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Major Histocompatibility Complex Class II Antigen Expression in Nerves in Leprosy; an Immunoelectronmicroscopical Study[1]

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Mycobacterium leprae has a predilection for living within Schwann cells, and there has been much interest over recent years in whether or not Schwann cells are capable of presenting *M. leprae* antigens to T lymphocytes in leprosy. Studies on cultured Schwann cells suggest that they may be induced to present antigen under certain circumstances. Samuel, *et al.* ([13, 14]) have shown that rat and human Schwann cells are able to express major histocompatibility complex (MHC) class II antigens when treated with gamma-interferon *in vitro*. This is the first requirement for any potential antigen-presenting cell to fulfill before antigen can be recognized by CD4+ T lymphocytes. Wekerle, *et al.* ([16]) have shown that such interferon-gamma-stimulated rat Schwann cells are capable of presenting endogenous myelin autoantigens and tuberculin purified protein derivitive (PPD) to antigen-specific T lymphocytes. Kingston, *et al.* ([6]) have demonstrated, under somewhat more physiological conditions, that rat Schwann cells can be induced to express class II antigens without pretreatment with interferon-gamma if they are cultured together with soluble *M. leprae* extract and sensitized syngeneic-T lymphocytes. However, class II inducibility seems to be a species-specific phenomenon, since Steinhoff, *et al.* ([15]) have shown that mouse Schwann cells are induced to express class I but not class II antigens by interferon-gamma.

Evidence for antigen presentation by Schwann cells *in vivo* has been limited to demonstrating class II expression *in situ* on frozen tissue sections. By this method, several researchers have reported class II-positive cells, presumed by their morphology or by staining serial sections with panels of antibodies to be Schwann cells, in a variety of inflammatory neuropathies ([3, 7, 10, 11]), including leprosy ([9]). However, resolution at the light-microscopic level is not sufficient to easily distinguish Schwann cells from fibroblasts and infiltrating cells. Only at the electron-microscopic (EM) level can Schwann cells be clearly identified and intercellular relationships observed. Various immunoelectronmicroscopy (immunoEM) techniques have recently been developed, but the processing schedule required for electron microscopy means that it is very difficult to study labile cell surface proteins such as MHC antigens and CD antigens. This is particularly problematic in neural tissue where strong fixation with glutaraldehyde and osmium is necessary to preserve the ultrastructure, especially of myelin. There is one report using immunoEM on nerves from Guillain-Barré syndrome patients ([10]); the technique did not use glutaraldehyde but involved freezing the tissue, leading to very poor ultrastructure.

Butter, *et al.* ([2]) have developed a post-fixation, pre-embedding immunoEM technique which gives clear labeling of leukocyte antigens in neural tissue. This technique was used by Cowley, *et al.* ([4, 5]) in a guinea-pig model of nerve damage in leprosy, and it was found that although immunostaining of frozen sections suggested that Schwann cells might be class II positive, at the EM level they were negative while infiltrating cells and some fibroblast-like cells were class II positive.

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THE TABLE. *Staining and processing schedule for nerve biopsies.*

At 4°C:
1. Buffer (1 × 15 min)
2. Phosphate buffered saline (PBS) (2 × 15 min)
3. Quench unreacted aldehyde groups with 0.5 M glycine in PBS (30 min)
4. PBS (1 × 15 min)
At room temperature on a rocking table:
5. Blocking serum from Vectastain ABC kit (Vector Laboratories, Peterborough) (1 hr)
6. Primary monoclonal antibody (overnight)
7. PBS + 0.05% Tween 20 + 0.1% bovine serum albumin (PBST) (3 × 15 min)
8. Biotinylated horse anti-mouse Ig (Vectastain) in PBST (2 hr)
9. PBST (3 × 15 min)
10. 0.3% H_2O_2 in PBS (30 min)
11. PBST (3 × 15 min)
12. ABC complex (Vectastain) in PBST (2 hr)
13. PBST (3 × 15 min)
14. 0.05% diaminobenzidine (DAB) in PBS (15 min)
15. 0.05% DAB + 0.03% H_2O_2 (15 min)
16. PBS (3 × 15 min)
17. 1% OsO_4 in PBS (overnight)
18. Wash in 70% ethanol; dehydrate and embed in araldite as for routine electronmicroscopy

