Isolation and characterisation of T lymphocytes from sural nerve biopsies in patients with Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy

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Abstract

Objectives—To characterise cultured T lymphocytes from nerve biopsies in patients with Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy (CIDP).

Methods—Sural nerve biopsies, obtained from six patients with Guillain-Barré syndrome, four with CIDP, and six controls with other neuropathies, were cultured with 20 U/ml recombinant interleukin-2 (IL-2) for eight weeks. Flow cytometry was used to determine the phenotype of cultured T lymphocytes. Their proliferative responses to a range of bacterial antigens were also examined.

Results—T cell lines were established from four of six patients with Guillain-Barré syndrome, one of four with CIDP, one patient with peripheral nerve vasculitis, and none of five controls with non-inflammatory neuropathies. One of these T cell lines from a patient with Guillain-Barré syndrome, preceded by *Campylobacter jejuni* infection, consisted entirely of $\gamma\delta$ TCR⁺ T lymphocytes. The peripheral blood of this patient also contained an increased frequency of $\gamma\delta$ T cells when stimulated with *C jejuni*. The nerve derived T cell lines failed to show a proliferative response to bacterial antigens or to a preparation of myelin proteins.

Conclusions—A new technique to isolate T cells from nerve biopsies in patients with Guillain-Barré syndrome and CIDP is reported. This technique may prove to be a useful tool in the investigation of the pathogenesis of other inflammatory neuropathies such as peripheral nerve vasculitis. The isolation of a $\gamma\delta$ TCR⁺ nerve T cell line is of interest because of the possibility that these cells might respond to glycolipid epitopes common to *C jejuni* and peripheral nerve gangliosides.

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Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy

(CIDP) are considered to be immune mediated disorders of peripheral nerve. Several studies have suggested that immune recognition of cross reacting epitopes on antigens common to organisms such as *C jejuni*, which cause antecedent infections, and gangliosides in peripheral nerve myelin might provide a possible mechanism by which the demyelination arises.^{1,2} Histological studies of peripheral nerve taken postmortem typically show evidence of an inflammatory infiltrate surrounding vessels consisting predominantly of lymphocytes.^{3,4} The histological findings are similar to those of the experimental model of Guillain-Barré syndrome, experimental allergic neuritis.⁵ Experimental allergic neuritis can be passively transferred with a rat T cell line reactive with the peripheral nerve protein P₂,⁶ suggesting that it is a primarily T cell mediated disease, although the efficiency of transfer and the degree of demyelination seems to be influenced by humoral factors.⁷ Histological studies of sural nerve biopsies from patients with Guillain-Barré syndrome or CIDP may show lymphocytic infiltrates although these are found less often than in motor nerves taken postmortem.³ Some studies have attempted to quantify and characterise these cells by immunohistochemistry.⁴ Such studies aim to improve understanding of the pathogenesis of demyelinating neuropathy by identifying the phenotype of tissue lymphocytes but this technique gives little information about their functional role. A more direct approach is to culture lymphocytes from these nerve biopsies, expand their numbers, and then examine their antigen specificity and cytokine expression. The number of lymphocytes in sural nerve biopsies is limited and we are not aware of any published reports of the isolation and culture of T cells from such small biopsy specimens. However, T cell lines have been cultured from brain material of patients with multiple sclerosis obtained postmortem⁸ and also from the cauda equina of rats with experimental allergic neuritis⁹ suggesting that it should be technically possible. We have therefore cultured sural nerve biopsies from patients with Guillain-Barré syndrome and CIDP, as well as control nerve material from other peripheral neuropathies, and produced T cell lines from these biopsies. We have then determined the phenotype and examined the antigen specificity of these T cell lines.

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Methods

PATIENTS

Permission was obtained to perform sural nerve biopsies in patients with Guillain-Barré syndrome and to use a portion of diagnostic sural nerve biopsies in patients with CIDP and other neurological conditions for this study. The remainder of each biopsy was submitted for neuropathological examination including light and electron microscopy, immunohistochemistry, and teased fibre preparations. All patients with Guillain-Barré syndrome were tested for serological evidence of preceding infection with *C jejuni* and cytomegalovirus and had stool cultures performed. Isolation of *C jejuni* from these stool cultures was considered evidence of current infection whereas a positive enzyme linked immunosorbent assay (ELISA) test at a titre of 1/160 for IgM antibody against *C jejuni* was considered evidence of past infection with that organism (*C jejuni* serology was performed by Dr Peter Wright, Public Health Laboratory Services, Preston, UK). A preceding infection with cytomegalovirus was considered likely if a positive ELISA for IgM antibody against cytomegalovirus (using the Vidas or IMX techniques) was obtained on a single sample of acute or convalescent phase serum.

