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Conduction block in immune-mediated neuropathy: paranodopathy versus axonopathy

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ABSTRACT

Background: Conduction block is a pathognomonic feature of immune-mediated neuropathies. The aim of this study was to advance understanding of pathophysiology and conduction block in chronic inflammatory demyelinating polyneuropathy (CIDP) and multifocal motor neuropathy (MMN). *Methods:* A multimodal approach was used, incorporating clinical phenotyping, neurophysiology, immunohistochemistry and structural assessments. *Results:* Of 49 CIDP and 14 MMN patients, 25% and 79% had median nerve forearm block, respectively. Clinical scores were similar in CIDP patients with and without block. CIDP patients with median nerve block demonstrated markedly elevated thresholds and greater threshold changes in threshold electrotonus (TE), while those without did not differ from healthy controls in TE parameters. In contrast, MMN patients exhibited marked

increases in superexcitability. Nerve size was similar in both CIDP groups at the site of axonal excitability. However, CIDP patients with block demonstrated more frequent paranodal serum binding to teased rat nerve fibres. In keeping with these findings, mathematical modelling of nerve excitability recordings in CIDP patients with block support the role of paranodal dysfunction and enhanced leakage of current between the node and internode. In contrast, changes in MMN likely resulted from a reduction in ion channel density along axons. *Conclusions:* The underlying pathology in CIDP and MMN are distinct. Conduction block in CIDP is associated with paranodal dysfunction which may be antibody-mediated in a subset of patients. In contrast, MMN is characterised by channel dysfunction downstream from the site of block.

INTRODUCTION

Disease mechanisms and antigenic targets remain largely elusive in chronic inflammatory demyelinating polyneuropathy (CIDP) and multifocal motor neuropathy (MMN). Correlating clinical phenotypes with pathophysiology will be required before targeted treatment approaches can be developed. Conduction block (CB), occurring when an action potential fails to propagate through an axonal segment, is a pathognomonic feature of MMN and is frequently observed in CIDP[1]. Understanding the mechanistic basis of CB will provide critical information to guide the development of diagnostic and treatment approaches.

Nodal and paranodal regions have been implicated as key antigenic targets in CIDP[2,3]. IgG4 antibodies directed towards protein complexes at the paranode, including neurofascin-155 (NF155), contactin-1 (CNTN1) and contactin-associated protein 1 (Caspr)[4-7] have been

described with patients tending to have a more severe phenotype, frequently refractory to standard first-line therapy[6,8]. Furthermore, IgM antibodies directed against GM1, a ganglioside enriched in the nodal and paranodal regions[9], are found in some patients with MMN, but their role in pathogenesis remains unclear[10].

To better understand the mechanism of CB and associated paranodal function, the present study incorporated comprehensive clinical assessments, neurophysiology, immunopathology and structural assessments to improve understanding of pathophysiological mechanisms underlying CIDP and MMN.

MATERIALS AND METHODS

Patient selection

Consecutive patients fulfilling criteria for definite or probable CIDP and MMN were prospectively recruited between April 2015 and January 2017[11, 12]. Patients testing positive for NF155, CNTN1 or NF186 IgG4 were excluded. All participants gave written informed consent to participate in the study. The study was approved by the Sydney Local Health District Ethics Review Committee (Royal Prince Alfred Hospital).

Assessment tools

Clinical assessments

Clinical assessments included assessment of muscle strength, grip strength and disability scores[13,14,15,16] (eMethods1, online).

Neurophysiological investigation

Comprehensive nerve conduction studies (NCS) were performed using Synergy software (Version 20.0). Patients with CB within the forearm segment of the median nerve were classified as CIDP (CB) or MMN (CB). As very distal and proximal CB can be difficult to accurately identify, this was not included in the CB group. The remaining CIDP patients without median nerve forearm block were classified as CIDP (eMethods2, online).

To assess axonal properties near the site of block, nerve excitability studies were undertaken on the median nerve at the wrist using the TRONDNF protocol within QTRACW software (© Institute of Neurology, University College London, UK)[17] in patients with a distal abductor pollicis brevis CMAP > 1mV. Patients with extremely elevated thresholds who did not tolerate the study were excluded from excitability analysis. Detailed methods and definitions of major excitability parameters have been previously described[17-20] (eMethods3 online). Results were compared with 29 age-matched healthy controls (HC).

Mathematical modelling

A mathematical model of the human motor axon was used to assist in interpretation of CIDP (CB) and MMN (CB) recordings[21-23]. This 'Bostock' model consists of nodal and internodal compartments connected by the Barrett-Barrett conductance (GBB) which corresponds to current leakage pathways through and under the myelin sheath. Voltage-dependent ion channels, ion pumps, capacitance and leak conductances are incorporated into the model[22]. After matching the model to healthy control data, parameters were adjusted sequentially to determine which single parameter change resulted in the greatest reduction in discrepancy between the patient data and model. The best fit by two parameter changes was also determined through sequential changes in model parameters.

