SHORT REPORT

Unusual T cell receptor phenotype V gene usage of $\gamma\delta$ T cells in a line derived from the peripheral nerve of a patient with Guillain-Barré syndrome

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Abstract

Guillain-Barré syndrome is considered to be an immune mediated disorder but the relative role of T cells and antibodies in its pathogenesis is unclear. As gut infection with *Campylobacter jejuni* is the most common antecedent infection it is possible that gut derived T lymphocytes might play a part in the development of the syndrome.

The T cell receptor phenotype (TCR) of a nerve $\gamma\delta$ T cell line obtained from a sural nerve biopsy taken from a patient with a demyelinating form of GBS was determined using polymerase chain reaction (PCR) and flow cytometry (FACS). This TCR was compared with the phenotype preferentially expressed in the peripheral blood of the same patient.

The T cell nerve line was found to express V γ 8/ δ 1 which represents an unusual T cell subset normally found on lymphocytes resident in epithelial tissue such as the gut. The peripheral blood $\gamma\delta$ T lymphocytes from the patient were of the V γ 9/ δ 2 subset, which is the phenotype predominantly expressed in the peripheral blood of healthy subjects.

In conclusion, the presence of this unusual population of V γ 8/ δ 1⁺ lymphocytes in nerve would be consistent with a pathogenic role for gut associated lymphocytes in the pathogenesis of Guillain-Barré syndrome associated with *C jejuni*.

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Up to 30% of all cases of Guillain-Barré syndrome are preceded by infection with *Campylobacter jejuni*. Structural similarities and antibody cross reactivity between the LPS of strains isolated from patients with Guillain-Barré syndrome and peripheral nerve gangliosides have been demonstrated.¹⁻³ *Campylobacter jejuni* infection originates in the bowel and persistent infection of stool is recognised in a proportion of patients with Guillain-Barré syndrome for some weeks after symptoms of the neuropathy are apparent. Gut T lymphocytes may therefore be important in the generation of

T cell responses against *C jejuni*. We have therefore analysed the $\gamma\delta$ T cell phenotype of a nerve derived T cell line from a patient with GBS associated with *C jejuni* to see if there was evidence of a TCR suggestive of gut origin.⁴

Methods

The patient fulfilled standard diagnostic criteria for an acute inflammatory demyelinating polyradiculoneuropathy (AIDP) form of Guillain-Barré syndrome.⁵ Full informed consent was obtained for the nerve biopsy and the study received ethical permission from our institution's local research ethics committee. A raised ELISA titre of 1/320 for IgM antibody against *C jejuni* was suggestive of a recent infection. The nerve biopsy was performed 10 days into the neuropathy when the illness was still progressing (disability grade 3⁶); the patient had subjective sensory abnormalities in the distribution of the sural nerve biopsied (sensory grade 1⁶).

Venepuncture was performed at day 6 of the neuropathy and peripheral blood mononuclear cells (PBMCs) were isolated and their proliferative responses assessed using tritiated thymidine incorporation as described previously.⁷ *C jejuni* (Penner serotype heat stable 4, phage group 146) was isolated from a stool sample of another patient with Guillain-Barré syndrome, cultured, and used as a source of antigen in proliferation assays at a concentration of 5 μ g/ml. *Salmonella agona* and *Yersinia enterocolitica* were obtained from the Department of Microbiology (Queen Elizabeth Hospital, Birmingham, UK) and used at a concentration of 3 μ g/ml and 1 μ g/ml respectively. The T cell mitogen phytohaemagglutinin (PHA, Murex Diagnostics, Dartford, UK) and purified protein derivative (PPD) of *Mycobacterium tuberculosis* were used at concentrations of 1 μ g/ml and 10 μ g/ml.

