AUTOIMMUNE NODO-PARANODOPATHIES OF PERIPHERAL NERVE: THE CONCEPT IS GAINING GROUND

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Word count: 3858; number of references: 78

Running title: autoimmune nodo-paranodopathies

ABSTRACT

Peripheral nerve disorders are classified as primarily demyelinating or axonal. Microstructural alterations of the nodal region are the key to understand the pathophysiology of neuropathies with antibodies to gangliosides and the new category of nodo-paranodopathy has been proposed to better characterize these disorders and overcome some inadequacies of the dichotomous classification. Recently the research in autoimmune neuropathies has been boosted by reports of patients carrying IgG4 antibodies against paranodal axo-glial proteins with distinct phenotypes and showing loss of transverse bands, terminal myelin loop detachment, nodal widening and axonal loss. These patients have been classified up to now as chronic inflammatory demyelinating polyradiculoneuropathy but, in our opinion, better fit into the nodo-paranodopathy category because nerve injury is due to dismantling of the paranode, segmental de-remyelination is absent and the pathogenic mechanism is not inflammatory. Evidence from nerve conductions and electron microscopy studies in patients and mutant animal models can reconcile the apparent contrast between the electrophysiological "demyelinating" features, explainable just by the paranodal involvement, and the axonal pathology. These patients broaden the autoimmune nodo-paranodopathy category and re-emphasize the utility of the term that pointing to the site of nerve injury reminds specific pathophysiological mechanisms, reconciles contrasting electrophysiological and pathological findings, and avoids misdiagnosis and taxonomic confusion. In our opinion the nodo-paranodopathy category better systematizes the neuropathies characterized by an autoimmune attack targeting and limited to the nodal region integrating the traditional classification of peripheral neuropathies.

Key words

Autoimmune nodo-paranodopathies, Guillain-Barré syndrome, chronic inflammatory demyelinating polyradiculoneuropathy, antiganglioside antibodies, antibodies to axo-glial proteins of paranodal junctions, reversible conduction failure.

INTRODUCTION

Peripheral nerve disorders are traditionally classified, on the basis of pathology and electrophysiology, as primary demyelinating or axonal. Microstructural changes restricted to the nodal region are the key to understand the pathophysiology of some peripheral neuropathies and the term nodo-paranodopathy was initially proposed to better characterize neuropathies with anti-gangliosides antibodies and overcome inadequacies of the dichotomous classification.[1] This categorization was later extended to include neuropathies of different etiology (autoimmune, inflammatory, ischemic, nutritional and toxic) in which the involvement of the nodal region is thought to be determinant in the pathogenesis.[2]

Recently the research in autoimmune neuropathies has been boosted by reports of patients carrying IgG4 antibodies against proteins of paranodal junctions and specific phenotypes.[3-5] These patients have been classified up to now as chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) but, in our opinion, better fit into the nodo-paranodopathy category because the site of nerve injury is focused and limited to the paranode, true de-remyelination is absent and the pathogenic mechanism is non-inflammatory.

In this personal view we consider the autoimmune neuropathies in which evidence from nerve conductions, pathology and animal models, indicates the involvement of the nodal region in the pathogenesis reemphasizing the utility of the nodo-paranodopathy concept.

THE NODAL REGION: FROM PHYSIOLOGY TO PATHOLOGY

The myelinated axons are organized in distinct domains each characterized by specific molecular arrangements: nodes of Ranvier, paranodes, juxtaparanodes and internodes (figure 1A). The nodal region is a crucial evolutionary structure of the nervous system ensuring, by saltatory conduction, the rapid and long distance transmission of impulses with the least expenditure of energy. At the node of Ranvier the myelin is interrupted and the axolemma presents the highest density of voltage-gated sodium (Nav) channels. The nodes are flanked by paranodal junctions composed of three

major proteins: contactin 1 (CNTN1) and contactin-associated protein 1 (Caspr1) on the axonal side, and neurofascin 155 (NF155) on the terminal myelin loops. The paranodal junction functions as an electrical and biochemical barrier restricting the mobility of ions and membrane proteins between the node and internode. At the juxtaparanodes, voltage-gated potassium (Kv) channels are anchored and clustered by contactin-associated protein 2 and transient axonal glycoprotein 1. Ganglioside GM1 and gangliosides with and *Gal(*b*1–3)GalNAc* moieties are enriched on nodal and paranodal axolemma, Schwann cell microvilli, and abaxonal myelin.[6,7]

