

Review Article

Can antiglycolipid antibodies present in HIV-infected individuals induce immune demyelination?

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Of the eight clinically defined neuropathies associated with HIV infection, there is compelling evidence that acute and chronic inflammatory demyelinating polyneuropathy (IDPN) have an autoimmune pathogenesis. Many non-HIV infected individuals who suffer from sensory-motor nerve dysfunction have autoimmune indicators. The immunopathogenesis of demyelination must involve neuritogenic components in myelin. The various antigens suspected to play a role in HIV-seronegative IDPN include (i) P₂ protein; (ii) sulfatide (GalS); (iii) various gangliosides (especially GM₁); (iv) galactocerebroside (GalC); and (v) glycoproteins or glycolipids with the carbohydrate epitope glucuronyl-3-sulfate. These glycoproteins or glycolipids may be individually targeted, or an immune attack may be raised against a combination of any of these epitopes. The glycolipids, however, especially GalS, have recently evoked much interest as mediators of immune events underlying both non-HIV and HIV-associated demyelinating neuropathies. The present review outlines the recent research findings of antiglycolipid antibodies present in HIV-infected patients with and without peripheral nerve dysfunction, in an attempt to arrive at some consensus as to whether these antibodies may play a role in the immunopathogenesis of HIV-associated inflammatory demyelinating polyneuropathy.

Key words: antibodies, galactocerebroside, gangliosides, human immunodeficiency virus, inflammatory demyelinating polyneuropathy, sulfatide.

INTRODUCTION

During the course of human immunodeficiency virus (HIV) infection at least 20% of infected individuals develop overt peripheral nerve disorders.^{1–3} A further 13–35% have been suggested to have an underlying subclinical neuropathy detectable only by nerve conduction studies.^{4,5} This review details investigations aimed at determining the antigenicity of peripheral myelin lipids in inflammatory demyelinating disorders associated with HIV infection.

Eight different forms of HIV-associated neuropathy have been described:⁶ (i) distal predominantly sensory neuropathy (DPSN); (ii) vasculitic neuropathy; (iii) lymphomatous neuropathy; (iv) cytomegalovirus (CMV); lumbosacral polyradiculopathy; (v) *Varicella zoster virus* (VZV) polyradiculopathy; (vi) acute and chronic inflammatory demyelinating polyneuropathy (IDPN); (vii) autonomic neuropathy; and (viii) neuropathy induced by di-deoxy-inosine (ddI) and di-deoxy-cytidine (ddC) therapy. Other minor forms of neuropathy associated with HIV infection have been documented.^{7–11}

DPSN is the commonest neuropathy and it occurs in up to 35% of individuals with advanced HIV infection.^{3,6} Although the pathology of DPSN has been determined as retrograde distal axonal degeneration ('dying back' axonopathy) and drop out of dorsal root ganglion neurons,^{12,13} little is known of its pathogenesis. In contrast, two other types of HIV-associated neuropathies, inflammatory demyelinating polyneuropathy (IDPN) (in both acute (AIDPN) and chronic (CIDPN) forms), and vasculitic neuropathy have clinical and pathological features that suggest an autoimmune pathogenesis. Both occur during phases of HIV infection in which T lymphocytes are not significantly depleted so that sensitization of T cells to peripheral nerve antigens can occur. Circulating antibodies of IgG and IgM isotypes against myelin have been found in the serum of HIV-seropositive individuals with IDPN and vasculitic

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neuropathy, and symptomatic improvement after plasmapheresis has been recorded in both groups.¹⁴ To date only a small number of individuals with HIV infection and symptoms of neuropathy has been studied. There are no data regarding the prevalence of antibodies against peripheral myelin and axonal epitopes in HIV-infected individuals without overt neuropathic symptoms. Anti-protein and antiglycolipid reactivity, however, has been demonstrated in various neuropathic syndromes (especially Guillain-Barré syndrome (GBS)) in individuals without HIV infection. Such data may assist in reaching a consensus on the significance of antimyelin antibodies in the pathogenesis of HIV-associated neuropathy.

HIV-ASSOCIATED INFLAMMATORY DEMYELINATING POLYNEUROPATHY

HIV-infected patients with AIDPN develop clinical symptoms which are similar to HIV-seronegative GBS.^{15,16} In addition to the presence of antibodies against HIV, cerebrospinal fluid (CSF) pleiocytosis is the most clearly distinguishing feature of HIV-associated AIDPN.¹⁵ This, however, is not specific for AIDPN because HIV-infected patients (with or without AIDPN) have up to 50 mononuclear leukocytes per mm³ in the CSF, whereas GBS patients rarely have more than 10 mononuclear leukocytes per mm³.¹⁷ The fact that CIDPN exhibits some clinical similarities with AIDPN and that they both respond favorably to plasmapheresis therapy is an indication that there is a common etiology.¹⁵

The predominant feature of HIV-associated AIDPN is primary demyelination. A small proportion of patients has axonal degeneration which is sometimes the predominant feature.^{15,18} Microscopic examination of nerve biopsies reveals moderate subperineurial edema reflecting breakdown of the blood-nerve barrier (BNB), small numbers of lymphocytes near endoneurial vessels and within the endoneurial space, and demyelinated and remyelinating fibers. There is usually prominent infiltration by macrophages containing myelin. More severely involved nerves display frequent fibers undergoing demyelination, often with macrophage-mediated myelin stripping. Extensive endoneurial mononuclear cell infiltrates and perivascular cuffs of inflammatory cells in the epineurium can also be seen.^{15,19} The CD4:CD8 ratio in the nerve has also been shown to reflect that of the peripheral blood, suggesting that many cells may enter the peripheral nerve lesions non-specifically secondary to a breakdown of the BNB.^{15,20,21}