Structural imaging

The cross-sectional area (CSA) of the median nerve was measured at the wrist crease and forearm[24] (eMethods4, online). Results were compared with 29 age and sex-matched HC.

Teased nerve fibre studies

Animal experiments were approved by the University of Sydney Animal Ethics Committee (2016/943). Indirect immunofluorescence studies on teased nerve fibres were performed on serum from all CIDP patients as previously described[7] (eMethods5 online). Where possible serum samples were collected before an IVIg dose (88% of CIDP patients).

Statistics

Statistical analysis and graph construction were performed using Graph Pad Prism 7 and IBM SPSS Statistics (Version 22) (eMethods6 online). Significance was defined by a p-value of <0.05 and adjusted for multiple comparisons where appropriate (defined as p<0.006 for excitability data). Clinical results are presented as mean ± standard deviation (SD) and excitability results as mean ± standard error of the mean (SEM).

RESULTS

The patient cohorts demonstrated clinical phenotypes that were typical of CIDP and MMN (Table 1). There was no difference in age (p=0.83) or disease duration (p=0.09) across groups. There were no differences in clinical scores between patients in the CIDP (CB) and CIDP groups (MRC SS:p=0.49; grip strength:p=0.49; INCAT:p=0.80). Six out of 11 (55%) of MMN patients tested positive for anti-GM1 IgM.

Neurophysiological profiles

Median nerve forearm CB was identified in 11 CIDP and 11 MMN patients and 33 CIDP patients were without median nerve forearm CB (Table 1). There was no difference across groups in mean distal median CMAP amplitudes (p=0.18). As expected, CIDP patients had longer distal motor latencies (DML) compared with MMN (CB) patients (CIDP: 4.6 ±1.8ms MMN:3.3 ± 1.0ms; p<0.05). CIDP (CB) patients had a more severe reduction in forearm conduction velocity compared with MMN (CB) (p<0.05) and CIDP (p<0.05) patients. Although patients in the CIDP group did not have median nerve forearm conduction block,

94% had abnormal NCS of the median nerve with 82% fulfilling EFNS/PNS criteria for demyelination in the studied nerve[11].

Threshold properties

CIDP (CB) patients exhibited a marked increase in threshold compared with healthy subjects (p<0.0001) (Table 2, Figure 1,2A) requiring greater than three times the current to achieve 50% of the peak response. The CIDP group without median nerve forearm block demonstrated a smaller increase in threshold compared with HC (p=0.009). In contrast, threshold was not significantly increased in the MMN (CB) group compared with HC (p=0.19).

Internodal properties

MMN (CB) patients demonstrated a "fanned out" threshold electrotonus compared to HC with changes in both depolarising and hyperpolarizing directions (Figures 1A, 2B, 2C). CIDP (CB) patients exhibited a similar pattern of changes in TE (Figures 1C, 2B, 2C), with greater threshold change to subthreshold hyperpolarising current evident (Figure 1E). In contrast, in CIDP patients without median nerve block in the intermediate segment, there were no significant TE changes compared HC (Figure 1G, 2B, 2C).

Recovery Cycle

Superexcitability was significantly increased in MMN (CB) patients when compared to CIDP (CB) patients (Fig 1F), CIDP patients without median nerve block and HC (Fig 1B, 2D). In

contrast, patients in both CIDP groups demonstrated no difference in superexcitability when compared with HC (Figures 1D, 1H, 2E).

Mathematical modelling

Chronic inflammatory demyelinating polyneuropathy

The single parameter change resulting in the greatest discrepancy reduction between CIDP (CB) patient and modelled data involved an increase in the Barrett-Barrett conductance (GBB) across the myelin sheath by 40.8%, which reduced the discrepancy by 63.3%. The best fit by two combined parameters was a 58% increase in GBB and 62% increase in fast K^+ conductance resulting in a 78.1% discrepancy reduction.

Multifocal motor neuropathy

The single parameter change resulting in the greatest discrepancy reduction between MMN and modelled data was hyperpolarisation with 0.027nA current, which reduced the discrepancy by 73.1%. A better fit was achieved by two parameters combining a 41.1% reduction in K^+ permeability and 56.1% reduction in persistent Na^+, which reduced the discrepancy by 92%.

