



NEUROLOGY AND AUTO IMMUNITY

Editorial

Autoantibodies can be found in some cases of peripheral neuropathy (about ten per cent of the total). In recent years, major progress has been made in the systematization of these forms of neuropathy, partly based on the classification of autoantibodies which recognize antigenic determinants in the nervous system.

This progress means that now, ordering a test for such antibodies is warranted in three situations :

- Peripheral neuropathy associated with plasma cell dysplasia.
- Neuropathy involving the peripheral nervous system-either acute (acute idiopathic polyneuritis) or chronic-associated with anti-ganglioside antibodies.
- Certain types of paraneoplastic polyneuropathy.

This, the fifty seventh issue of the Letter is dedicated to these three different types of neuropathy and the autoantibodies involved in their pathogenesis.

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Peripheral neuropathies associated with monoclonal immunoglobulin

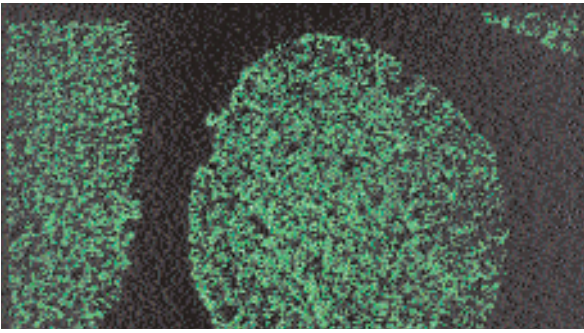
A monoclonal immunoglobulin is identified in ten to fifteen per cent of all cases of neuropathy, and it is often the neuropathy which is the factor which leads to discovery of the dysglobulinemia. In most cases, it is a Monoclonal Gammopathy of Undetermined Significance (MGUS) but it may also be Waldenström's macroglobulinemia, about five per cent of cases of which are complicated by peripheral neuropathy.

Testing for autoantibodies is justified if the abnormal antibody is of the IgM class. Most commonly, the autoantibody is directed against myelin but sometimes, the structure recognized is the axonal cytoskeleton or a component of the extracellular matrix. Testing for autoantibodies against myelin is recommended when the neuropathy is sensory (or sensorimotor with a predominantly sensory component) and symmetrical. Such neuropathy most commonly affects males in their sixties, begins in the legs, and develops along chronic lines. The symptoms are due to demyelination which can be detected by electrophysiological testing and anatomical analysis.

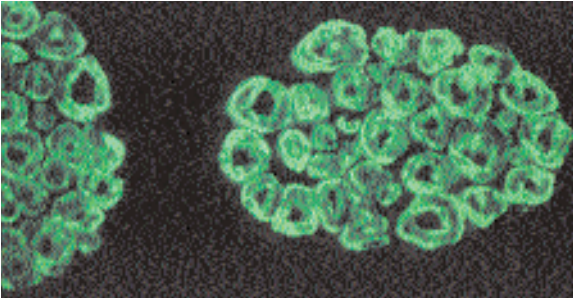
In the example, anti-myelin antibodies were detected using IF staining of sections of monkey sciatic nerve tissue (Photographs 1 and 2). The pattern is one of more or less uniform staining throughout the myelin sheath, with no binding to the axon in the middle. There is no relationship between the level of the antibody and the seriousness of the clinical symptoms so a purely qualitative assay of may be sufficient here.

It can be useful to characterize the actual antigen which is recognized. Western blotting or ELISA testing usually shows the target to be Myelin-Associated Glycoprotein (MAG). The affinity of the antibody for the glycoprotein usually correlates with the intensity of the IF staining (Photograph 3). There is also usually cross-reactivity with other peripheral nervous system





Photograph 1 : Anti-myelin IgM antibodies detected on sectioned monkey sciatic nerve tissue
(The Binding Site Company)



Photograph 2 : Anti-myelin IgM antibodies detected on sectioned monkey sciatic nerve tissue (serum diluted 50-fold) (micrograph taken at higher magnification).
(The Binding Site Company)

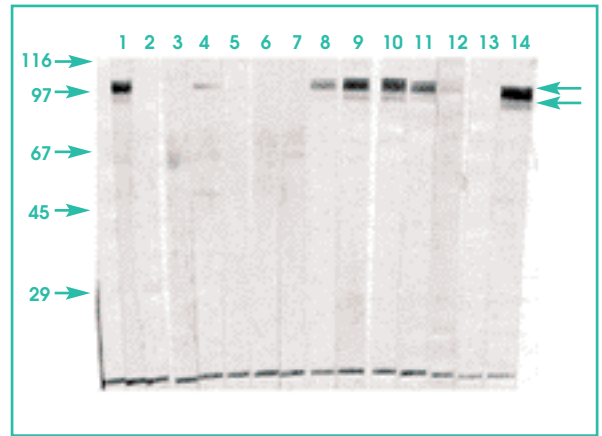
glycoproteins and sulfoglycolipids (SGPG - SGPLG) which are detected by immuno-footprinting after thin layer chromatography. In fact, the actual epitope which is recognized is an oligosaccharide which is found on all these antigens. In some cases, the antibody recognizes SGPG and/or other glycoproteins without reacting at all with MAG. In this case, the clinical picture and anatomical characteristics can be variable. Anti-SPGP antibodies have been reported in many different clinical syndromes, even in the absence of monoclonal gammopathy.