It took almost eight decades, since the original description by Ranvier in 1871, to demonstrate that the nodes are the sites where inward membrane currents are generated to sustain saltatory conduction,[8] and only in the last two decades the nodal region has been recognized as a possible site of specific autoimmune attack in peripheral neuropathies. Figure 1B summarizes the acute and chronic neuropathies with antibodies against gangliosides and axo-glial proteins of the paranodal junctions with the current evidence that these antibodies induce dysfunction/disruption limited to the nodal region.

AUTOIMMUNE NODO-PARANODOPATHIES: THE PREMISES

In the early 90s the connection among an acute, motor, primarily axonal subtype of Guillain-Barré syndrome (GBS) with a preceding *Campylobacter jejuni* infection and antiganglioside antibodies was established.[9,10] Pathological studies of acute motor axonal neuropathy (AMAN) patients showed Wallerian-like degeneration with no demyelination and inflammation, and with the earliest changes consisting in lengthening of the node of Ranvier.[11,12] Most importantly it was found that IgG and complement deposition at the nodes of Ranvier preceded the development of Wallerian-like degeneration.[13] It was suggested that the simple binding of antibody could be sufficient to impair nerve conduction resulting in paralysis with little structural changes and with potential rapid recovery.[13]

Nonetheless, for long time, AMAN was thought to be characterized simply by axonal degeneration and its electrodiagnosis was based on the absence of demyelinating features and reduced compound

muscle action potential (CMAP) amplitudes.[14,15] During the years, GBS patients with anti-GM1 and -GD1a antibodies showing conduction block (CB) and or conduction slowing that promptly resolved without the development of excessive temporal dispersion of CMAPs characteristic of de-remyelination were reported (figure 2A, B).[16,17] This rapid recovery of CB and conduction slowing was thought to be caused by a temporary conduction failure at the nodes of Ranvier possibly due to loss of Nav channels and named reversible conduction failure (RCF) to distinguish it from the classical demyelinating CB.[16] In the common belief slow conduction velocity is thought to be characteristic of a demyelinating neuropathy and in axonal neuropathies conduction velocity is usually considered to be normal or slightly slow. However, inactivation of Nav channels in humans by intravenous infusion of lidocaine or tetrodoxin intoxication reduces conduction velocity, even reaching the demyelinating range,[18,19] possibly by increasing the rise time of the action potential and the internodal conduction time. The description of AMAN patients with conduction failure evolving to axonal degeneration or showing RCF and axonal degeneration co-occurring in the same or different nerves,[20-22] ultimately established that AMAN was electrophysiologically characterized not only by axonal degeneration but also by a reversible failure of conduction and that both processes could be ascribable to the same immune attack to the nodal axolemma.

At this point the foundations for the concept of autoimmune nodo-paranodopathy were laid.

THE CONTRIBUTION OF ANIMAL MODELS

AMAN is associated with anti-GM1 and –GD1a antibodies and in mutant mice lacking these gangliosides the paranodal loops fail to attach to the axolemma with widening of the node, Nav channel clusters are broadened, and juxtaparanodal Kv channels are mislocated to the paranode.[23] The sensitization of rabbits with GM1 induced weakness, high titres of anti-GM1 antibodies that bound to the nodes of Ranvier and activated complement resulting in the formation of the membrane attack complex, destruction of Nav channels, paranodal detachment of myelin loops and widening of the nodes.[24,25] In an *ex vivo* mouse preparation it was shown that the nodes of Ranvier

of distal motor axons were targeted by anti-GD1a antibody.[26] Complement deposition was associated with loss of nodal proteins, including Nav channels, and loss of inward Na^+ and outward K^+ currents. Both morphological and conduction abnormalities were prevented by eculizimab, a monoclonal humanized mouse monoclonal antibody that prevents the formation of the membrane attack complex, whereas inhibition of the protease calpain preserved the immunostaing profile of node of Ranvier without protecting nerve conduction.