Structural nerve assessment

There was no difference in nerve CSA between the two CIDP groups at either the wrist [CIDP:12.9 ± 4.5 mm^2; CIDP (CB):11.1 ± 1.6 mm^2; p=0.3) or forearm [CIDP:10.0 ± 9.5

mm^2; CIDP (CB):11.2 ± 6.4 mm^2; p=0.2;eFigure1A-B online) suggesting that the marked increase in thresholds and TE changes in CIDP (CB) patients were unlikely to be primarily due to nerve enlargement. CIDP patients (with and without median nerve forearm CB), exhibited greater nerve CSA compared with MMN patients at the wrist (all CIDP:12.5 ± 4.0 mm^2; MMN:9.4 ± 1.7 mm^2; p=0.007), and forearm (all CIDP:10.3 ± 8.8 mm^2; MMN:6.1 ± 1.1 mm^2; p=0.028) as well as HC at the wrist (10.1 ± 1.6 mm^2; p<0.005), and forearm (6.2 ± 0.8 mm^2; p<0.005;eFigure1C-D online).

Teased fibre studies

Nine out of 11 CIDP (CB) patients demonstrated binding at either the node, paranode and/or myelin compared with only 13 out of 33 CIDP patients (81.8% vs.39.4%; p<0.05) (Figure 3) While there was no difference in the proportion of patients demonstrating nodal binding between the two groups (p = 0.09), patients with CIDP (CB) more frequently demonstrated paranodal binding compared with CIDP patients (36.4% vs.6.1%; p<0.05) suggesting antibodies targeting paranodal structures may be linked to neurophysiological evidence of paranodal dysfunction. In contrast, only one patient with MMN demonstrated paranodal binding (1/11; 9.1%), arguing against a primary paranodal mechanism in MMN.

DISCUSSION

The present study utilised a multimodal approach to examine pathophysiological mechanisms in immune-mediated neuropathy, linking neurophysiological evidence of paranodal dysfunction with an antibody response directed towards unidentified antigens in the paranodal region. There was no alteration in nerve size between the two CIDP groups on

ultrasound, while CIDP nerves were enlarged compared to MMN, indicating distinct structural nerve profiles. Furthermore, distinct axonal excitability profiles were identified in MMN compared with CIDP associated with conduction block, highlighting significant distinctions in underlying pathology.

Excitability results suggest that an increase in the conductance through and under the myelin sheath (GBB)[22], associated with an increase in fast potassium conductance may be a contributor to peripheral nerve dysfunction and conduction block in CIDP. Critically, both conductances are anatomically associated with the paranode or juxtaparanodal regions[19]. Loosening of the paranodal seal and greater leakage of current into the internode associated with paranodal demyelination would likely produce an increase in GBB and expose juxtaparanodal potassium channels, producing the pattern of changes demonstrated in CIDP patients with block, including higher stimulus thresholds for excitation and changes in TE. While an increase in GBB is typically associated with increased superexcitability (as seen in NF155 neuropathy)[3], this may have been counteracted by the reduction in superexcitability which occurs with an increase in fast potassium conductance[25]. Interestingly, patients with CIDP without block in the region of study did not demonstrate changes in TE and superexcitability parameters despite most demonstrating demyelinating features and nerve enlargement. Simulated models have demonstrated that axonal excitability properties may be normal when the degree of demyelination does not cause CB[26].

A different pattern of excitability abnormalities was demonstrated in MMN with conduction block, consistent with previous findings[27]. Mathematical modelling suggested that these changes were best modelled by reduction in both Na^+ and K^+ channel function. Marked

reduction in motor unit numbers associated with strikingly enlarged motor units have been identified distal to the site of block in patients with MMN[28]. Furthermore, axonal degeneration has been shown to be strongly associated with block suggesting that the same pathophysiology is likely to account for both processes[29]. Accordingly, a reduction in Na^+ and K^+ channel function may represent a process of a generalised reduction in channel density along enlarged axons with an increased load. A similar pattern of excitability change has been reported in single unit studies undertaken in patients with motor neuron disease, attributed to reduced channel densities along an enlarged axon supplying an abnormally large load of muscle fibres, and impaired axonal transport of membrane proteins and channels resulting from the disease process[30].

Furthermore, excitability studies in motor axons undergoing degeneration due to proximal nerve transection demonstrated similar changes in TE and superexcitability attributed to a reduction in Na^+ and K^+ channel function[31]. Hence, there may be a contribution of degenerating axons (which are still able to conduct), to the axonal abnormalities seen in MMN.

A further consideration to account for the excitability changes in MMN is the possibility of paranodal dysfunction given that GM1 is abundant in the nodo-paranodal region[9]. However, an elevation in motor thresholds was not observed in MMN as would be expected with paranodal dysfunction due to the effect on GBB. Furthermore, paranodal dysfunction was not evident in MMN patients on teased nerve fibre studies.

