret. Both methods of illumination were used simultaneously.

(2) OTHER TESTS

The effect of absorption with BCG 0.2 mg moist weight, grown on the surface of Sauton's medium and twice washed, was investigated in 6 sera from patients with lepromatous leprosy and 4 sera from patients with post-infective poly-neuropathy. The efficacy of the process was assessed by testing the sera for

specific antibody before and after absorption, using immunoplates containing BCG antigen. Five sera from patients with lepromatous leprosy were absorbed with AB substance. Further absorption studies using sheet red blood cells were carried out on these sera. Immunoglobulin levels were determined in 20 sera from patients with lepromatous leprosy, using the radial diffusion method (Mancini *et al.*, 1965).

## Results

Immunofluorescence was detected on the axons of the sciatic nerves of rats in 23 of the 59 sera from patients with lepromatous leprosy including 3 out of 3 with borderline leprosy from Malaysia, 2 out of the 10 patients with tuberculoid leprosy, and in 4 of the 6 sera from patients with acute post-infective polyneuropathy (Table 3). In most cases, the titre was low (1:30); the highest was 1:100. Only 6 out of the 271 sera from control cases tested (see Table 1) and one out of the 25 sera from patients with other neurological disease and drug peripheral neuropathy gave a positive axonal fluorescence. There was no statistical difference between the number of positives in the patients with neurological diseases, Malaysian control group, and the diverse control group ($P > 0.7\chi^2$ test). Two of the positive reactions in this group occurred in healthy adults, one from the blood donor group and one from a healthy patient with anti-nuclear factor present in his serum, while the other two positives occurred in the hospital in-patient group, one with coronary insufficiency and the other with disseminated lupus erythematosis. In the neurological control group, the positive test was found in a patient with neurosyphilis. In contrast, axonal staining was found only in one serum out of 12 tested, from patients (from Ethiopia) with nerve damage due to leprosy. There was no statistical difference in the incidence of axonal staining between this group and that of the non-leprosy relapsing fever Ethiopian control group and the diverse general controls (Table 3).

TABLE 1

*Neurological diseases group*

| | Total |
|---|---|
| 1. *Peripheral neuropathy* | |
| 1. Post-infective neuritis persistent | 5 |
| 2. Post-infective neuritis (resolved) | 1 |
| 3. Vincristine neuropathy | 1 |
| 4. Nitrofurantoin neuropathy | 1 |
| 2. *Other Neurological diseases* | |
| Tabes | 3 |
| Meningovascular syphilis | 1 |
| Disseminated sclerosis | 9 |
| Cerebrovascular accident | 4 |
| Motor neurone disease | 2 |
| Epilepsy | 3 |
| Headaches | 3 |
| | 33 |

TABLE 2

*Distribution of "Axonal" staining in control series*

| Source | Positive 1/10 | 1/30 | Total tested |
|---|---|---|---|
| Blood donors | 6 | 1 | 90 |
| General hospital in-patients | 19 | 1 | 40 |
| Relapsing fever (lyophilized), Ethiopian | 5 | 0 | 40 |
| Non-leprosy Malaysian-Chinese (in-patients) subjects | 0 | 2 | 38 |
| Autoantibody sera | | | |
| 1. Antinuclear antibody | 7 | 2 | 24 |
| 2. Gastric parietal antibody | 1 | 0 | 10 |
| 3. Thyroid microsomal antibody | 2 | 0 | 10 |
| 4. Reticulin antibody | 1 | 0 | 5 |
| 5. Mitochondrial antibody | 1 | 0 | 10 |
| 6. High titre agglutinins to AB blood groups | 1 | '0' | 4 |
| | 43 (15.8%) | 6 (2%) | 271 |

TABLE 3

*Distribution of "Axonal" staining at a serum dilution of 1/30*

| Source | Positive | Total |
|---|---|---|
| Lepromatous leprosy from Malaysia | | |
| 1. Active Neuritis present | 5 | 6 |
| 2. Active Neuritis absent | 18 | 53 |
| Tuberculoid leprosy from Malaysia | 2 | 10 |
| Malaysian non-leprosy subjects | 2 | 38 |
| Leprosy with Neuritis from Ethiopia | 1 | 12 |
| Non-leprosy (relapsing fever) from Ethiopia | 0 | 40 |
| Acute post-infective polyneuritis | 4 | 6 |
| Other neurological diseases | 1 | 29 |
| Control series (see Table 2) | 6 | 271 |

The presence of axonal staining in the sera from Malaysian lepromatous patients was highly significant when compared with sera from the control group ($P< 0.005\ \chi^2$), as was the presence of this reaction in sera from patients with acute post-infective polyneuropathy, bearing in mind the small number of observations, when compared either with the control group derived from the neurological department ($P< 0.005\ \chi^2$) or with the general control group ($P< 0.0005\ \chi^2$ ). The finding of the axonal antibody in 5 out of the 6 sera from patients with lepromatous leprosy and active ENL neuritis was suggestive of an association but did not quite reach a level of statistical significance, since the axonal antibody was also present in 18 of the sera from 53 lepromatous patients in whom no evidence of acute or subacute episodes of ENL neuritis was found ($P< 0.02\ \chi^2$).