The above findings indicate that the early stage of AMAN is characterized by an attack to the nodal region with destruction of Nav channel cluster and failure of nerve conduction which may still be reversible. If the immune attack progresses, calcium enters into the axon and protease activation induces axonal damage and Wallerian-like degeneration.[1] The reevaluation of experimental models associated with antibodies to GM1, GD1a and GD1b indicated in all a common pathophysiologic mechanism characterized by complement mediated dysfunction/disruption at the node of Ranvier.[27] The crucial role of complement demonstrated in animal models prompted two ongoing randomized controlled trials "Inhibition of complement activation in Guillain-Barré syndrome: the ICA-GBS study" and the "Japanes eculizimab trials for Guillain-Barré syndrome (JET-GBS)" aiming to answer the question of whether eculizimab, given in addition to IVIg, is of benefit in GBS. [28,29(Davidson, Yamaguchi)] The preliminary results of the Japanese trial showed a greater percentage of patients able to run 24 weeks after GBS onset in the Eculizimab treated group compared to placebo (74 vs 18%) (S. Kuwabara, personal communication).

ACUTE AUTOIMMUNE NODOPATHIES: THE PROPOSAL OF THE CONCEPT

The recognition of RCF led to reconsider the diagnostic accuracy of the most commonly used criteria sets in the electrodiagnosis of GBS subtypes.[30] In Italian and Far East GBS cohorts 24-38% of patients changed the initial electrodiagnostic classification after serial recordings.[20, 31-33] The majority of shifts were from acute inflammatory demyelinating polyradiculoneuropathy (AIDP) and unclassifiable diagnosis to axonal GBS, and the main reason was the recognition of the RCF as

expression of axonal pathology. RCF is not rare as it is detectable by serial studies in distal, intermediate or proximal segments in at least two motor nerves of 46.6% of patients with axonal GBS (figure 2).[34] Not considering the possibility of distal RCF may induce, on the basis of a single test showing reduced distal CMAP amplitudes, to erroneously formulate a poor prognosis. Moreover, by a single test, patients with AMAN and RCF might be fallaciously classified as AIDP. These observations also explain why antibodies to gangliosides were thought to be associated not only to AMAN but also to AIDP.[14,35]

RCF is an ‘‘a posteriori” diagnosis as it can be recognized only by serial studies and is not included in the currently employed electrodiagnostic criteria.[14,15] This questioned the utility of nerve conduction studies in distinguishing the GBS subtypes by a single test in the early stages of disease.[30,31] However, a recently proposed criteria set considering proximal/distal compound muscle action potential amplitude ratio <0.7, without other features of demyelination, as indicative of axonal GBS and additionally evaluating CMAP duration and sural sparing pattern as well as an innovative statistical-mathematical method of classification greatly increased the electrodiagnostic accuracy of GBS subtypes at first electrophysiologic test.[34]

RCF is not only detectable in motor fibers. RCF in sensory nerves as well as abnormally elongated nodes in the dorsal roots were described in patients with acute motor and sensory axonal neuropathy.[36,37] RCF in motor and sensory fibers has been also reported in patients with the pharyngeal-cervical brachial subtype of GBS.[38,39] Finally, RCF restricted to sensory fibers has been reported in patients with promptly reversible acute sensory ataxic neuropathy and in Miller Fisher syndrome (figure 2).[40,41] The first ever reported sensory nerve biopsy of an anti-GQ1b positive patient with Miller Fisher syndrome revealed lengthening of nodes of Ranvier, myelin splitting at the paranode and macrophage invasion of the internodal axon without any features of segmental demyelination similar to the pathologic features found in motor fibres of AMAN patients.[42]

At last the description of a patient with the clinical picture of GBS and severe neuropathic pain, RCF and IgG3 anti-Caspr1 antibodies activating complement suggested that reactivity to paranodal