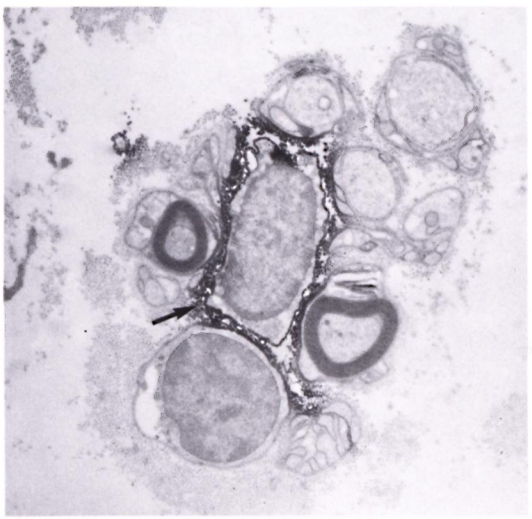

FIG. 1. Electronmicrograph of a normal, human tibial nerve stained with LN3 (antibody against HLA-DR), showing a class II-positive cell (arrow) adjacent to several class II-negative Schwann cells associated with both myelinated and unmyelinated axons (×12,000).

This study uses this immunoEM technique to investigate class II expression in leprosy nerve biopsies in humans.

MATERIALS AND METHODS

Patient Material. Biopsies of the sural or radial cutaneous nerves used for this study were taken from patients attending the All Africa Leprosy and Rehabilitation Training Centre (ALERT), Addis Ababa, Ethiopia. The study was approved by the ALERT/Armauer Hansen Research Institute (AHRI) Research Committee. Eight biopsies were from new, untreated patients and two were from patients who had been treated many years previously. All patients were undergoing nerve biopsies primarily for diagnostic purposes, usually for suspected neural leprosy where skin biopsies were uninformative. Ten suitable biopsies were obtained in which leprosy was subsequently diagnosed by histological examination of the nerve and where at least some neural tissue remained identifiable (very fibrosed or enormously infiltrated nerves were not used). According to the bacterial index (BI) within the granuloma of the nerve, the nerve lesions were classified as multibacillary (BI > 2, four cases) or paucibacillary (BI ≤ 2, six cases) ([8]). The BI within the granulomas was assessed in a 5-μm thick section in exactly the same way as in a skin smear. Three tibial nerves from amputations due to vascular disease and one normal radial cutaneous nerve biopsy served as normal control specimens.

Immunoelectronmicroscopy. The nerve biopsies were immediately fixed in cold 1% monomeric glutaraldehyde (Polysciences, U.K.) in Sorensen's reagent for 30 min. They were then supported in 2% agar and 200 μm transverse sections were cut, under fixative, on a Vibroslicer (Campden Instruments, U.K.). After a further 30 min in fixative, the sections were taken through the staining and processing schedule given in The Table. Transverse sections (1 μm) were cut and counterstained with toluidine blue for light microscopy, and suitable areas for ultrathin sectioning were selected. The sections were dried onto 200-mesh copper/rhodium grids and viewed, unpoststained, on an AEI 275 Corinth transmission electron microscope.

Monoclonal antibodies. All of the antibodies used were screened for suitability for this staining technique by preliminary Facscan analysis of antibody binding to glutaraldehyde-fixed human leukocytes (fresh and

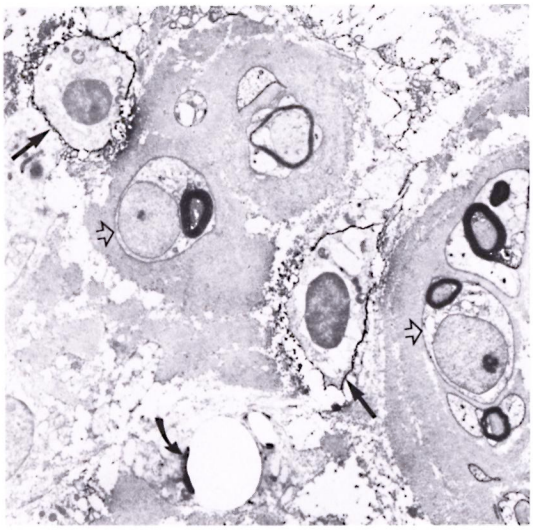

FIG. 2. Multibacillary leprosy nerve biopsy stained with SN130 (antibody against the restricted leukocyte common antigen), showing two positively stained leukocytes (straight arrows) in the vicinity of myelinating Schwann cells which are surrounded by dense collagen (hollow arrows); curved arrow = bacilli (×4500).