There was no obvious association between ENL and the presence of axonal antibody, as the axonal fluorescence was detected in 17 out of the 45 patients

with present or past ENL and 6 out of 14 in whom ENL had never occurred. Of the 12 patients who had second specimens of sera tested, 5 were positive initially and negative when subsequently tested; however, 2 of the 7 patients who were initially negative were found to be positive when their second specimens were tested. In no patient was the immunofluorescent finding unchanged, perhaps indicating the transient nature of this reaction. The two patients without antibodies initially but who were positive on re-testing, were included in the positives referred to above.

There was no relationship between the presence of testicular antibodies and the axonal antibodies described above, since axonal antibodies were found in 17 out of the 52 leprosy sera with testicular antibodies and 6 of the 19 without ($P < 0.3\ \chi^2$). The prevalence of antinuclear factor using anti-IgG conjugates was low (2%) but with anti-IgM 37% of the sera were positive.

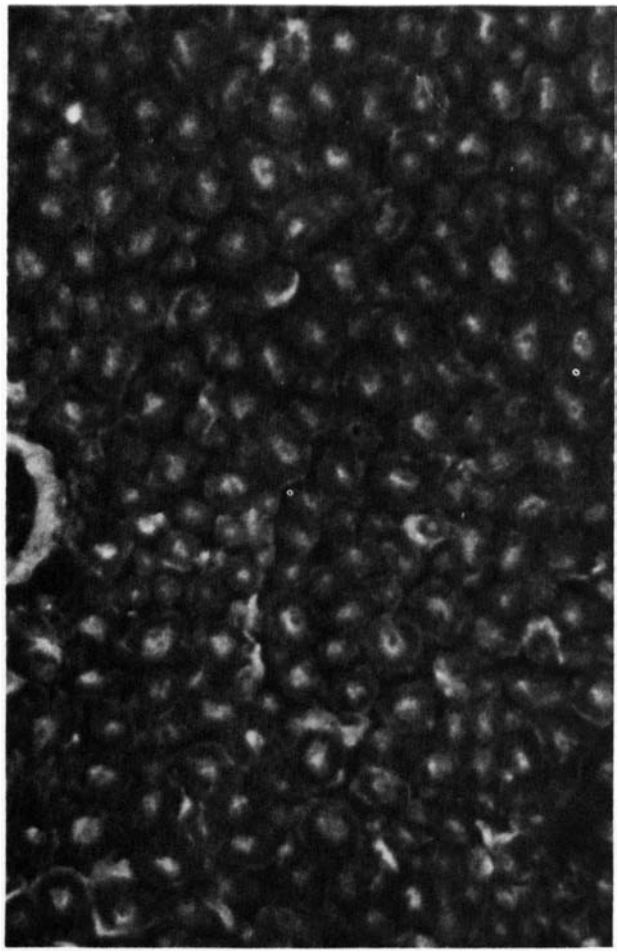

Fig. 1. Axonal fluorescence demonstrated by an anti-IgG fluorescent conjugate on rat sciatic nerve (acetone-ether fixed). (X 400.)

The immunofluorescent staining obtained with a proportion of the human sera tested was found on the sciatic nerve and was confined to the axon, staining it diffusely (Fig. 1). When nerve sections were cut longitudinally, the axonal staining appeared more filamentous than that produced by antimyelin antibodies. This immunofluorescence was not found on the axons of nerves in the cerebellum or those of the myenteric plexus. It is not definitely known whether this staining pattern was purely axonal or whether a component of myelin was also involved. Nevertheless, the participation of myelin is unlikely because in contrast, when sera containing guinea pig anti-rabbit myelin antibody was tested, the central core of the nerve fibre was left unstained but the myelin layer of the cerebellum (the medulla) fluoresced brightly. Denser axonal patterns were obtained in acetone-ether treated sections than in those left unfixed. The latter possessed the disadvantage that they gave patterns of staining which were blurred so that occasionally results were equivocal.