CELL CULTURE

A section (2–3 mm) of the sural nerve biopsy material was washed in 1 ml of a 10 mg/ml solution of gentamicin (Sigma, Poole, UK) to sterilise the tissue and to remove any excess blood. The tissue was cut with a sharp scalpel, gently teased with two needles, and then cultured in "T cell medium" with 20 U/ml IL-2 (Chiron UK Ltd, Harefield, UK) for a period of eight weeks at 37°C and in an atmosphere of 5% CO₂. "T cell medium" consisted of RPMI-1640 medium (Gibco BRL, Paisley, UK) supplemented with 5% heat inactivated human AB⁺ serum, 2 mM L-glutamine + 100 U/ml penicillin + 100 µg/ml streptomycin (Sigma), 1% non-essential amino acids (Sigma), 1% HEPES buffer (Sigma), and 1% sodium pyruvate (Sigma).

T Lymphocytes isolated from these cultures were expanded by coculturing the cells with 1 µg/ml of the T cell mitogen phytohaemagglutinin (PHA, Murex Diagnostics, Dartford, UK), 50 U/ml IL-2, and 10⁶/ml irradiated (3000 rad) allogeneic peripheral blood mononuclear cells (PBMCs) as antigen presenting cells.

CHARACTERISATION OF T CELLS

The surface phenotypes of all nerve derived T cell lines were assessed by flow cytometry with an EPICS XL benchtop flow cytometer (Coulter Electronics Ltd, Luton, UK). Aliquots of 10⁵ cells were stained with directly conjugated fluorescent mouse antihuman monoclonal IgG1 antibodies to the following cell markers: CD3 (UCHT1, Dako Ltd, High Wycombe, UK), CD4 (Leu3A, Becton Dickinson Ltd, Oxford, UK), CD8 (RFT8γ, obtained from Dr A Akbar, Royal Free Hospital, London, UK), αβ TCR (Becton

Dickinson), and γδ TCR (Becton Dickinson). A fluorescent conjugated mouse IgG1 antibody (Dako) was used as a control.

ANTIGENS

A peripheral nerve antigen containing myelin was prepared from cauda equina obtained at necropsy, essentially as described by Winer.¹⁰ Briefly, protein material was obtained after lipid extractions using chloroform and methanol and solubilised in phosphate buffered saline (PBS) containing 1.5% SDS. The protein content of this final preparation was assessed with a Bio-Rad protein estimation kit (Bio-Rad, Hemel Hempstead, UK) as 2.5 mg/ml. This sample was used at a concentration of 2.5 µg/ml (containing 0.0015% SDS) in proliferation assays, the optimal concentration previously shown to maximally stimulate proliferation of PBMCs without a toxic effect.

C jejuni isolated from the stool sample of a patient with Guillain-Barré syndrome (patient 2) was cultured and used as a source of antigen in proliferation assays at a concentration of 10 µg/ml. The antigen was prepared by heat inactivation of the bacteria at 100°C for 40 minutes, followed by brief sonication for two minutes at 11 W. The protein concentration of this preparation was assessed with a Bio-Rad protein estimation kit (Bio-Rad, Hemel Hempstead, UK).

Purified protein derivative (PPD) of *Mycobacterium tuberculosis* (Statens Serum Institut, Copenhagen) was used at a concentration of 10 µg/ml. *Salmonella agona* was obtained from Dr G Clarke, Department of Microbiology, University of Birmingham and killed with γ irradiation. This preparation was used at a concentration of 2 × 10⁸ organisms/ml. Heat killed *Yersinia enterocolitica* 0:3 was obtained from Dr K Granfors, University of Turku, Finland and used at a concentration of 2 × 10⁶ organisms/ml.