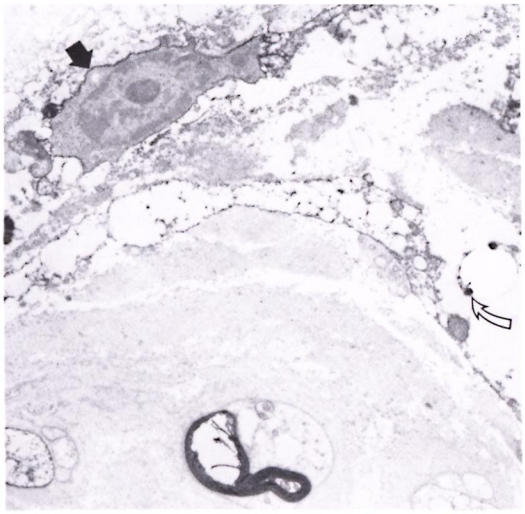

FIG. 3. Multibacillary nerve stained with UCHT1 (antibody against the CD3-complex on T cells), showing a positively stained T cell (straight arrow). Schwann cells are thickly surrounded by collagen; curved arrow = bacilli (×7500).

phytohemagglutinin-stimulated peripheral blood leukocytes) and also by immunostaining of human lymphoid tissue at the light- and electron-microscopic level (results not shown). Two monoclonal antibodies specific for MHC class II antigens were selected which were obtained from Biotest (W. Germany): LN3 (mouse IgG2a) reacts with a nonpolymorphic region of the HLA-DR region; Clonab DP/DR (mouse IgG2a) reacts with products of the HLA-DP region and also with the products of the DR region in some haplotypes. Monoclonal antibodies detecting other leukocyte cell-surface antigens were also used: UCHT1 (reacts with the CD3 complex on T cells); 2D1 (detects CD45, "leukocyte common antigen"); SN130 (detects the restricted form of the leukocyte common antigen, CD45R). Appropriate antibody and buffer controls were included in each processing run.

RESULTS

At the light-microscopic level, positively stained cells were identifiable by a brown ring, as with conventional immunoperoxidase techniques developed with diaminobenzidine (DAB). Under the electron microscope the reaction product was visible as an electron-dense (i.e., black) deposit on the cell surface. The sections incubated with control reagents were unstained except on the periphery of the block.

In normal, control nerves the only cells staining positively with the anti-class II antibodies were the luminal surface of endothelial cells and occasional endoneurial cells which were not associated with axons and did not have basement membranes (Fig. 1). There was no significant staining of normal nerves with any of the other antibodies.

In the diseased nerves, T cells and leukocytes were scattered throughout the endoneurium, as identified using the antibodies UCHT1, 2D1 and SN130 (Figs. 2 and 3). Although the infiltrating cells were found in the general vicinity of the remaining resident Schwann cells and axons, it was very rare to see a lymphocyte in close contact with a Schwann cell. In the multibacillary cases, there was dense collagen surrounding the neural cells which the infiltrating lymphocytes were not often seen to penetrate.

Staining with the anti-class II antibodies showed many positive macrophages (Figs. 4–6), lymphocytes (Fig. 7), and some positive fibroblast-like cells. No class II-positive Schwann cells, whether associated with myelinated, demyelinated or unmyelinated axons, were seen in any of the multibacillary or paucibacillary leprosy nerves studied

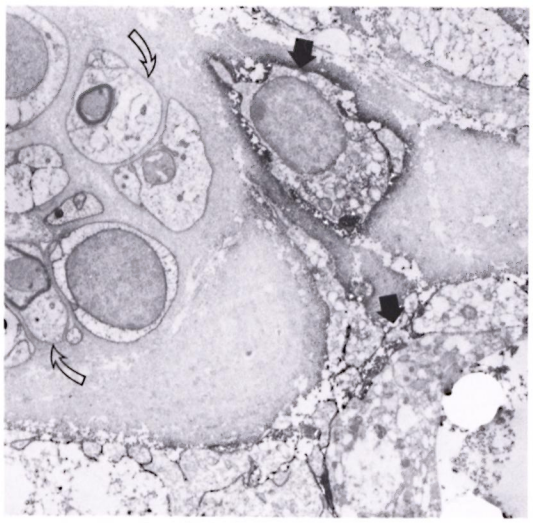

FIG. 4. Multibacillary nerve stained with LN3, showing positive macrophages (straight arrows) and negative Schwann cells (hollow arrows) surrounded by thick collagen (×7500).