Because this disorder generally occurs in HIV-infected individuals with a CD4 count > 500/μL or at the stage of seroconversion, and there is an underlying pathology of primary demyelination with active infiltration of

immunocompetent leukocytes similar to its non-HIV counterpart, this disorder has been suggested to be immune mediated.¹⁵ Further evidence comes from data showing high titers of circulating antiperipheral nerve myelin antibodies elevated acutely and decreased during convalescence,^{22,23} and perineurial IgM deposits in HIV-infected patients with neuropathy.¹⁴ The strongest argument for this hypothesis is favorable patient responses to plasmapheresis, corticosteroid and intravenous immunoglobulin treatment.^{24,25}

ANTIBODIES AGAINST MYELIN CONSTITUENTS

Antibodies that react with myelin constituents have been frequently demonstrated in the circulation of patients with a variety of demyelinating neuropathies and other neurological conditions, in particular AIDPN, CIDPN and polyneuropathy associated with monoclonal IgM paraproteinemia.²⁶⁻³⁶ Clinical improvement in some AIDPN patients, and declining serum titers of antimyelin antibodies after plasmapheresis, suggest that the pathogenesis, at least in part, is likely to involve disordered humoral immunity.^{15,37-39}

Many investigators have reported immunoreactivity against various glycolipids of peripheral nervous system myelin in patients with IDPN.^{34,40-43} This immunoreactivity has implicated these glycolipids in the pathogenesis of immune demyelinating polyneuropathies and some motor neuropathies, in particular multifocal motor neuropathy (MMN).⁴²⁻⁴⁴

Complex carbohydrates which are constituents of peripheral nerve myelin contain repetitive hexose units. These can directly activate B cells by cross-linking surface immunoglobulin receptors in an antigen-specific manner. This may augment a non-specific T-cell response.⁴⁵ These complex carbohydrates are also capable of eliciting a polyclonal B-cell response with the generation of specific IgM antibodies.

For an IgG response to occur, the antigen must be processed by an antigen-presenting cell and presented to T cells via the major histocompatibility complex (MHC) class II.^{46,47} Glycolipid molecules such as gangliosides cannot be processed intracellularly and presented to the immune system in this manner.⁴⁸ For these glycolipids to be neuritogenic they need either to be closely associated with a protein or contain an epitope that cross-reacts with a glycoprotein, unless they are T-cell-independent antigens.^{45,49} CD5-expressing B cells react with carbohydrates and may be able to process and present a peptide fragment thereby eliciting T-cell help.⁵⁰⁻⁵² This process could then lead to the generation of IgG antibodies against glycolipid carbohydrate moieties.

There exists, however, a subpopulation of T cells bearing the $\alpha\beta$ T-cell receptor (TCR) that recognizes non-peptidic components of *Mycobacterium tuberculosis*.⁵³ These T cells are specific for lipids and glycolipids from the mycobacterium cell wall, recognizing the carbohydrate moiety of the glycolipid. More importantly, these T cells recognize glycolipids by a non-MHC-type mechanism, involving the CD1b molecule which can present glycolipid to the T-cell receptor.⁵⁴ Of major significance were the recent findings of Khalili-Shirazi *et al.*, who demonstrated strong labeling of CD1b on endoneurial macrophages and on myelinated nerve fibers in AIDPN biopsied nerves but could not see such patterns in other diseased (various peripheral neuropathies) and normal nerves, suggesting a strong association between the upregulation of CD1 and inflammatory neuropathy.⁵⁵ Further evidence for this association were the recent results presented by Shamshiev *et al.* who showed that circulating T cells isolated from multiple sclerosis (MS) patients with either active or stable demyelinating lesions were reactive against various glycolipids with different clones showing intense reactivity with gangliosides, GalC and GalS.⁵⁶ Importantly, one specific CD1b-restricted T-cell clone, reacting against the monosialoganglioside GM₁, was shown to be specifically recognized by the TCR and is able to discriminate between small variations in the carbohydrate moieties. These investigators went further to show that the glycolipid reactive T cells more often show a T helper (Th)1-like functional phenotype because they release the pro-inflammatory cytokines tumor necrosis factor (TNF)- α and interferon (IFN)- γ . These findings would suggest that in MS there is an expansion of glycolipid-reactive T cells and that these may be responsible for the onset and progression of inflammatory demyelinating lesions seen in this disease. It is possible that B cells generating specific antiglycolipid antibodies may be activated by these CD1b-positive, glycolipid-reactive T cells.

IgM paraproteinemic neuropathy was one of the first disorders to implicate antiglycolipid antibodies in the pathogenesis of a peripheral demyelinating disorder.^{57,58} It was found that the myelin-associated glycoprotein (MAG) carbohydrate moiety of peripheral myelin is also present on the acidic peripheral myelin glycolipid sulfoglucuronyl paragloboside (SGPG).⁵⁹⁻⁶² Antibodies to SGPG have since been shown to cause demyelination and other pathological changes such as widening of myelin lamellae in rats, when injected intraneurally^{63,64} or by systemic passive transfer of immune serum into chickens.⁶⁵ The sulfoglucuronyl moiety or a similar carbohydrate residue has been shown to be essential for antibody binding. This carbohydrate moiety is shared by several other nervous system glycoconjugates besides MAG and P₀, including the adhesion molecules J1 and neural cell adhesion molecule

(NCAM) and several 20–26-kDa peripheral nervous system (PNS) glycoproteins.⁴² It is possible that the pathological findings seen in patients with these circulating autoantibodies might arise from interference with the structural integrity of PNS myelin. Also, these antibodies may disrupt critically important interactions between the PNS neurons and the accompanying Schwann cells by binding to cell adhesion molecules.⁴² These antibodies have never been demonstrated in HIV-infected patients with neuropathy.