Caspr1, with a mechanism similar to anti-ganglioside antibodies, could be also involved in acute autoimmune neuropathies.[5]

The traditional classification of neuropathies into axonal or demyelinating might generate confusion in the diagnosis of GBS subtypes. AMAN is classified as an axonal neuropathy because the primary attack is directed towards the nodal axolemma leading ultimately to axonal degeneration. However, the term axonal may be misleading as in the common view it is strictly associated to Wallerian-like degeneration, evokes poor prognosis and not the possibility of a prompt recovery. On the other hand, although the pathogenic mechanism is focused on the node, some "demyelinating-like" features such and detachment of myelin terminal loops and widening of the nodes (but never true segmental demyelination) have been shown in AMAN. To overcome these nosologic difficulties GBS subtypes with anti-gangliosides antibodies could be better classified as acute nodopathies because of the common pathophysiologic mechanism at the node with the key initial involvement of Nav channels and a pathophysiological continuum from RCF to axonal degeneration (figure 1B). The advantages are that the term nodopathy points directly to the site of nerve injury reminding the specific pathophysiologic mechanism, circumvents the apparent paradox that axonal neuropathies may be promptly reversible and have a good prognosis, reconciles the contrast between electrophysiology and morphology avoiding the confusing situation that the same patients, in spite of the common site of nerve damage and pathophysiological mechanism, might be electrophysiologically classified as demyelinating or axonal.

CHRONIC AUTOIMMUNE PARANODOPATHIES: EXPANDING THE CONCEPT

CIDP is a clinically heterogeneous disorder thought to have an autoimmune basis and its hallmark is inflammatory-mediated demyelination.[43,44] The pathological criteria for diagnosis require unequivocal evidence of demyelination or remyelination with at least 5 demyelinated fibers in semithin or ultrathin sections or more than 12% of teased fibers (minimum of four internodes each) showing de-remyelination.[45,46] Supportive criteria include mononuclear cell infiltration and presence of onion bulbs.

Because of the similarity of CIDP with experimental allergic neuritis, induced in rats by sensitization with purified P0, P2 and PMP 22, the proteins of compact myelin have been thought to be possible autoantigens but after many years there is little evidence for a pathogenic role of a humoral response to these proteins in the majority of CIDP patients.[47] An innovative avenue was opened by Devaux and colleagues in 2012 reporting that IgG from 30% of CIDP patients bound at the nodal region of rat nerve.[48] These findings re-boosted the investigation in the field and patients with antibodies targeting the axo-glial component of paranodal junctions were soon described. Querol and colleagues showed that 6.5% of 45 Spanish CIDP patients carried IgG to CNTN1 or CNTN1 and Caspr1.[3] These patients shared a phenotype characterized by advanced age, aggressive onset, severe axonal damage, motor predominance, and poor response to intravenous immunoglobulin (IVIg). Anti-CNTN1 antibodies were found also in 2.4% of 500 Japanese CIDP patients presenting subacute onset, sensory ataxia and poor response to IVIg and in 8% of 53 German patients showing acute onset, prevalently motor neuropathy and tremor.[49,50] The Spanish group reported also four patients with antibodies to NF155 presenting severe distal sensory-motor neuropathy, disabling tremor and poor response to IVIg.[4] This observation was confirmed in 7% of 533 Japanese CIDP patients showing ataxia, tremor, poor response to IVIg and, in three patients, CNS demyelination.[51] Finally antibodies directed exclusively to Caspr1 were described in one CIDP patient with prominent neuropathic pain.[5] Overall the patients harboring antibodies against proteins of paranodal junctions account for about 10% of all patients classified as CIDP.

The axonal cell adhesion molecules CNTN1 and Caspr1, and the glial NF155 form a ternary complex essential in the formation and stability of paranodal junctions and responsible, through the transverse bands, of the adhesion of terminal myelin loops to axolemma. Paranodal junctions act also as a fence for the juxtaparanodal segregation of Kv potassium channels and overall contribute to the saltatory conduction in myelinated fibers (figure 1).