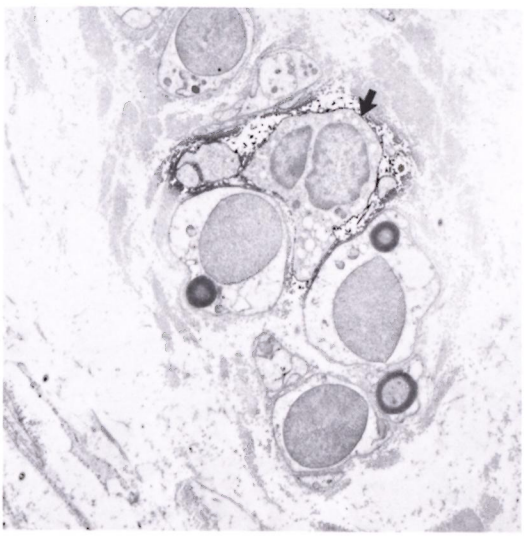

FIG. 6. Paucibacillary nerve stained with LN3, showing a class II-positive macrophage (arrow) among a group of Schwann cells associated with unmyelinated and small myelinated axons (×7500).

(Figs. 4–7). This was not due to inaccessibility of the cells to label, since often a positively labeled mononuclear cell would be in the vicinity of negative Schwann cells (as in Fig. 4).

DISCUSSION

Immunohistochemistry is always a compromise between good tissue preservation and good antigen labeling. In frozen sections, for example, antigen preservation is good but morphological information is very limited. With EM, the range of antibodies that can be used is restricted to those which can detect glutaraldehyde-fixed antigen. The technique we have employed uses relatively weak glutaraldehyde fixation and this, to-

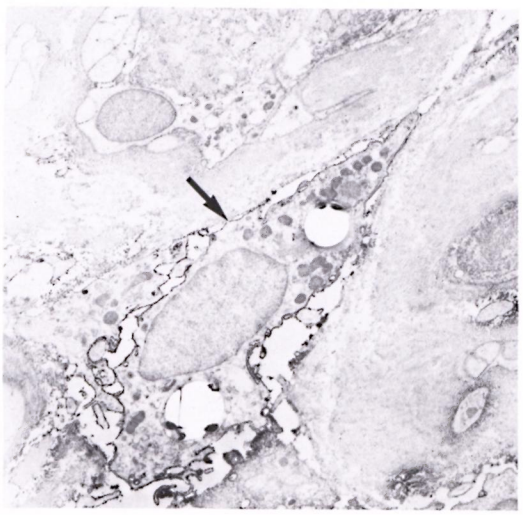

FIG. 5. Class II-positive macrophage containing bacilli (arrow) in a multibacillary nerve biopsy (×7500).

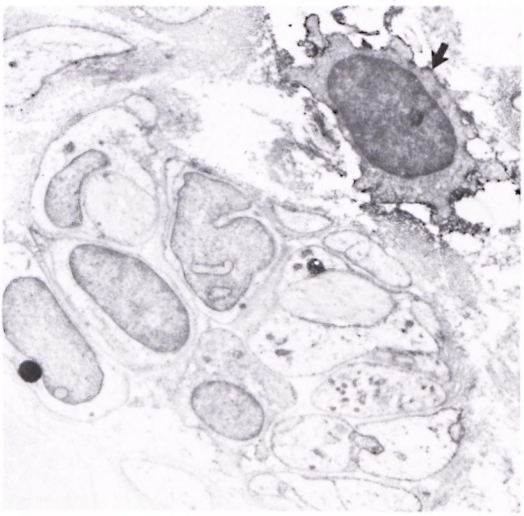

FIG. 7. Class II-positive lymphocyte (arrow) adjacent to Schwann cells and unmyelinated axons in a paucibacillary nerve. There is far less collagen than in the multibacillary nerve biopsies (×7500).

gether with the fact that EM sections are not counterstained with uranyl acetate and lead citrate (since this obscures the electron-dense immunostain), means that some definition is sacrificed in the final electronmicrographs. Despite these restrictions, immunoEM can yield much more information than standard immunohistochemistry on frozen tissue. In this and a previous study ([4]) we have shown that although class II-positive cells in leprosy and experimental granulomatous nerve lesions may appear to be Schwann cells by light microscopy, ultrastructural observations show that Schwann cells, easily identifiable at this level by their basement membranes and/or association with axons, are class II negative.