ANTI-GALS ANTIBODIES

Anti-sulfated carbohydrate antibodies have been detected frequently in patients with distal sensory polyneuropathies without HIV infection. These antibodies are present to a lesser degree in proximal polyneuropathies and very little reactivity is seen in patients with sensory ganglionopathy.⁶⁶ Patients with anti-GalS reactivity exhibit predominantly sensory neuropathic symptoms. There has been much speculation surrounding the involvement of anti-GalS antibodies in sensory neuropathies. Quattrini *et al.* screened 200 plasma samples from patients with various peripheral neuropathies and other neurological and immunological diseases.⁶⁷ Five of these samples contained a high anti-GalS antibody titer. All five samples were from patients with sensory impairment, one patient also suffering from MS. Pre-absorption with GalS and not GalC eliminated the tissue-binding activity of these antibodies. The specificity of the anti-GalS antibodies is largely directed at the sulfate (SO₃) and hydroxy (OH) groups of the sugar residue.⁶⁸ Antibodies against GalS were also found to bind only to fixed sections of peripheral and central myelin.⁶⁷ This tends to suggest that the GalS epitope is shielded normally but may be exposed in some types of nerve injury and during tissue fixation.

Anti-GalS IgM antibodies are restricted to the lambda (λ) subclass; this restriction may result from preferential utilization of particular immunoglobulin variable-region genes by these antibodies⁶⁷ similar to that reported for antibodies with anti-DNA, rheumatoid factor, cold agglutinin or anti-MAG reactivity.⁶⁹⁻⁷² It has been suggested that low levels of anti-GalS antibodies may be common constituents of the normal human immune repertoire and may be secreted by the CD5-positive subset of B cells, similar to other naturally occurring anti-carbohydrate IgM antibodies.⁷³ These B cells might be activated by GalS incorporated into the envelope of a budding virus,⁷⁴ by cross-reactive foreign antigens or antigen complexes, and may be excessively produced by expanded B cell clones in monoclonal gammopathies.

Further evidence that these antibodies cause sensory symptoms comes from studies of the relative permeability

of the BNB in dorsal root ganglia (DRG).⁷⁵ This exposes DRG neurons as potential targets of anti-GalS antibodies. Anti-GalS antibodies have been shown to bind to the surface of unfixed rat DRG neurons.⁶⁷ The high content of GalS⁶⁷ and the BNB permeability in DRG support the theory that these antibodies may contribute to sensory syndromes. It is possible that the antibodies could also disrupt the interaction of GalS with extracellular adhesion molecules. This would, in turn, cause disruption of the close contact between axons and Schwann cells that is necessary to maintain the integrity of myelinated axons.⁷⁶ Anti-GalS antibodies could also interfere with the function of associated β -endorphin receptors,⁷⁷ or damage cells through complement fixation.

Anti-GalS antibodies are frequently found in patients with IDPN.^{41,42} As has been already described, GalS appears to be concentrated on the external surface of the myelin sheath.⁷⁸ This would suggest that anti-GalS autoantibodies could play a role in the pathogenesis of motor neuropathies such as GBS. Anti-GalS antibodies have been detected in 65% of patients with GBS and also in 87% of patients with CIDPN; but these antibodies are found in only 15% of control sera.⁴¹

Another study has shown a direct relationship between anti-GalS antibodies and the process of immune demyelination in the PNS.⁷⁹ Passive transfer of purified monoclonal anti-GalS IgM antibodies from a patient with benign IgM- λ paraproteinemic demyelinating polyneuropathy to newborn rabbits produced demyelinating nerve lesions similar to those seen in the donor. The experimental lesions showed direct binding of anti-GalS antibodies to Schmidt-Lanterman incisures and nodes of Ranvier. These antibodies were also shown to bind to satellite cells in DRG, suggesting a possible pathogenetic role of anti-GalS antibodies in sensorimotor syndromes.

Anti-GalS antibodies have been demonstrated in the serum and CSF of HIV-infected patients with neurological involvement, particularly those patients with peripheral nerve dysfunction.⁸⁰ These investigators found that six of the seven CSF samples obtained from HIV-associated peripheral neuropathy patients contained IgG anti-GalS antibodies. They concluded that anti-GalS antibodies present within the CSF of these patients could be synthesized intrathecally by infiltrating peripheral blood plasma cells. These could also enter peripheral nerves through an altered BNB. Alternatively, these antibodies may bind to DRG tissue components after passing through the permeable DRG-BNB.

Recently Gisslen *et al.* described anti-GalS IgG antibodies present in HIV-infected individuals both with and without neurological involvement.⁸¹ These investigators studied 25 patients who were HIV seropositive, of which nine had documented neuropsychiatric symptoms (three

with AIDS dementia complex; two with cerebral toxoplasmosis; two with progressive multifocal leukoencephalopathy; one with vacuolar myelopathy; one with new-onset psychosis). Twenty-four of the 25 HIV-infected patients had GalS antibody titers ranging from 1 : 3200 to 1 : 25 600. No antibodies were found in the CSF but the majority of patients had signs of intrathecal immunoglobulin production, with 12/25 showing an increased IgG index and 22/25 showing two or more oligoclonal bands enriched in CSF. Gisslen *et al.* concluded that anti-GalS antibodies did not seem to be involved in the pathogenesis of central nervous system (CNS) myelin damage in HIV-1 infection.⁸¹ But they did discuss the possibility that these antibodies may induce immune demyelination preferentially in the PNS if there exists a difference in the structural location of GalS in central and peripheral myelin membranes.