Interestingly antibodies to axo-glial proteins are predominantly or exclusively of the IgG4 isotype that has been originally considered to be immunomodulatory as it is unable to activate complement

and bind to immunoglobulin Fc receptor.[52] In allergies IgG4 antibodies dampen the inflammatory response and induce tolerance to allergens after repeated challenge.[53] Up to now IgG4 antibodies have been reported only in a few non-allergic diseases as pemphigus myasthenia gravis with antibodies to muscle-specific tyrosine kinase and in these disorders are thought to be pathogenic by blocking critical functions of the target antigen.[52] In the specific, IgG4 anti-CNTN1 prevented, in an *in vitro* model, the binding of the CNTN1/Caspr1 complex to NF155 and disrupted the node of Ranvier structure in absence of complement and inflammatory cells.[54] After intraneural injection *in vitro,* IgG4 anti-CNT1 diffused into the paranode and induced, when passively transferred in rats immunized with P2, progressive deterioration of the experimental neuropathy.[55]

Electrophysiological studies of patients with antibodies to axo-glial proteins of paranodal junction showed prolonged distal motor latencies and conduction slowing in the demyelination range, conduction block and some temporal dispersion but also low amplitude distal compound muscle action and spontaneous activity at needle electromyography indicative of significant axonal degeneration.[3-5]

Immunohistochemistry studies of dermal myelinated fibers of patients with anti-CNTN1 and -Caspr1 antibody revealed destruction of paranodal Caspr1 and neurofascin immunoreactivity and elongated nodes.[5,50] Optic microscopy of sural biopsy of patients carrying antibodies to CNTN1, NF155 and Caspr1 showed axonal degeneration and loss with regenerating fibers, few fibers with thin myelin sheaths but no inflammatory infiltrates or onion bulbs.[5,50,51,56] A teased fiber study of nine patients with ab to NF155 showed widening of the nodes and “apparent demyelination” in three patients but the percentages of de-remyelinated fibers in the whole group was no different from normals.[57] At last, ultrastructural studies in patients with anti-NF155 antibodies displayed detachment of terminal myelin loops due to lack of transverse bands, abnormal widening of periaxonal space with in some instances Schwann cell processes interposed between myelin loops and axon, and widened nodes (figure 3).[56,57] It should be underlined that the above alterations do not fulfill the pathologic criteria for diagnosis of CIDP, and an axonal neuropathy with no features of

de-remyelination was the final pathological diagnosis in three patients with anti-CNTN1 and in one with anti-Caspr antibodies.[5,50]

In the mutant mice models of CNTN1, NF155, and Caspr1 myelin thickness and the g-ratio are not different from the wild type mice, the structure of compact myelin is normal and the ultrastructural alterations found at paranode are identical to those reported in patients.[58-60] Kv channels are aberrantly expressed at the paranode whereas sodium channels retain their nodal distribution.[60]

From the electrophysiological point of view in the mutant animals there is a 2-3 fold decrease in motor nerve conduction, the threshold of nerve stimulation and the refractory period are increased whereas the CMAP amplitude is decreased.[58-60]

In both patients and mutant animals we are facing a condition in which the electrophysiology is clearly "demyelinating" whereas pathology shows only paranodal dismantling without evidence of true de-remyelination. This is only an apparent contradiction. The involvement of paranodal junctions with loss of transverse bands induces terminal myelin loop detachment and retraction resulting in widening of the node with consequent increased nodal capacitance and dilution of the capacitive current over a larger surface (figure 4). Myelin loop detachment and increased periaxonal space may reduce the paranodal transverse resistance with increased current leakage, radial shunting and backflow of the current to the paranode instead of longitudinal progression to the following node (figure 4).[58] Moreover the abnormal displacement of Kv channels at the paranode may shifts membrane polarization to more negative values. All these changes increase the time necessary to depolarize the next node producing slowing of conduction and, if the current is insufficient to reach the threshold, failure of impulse transmission. Overall these considerations well explain the electrophysiological "demyelinating" features in presence of only paranodal disruption and dysfunction.