Autoantibodies against gangliosides and neuropathies

Testing for antibodies directed against gangliosides is warranted in five clinical conditions.

■ Multifocal Conduction Block Neuropathy (MCBN) :

The onset of motor problems with or without muscular wasting but without any sensory component, with steadily progressive, asymmetric involvement justifies testing for anti-GM1 antibodies, either by ELISA or by thin layer chromatography. Diagnosis depends on defining persistent conduction blocks in electrophysiological tests. Autoantibodies are found in about eighty per cent of cases of MCBN. The abnormal antibody is of the IgM class and it recognizes an epitope which is carried on both asialo-GM1 and GD1b. If the GM1 test gives a positive result, it may be worth testing separately for antibodies against GD1a, GD1b and asialo-GM1 to generate a "profile" of anti-ganglioside reactivity.



Photograph 3 : Myelin Western blot developed with alkaline phosphatase-conjugated anti-human IgM. Developed in the presence of NBT/BCIP (serum diluted 200-fold). Two different proteins are bound, one at 100 kDa (MAG) and another lighter band at 90 kDa (d-MAG). The differential intensity of the labelling is evident (lanes 1, 4, 8, 9, 10 and 11).

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The administration of IV antibodies may palliate the symptoms although, since this kind of treatment does not alter the actual concentration of the antibody (even when clinical improvement is observed), regular follow-up testing is not necessary.

■ Amyotrophy without detectable conduction block and no pyramidal symptoms : differential diagnosis of amyotrophic lateral sclerosis (Lou Gehrig Disease) :

In some cases, anti-GM1 antibodies can be detected, usually belonging to the IgG class. Again, immunoglobulin treatment may be effective but this is less often the case with this pathology.

■ Acute idiopathic polyneuritis involving only motor nerves :

Certain forms of acute idiopathic polyneuritis are characterized by severe primary axonal involvement and distinction is made between these forms and the more classic forms which are associated with demyelination. The anti-GM1 antibodies detected are either of the IgA or IgG class, and may be elicited as a result of *Campylobacter jejuni* infection because the lipopolysaccharide of this bacterium contains sugar sequences which are identical to those found in certain gangliosides. Autoantibody levels are at their highest at the beginning of the acute phase and tend to fall over the ensuing weeks.

■ Miller-Fisher Syndrome :

This is a variant of acute idiopathic polyneuritis associated with ophthalmoplegia in which the ophthalmic component may be the only symptom. This syndrome is very specifically associated with IgG antibodies directed against GQ1b (which also contains groups identical to antigenic determinants found on certain strains of *Campylobacter jejuni*) which appear and then rapidly drop off.



■ **Chronic sensory neuropathies :**

Anti-GD1b antibodies have been reported in some cases of chronic sensory neuropathy. These are of the IgM class, often monoclonal, and do not cross-react with GM1.

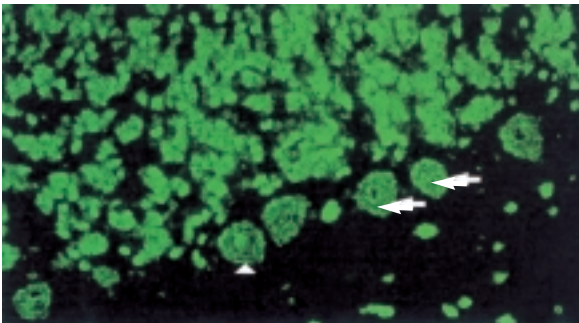
Peripheral paraneoplastic polyneuropathies

Neurological paraneoplastic polyneuropathy is an extremely rare syndrome which is not associated with either metastatic invasion or infectious or metabolic complications. Such syndromes are associated with the presence of autoantibodies directed against an antigen carried on the malignant clone which is also expressed in nervous tissue. Most neurological paraneoplastic syndromes involve the central nervous system.

In a few cases, it is the peripheral nervous system which is affected; the best characterized of these is hereditary sensory radicular neuropathy (Denny Brown).