We have previously shown peripheral nerve immunoreactivity by enzyme-linked immunosorbent assay (ELISA) in a cohort of HIV-positive plasma samples.⁸² Eighteen of the 35 plasma samples screened contained antiperipheral nerve myelin (PNM) IgM antibodies and 11 had IgG anti-PNM antibodies. Thin layer chromatography (TLC)-immunostaining and western immunoblotting determined reactivity to be purely against glycolipid components of PNM. The majority of IgM and IgG antibody reactivity was found to be directed against GalS (Fig. 1). Of the 35 HIV-positive samples screened by ELISA, five were considered to have raised anti-GalS IgM antibody titers (i.e. > 1000 arbitrary units (AU)/L) and six samples had raised anti-GalS IgG antibody titers (> 1000 AU/L; Tables 1,2).⁸³ The reactivity of these antibodies, which was directed specifically against GalS, was localized by indirect immunofluorescence to myelin (Fig. 2a,b). In teased nerve fiber preparations it was clear that although staining of myelin was diffuse, staining at the paranodal regions and Schmidt-Lanterman incisures was more intense (Fig. 2c,d).⁸⁴

These anti-GalS antibodies were present in HIV-infected individuals with and without immunosuppression.⁸² This suggests that in immunosuppressed HIV-positive individuals (CD4 lymphocyte counts < 200/ μ L of peripheral blood), CD5-positive B cells may be responsible for producing autoantibodies against the carbohydrate moieties of peripheral myelin glycolipids because high-titer antibodies were present in these patients. Alternatively, these antibodies may be generated during hypergammaglobulinemia⁸⁵ because polyclonal generation of immunoglobulins is common in HIV infection even when immunosuppression is severe.^{85,86} A significant association between the presence of autoantibodies in HIV-infected patients and the depletion of CD4 lymphocyte counts has been documented.⁸⁷ Massabki *et al.*, however, found that

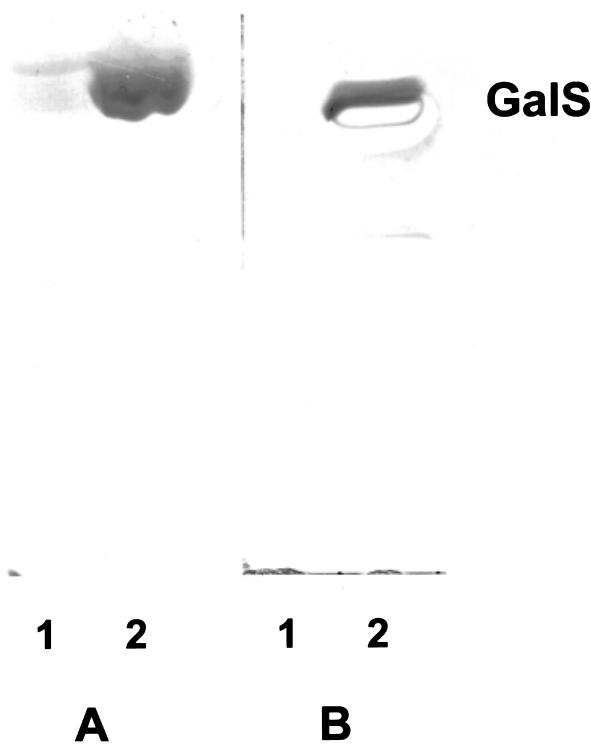


Fig. 1 Thin layer chromatography immunostaining procedure detecting both IgM and IgG anti-GalS antibody reactivity in plasma from HIV-infected individuals. Each lane was loaded with 5 µg of either neutral (lane 1) or acidic (lane 2) glycolipids extracted from human sciatic nerve myelin. IgG anti-GalS reactivity (a) and IgM anti-GalS reactivity (b) both from different HIV-infected individuals.

Table 1 Correlation of IgM anti-GalS titer in HIV patient plasma with CD4 lymphocyte count

CD4 count	Titer (AU/L)				Total no. reacting
	200	500	1000	2000	
> 500	2	1	3	3	9
200-500	4	1	1	2	8
< 200	-	1	1	-	2
Total	6	3	5	5	19

AU, arbitrary units.

Table 2 Correlation of IgG anti-GalS titer in HIV patient plasma with CD4 lymphocyte count

CD4 count (µL of peripheral blood)	Titer (AU/L)					Total no. reacting
	200	500	2000	5000	10000	
> 500				1	1	2
200-500			1			1
< 200	3	2	1	1	1	8
Total	3	2	2	2	2	11

AU, arbitrary units.

patients with low-titer autoantibodies do not develop autoimmune disease.⁸⁷ In contrast, our findings indicate that certain antiglycolipid antibodies (specifically IgG anti-GalS) can be present in very high titers.

In the present study, titers of IgG anti-GalS antibodies in HIV-infected patients were as high as 10 000 AU/L compared with IgM anti-GalS antibody titers of up to 2000 AU/L. The higher IgG titers may reflect recurrent episodes of subclinical damage to myelin. A similar profile was found in two HIV-negative patients with demyelinating neuropathy associated with IgM paraproteinemia. In these, the anti-GalS IgM antibody titers detected were 10 000 and 1 000 000 AU/L, respectively.³⁵ But antibody titers in plasma samples from HIV-infected patients with neuropathy were moderate and could not be regarded as raised. The highest anti-GalS IgG and IgM antibody titers in these patients were 1000 and 500 AU/L, respectively.⁸³

Experimental polyneuropathy has been produced by Qin and Guan in guinea-pigs using GalS as the neuritogen.⁸⁸ These investigators correlated IgG antibody titer levels with the pathological findings in nerves of 13 animals injected with bovine brain GalS. All but two animals were symptomatic. These two had only moderately raised titers (1 : 800, 1 : 1600). The 11 symptomatic animals showed typical GBS and the intensity of their motor deficits correlated significantly with their serum antibody titers. Demyelination (especially in nerve rootlets) was the major pathology observed. Deposits of IgG were found at nodes of Ranvier and in myelin sheaths. The Neuro-pathology Research Laboratory at Royal Melbourne Hospital has been able to show that HIV-positive plasma samples with raised IgM or IgG anti-GalS antibody titers showed strong binding to myelin but no binding to the axonal membrane.⁸⁴ It is possible that raised anti-GalS antibodies may predict a higher likelihood of neuropathic disease in HIV-infected patients.