PROLIFERATION ASSAYS

Aliquots of 10⁵ PBMCs were cultured in triplicate U bottomed 200 µl well plates, with or without antigens, at 37°C and 5% CO₂ for six days. For nerve derived T cell assays, aliquots of 10⁵ T cells were similarly cultured with 10⁵ irradiated (3000 rad) autologous PBMCs (as antigen presenting cells) for the shorter period of three days. For both PBMC and nerve T cell assays, 0.15 µCi tritiated thymidine was added to each well for the last six hours of culture. The cells were then harvested and thymidine incorporation measured with a liquid scintillation counter. Results were recorded as mean cpm (SEM) and are presented as stimulation indices (SI), where SI = (cpm of cells + antigen)/(cpm of cells only).

When PBMCs depleted of T cells were used as antigen presenting cells, aliquots of 2 × 10⁵ cells were cultured in 96 U bottomed plates overnight (16 hours). The wells were gently washed twice to remove the non-adherent cells and 100 µl medium was then added to each well. 10⁵ T cells in 100 µl and the appropriate antigens were then added and the

proliferation assays carried out as described above.

GENERATION OF *C JEJUNI* RESPONSIVE T CELL LINE

10^5 PBMCs were cultured in U bottomed 200 μ l well plates with 10 μ g/ml *C jejuni* antigen at 37°C and 5% CO₂ for 12 days. The culture medium was replaced twice weekly and the cells split 1 in 2, when confluent, into new wells. These cells were then analysed for T cell phenotype by flow cytometry.

CYTOTOXICITY ASSAY

Assays were performed to assess lectin mediated lysis of the $\gamma\delta$ and CD8 T cell lines isolated from patients 6 and 7.¹¹ 10^6 target cells (EBV transformed B cells) were labelled with 50 μ Ci ⁵¹Cr (Amersham, Aylesbury, UK) in 200 μ l for one hour at 37°C in 5% CO₂. The cells were then washed thoroughly and 1 μ g/ml PHA (lectin) was added. Aliquots of 10^4 cells per well were dispensed into 200 μ l V well plates in triplicate and incubated with 5×10^4 effector T cells per well at 37°C and 5% CO₂ for six hours (effector: target ratio of 5:1). At the end of the assay, 100 μ l supernatant from each well was removed for γ counting. Triplicate wells containing effector cells only or effector cells lysed with 1% Triton X-100 in water were used to determine minimum and maximum release respectively. The percentage specific cytotoxicity was calculated as:

$$\% \text{ specific cytotoxicity} = \frac{(\text{mean test release} - \text{mean spontaneous release})}{(\text{mean maximum release} - \text{mean spontaneous release})} \times 100$$

Results

PATIENTS

Sural nerve biopsy material was obtained from six patients with Guillain-Barré syndrome. Patients were classified as having acute inflammatory demyelinating polyneuropathy (AIDP, patients 1, 2, 3, 4, and 6) or acute motor axonal neuropathy (AMAN, patient 5) subtypes of Guillain-Barré syndrome on the basis of electrophysiology, sural nerve biopsy morphology, and clinical presentation. At the time of biopsy, patients 1, 2, 3, and 4 were confined to bed (functional scale 4)¹² and patients 5 and 6 required ventilation (functional scale 5).¹² In all the patients with Guillain-Barré syndrome, course of disease was at its nadir at the time of the biopsy.

Patient 1 developed fever and deranged

liver function tests 52 days after transplantation with a cytomegalovirus positive donor heart and was noted to have IgM antibodies against cytomegalovirus, having previously been IgG and IgM negative. Guillain-Barré syndrome developed 23 days later and a biopsy was obtained at day 38 after diagnosis. Another patient with Guillain-Barré syndrome, patient 6, was found to have IgM antibodies against cytomegalovirus when serum from the acute phase of the disease was tested. This patient was biopsied at day 23 after diagnosis of Guillain-Barré syndrome. Three of the other patients (patients 2, 3, and 5) had serological evidence of previous infection with *C jejuni*. These patients had a sural nerve biopsy performed at days 17, 18, and 16 respectively, after diagnosis of Guillain-Barré syndrome. A stool culture of *C jejuni* was also obtained from patient 2 and this was used as a source of antigen in the proliferation assays. No serological evidence of cytomegalovirus or *C jejuni* infection was seen in Guillain-Barré syndrome patient 4, who was biopsied at day 29 after diagnosis.