Patients with IgG4 antibodies to proteins of paranodal junctions show acute-subacute onset, usually a severe course with axonal degeneration and poor response to IVIg.[3,4,49] As major mediators of IVIg effect are inhibition of complement pathway and binding to the inhibitory immunoglobulin

receptor the peculiar characteristics of IgG4 antibodies may explain the poor response to IVIg in these patients.[61-63] The paranodal junction is thought to play a role in molecular communication between the axon and the Schwann cell. Nodal and paranodal axoplasm of NF155 and Caspr1 mutant mice contain large mitochondria and abnormal accumulation of cytoplasmic organelles indicating an altered axonal transport and suggesting that the disrupted axo-glial interaction may induce, in a still unknown way, axonal degeneration.[59,60] B-cell-depleting therapy, as rituximab, resulted effective in patients not responding to conventional treatment above all in patients with short disease duration possibly because of less axonal damage.[5,50,64] Therefore identifying the target antigens in these autoimmune neuropathies is important, not only to understand their pathogenic mechanisms, but also to correctly diagnose and treat the affected patients.

Because of the localization and function of the axo-glial proteins and the ultrastructural alterations restricted at the paranode without de-remyelination, the neuropathies with antibodies to paranodal junction components should be better classified, in our opinion, as chronic paranodopathies (figure 1B). The advantages are that the term, focusing on the primary site of nerve injury, reconciles the contrasting electrophysiological and microscopical findings and separates these autoimmune neuropathies from CIDP as there is no evidence of inflammatory infiltrates, complement activation, and of de-remyelination (namely the I and D of the CIDP acronym).

Very recently five patients classified as CIDP and harboring antibodies against NF186 and NF140, the nodal isoforms of neurofascin, have been reported.[65] Nerve conductions showed axonal features in two patients, conduction block and demyelinating features in three patients although, after normalization of the electrophysiological findings, only one patient fulfilled the EFSN/PNS electrodiagnostic criteria for definite CIDP. Nerve biopsy in this patient showed only mild axonal loss without demyelination, onion bulbs, or cellular infiltration. Four patients had predominantly IgG4 antibodies and one presented IgG3 antibodies that in *vitro* activated complement. IVIg and corticosteroids were effective in three patients and one patient remitted following treatment with rituximab. Remission was associated with autoantibody depletion and with recovery of conduction

block and distal CMAP amplitudes. Although reactivity to nodal NF 186 needs to be confirmed in further studies and there are no available data about ultrastructural alterations we think that also these patients could be better described as chronic nodo-paranodopathy than CIDP.

Multifocal motor neuropathy (MMN) is characterized by asymmetrical, predominantly distal limb weakness, no sensory loss, motor CB (often persistent) with or without temporal dispersion of compound muscle action potential, presence of IgM to GM1 in about 50% of patients and response to IVIg in up to 90% of patients suggesting an immune-mediated etiology.[66] In addition to CB, MMN is characterized by axonal degeneration which is the main determinant of the progressive course.[67] It is controversial whether MMN is a primary demyelinating or an axonal disorder and the results of the few pathological studies are contradictory.[68,69] An active immunization model is lacking but injection of human sera containing IgM anti-GM1 antibodies and fresh complement into the rat sciatic nerve caused CB, immunoglobulin deposition at the nodes of Ranvier, nodal widening and some paranodal disruption.[70,71] Application to rat single myelinated axons of high concentration anti-GM1 sera without complement increased K^+ current whereas in presence of complement Na^+ currents decreased and leakage current increased.[72] These findings suggested that anti-GM1 antibodies by themselves can uncover K^+ channels in the paranodal region while anti-GM1 antibodies bound to the nodal membrane in the presence of complement may block Nav channels and disrupt the membrane at the node **of** Ranvier. However, it should be mentioned that a following study did not confirm these effects.[73]