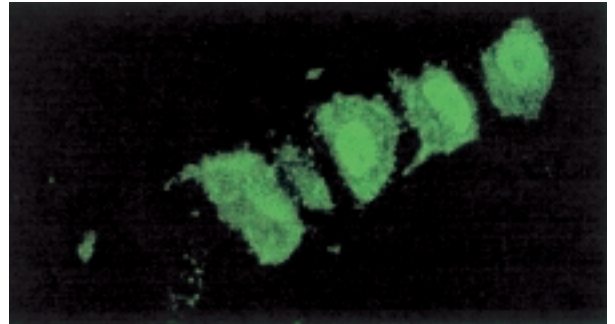
The autoantibodies concerned are called anti-Hu or ANNA-1 (an for Anti-Neuronal cell Antibody type 1). The most common kind of cancer associated with these antibodies is undifferentiated small cell lung carcinoma (80 % of cases) but other malignancies may be implicated, including neuroblastoma, prostate cancer, rhabdomyosarcoma and seminoma. Anti-Hu antibodies are also associated with other clinical situations such as limbic encephalitis and spinal problems. These antibodies should be tested for in any patient who has unexplained acute or sub-acute neurological symptoms and, if there is central nervous system involvement, a CSF assay may be justified in order to determine whether the autoantibody is being synthesized intrathecally or not.

The assay involves staining sectioned cerebellar tissue (Photograph 4). The antibody binds the nuclei of the neurons and both the cytoplasm and the nuclei of Purkinje cells. All the neurons are labelled, including those of the peripheral nervous system, in contrast to the pattern seen with anti-R1 antibodies

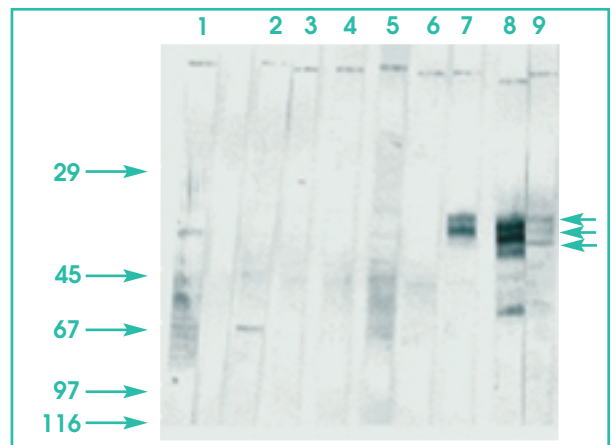


Photograph 4 : Anti-Hu antibody staining. Detected by IF on sectioned cerebellar tissue. There is binding in both the nuclei (▲) and cytoplasm (◄) of the Purkinje cells. (The Binding Site Company)

(see the IF staining of the neurons of the mesenteric plexus of rat stomach tissue in Photograph 5). The possibility of such anti-nuclear antibodies being present has to be excluded using appropriate substrates, and the result can be confirmed by Western blotting using either a protein extract or a recombinant protein (Photograph 6). Such antibodies are often detected before the underlying tumor has been discovered. Sometimes, the tumor cannot be found immediately but nevertheless monitoring should be kept up the tumor may eventually be found months or years later, or it may never be found at all (tumor growth is usually retarded in patients with this kind of autoantibody). In general, treatment of the tumor does not help with the neurological symptoms which advance independently of the malignancy.



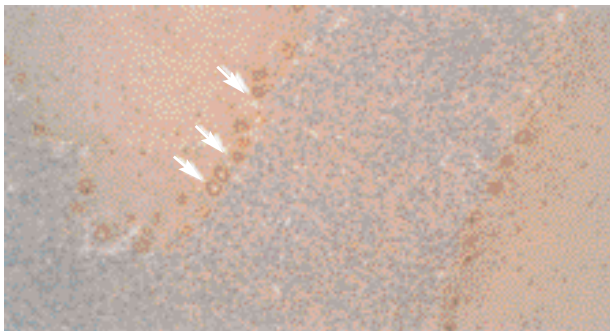
Photograph 5 : IF staining of the rat mesenteric plexus. Anti-Hu antibodies. (The Binding Site Company)



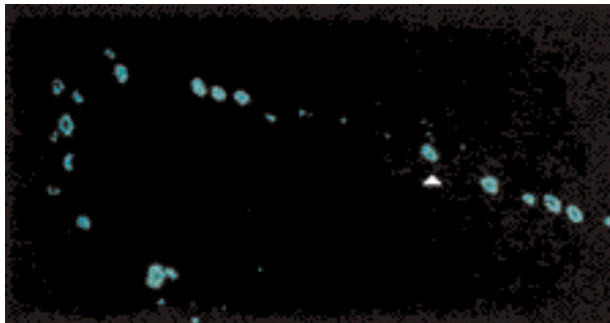
Photograph 6 : Western blot of neuronal anti-nuclear factors. There is intense anti-Hu labelling in lane 8 (three bands between 35 and 40 kDa) (serum samples diluted 200-fold). Band 7 was tested against CSF (diluted 2-fold) taken from the same patient and binding was very weak so it can be concluded that anti-Hu antibodies are not being synthesized intrathecally. José Boucraut (Immunology Department, Conception Hospital, Marseille)