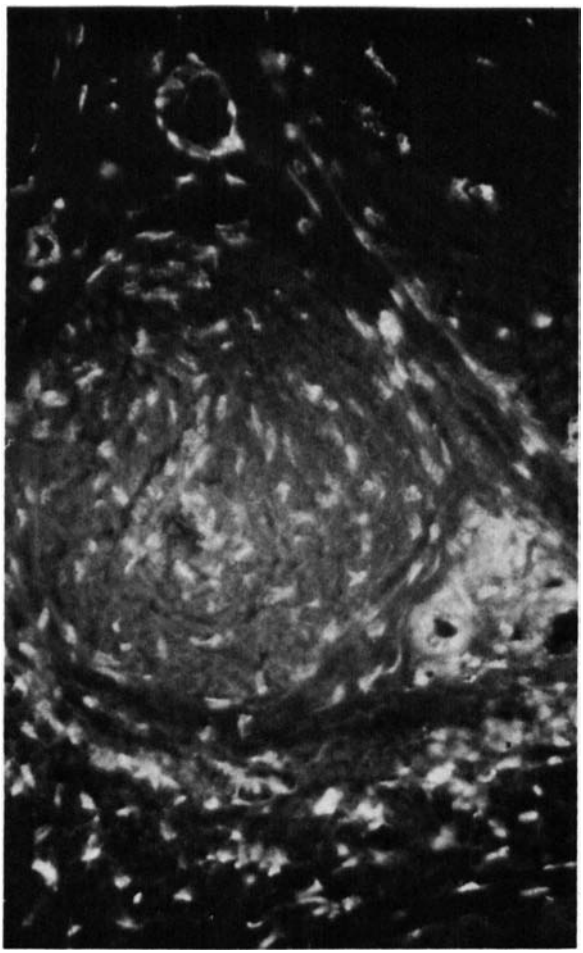

Fig. 2. Antinuclear factor staining nuclei of Schwann cells, as above. (X 250.)

The pattern of staining found on peripheral nerve was distinct from that obtained with antinuclear factor, which stained only the nuclei of the Schwann cells (Fig. 2). The presence of antinuclear factor in the appropriate sera was confirmed by observing that these picked out the nuclei of the granular layer of the cerebellum (Fig. 3), an appearance not to be confused with the cytoplasmic diffuse staining of the granular layer found with sera containing any of the mitochrondrial antibodies (Fig. 4). No consistent pattern of staining of peripheral nerve fibres was found when sections were treated with sera from any of the autoimmune disease control patients. (See Table 2.) Treatment of the nerve sections with anti-A and anti-B sera did not reproduce the staining pattern, nor could axonal fluorescence be inhibited by absorption of axonal positive sera with blood group substance; hence the fluorescence produced by leprosy sera was not due to anti-B blood group antibody. Sheep red cells were also ineffective in

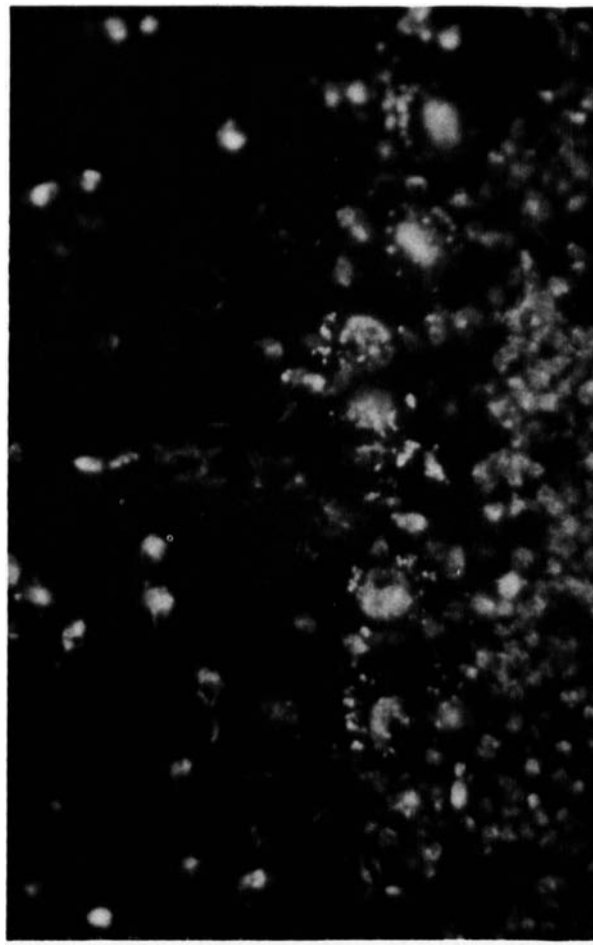

Fig. 3. Antinuclear factor staining the nuclei of granular layer of rat cerebellum. (Note staining of Purkinje cells), as above. (X 400.)

abolishing the axonal staining pattern indicating that this was not due to Forssmann-type antibodies.