The nerve T cell line was generated as described previously.⁸ Cells were stained with IgG₁ isotype control, CD3, CD4 (DAKO Ltd, Bucks, UK), CD8, $\alpha\beta$, $\gamma\delta$ (Becton Dickinson, Oxford, UK) and to the following $\gamma\delta$ subsets: V γ 1, V γ 9, V δ 2, V δ 3 (Immunotech/Coulter, Luton, UK) and V γ 8 (gift from Professor R Kiessling, Karolinska Institute, Sweden), for 30 minutes at 4°C and analysed on an FACS (Coulter, Luton, UK). Analysis of other V γ /V δ

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PCR analysis of $V\gamma/V\delta$ subsets of T lymphocytes. Nerve derived T cell line and PBMC analysed by PCR for $V\gamma/V\delta$ transcripts. DNA ladder marker was (174Hae (Gibco BRL) with 603 bp and 310 bp indicated from the left. $V\gamma$ and $V\delta$ TCR products are between 213–371 bp.

subsets were not investigated by flow cytometry due to lack of availability of antibodies, but were studied by PCR.

RNA was extracted from 5×10^6 – 10^7 PBMCs and nerve derived T lymphocytes using a Qia-gen RNeasy Minikit (Qiagen, West Sussex, UK), following the manufacturer's instructions. Reverse transcription was carried out using oligo dT primers (Gibco BRL, Paisley, UK) at 70°C for 5 minutes then 37°C for 1 hour with 200 U Reverse Transcriptase (Superscript II; Gibco BRL).

The PCR was by a standard protocol as described previously,⁷ with minor modifications. Briefly, cDNA was amplified using a variable region (V) 5' primer ($V\delta 1$ –5, $V\gamma 2$ –5, $V\gamma 8$ –10) and either a constant (C) $C\gamma$ or $C\delta$ region 3' primer. The primer sequences have been described previously by Söberström *et al.*⁴ Cycle conditions were as follows; denaturation 94°C for 60 seconds, annealing 55°C for 60 seconds, and extension 72°C for 60 seconds; PCR samples were amplified for 42 cycles and were sampled every three cycles from cycle 20 by removing 10 μ l aliquots. Samples were analysed by 1.5% agarose gel electrophoresis and stained with ethidium bromide. Actin was used as a housekeeping gene and concentrations of cDNA were adjusted to maintain optimal and equal concentrations for the primers used.

Results

The proliferative response of PBMCs obtained from this patient with *C jejuni* was mean cpm=13 855, SI=37. PBMC responses to two other gram negative bacteria, *Salmonella* and *Yersinia* were lower than to *C jejuni* (cpm=11367, stimulation index (SI)=30 and cpm=7051, SI=19, respectively). This patient also exhibited high proliferative responses to PHA (cpm=19 533, SI=53) and PPD (cpm=17242, SI=46.9).

Analysis of the nerve derived T lymphocytes from this patient showed that the cells were $CD3^+$ T cells (99.8%), 23% were $CD4^+$ and 74.6% were $CD8^+$, 64% expressed the $\alpha\beta$ TCR and 27% expressed the $\gamma\delta$ TCR. After the in vitro expansion of the nerve derived T cells with PHA and interleukin 2⁸ the cells showed no ability for specific proliferation to *C jejuni*. (Data not shown.) However, analysis of the

$V\gamma/V\delta$ usage by flow cytometry showed marked skewing of their TCR phenotype so that all the $\gamma\delta^+$ T lymphocytes expressed the $V\gamma 8$ subset (27%). No staining above background concentrations was seen when the nerve T cells were stained with $V\gamma 1$, $V\gamma 9$, $V\delta 3$. A proportion (4.8%) were positive at the cell surface for $V\delta 2$. Analysis of the peripheral blood lymphocytes showed that they were 7.4% $\gamma\delta$ TCR⁺, expressing predominantly $V\gamma 9/\delta 2$. No staining was seen for $V\gamma 8$.