Other neurological paraneoplastic syndromes have been described, with the following being the most commonly implicated autoantibodies :

- Anti-Yo (or PCA-1 for anti-Purkinje Cell Antibody type 1): a syndrome affecting the cerebellum, predominantly (90 % of cases) associated with gynecological malignancies (ovarian and breast cancer) but occasionally with undifferentiated small cell lung carcinoma (Photographs 7 and 8).
- Non-Yo anti-Purkinje cell antibodies (or PCA-2): a syndrome affecting the cerebellum, associated with lymphoma.
- Anti-R1 (or ANNA-2): myoclonia, usually associated with breast cancer.



Photograph 7 : Sectioned cerebellar tissue labelled with immunoperoxidase-conjugated antibody and developed with alpha ethyl carbazole (serum diluted 200-fold). There is only binding in the cytoplasm of the Purkinje cells.
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Photograph 8 : staining of Purkinje cells by indirect immunofluorescence on sectioned monkey cerebellar tissue (serum diluted 50-fold).
(The Binding Site Company)

- Anti-calcium channel antibodies: Eaton-Lambert syndrome, a myasthenia-like syndrome which is most commonly (70 % of cases) associated with small cell lung carcinoma.
- Anti-CV2, anti-amphysin, anti-GAD, anti-Ma1, anti-Ma2 or anti-To antibodies, associated with a variety of clinical pictures and different tumors.

Autoantigens

FURTHER INFORMATION...

The **glycosphingolipids** are a family of glycolipids made up of :

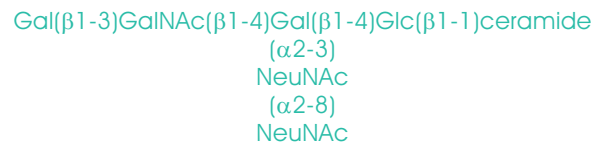
- a lipid-the ceramide portion which consists of a fatty acid attached to a sphingosine base,
- carbohydrate (hexose sugars).

The ceramide group anchors the glycosphingolipid in the plasma membrane with the sugar(s) projecting out into the extracellular environment.

Cerebroside consists of a ceramide attached to a single sugar (i.e. a mono-hexosyl ceramide). The most widespread in humans is galactocerebroside which, when sulfated at C3, forms the **sulfatide** which is the major component of myelin in the periphery and which might act as an autoantigen in certain forms of sensory neuropathy (although this is controversial).

The gangliosides are complex glycosphingolipids which contain at least one residue of sialic acid (or N-acetylneuraminic acid in humans). The oligosaccharide chain contains between one and four hexose sugar residues in the following (maximum) sequence: ceramide-glucose-galactose-N-acetyl galactosamine-galactose. The terminology used to describe this family is G for glycosphingolipid then M, D, T or Q depending on the number of residues of sialic acid (mono-, di-, tri- or quadri-) followed by a number and, in some cases, a lower case letter to indicate the numbers of hexose sugars.

Thus, for example, GD1b has the following structure:



Paragloboside is a neutral glycolipid (Cer-Gal-GlcNAc-Gal) which can be sialylated at its terminal glucose to generate sialosylparagloboside, SGP (or **LM1**), an important glycolipid in peripheral nerves. Substitution of the terminal sialic acid by a sulfated glycuronic residue results in the formation of **sulfated glycuronyl paragloboside** (SGPG).

Autoantibodies recognize carbohydrate groups on these molecules. Different glycolipids, different glycoproteins and other carbohydrate-rich molecules (e.g. bacterial lipopolysaccharides) may contain the same antigenic determinants and therefore there is extensive cross-reactivity. The $\text{Gal}(\beta 1-4)\text{GlcNAc}$ so-called **HNK-1** epitope which is thus named because it is marker for a certain sub-population of Natural Killer cells-is represented in SGPG, Myelin-Associated Glycoprotein (MAG), the PO and PMP-22 proteins of compact peripheral myelin, as well as various adhesion molecules, including NCAM or L1. Whereas SGPG is specific for the peripheral nervous system, MAG is found in all nervous tissue although the demyelinating activity of anti-MAG IgM monoclonal antibodies never impinges on the central nervous system.

Autoantibodies

In normal physiology, most endogenous antigens have corresponding autoantibodies.

The difference between a healthy autoantibody and a pathological one depends on its affinity for the antigen and its level.

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Reference list available on request