Sural nerve biopsy material was also obtained from four patients with CIDP and six control patients with other types of neuropathies—namely, IgG paraproteinaemia, hereditary motor and sensory neuropathy type I, multifocal motor neuropathy, paraneoplastic neuropathy, and peripheral nerve vasculitis.

ESTABLISHMENT OF T CELL LINES

After one to two weeks in culture, fibroblasts could be seen growing out from the biopsy fragment of peripheral nerve tissue. When T cells were obtained from the cultures, these were only seen after four to six weeks in culture and were in close association with the biopsy material. T cell lines were established from sural nerve biopsy material from four out of the six patients with Guillain-Barré syndrome (patients 1, 2, 3, and 6), one patient with CIDP (patient 7), and one patient with a peripheral nerve vasculitis (patient 16), but not from any of the non-inflammatory control nerves.

CHARACTERISATION OF T CELL LINES

The phenotype of the nerve derived T cell lines was assessed by flow cytometry and table 1 shows the results. Predominantly CD4⁺ $\alpha\beta$ TCR⁺ T cell lines were obtained from three patients with Guillain-Barré syndrome (patients 1, 3, and 6), a CD8⁺ $\alpha\beta$ TCR⁺ T cell line was obtained from CIDP patient 7 and a $\gamma\delta$ TCR⁺ T cell line was obtained from patient 2.

The proliferative responses of PBMCs obtained from Guillain-Barré syndrome patients 1, 2, 6, and CIDP patient 7 were assessed to *C jejuni* and to a cauda equina extract containing myelin (fig 1) and compared with proliferative responses of the nerve derived T cell lines (fig 2). Proliferative responses to *C jejuni* were seen in PBMCs from patients 2 and 6 (fig 1B patient 2, SI = 64; fig 1C patient 6, SI = 108) and in these patients PBMC responses to other Gram nega-

Table 1 Phenotypes of the nerve derived T cell lines

Patient	Diagnosis	Phenotype of cultured nerve T cells (% total population)				
		CD3 ⁺	CD4 ⁺	CD8 ⁺	$\alpha\beta$ TCR	$\gamma\delta$ TCR
1	GBS	100	85	14	96	0
2	GBS	97	0	35	2	99
3	GBS	99	98	0	99	0
6	GBS	99	87	10	98	0
7	CIDP	100	6	97	100	0

GBS = Guillain-Barré syndrome; CIDP = chronic inflammatory demyelinating polyneuropathy.

Figure 1 Proliferative responses of PBMCs from patients with Guillain-Barré syndrome (A) 1, (B) 2, (C) 6, and (D) from CIDP patient 7. Results are the mean cpm ($\times 10^3$) of triplicate wells (SEM). Only a limited assay was performed on the PBMCs from patient 1 and the results of two separate experiments are shown for PBMCs from patient 2 as responses to the myelin preparation was not tested in the first experiment; responses to *Salmonella* and *Yersinia* were not assessed in the second experiment. ND = not done.

tive bacteria such as *Salmonella* and *Yersinia* were lower than to *C jejuni*. Significant proliferative responses to the myelin preparation were not found, with only a weak proliferative response in PBMCs from patients 1 (fig 1A; SI = 3) and 6 (fig 1C; SI = 2). In all cases tested, PBMC responses to PPD were greater than to other antigens. In the light of the fact that the nerve derived T cells from patient 2 were predominantly $\gamma\delta$ TCR⁺, and this popu-

lation makes up only a small percentage of circulating T cells,¹³ the phenotype of the *C jejuni* responsive T cells in the peripheral blood of patient 2 was assessed and table 2 shows the results. Compared with unstimulated peripheral blood cells, in which there were only 2% $\gamma\delta$ TCR⁺ T cells, stimulation of PBMCs from patient 2 with *C jejuni* for 12 days generated a T cell line that contained 39% $\gamma\delta$ TCR⁺ cells.