Anti-GM1 IgM antibodies from patients with MMN, activate complement in vitro and efficient complement activating properties are determinant of weakness and axonal loss.[74-76] By analogy with AMAN, it has been hypothesized that IgM anti-GM1 antibodies induce complement mediated disruption of ion channels and paranodal structures compromising nerve conduction and eventually causing axonal degeneration.[66]

Studies of antibodies against nodal proteins have generated conflicting results in MMN. One study found that 62% of patients had IgG autoantibodies against gliomedin or NF186 in combination with

anti-GM1 IgM or alone in 10% of patients.[77] In the same study antibodies to gliomedin or NF186 were found in only 1% of CIDP patients. A following study failed to replicate the association between MMN and anti-NF186 antibodies and did not find anti-NF155 or anti-CNTN1 antibodies either.[78] Although the discrepancy of results may be explained by different proteins used for ELISA the potential association between MMN and an antibody response against nodal proteins may be worthwhile of further investigation.

In conclusion although the pathologic evidence is still scanty and the pathophysiology not fully understood we think that also MMN could be better classified as a chronic autoimmune nodo-paranodopathy than a demyelinating neuropathy (figure 1B).

CONCLUSIONS

The nodo-paranodopathy concept seems appropriate to several acute and chronic neuropathies associated with antibodies to ganglioside and to paranodal axo-glial protein. It has the advantage to focus on to the site of primary nerve injury reminding specific pathophysiologic mechanisms, reconciles contrasting morphological and electrophysiological findings finally avoiding misdiagnosis and taxonomic confusion. We think that the category of the autoimmune peripheral nodo-paranodopathy that we propose better systematizes the neuropathies characterized by an autoimmune attack targeting and limited to the nodal region and integrates the traditional classification of peripheral neuropathies into demyelinating and axonal.

Authors' contribution

AU conceived the review and wrote the first draft. JMV contributed with the electron microscopy studies and critically reviewed all the versions of the manuscript.

Competing of interests

The authors declare no conflicts of interest.

Funding source

None.

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Figure legends

Figure 1

(A) Simplified molecular organisation at nodes, paranodes, and juxtaparanodes. NF, neurofascin; CNTN1, contactin 1; Caspr, contactin associated protein; TAG1, transient axonal glycoprotein 1; GM1, ganglioside GM1.

(B) Autoimmune nodo-paranodopathies. AMAN, acute motor axonal neuropathy; AMSAN, acute motor-sensory axonal neuropathy; ASAN, acute sensory ataxic neuropathy; PCB, pharyngeal-cervical-brachial subtype of Guillain Barré syndrome; MFS, Miller Fisher syndrome; MMN, multifocal motor neuropathy. The association of neuropathies with antibodies against gangliosides and proteins of paranodal junctions with the evidence supporting the nodal and paranodal involvement are shown. The main IgG subclasses, when known, are reported. Strength in association with antibodies and evidence are rated as: black, strong; dark gray, medium; light gray, weak; blank none. Pathology indicates immunohistochemical and ultrastructural evidence of involvement of the nodal region in humans. Autoimmune model indicates evidence of dysfunction/disruption of the nodal region in animals by active immunization or by passive transfer of antibodies. Mutant model indicates evidence of alteration of nodal region in mutant mice lacking gangliosides or axo-glial proteins of paranodal junctions. Modified from Uncini et al. 2013.[1]

Figure 2

Upper: examples of reversible conduction failure (RCF) in motor fibers of different patients (A, B, C, D, E). (A) Distal RCF in the median nerve. On day six, distal and proximal CMAP amplitudes were reduced (2.6 mV) with slightly prolonged DML (4.8 ms). On day 25 distal CMAP was 280% increased without development of temporal dispersion. The patient had IgG anti-GD1b. (B) RCF in intermediate nerve segment of the ulnar nerve. On day 10 CMAP amplitude ratio from stimulation above and below the elbow was 0.3 and improved in the following recordings up to 0.7 on day 27 without the development of temporal dispersion. Conduction velocity across the elbow was slow

