CD1 expression in human peripheral nerve of GBS patients

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Guillain-Barré syndrome (GBS) is an acute inflammatory disorder of the peripheral nervous system (PNS) with a heterogeneous pathogenesis, involving both cellular and humoral immunity. It can be caused by acute inflammatory demyelinating polyradiculoneuropathy (AIDP), or by acute motor or motor and sensory axonal neuropathy (AMAN or AMSAN) [1]. Acute lymphocytic infiltration of the endoneurium may be prominent [2] and circulating activated T cells and serum concentration of soluble IL-2 receptors are increased [3,4]. Immunocytochemical studies have revealed lymphocyte and macrophage to accumulate in a focal and perivascular distribution throughout the PNS from root to motor terminals. In vitro Schwann cells can present antigen and be direct targets for autoreactive CD4+ or CD8+ T cells. The blood nerve barrier (BNB) normally separates the immune components from the nerve and circumstantial evidence implicates T cells as the immunological triggering factor in the pathogenesis of GBS. T cells need to be activated in the periphery and adhesion molecules need to be upregulated in order for the T cells to cross the BNB and initiate the local inflammatory process. This process has already been observed in the animal model of GBS, experimental allergic neuritis. The infiltration of T cells and their focal activation leads to the production of cytokines and chemokines leading to further breakdown of the BNB. Various attempts have been made to identify the nerve antigens in GBS to which T cell responses are directed, but no conclusive candidate antigen has been found, nor has a restricted T cell receptor (TCR) gene usage [5,6]. Antibodies to P0, P2 proteins and to myelin glycolipids have been found in some patients [7,8].

There is evidence of antibodies to gangliosides in GBS, there is no doubt that anti-glycolipid immune response plays a significant role in the pathogenesis of GBS. 25% of GBS and 90% of its variant form Miller Fisher syndrome patients have IgG anti-glycolipid antibodies [9,10]. These IgG antibodies are of IgG1 or IgG3 subclass. IgG1 production is a T cell dependent process and the general consensus is that carbohydrate haptons do not participate in HLA-TCR interactions, because they are too “bulky” to fit the MHC binding site. Therefore an alternative system must be responsible for these observations. The non-MHC encoded CD1 family has recently been recognised as an antigen-presenting system that is entirely different from either class I or class II related molecules. There are 5 non-polymorphic CD1 genes in man [11] and human T cell clones can recognise specific isoforms of human CD1. Recently human CD1b and CD1d have been shown to act as restriction elements in the presentation of various mycobacterial lipid antigens to T cells, that can be either double negative (DN), CD4+ or CD8+ T cells [12]. It is reasonable to propose that CD1-mediated response to lipids or hydrophobic antigens may be found in some nervous system diseases. CD1b has recently been reported to be present on inflammatory cells, hypertrophic astrocytes and macrophage in multiple sclerosis lesions [13]. Considering the presence of the prominent immune responses to lipid antigens in GBS, we carried out some preliminary studies to investigate the presence of CD1b molecule in nerve biopsies from GBS patients, as well as controls.

Sural nerve biopsies were taken from GBS, paraproteinemia neuropathy patients and controls. Frozen sections of 6μ thickness were cut and fixed for 10 minutes in chilled acetone. Serial sections were stained with different markers and an avidin/biotin/peroxidase system (Vector Elite Kit, Vector Labs, UK). As negative controls an irrelevant antibody, an IgG2b anti-E2 protein of Semliki Forest virus (SFV) monoclonal antibody was used. As positive tissue for the antibodies, frozen sections of human tonsil were used. For CD1b, three different anti-CD1b monoclonal antibodies of IgG and IgG2b were used (gift from Dr M Londie, SUNNY RESEARCH INSTITUTE, London W6, UK, SEROTEC, UK, ANCELL, UK). In addition anti-CD2, CD4, CD8 monoclonal antibodies, as markers for T cells, anti-CD68 for macrophage, and anti-HLA-DR monoclonal antibodies were used (SEROTEC, UK). Briefly, non-specific binding was blocked with normal horse serum and optimal dilution for each antibody was found. After incubating for 40 minutes with the first antibody, the sections were washed and incubated with biotinylated anti-mouse immunoglobulin antibody for 40 minutes. Further washes and incubations with reagents were carried out according to manufactures instructions, and the sections finally counter-stained with hematoxylin. In normal nerve, positive staining for HLA-DR was seen but only weakly for CD2 and very marginally for CD1b was observed. A similar staining pattern was seen in paraproteinemic neuropathy patient's nerve, despite the existence of demyelination. In GBS nerve with inflammatory demyelination, prominent infiltration of T cells and macrophage was observed. These sections were positive for CD2, CD4, CD8, CD68 and HLA-DR. The expression of CD1b was enhanced and upregulated in these biopsies, especially within macrophages. Whether this expression is also present within Schwann cells remains to be proven. The staining with anti-CD1b antibody was similar with all the three antibodies tested. However, the cleanest staining was observed with the anti-CD1b from Ancell Laboratories and the gift from Dr Londie. All the sections treated with the anti-E2 protein of SFV were negative. To establish the relationship between T cells and cells expressing CD1b double stained preparations will be used. The purpose of this investigation was to study CD1, the putative antigen presenting molecule for glycolipid and lipid antigens, in the PNS and to test the hypothesis that CD1 expression may be differentially enhanced on endoneurial macrophage or Schwann cells in patients with inflammatory neuropathy and prominent anti-glycolipid responses and so be involved in a recognition of glycolipids by T cells. Such a T cell recognition of glycolipid antigens could be responsible for some of the observed inflammation in GBS and other inflammatory diseases of the nervous system.

References  

Acknowledgements: We thank Dr M Londie and Ancell for the gift of antibodies.