A proliferative response was seen to PPD in

Figure 2 Proliferative responses of nerve derived T cell lines from patients with Guillain-Barré syndrome, (A and B) patient 2, (C) patient 6, and (D) from CIDP patient 7. Results are the mean cpm ($\times 10^3$) of triplicate wells (SEM). The nerve T cell line from patient 1 was not assayed. Nerve derived T cells from patient 7 showed no lymphoproliferative responses above background and the line was subsequently shown to be cytotoxic. The T cell line from patient 2 was subsequently retested with PBMCs depleted of T cells to assess the nature of the response to PPD and the results are presented as mean (SEM) cpm ($\times 10^3$). ND = not done; APC = antigen presenting cells.

the nerve derived T cells from patient 2 (fig 2A). However, in a separate experiment, this was shown to be most likely due to "back stimulation" (the production of IL-2 by PPD responsive T cells within the irradiated PBMC population which then acts to cause the proliferation of the nerve derived T cells), because when PBMCs depleted of T cells were used as antigen presenting cells, the T cell response to PPD was abolished (fig 2B). No proliferative responses were seen in the nerve derived T cells in the patients to any of the other antigens tested, although autologous antigen pre-

senting cells were shown to elicit some degree of responsiveness in the nerve derived T cells from Guillain-Barré syndrome patient 6. Moreover, no proliferation above the control level (antigen presenting cells only) was detected using nerve derived cells from patient 7 (fig 2D) which was expected as this cell line was dominated by CD8⁺ cells. The proliferative responses of Guillain-Barré syndrome patients 3 (PBMCs and nerve T cells), 4 (PBMCs), 5 (PBMCs), and 1 (nerve derived T cells) were not assessed in this series of experiments.

Table 2 Phenotypes of unstimulated and day 12 stimulated peripheral blood cells from Guillain-Barré syndrome patient 2

Cells	Phenotype of cultured nerve T cells (% total population)				
	CD3 ⁺	CD4 ⁺	CD8 ⁺	$\alpha\beta$ TCR	$\gamma\delta$ TCR
Unstimulated PBMCs	81	79	13	78	2
<i>C jejuni</i> stimulated PBMCs	83	29	12	37	39

PBMCs = peripheral blood mononuclear cells.

Lectin mediated cytotoxicity assays were performed on the nerve derived T cell lines obtained from patients 7 (CD8⁺, $\alpha\beta$ TCR⁺) and 2 ($\gamma\delta$ TCR⁺) using autologous EBV-transformed B cells + PHA as targets. The CD8⁺ T cell line (patient 7) was found to be cytotoxic (mean of three separate assays = 35% specific cytotoxicity; effector : target ratio = 5:1), but no cytotoxic activity was found in the $\gamma\delta$ T cell line from patient 2.

Discussion

Our results have shown that it is possible to isolate T lymphocytes and establish T cell lines from nerve biopsy specimens of patients with inflammatory demyelinating neuropathies. We believe that this is the first report of in vitro isolation and characterisation of T cells in nerve biopsy material from patients with Guillain-Barré syndrome and CIDP.

Although we cannot completely exclude the possibility that the cultured T cells could have arisen from passenger peripheral blood lymphocytes from the blood vessels in the biopsy samples we think that this is very unlikely for several reasons. Firstly, T cell lines were not seen following the culture of peripheral nerves from non-inflammatory control biopsy material. More importantly, the T cell outgrowth occurred only after an unusually prolonged period of several weeks of culture. This is contrary to our experience of culturing lymphocytes from other tissues including synovial membrane¹⁴ muscle and colonic mucosa (unpublished data) and work from other laboratories on the culture of T cells from other tissues such as liver¹⁵ and skin.¹⁶ In all of these cases, the outgrowth of T lymphocytes occurred within a few days and not after four weeks as we found with T cells emerging from our Guillain-Barré syndrome nerve biopsies. Moreover, in the control biopsy from patient 16, who was thought to have a vasculitic neuropathy, T lymphocytes were found after only 10 days of culture. The prolonged period before T cells became visible in our Guillain-Barré syndrome and CIDP biopsy cultures might be explained by firm adherence to the nerve tissue. There is evidence that T lymphocytes in experimental autoimmune neuritis express specific integrins¹⁷ and that their ligands can be expressed by the extracellular matrix,¹⁸ endoneurial macrophages and activated endothelial cells,¹⁹ providing a possible mechanism by which adherence to nerve tissue could be mediated.