This *in vivo* evidence does not necessarily conflict with the *in vitro* studies which demonstrate class II inducibility and antigen presentation by Schwann cells ([6, 13–16]). Schwann cells in culture do not have basement membranes, are not associated with axons, and do not produce myelin, unlike their well-differentiated *in vivo* counterparts ([1, 12]). It is possible that only relatively undifferentiated Schwann cells will respond to cytokines by synthesizing class II antigens. This question might be resolved by *in vitro* studies using organized nerve cultures, where Schwann cells associate with axons and produce basement membranes and myelin ([1]).

It is also possible that in nerve lesions local levels of cytokines are not great enough to induce class II expression by Schwann cells. Although we have shown that T lymphocytes are present in areas of remaining Schwann cells, not many T cells were observed in very close proximity to Schwann cells. There was often a thick layer of collagen around the Schwann cells, especially in multibacillary nerves, which may restrict interaction with infiltrating cells and accessibility of cytokines. It is also unknown whether the Schwann-cell basement membrane interferes with immune interactions.

This study has been restricted to cases of pure neuritic leprosy because of ethical considerations when taking nerve biopsies and does not, therefore, represent all cases of leprosy. The immunological events in nerves in early leprosy may differ from those in the established disease where the nerves have become chronically damaged and fibrosed.

SUMMARY

A technique for immunoelectronmicroscopy has been used to investigate major histocompatibility class II expression in leprosy nerves. In normal nerves, endothelial cells and occasional endoneural cells (not Schwann cells) were constitutively class II positive. In both paucibacillary and multibacillary leprosy nerve biopsies, infiltrating leukocytes were positive but class II-positive Schwann cells were not seen. These observations indicate that Schwann cells may not be involved in presenting *Mycobacterium leprae* antigens to T cells in leprosy. This conflicts with evidence from *in vitro* studies, but may be explained by the fact that *in vivo* Schwann cells are surrounded by basement membranes and are closely associated with axons.

RESUMEN

Se usó una técnica de inmunomicroscopía electrónica para investigar la expresión de antígenos de histocompatibilidad clase II en los nervios de casos con lepra. En los nervios normales, la células endoteliales y las células endoneurales ocasionales (no las células de Schwann) fueron constitutivamente clase II-positivas. En las biopsias de nervios de casos de lepra tanto paucibacilares como multibacilares, los leucocitos infiltrantes fueron positivos pero no se observaron células de Schwann clase II-positivas. Estas observaciones indícan que las células de Schwann pueden no estar involucradas en la presentación de antígenos del *Mycobacterium leprae* a las células T en la lepra. Los hallazgos están en conflicto con los resultados de los estudios *in vitro*, pero ésto puede explicarse considerando que las células de Schwann *in vivo* están rodeadas por membranas basales, además de que se encuentran íntimamente asociadas con axones.

RÉSUMÉ

Une technique de microscopie électronique a été utilisée pour étudier l'expression de l'histocompatibilité majeure de classe II dans des nerfs de lépreux. Dans les nerfs normaux, des cellules endothéliales et d'occasionnelles cellules endoneurales (mais pas des cellules de Schwann) étaient par constitution positives pour la classe II. Dans des biopsies de nerfs de lèpre paucibacillaire et multibacillaire, des leucocytes infiltrants étaient positifs, mais on n'a pas trouvé de cellules de Schwann positives pour la classe II. Ces observations indiquent que les cellules de Schwann pourraient ne pas être impliquées dans la présentation des antigènes de *Mycobacterium leprae* aux cellules T au cours de la lèpre. Ceci est en contradiction avec ce qui a été

vu dans des études *in vitro,* mais pourrait être expliqué par le fait que les cellules de Schwann sont *in vitro* entourées par des membranes basales, et sont étroitement associées aux axones.

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