ANTIGANGLIOSIDE ANTIBODIES

Antibodies against gangliosides have been found in a variety of human neurological disorders of both immune and non-immune pathogenesis,⁸⁹⁻⁹² but their contribution to inflammatory demyelination is questioned.⁹³ Despite inconsistent data linking various antiganglioside antibodies to certain neuropathies and neurodegenerative conditions, two neuropathies stand out with regard to their association with specific antiganglioside autoantibodies.

Patients with multifocal motor neuropathy (MMN) commonly have anti-GM₁ antibodies present in high titers.^{44,94} One study reported anti-GM₁ IgG and IgM antibodies in 90% of patients with MMN.⁹⁴ Among these, 60% of patients had high titers. Another study reported an ability to stimulate the production of anti-GM₁ antibodies

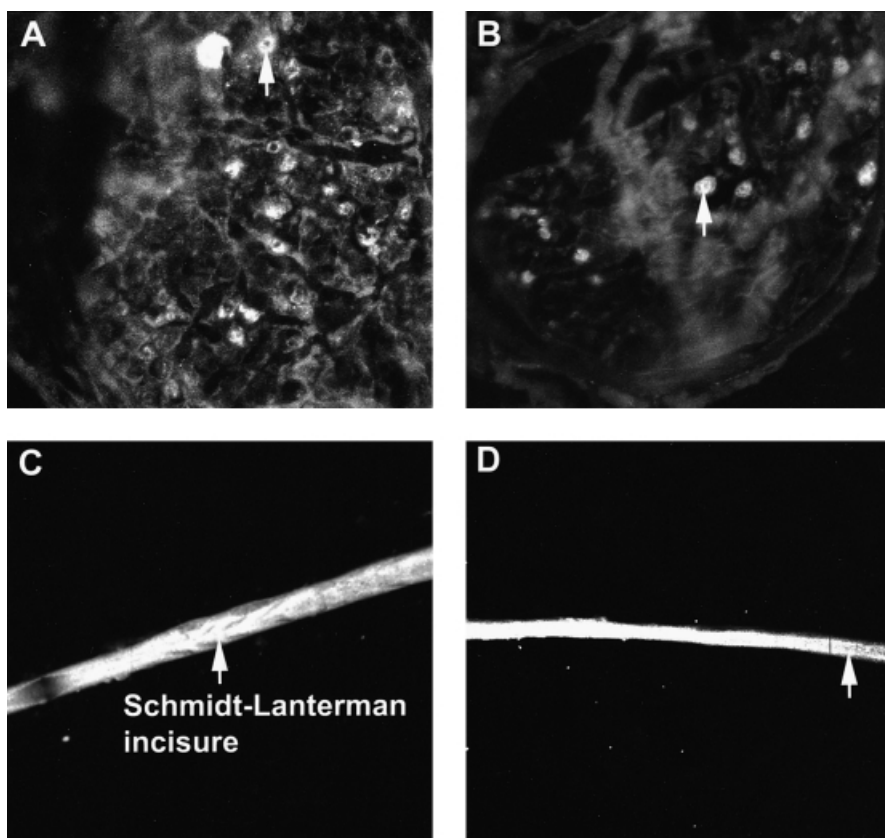


Fig. 2 Immunofluorescence of unfixed human sciatic nerve sections and fixed teased nerve fibers. IgM anti-GalS antibody reactivity from plasma obtained from an HIV-infected individual (**a**); IgG anti-GalS antibody reactivity from plasma obtained from a different HIV-infected individual (**b**). IgM anti-GalS antibody reactivity from plasma obtained from an HIV-infected individual (**c**); IgG anti-GalS antibody reactivity from plasma obtained from a different HIV-infected individual (**d**). Arrows show myelin staining.

from peripheral blood mononuclear cells (PBMNC) isolated from MMN patients.⁹⁵ Because GM₁ is concentrated at the nodes of Ranvier,⁴⁴ it remains plausible that these antibodies may cause both electrophysiological abnormalities such as conduction block and pathological abnormalities, in particular paranodal demyelination. Injection of serum containing high titers of anti-GM₁ antibodies from a MMN patient into rat sciatic nerve resulted in conduction block with temporal dispersion, demyelination in 6.5% of the fibers and immunoglobulin deposition at the nodes of Ranvier.⁴⁴

The other disorder showing antiganglioside reactivity is the Miller-Fisher syndrome. This syndrome is strongly associated with anti-GQ_{1b} IgG antibodies.⁹⁶⁻⁹⁸ GQ_{1b} ganglioside is largely concentrated in the oculomotor, trochlear and abducens nerves in paranodal regions.⁹⁶⁻⁹⁸ Most patients have a favorable response to plasma exchange.

Apart from these two disorders, there has been conflicting evidence regarding the role of gangliosides as possible antigens in GBS. In a study by Ilyas *et al.* five of 26 patients with GBS had high titers of antibodies to gangliosides.⁹⁹ One of these patients had strong IgG reactivity against the most predominant ganglioside of PNS myelin, LM₁. No antiganglioside antibodies were found in

sera of healthy controls. Alternatively, Svennerholm and Fredman found antiganglioside reactivity in 39 of 50 patients with GBS, but also in 47 of 197 normal control individuals.¹⁰⁰

Significant levels of antibodies against asialo-GM₁ neutral ganglioside have been reported in 36% of 31 homosexual men with AIDS.¹⁰¹ Whether these antibodies participate in the pathogenesis of any of the various peripheral neuropathies seen in patients infected with HIV remains to be determined. Sorice *et al.* detected both IgG and IgM antiganglioside antibodies in the CSF in three AIDS patients with progressive encephalopathy and signs of hypomyelination.¹⁰² Two of the samples were shown to react specifically with GM₃, GM₁, and GD_{1a}, and one with GD_{1a} alone. None of the sera from HIV-positive patients without encephalopathy contained antiganglioside reactivity, nor did any of the HIV-seronegative control samples with and without CNS disease. Whether these patients had concomitant peripheral nerve demyelination is not known, but these data demonstrate that antiganglioside antibodies can be present in patients with advanced HIV infection.