The isolation of a $\gamma\delta$ T cell line from the nerve of patient 2 who developed Guillain-

Barré syndrome after *C jejuni* infection is of particular interest. $\gamma\delta$ T Cells make up only a small proportion of the circulating T cell population, the rest being $\alpha\beta$ T cells.¹³ Some antigen specific $\gamma\delta$ T cells recognise non-protein bacterial antigens such as prenylpyrophosphate derivatives and other components of bacterial cell walls.²⁰ Many antigen specific $\gamma\delta$ T cells show non-classical MHC restriction by molecules such as CD1,²¹ unlike conventional $\alpha\beta$ T cells which recognise peptide antigens only in the context of class I and II HLA antigens. In fact, there have been reports that antigen recognition by $\gamma\delta$ T cells may not always require any kind of restricting element²² or, in other cases, may be more similar to recognition of antigen by immunoglobulin.²³ This fact is of interest as there have been recent reports of sequence similarity between the lipopolysaccharide of *C jejuni* strains isolated from patients with Guillain-Barré syndrome and the peripheral nerve gangliosides GM1, GQ1b, and GD1a, and antibodies to these gangliosides have been found to show cross reactivity to *C jejuni* LPS.^{24,25} Axonal forms of Guillain-Barré syndrome have been described after parenteral administration of gangliosides²⁶ and secretion of interferon- γ in response to GM1 by T cells from the lymph nodes of rats with antigen presenting cells has been reported.²⁷ It is possible, therefore, that $\gamma\delta$ T cells responding to an initial *C jejuni* infection could cross react with antigens of peripheral nerve such as gangliosides and mediate peripheral nerve demyelination. Moreover, we have also shown that *C jejuni* specific T cell lines derived from the peripheral blood of patient 2 were greatly enriched for $\gamma\delta$ TCR⁺ T cells. The nature of this peripheral blood $\gamma\delta$ T cell response to *C jejuni* is therefore of interest. Although no proliferative response to *C jejuni* antigen was seen in the nerve derived $\gamma\delta$ T cell line, it is possible that a reactive subpopulation may be present within the T cell line which would be disclosed by cloning studies. Alternatively, the relevant *C jejuni* antigen (which may be different from the one involved in the PBMC response) may have been lost during the heat inactivation and sonication procedures used to prepare the antigen. Both of these possibilities are currently under investigation.

In our other patients with Guillain-Barré syndrome, we were able to culture T cell lines with both CD4⁺ and CD8⁺ phenotypes. This may reflect differences in the pathogenesis of different types of Guillain-Barré syndrome or could simply reflect differences in the time course of the disease at the time of nerve biopsy.

The nerve derived T cell lines from Guillain-Barré syndrome patients 1 (who developed Guillain-Barré syndrome after infection with cytomegalovirus) and 6 (who had IgM antibodies to cytomegalovirus) both had a predominantly CD4⁺ T cell phenotype. Infection with cytomegalovirus is associated with CD4⁺ and CD8⁺ proliferative and cytotoxic antigen specific T cell responses²⁸ and sequence similarity between cytomegalovirus

antigens and P₀ protein has been reported²⁹ providing a possible mechanism by which these nerve infiltrating CD4⁺ T cells could be involved in demyelination. It is of interest that a significant proliferative response to *C jejuni* was detected in the PBMCs from patient 6 despite no serological evidence of a preceding *C jejuni* infection. It is possible that patient 6 had a previous, undetected *C jejuni* infection; however, the relevance of *C jejuni* responsiveness to disease pathogenesis is unclear.

The technique of culturing lymphocytes from nerve biopsy tissue may have a wider application in the assessment of the relevance of T cell infiltrates in other inflammatory peripheral nerve disease. One of our control biopsies was obtained from a patient (patient 16) with a multifocal neuropathy, systemic indices of inflammation, and a biopsy showing prominent inflammatory infiltrates and who was considered to have a vasculitic illness. It was possible to develop a T cell line from this biopsy, providing a possible technique to approach the investigation of the pathogenesis of peripheral nerve vasculitis.

The factors that influence the success of the T cell culture techniques from these nerve biopsies are uncertain. The success of culture may depend on the total number of the lymphocytes available in the biopsy material. Alternatively, the culture conditions may favour the proliferation of activated cells in preference to resting or passenger T cells. During the course of this study T lymphocytes were successfully cultured from the peripheral nerves of patients with leprosy,³⁰ confirming the success of this approach in the investigation of other neuropathies associated with lymphocytic infiltrate within nerve. We are currently attempting to quantify the numbers of lymphocytes in our biopsy material to answer some of these questions.

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