Axonal fluorescence was restricted to the IgG class of immunoglobulin, since no staining was found when anti-IgM or anti-IgA conjugates were used. Fixation of complement was found in 4 out of 16 sera examined using anti-βic/βia conjugates. The immunofluorescent pattern was not abolished by repeated freezing and thawing or heating to 56°C. The staining pattern was not species-specific, identical fluorescence being found on rat and guinea-pig peripheral nerve substrates. The fluorescence was not due to non-specific absorption of circulating immunoglobulin, since there was no significant difference between the mean levels of IgG, IgM or IgA of sera with axonal antibodies and those without antibodies. The application of anti-Fic conjugate on the sciatic nerve sections did not result in axonal staining.

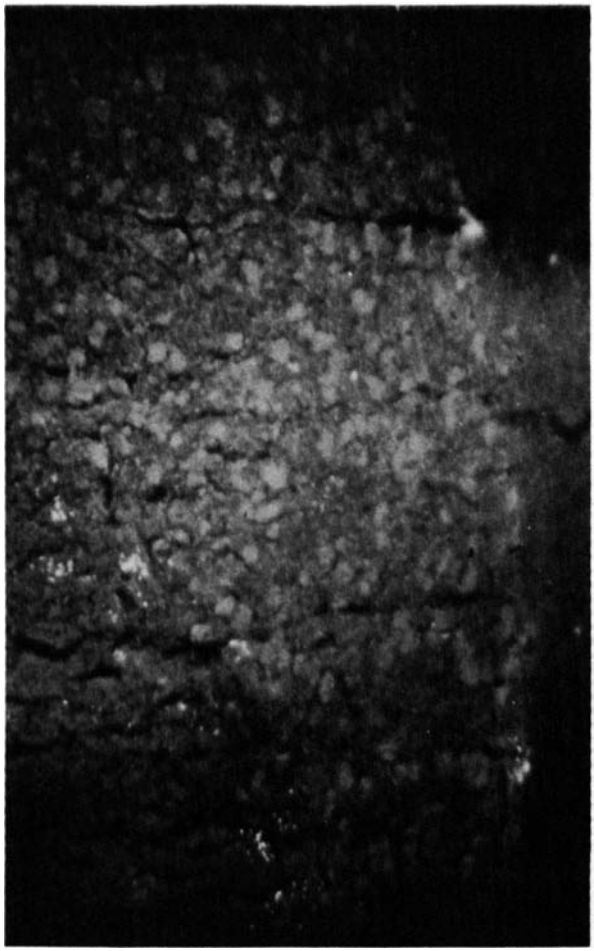

Fig. 4. Mitochondrial antibody staining the cytoplasms of the cells of the granular layer of rat cerebellum, as above. (X 250.)

When positive sera from patients with lepromatous leprosy or post-infective polyneuropathy were repeatedly absorbed with BCG, there was no resulting loss of axonal immunofluorescence or reduction in titre. This implied that the peripheral nerve pattern of staining was not restricted to leprosy or to mycobacterial disease, nor did the leprosy bacillus appear to be a specific inducer of this pattern.

## Discussion

The selective binding of antibody to peripheral nerve axons has not been previously described. It is surprising that the axons of the nerves of the autonomic and central nervous system are not stained by this antibody, but this could be because of subtle antigenic differences in the molecular structure or denaturation products of peripheral nerve axon (Knowles *et al.*, 1969). It is possible that if tests were carried out on central nervous system tissue substrates using a serum containing antibody of high titre, axonal staining might be observed as clarity is sometimes difficult to achieve. There was certainly no correlation between the occurrence of axonal antibodies and antinuclear antibodies or anti-testicular antibodies, both present in leprosy (Wall and Wright, 1974). Reports in the past, using crude animal organ extracts, implied that there was a degree of immunological cross-reactivity between such disparate organs as testis and brain (Lewis, 1934), or thyroid and spinal chord (Beutner *et al.*, 1958). This has not been confirmed using more refined methods (Whittingham *et al.*, 1972). Similarly, the axolemmal staining described by Tomasi and Kornguth (1968) using an immunofluorescent method was quite distinct from axonal staining. Furthermore (their suggestion that axolemmal staining cross reacts with nuclei in the central nervous system was not confirmed) using anti-IgG conjugate in almost all our preparations.