We have shown that antiganglioside antibody titers in HIV-positive plasma samples do not appear to have any clinical significance. Only one sample contained a relatively

Table 3 Correlation of IgG anti-ganglioside titer in HIV patient plasma with CD4 lymphocyte count

CD4 count	Titer (AU/L)				Total no. reacting
	100	200	500	2000	
> 500	–	–	1	–	1
200–500	–	–	1	–	1
< 200	2	1	1	1	5
Total	2	1	3	1	7

AU, arbitrary units.

raised titer of 2000 AU/L and only seven of the 35 HIV-positive plasma samples showed some reactivity against the various gangliosides, and so it is reasonable to conclude that these glycolipids are not candidate autoantigens in HIV-infected individuals (Table 3; Petratos *et al.* unpubl. data, 1998). Data linking antiganglioside antibodies to peripheral neuropathy have demonstrated these antibodies only in patients with motor deficits. The most predominant neuropathy in HIV infection, however, is DPSN, a sensory disorder.^{103–106} This may explain the absence of antiganglioside antibodies in the HIV-positive plasma samples tested in the present study.

ANTI-GALC ANTIBODIES

There have been few data published regarding a possible role for GalC and other minor glycolipids in the pathogenesis of immune demyelinating neuropathies. Furthermore, no such data have been published for HIV-infected individuals with and without peripheral neuropathy. There has been wide documentation of anti-GalC antibody-induced experimental allergic neuritis (EAN), which closely depicts the pathology of IDPN. The only difference from IDPN is that this type of EAN does not show T lymphocyte infiltration of affected nerves.¹⁰⁷ Considering that GalC is the most predominant glycolipid in PNS myelin,^{108–110} it is possible that the presence of anti-GalC antibodies may be an early indication of a propensity for demyelination. Investigators have searched for anti-GalC antibodies in plasma samples from GBS patients but have not been able to detect elevated antibody levels relative to healthy donor samples.⁴² The only group that found some antibody activity was able to detect IgM reactivity against GalC in only two samples obtained from 52 acute GBS sera.⁸ These data would suggest that anti-GalC antibodies are not elevated in human immune demyelinating neuropathies.^{111,112}

We have shown only three of 35 HIV-positive plasma samples screened by ELISA to have relatively raised antibody titers of 2000 AU/L (Table 4; Petratos *et al.* unpubl. data, 1998). Of interest, these were from patients with CD4 lymphocyte counts < 200/ μ L and the antibody class was IgG. This pattern was similar to that seen with the

Table 4 Correlation of IgG anti-GalC titer in HIV patient plasma with CD4 lymphocyte count

CD4 count	Titer (AU/L)		Total no. reacting
	500	2000	
> 500	–	–	0
200–500	1	–	1
< 200	2	3	5
Total	3	3	6

AU, arbitrary units.

anti-GalS IgG titers described earlier. A similar pathway augmenting anti-GalC IgG antibodies could have been operating in these three HIV-infected individuals.

Other neutral glycolipids have been proposed as neuritogens involved in immune demyelinating disorders. Koski *et al.* reported the presence of antineutral glycolipid antibodies in all of the 10 GBS patients studied whereas none of the control group contained these antibodies.¹¹³ Five GBS sera showed reactivity against a minor neutral lipid migrating between paragloboside and neolacto-hexaosesyl ceramide. Ilyas *et al.* demonstrated intense reactivity in a proportion of sera obtained from GBS patients against purified Forssman glycolipid and a number of glycolipids in higher neutral glycolipid-enriched fractions of human cauda equina and dog sciatic nerve.⁴² But similar results were found in the control group. These results contrast with the findings of Koski *et al.*, who demonstrated high IgM antibody titers against Forssman neutral glycolipid in GBS sera but could not find the same reactivity in their control group.²⁸

The majority of these glycolipids are not exclusively found in the PNS. Glycolipids such as the gangliosides, GalC and GalS are major components of central myelin.¹¹⁴ Hence, the question arises as to why IDPN is localized to the PNS without concomitant CNS dysfunction. Studies have shown, however, that some patients with CNS demyelination may have concomitant PNS demyelination.^{115,116} Kinoshita *et al.* showed at autopsy that a patient who had died from acute disseminated encephalomyelitis also had inflammatory demyelinating polyradiculitis.¹¹⁶ This raises the question of a shared immunological epitope between the CNS and the PNS. There have been other reports of this type of finding in CNS disorders.^{115,117,118} Adding to the body of evidence supporting this shared epitope hypothesis is the finding of active peripheral demyelinating lesions in MS patients.^{119–123} In HIV infection both CNS and PNS compartments may be infected, leading to concomitant lesions.¹²⁴

THE AUTOIMMUNE HYPOTHESIS

In the early stages of HIV infection, autoimmune diseases such as immune thrombocytopenia and vasculitis can

complicate the disease.¹²⁵ These autoantibodies are usually produced as part of the immune dysregulation induced by HIV infection.^{85,86} Neurological disorders such as those seen for T cell depletion during HIV infection¹²⁶ may be in part due to neuroimmunological responses to HIV infection.¹²⁷ Dysregulation of the immune system may play a role in HIV-associated neuropathies. Immunological phenomena such as antilymphocyte antibodies, circulating immune complexes, cross-reacting antigens to myelin, and abnormally expressed human leukocyte antigens (HLA) may play significant roles in determining neurological outcomes in HIV-infected patients.¹²⁶⁻¹³⁰ Also, during immunosuppression in HIV-infected individuals, depletion of Th cells may impair the generation of suppressor cells, allowing a cell-mediated autoimmune response similar to that seen in the P₂-specific EAN model.¹³¹ HIV-associated IDPN has the same clinical and pathological features as in non-HIV-associated GBS and the experimental animal model, EAN.¹⁵ The inflammatory lesions are frequently associated with foci of demyelination or tissue necrosis, similar to those seen in sheep infected with another of the lentiviruses, visna.¹³² It is also possible that mononeuropathy multiplex may be of autoimmune pathogenesis because similar lesions may occur in immune complex-mediated vasculitis involving peripheral nerves.¹³³