Analysis by PCR showed that the nerve derived T lymphocytes were positive for $V\gamma 2$, $\gamma 5$, $\gamma 8$, $\gamma 9$, and $V\delta 1$ transcripts. Sampling of these reactions showed that the first PCR products to be detected at cycle 32 were $V\gamma 2$, $\gamma 5$, and $\gamma 8$. Reverse transcriptase-PCR showed that PBMCs were positive for $V\gamma 2$, $\gamma 5$, $\gamma 8$, $\gamma 9$, $\gamma 10$, $V\delta 1$, $\delta 2$, $\delta 3$, and $\delta 5$ (figure). $V\gamma 10$ has previously been shown to be a pseudogene in humans.⁹ No PCR products could be detected in the negative controls (no cDNA) using any of the primer pairs (data not shown).

Discussion

Here we report that nerve derived T lymphocytes isolated from the peripheral nerve of a *C jejuni* positive patient with Guillain-Barré syndrome preferentially expressed a $V\gamma 8/V\delta 1$ phenotype. Although transcripts were detected in the nerve T cells for $V\gamma 9$, this V region could not be detected above background concentrations at the cell surface by flow cytometry. Due to the limited number of $V\gamma$ TCR specific antibodies we could not seek expression of $V\gamma 5$ directly, although transcripts for $V\gamma 5$ were detected by PCR. Almost all the $\gamma\delta$ T cells derived from nerve, detected by flow cytometry, expressed $V\gamma 8$ making it unlikely that any significant number of T cells expressed $V\gamma 5$ despite the presence of message. This latter TCR is of interest as transcripts for this TCR γ chain were detected by PCR in the nerve derived $\gamma\delta$ T cell line from a patient that we have previously reported.⁷ A very small percentage seemed to express $V\delta 2$ but we could not detect any transcript for this gene nor for $V\delta 3$ (figure). These nerve derived T cells were distinct from those found in the peripheral blood, which contained transcripts for some of the $V\gamma/\delta$ subsets but were predominantly

V γ 9/ δ 2 by flow cytometry. This confirms that the cells generated from the nerve culture were not simply lymphocytes from any contaminating peripheral blood.

$\gamma\delta$ T cells of the human gut have been shown to preferentially express V γ 8/V δ 1⁴ and V γ 8/ δ 1 cell numbers are also increased in blood from patients with inflammatory bowel disease.¹² We have previously shown that $\gamma\delta$ T lymphocytes could be cultured from nerve biopsies,⁷ and postulated a role for $\gamma\delta$ cells in infection and Guillain-Barré syndrome pathogenesis.⁸ Data presented here extend those findings by providing evidence that $\gamma\delta$ T lymphocytes typical of an intraepithelial origin and thus possibly resident in gut may be selectively recruited to nerve and thus available to play a part in demyelination.

Recruitment of T cells to inflamed tissues requires expression of adhesion molecule and chemoattractant chemokines by vascular endothelial cells at the inflammatory site.^{11,12} The expressed molecules may vary in a tissue specific manner, over time and in response to proinflammatory cytokines and other mediators. The array of counter ligands for adhesion molecules and of chemokine receptors governs the T cell subsets that are recruited. This implies that selection of V γ 8/ δ 1 T cells for recruitment to peripheral nerve requires selectivity at, for example, the level of nerve endothelial expressed adhesion molecules or chemokines and/or specific expression of adhesion counter ligands or chemokine receptors on the V γ 8/ δ 1 T cells. As yet, there is no information on nerve specific or $\gamma\delta$ -directed T cell recruitment, although this will be a valuable approach for further studies. Once recruited, there is evidence that $\gamma\delta$ T cells can contribute to the chemokine pool within inflamed tissue, at least in the CNS.¹³

Direct proliferation of nerve derived lymphocytes to either *C jejuni* or ganglioside

antigens has not so far been demonstrated but prolonged culture of lymphocytes, in these conditions, may impair antigen specific responses and thus render it impossible to detect such specific proliferation.

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