The clinical association of axonal antibodies both with possibly post-infective neuropathy, a demyelinating condition, and with lepromatous leprosy, in which the site of the primary pathological change is not axonal but endoneurial, or in ENL, perineurial (Pearson, 1972), indicates that the pathogenesis is non-specific. The inability to absorb out fluorescence with mycobacteria provides further evidence that these antibodies may arise following injury to peripheral nerve. When nerve damage occurs in lepromatous leprosy, the Schwann cells and perhaps the perineurial cells release *Myco. leprae*, (Pearson, 1974; Weddell *et al.*, 1964) which may then lie either in close proximity to nerve axons or rarely in their interior (Boddingius, 1974). The unmasked axons combined with the adjuvant potential of the leprosy bacilli (Stewart-Tull and Davies, 1972) may provide a weak yet important antigenic stimulus for the production of axonal antibodies.

The detection of axonal antibodies in a proportion of leprosy patients in whom obvious progressive clinical neuropathy could not be detected suggests that sub-clinical nerve damage was occurring and further, that serial axonal antibody estimations may be helpful in the study of the incidence, natural history and treatment of nerve damage in these patients. The failure to relate axonal antibody to ENL is interesting, but must be taken with some reserve in view of the small number of lepromatous patients included in our series who had never suffered from this complication. The occasional positive reaction detected in our control series may also have been due to sub-clinical nerve damage, of uncertain aetiology. The virtual absence of axonal antibody from patients with leprosy neuropathy

from Ethiopia, may alternatively reflect racial variation in auto-antibody responses rather like that found with thyroid auto-antibodies in leprosy (Wright, 1973).

The finding of axonal antibodies is a rarity, whether in a Malaysian, European or Ethiopian population. Edgington and Dalissio (1970) did not detect any axonal staining in their normal control series, although they did report a high incidence of antimyelin antibodies, a finding which we could not confirm. A possible explanation is that they carried out their immunofluorescent tests using either neat sera or with sera in very low dilutions, just as, at a titre of 1/10, our control series sera more often showed axonal staining than at 1/30.

Allerand and Yahr (1964) could not demonstrate any affinity of immunoglobulin for normal central nervous system tissue, although this may not be true for the peripheral nerves (Abe, 1973). Furthermore, high levels of immunoglobulin, especially in such conditions as lepromatous leprosy (Bullock *et al.*, 1970), may well predispose to non-specific deposition of immunoglobulin on nerve fibres. However, direct measurements of the immunoglobulin levels were similar whether axonal staining was present or not. Thus the non-specific deposition of immunoglobulins on nerve fibres in leprosy is very unlikely, especially as sera from the majority of the patients who had ENL, the immune complex reaction of leprosy (Wemambu *et al.*, 1969), gave no neuronal immunofluorescent staining. Previously, the only instance of binding of immunoglobulin to human peripheral nerve was demonstrated by Luijten *et al.* (1972), who found that complement-binding IgM antibodies were deposited along the myelin sheaths of the autochthanous nerve fibres in four out of six cases of the Guillain-Barré syndrome, in which the dominant pathological change is demyelination. This finding could well have been due to the unmasking of axonal antigens following demyelination or perhaps related to axonal regeneration (McGuire and Grafstein, 1973) following injury, and similar mechanisms could explain the changes in lepromatous leprosy.

Like Diedreichsen and Pyndt (1968), we were unable to find anti-myelin antibodies or even any axonal staining with anti-IgM conjugates in the Guillain-Barré syndrome. This was surprising as myelin or myelin constituents, such as myelin basic protein, can evoke a humoral response especially in animal models such as allergic encephalitis (Mackay *et al.*, 1973; McFarland, 1970; Field *et al.*, 1963; Sherwin *et al.*, 1961) and experimental allergic neuritis (Winkler, 1965). However, this may bear no relation to the Guillain-Barré syndrome.

Furthermore, a defect in cell-mediated resistance is described in lepromatous leprosy (Dwyer *et al.*, 1973; Godal *et al.*, 1972; Rees and Waters, 1972). There is some evidence that auto-immune disorders may be associated with pathological processes in the thymus and that the immunological defect is due to a reduction in the level of thymic hormone. Reduced titres having been found in the auto-immune state in NZB mice. It has been suggested that thymic hormone prevents sensitisation to self-antigens, especially as normal adult lymphocytes have now been shown to have receptors for self-recognition (Trainin *et al.*, 1973). In this respect, the lack of inhibitor in leprosy described by Abe (1973) may be relevant.

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