Alternatively, antibodies reacting against peripheral nerve constituents could possibly be generated from the productive infection of cells outside the peripheral nerve compartment. Direct HIV infection of cells containing epitopes (such as glycolipids) similar to those expressed in the PNS may provoke an autoimmune response: (i) by altering the structural integrity of cell membrane components; or (ii) by the incorporation of host cell glycolipid into the viral coat (viral budding), allowing them to become antigenic.¹³⁴ Enveloped budding viruses (such as HIV) take host cell glycolipid into their coat (Fig. 3). Glycolipid presented in the virus coat to the immune system may be much more antigenic than glycolipid alone. HIV has been reported to infect cells containing cell membrane glycolipids, similar to those found in Schwann cells, myelin,¹³⁵ peripheral T cells,¹³⁶ *in vitro* differentiated monocyte-derived macrophages¹³⁷ and epithelial cells of the gastrointestinal system.^{138,139} It is therefore plausible to suggest that these glycolipids, incorporated into the membrane of budding virus and presented to the immune system, can provoke both cell-mediated and humoral antibody attacks against glycolipid present on PNS cells, Schwann cells and myelin.

Evidence for this pathogenic mechanism has been clearly demonstrated for other viruses. Almeida and Waterson have shown that only the viral protein spikes of corona virus derived from chicken fibroblasts can be labeled by immune serum.¹⁴⁰ But when the coronavirus

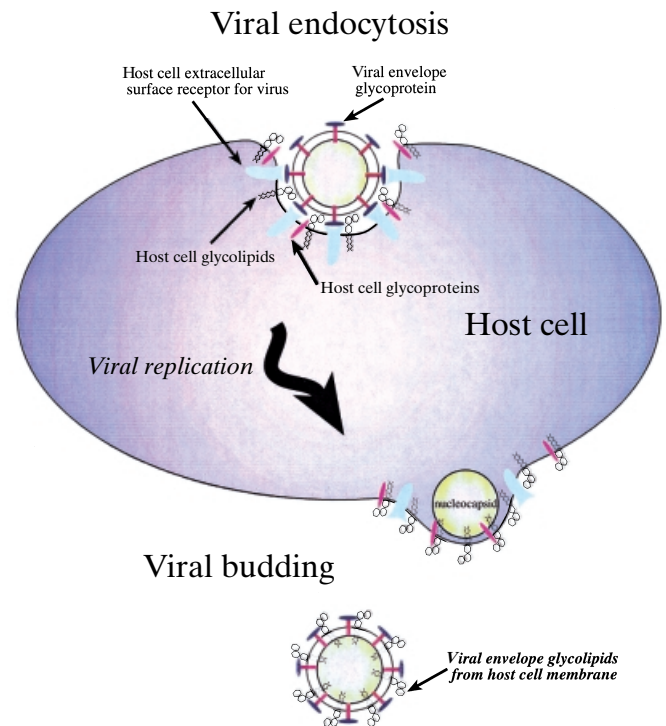


Fig. 3 Proposed incorporation of host cell glycolipid into budding viral particles. As the HIV replicates and subsequently buds off from a host cell membrane enriched in glycolipid, it incorporates these glycolipids into its glycoprotein coat. Upon presentation to the host immune system the glycolipid carbohydrate epitopes are recognized as 'foreign' and a primary immune response is elicited.

derived from chickens is used to make immune serum in rabbits, both the viral protein spikes and the intermediate membrane envelope of the virus are labeled, indicating that the chicken-derived virus is able to produce considerable antichickens host-membrane antibody in the rabbit. An example more pertinent to HIV is that of Friend leukemia virus (a retrovirus). This virus buds from the erythrocyte membrane, taking antigen into its coat,¹⁴¹ and can evoke an immune reaction that causes hemolysis of normal, uninfected erythrocytes.¹⁴²

Of interest is the finding that mice inoculated with the neurotropic strain of vaccinia virus produce antibodies that bind to normal, uninfected myelin and oligodendrocytes, indicating that the virus presents antigenic myelin and oligodendrocyte membrane components to the immune system.¹⁴³ This does not, however, occur when the dermatotropic strain of virus is used. A possible cellular immune destruction of neural tissue was demonstrated by Rook and Webb, who showed that cytotoxic T cells sensitized to tick-borne encephalitis (TBE) virus killed not only TBE-infected glial cells but also a significant percentage of normal, uninfected glial cells.¹⁴⁴

Experiments have shown that the lipid composition of the envelope of paramyxovirus SV5, cultured in four different host cells with different lipid compositions, very closely resembles the membrane of the host cell in which it was cultured.¹⁴⁵ The lipid composition of the viral envelope has been shown to resemble closely those of the host cells from which they are derived in mumps,¹⁴⁶ influenza,^{147,148} Sinbis,^{149,150} Venezuelan equine encephalomyelitis,¹⁵¹ and Semliki Forest viruses (SFV).^{152–154} The SFV (avirulent A7 (74) strain) has been used as a model for virus-induced demyelination in mice.¹⁵⁵ These mice develop pronounced antiglycolipid reactivity.^{156,157}

Monoclonal antibody (MAb) (373) raised against brain-derived 'inactivated' SFV has been shown to cross-react with GalS and GalC.¹⁵⁸ This was subsequently shown to neutralize SFV significantly.¹⁵⁸ This antibody also labels SFV, influenza, and measles virus, which replicate in the same brain cell cultures from which the SFV is derived.⁷⁴ This indicates that budding viruses take similar glycolipid into their coat if the original host cell of replication is the same.