(38 m/s) on day 10 but improved in parallel with the resolution of conduction failure (48 m/s on day 27). The patient had IgG anti-GM1, -GD1a and -GD1b. (C) RCF in intermediate and distal nerve segments of the ulnar nerve. The abnormal CMAP amplitude ratio (0.2) across the elbow on day five rapidly resolved on day 11 without the development of temporal dispersion. Distal CMAP amplitude was also 240% increased. The patient had IgG anti-GM1. (D) Improvement of RCF in distal segment of the median nerves reveals an abnormal amplitude reduction in the intermediate segment. At day 5 DML was slightly prolonged (4.8 ms), distal CMAP amplitude was 7.6 mV and p/d CMAP amplitude ratio was 0.92. At day 25 distal CMAP amplitude was 158% increased and p/d CMAP amplitude ratio was 0.68 revealing a conduction block in the intermediate nerve segment. The patient had IgG anti-GD1a, -GalNac-GD1a and -GT1a. (E) Isolated F wave absence in the ulnar nerve in a patient with otherwise normal conductions as example of RCF in proximal nerve segments. Persistence of F wave is markedly reduced at day 2 (only one response in 16 trials). At day 40 F waves were normally represented with normal minimal latency (27.7 ms). At both recordings CMAPs amplitude and duration, DML, CVs and p/d CMAP ratios with stimulation up to the ERB's point were normal. The patient had IgG anti-GM1. From Uncini et al. 2017.[34]

Lower: reversible conduction failure in sensory fibers. Sensory conductions recorded antidromically in a patient with acute sensory ataxic neuropathy. Calibration is 10 μV/2 ms in all tracings. Sensory nerve action potential amplitudes were already reduced at day 1 after the onset of symptoms in median and ulnar nerves and improved on day 4 by respectively 270% and 516%. Sural nerve sensory action potential was still in normal range at day 1 (7 μV) but improved by 159% at day 4. Motor conductions studies were normal at both recordings. The patient had IgG anti-GD1b and completely recovered in 10 days without treatment.

Figure 3

Ultrastructural consequences of antibodies to axo-glial proteins of paranodal junctions. Sural nerve biopsy, longitudinal ultrathin sections of the nodal region. (A) Normal paranode in a control showing myelin terminal loops closely attached to the axolemma by transverse bands (arrow). (B, C, D) Ultrastructural alterations at paranodes in a patient with antibodies to NF155. (B) Lack of transverse bands and enlargement of the space between the axon and the paranodal loops (arrow). (C) Cellular processes (arrow) are interposed between the paranodal myelin loops (ml) and the axon (a). (D) A widened node.

Figure 4

Schematic representation of the morphological and hypothesized electrophysiological effects of antibodies to axo-glial proteins of paranodal junction. (A) Normal structure of node and paranode, Nav channels are clustered at the node and Kv channels at the juxtaparanode. In the insert the axo-glial proteins forming the paranodal junctions are shown. Modified from Boyle et al. 2001[58]. (B) Antibodies to axo-glial proteins induce loss of transverse bands with consequent terminal myelin loop detachment, increased periaxonal space, nodal widening and expression of Kv channels at paranode. (C) Saltatory conduction in normal nerve fibers. At the active node (left), transient Nav channels are open, inducing an inward ionic named action current. This causes a current circuit with the current flowing at the following node to be activated (right) where positive charges accumulate at the inside and are withdrawn from the outside of the nodal membrane (driving current). As soon as the nodal membrane is depolarised to threshold, Nav channels open, Na^+ enters into the axon inducing further depolarization and the driving current converts into an action current generating an action potential. Note that myelin is not a perfect insulator and a relatively small amount of current flows across the axonal membrane at the internode and completes the circuit by flowing radially through the myelin sheath or through the axo-glial junction at the paranode. D) Antibodies to axo-glial proteins, because of terminal myelin loop detachment, induce nodal widening increasing nodal

and paranodal capacitance and diluting the current over a larger surface. Loops detachment and increased periaxonal space reduce the paranodal transverse resistance with increased current leakage across the terminal myelin loops, radial shunting and backflow of current to the paranode instead of longitudinal progression to the following node (note the relative thickness of the lines compared to C).