Prospective studies are essential to link antiglycolipid antibodies with peripheral nerve dysfunction in HIV infection. Data published by De Gasperi *et al.* have demonstrated such a link with serum and CSF antibodies.⁸⁰ But they believe that the anti-GalS antibodies present in serum are non-specific, noting that anti-GalS antibodies are present in the serum of patients without HIV infection and other neurological and immunological disorders,^{41,42,66,67,159–164} and that their serum sample group did not show any correlation between IgG anti-GalS levels and CD4 lymphocyte count. As already explained, however, IgG levels do not necessarily decline with declining CD4 lymphocyte count, because the epitope it recognizes is a glycoconjugate (in this case a glycolipid). The CD5-positive B cells are most likely responsible for the production of antiglycolipid antibodies in HIV-positive individuals.^{38,165} Activation of these cells may be due to the glycolipid (most likely GalS) being presented to the immune system in an altered fashion. GalS may become incorporated into viral envelope protein during the course of productive infection (i.e. cells containing GalS within their membrane) and the viral budding process.⁷⁴ The integration of GalS into the viral membrane may be the trigger for the immune system to begin synthesizing autoantibodies against the carbohydrate moiety of the molecule.

Recently, evidence has shown that budding HIV-1 particles produced by infected T cell lines occurs selectively from glycolipid enriched membrane regions ('lipid rafts') which include GM₁.¹⁶⁶ These investigators demonstrated by confocal microscopy that lipid raft-associated molecules, including the glycosylphosphatidylinositol (GPI)-anchored

proteins Thy-1 and CD59 and GM₁, colocalize with HIV-1 proteins. Through virus phenotyping using monoclonal antibodies these investigators were also able to indicate that these molecules were incorporated into HIV-1 particles. Their data suggest that budding of HIV virions through these membrane lipid rafts provides for the incorporation of host cell glycolipids into the viral envelope. This now provides direct evidence that glycolipids (which are possible peripheral nerve myelin neuritogens) are incorporated in budding HIV. Whether this presentation to the peripheral immune cells can elicit an antiglycolipid autoantibody response is yet to be determined.

Experiments in laboratory animals are now required to determine if these antiglycolipid antibodies are capable of directly damaging myelin and whether other factors such as complement, oxygen reactive species and cytokines are also involved in the pathology of inflammatory demyelination in HIV infection. Future work could test the hypothesis that antibodies against peripheral myelin glycolipids in HIV-infected people induce inflammatory demyelination. Specifically, this research would involve the induction of EAN in Lewis rats by injection of purified antiglycolipid antibodies from HIV-infected individuals with and without neuropathy. Further experiments would correlate the electrophysiological and morphological data indicating myelin damage.

If these antiglycolipid antibodies are capable of inducing immune demyelination then direct immunofluorescence would show antibodies bound to myelin, and morphological assessment would show intramyelinic swelling and vacuolation. This would most likely be initiated at paranodal areas and Schmidt-Lanterman incisures for anti-GalS antibodies.⁷⁹ These antibodies have also been shown to cause concomitant widening of the outer and inner myelin lamellae of Schwann cell cytoplasm.⁷⁹ Electrophysiological abnormalities would include absent F-waves with preserved distal compound muscle action potential (CMAP) amplitudes ($\geq 75\%$ lower limit of normal) as evidence of proximal conduction block,¹⁶⁷ conduction latency, as a sign of axonal degeneration, recorded distally,¹⁶⁸ or similar conduction slowing in both distal and proximal nerve segments for demyelinating neuropathies.¹⁶⁹

CONCLUSION

If antiglycolipid antibodies cause immune demyelination in HIV-infected patients, a screening ELISA could be used to predict the likelihood of individuals developing peripheral neuropathy. It may also assist in the diagnosis of HIV-infected patients with underlying subclinical neuropathy. Such patients may benefit by the early institution of treatment such as plasmapheresis and intravenous

immunoglobulin. A serological screening test may also be of benefit in monitoring disease progress by measuring antiglycolipid antibody titers. It may also be possible to differentiate the neuropathic syndrome according to the specific glycolipid against which antibodies are directed. An example of this is sensorimotor dysfunction with raised anti-GaIS antibody titers.

Of further benefit to the patient would be the identification of the specific CD5⁺ B cell generating antiglycolipid antibodies. If future research is able to specifically identify these cells it may be also possible to cyto-apheresis patients (specifically for this cell type) with raised titers of antiglycolipid antibodies.

Despite the data presented throughout this report, two main issues still remain to be resolved in identifying the pathogenesis of HIV-associated inflammatory demyelination: (i) whether the disease is autoimmune; and (ii) whether antiglycolipid autoantibodies are pathogenetic. There exist a set of working criteria which have been derived from a variety of diseases that are generally believed to have antibody-mediated autoimmune mechanisms.¹⁷⁰ These include: (i) antibody is present in patients with the disease; (ii) antibody interacts with the target antigen; (iii) passive transfer of the antibody produces the disease; (iv) immunization of animals with the antigen produces disease; and (v) reduction of the antibody ameliorates the disease. All of the aforementioned criteria have been described in assessing the role of anti-GaIS antibodies in peripheral neuropathy. Only criteria 1 and 2 were described by De Gasperi *et al.*,⁸⁰ Petratos *et al.*⁸²⁻⁸⁴ and Gisslen *et al.*⁸¹ in HIV-infected patients. Passive transfer of these antibodies in HIV-infected patients into animals would address criterion 3 and has been described in HIV-negative patients with immune demyelination.⁷⁹ Criterion 4 has already been clearly defined in animals⁸⁸ and criterion 5 could be trialed in HIV-infected patients once a clear pathogenetic link of these autoantibodies has been established. For the time being, further research is essential to develop a direct immunopathogenic link for these antiglycolipid antibodies in HIV-associated peripheral neuropathy. Only then will these diagnostic and therapeutic regimens be implemented within the neurological clinic.

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