Indirect immunofluorescence results from the present study demonstrate that conduction block in CIDP is associated with a high incidence of serum binding to teased nerve fibres, suggesting that yet-to-be identified antibodies are targeting peripheral nerve components. A subset of patients tested negative for binding at the node, paranode or myelin. This group may include those in whom disease is primarily mediated by cellular immunity, rather than antibody response, in addition to patients with low-level antibodies or antibodies that have been cleared following treatment.

The present study has demonstrated the heterogeneity in clinical phenotypes in immune-mediated neuropathies. The tools used in the present series can be utilised to further understanding of disease pathophysiology in the inflammatory neuropathies. While imaging techniques can provide information on nerve structure, axonal excitability techniques are an in vivo method of assessing axonal function with mathematical modelling representing a tool to assist in interpretation of excitability results.

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Table 1. Clinical features and median NCS of disease cohorts*

	MMN (CB) (n=11)	CIDP (CB) (n=11)	CIDP (n=33)
Age (yrs)	54.82 ± 11.7	56.4 ± 15.9	57.8 ± 15.1
Sex (M:F)	6:5	4:7	20:13
Disease duration (yrs)	12.4 ± 7.3	15.9 ± 13.7	8.7 ± 8.3
CIDP Phenotype			
Typical [n(%)]	–	7(64%)	16(48%)
DADS [n(%)]	–	0(0%)	3(9%)
MADSAM [n(%)]	–	1(9%)	7(28%)
Focal [n(%)]	–	0(0%)	2(6%)
Motor predominant [n(%)]	–	2(18%)	1(3%)
Sensory predominant [n(%)]	–	1(9%)	4(12%)
Treatment			
IVIg (%)	11(100%)	9(82%)	32(97%)
Additional immunosuppression[#]	1(9%)	3(27%)	10(30%)
Clinical scores			
MRC sum-score (/70)	N/A	64.8 ± 5.1	66.2 ± 4.4
E-MRC sum-score (/150)	134.7 ± 8.4	N/A	N/A
Grip strength (kg force)	19.4 ± 11.5	19.6 ± 11.4	23.1 ± 10.8
INCAT ODSS (/12)	N/A	2.8 ±1.0	2.9 ± 1.4
Median NCS results			
DML (ms)	3.3 ± 1.0	4.5 ± 1.7	4.6 ± 1.9
CMAP (wrist) (mV)	7.2 ± 2.8	5.6 ± 3.7	6.9 ± 3.1
CMAP (elbow) (mV)	2.3 ± 1.4	2.2 ± 1.6	6.2 ± 2.2
Forearm CV (m/s)	42.5 ± 13.0	31.8 ± 7.5	44.3 ± 9.1
Wrist to elbow area reduction (%)	56.7 ± 21.1	58.8 ± 22.3	4.7 ± 12.2

*mean±SD

[#] patients administered prednisone, mycophenolate, azathioprine in addition to IVIg

MRC sum-score:Medical Research Council sum-score;E-MRC:expanded Medical Research Council sum-score;

INCAT ODSS:INCAT overall disability sum score;DML:distal motor latency;CMAP:compound muscle action

potential;CV:conduction velocity

Table 2. Excitability measurements*

Excitability measure	Healthy Controls (n=29)	MMN (CB) (n=9)	CIDP (CB) (n=7)	CIDP (n=26)	P-value MMN(CB) vs CIDP(CB)	P-value CIDP(CB) vs CIDP
Peak (mV)	8.6 ± 0.6	7.5 ± 0.5	6.9 ± 1.3	7.9 ± 0.6	ns	ns
Threshold properties						
Stim for 50% response (mA)	3.7 ± 0.3	5.1 ± 0.7^{ns}	$13.0 \pm 2.1^{****}$	$6.4 \pm 0.8^{**}$	ns	ns
SR slope	4.8 ± 0.3	4.3 ± 1.1^{ns}	3.0 ± 0.7^{ns}	4.6 ± 0.6^{ns}	ns	ns
Rheobase (mA)	2.5 ± 0.2	3.2 ± 0.3^{ns}	$9.0 \pm 1.7^{***}$	4.1 ± 0.6^{ns}	ns	ns
Internodal function						
TEh (90-100) (%)	-113.3 ± 3.3	$-147.6 \pm 8.1^{****}$	$-194.0 \pm 13.4^{****}$	-119 ± 2.4^{ns}	<0.0001	<0.0001
TEd (90-100) (%)	44.7 ± 0.7	$53.1 \pm 1.8^{****}$	$52.4 \pm 1.5^{***}$	46.8 ± 0.8^{ns}	ns	<0.05
TEd20 (10-20) (%)	34.4 ± 0.5	$39.6 \pm 1.3^{**}$	$44.7 \pm 2.1^{***}$	35.0 ± 0.6^{ns}	ns	<0.001
TEh20 (10-20) (%)	-37.2 ± 0.9	-39.3 ± 1.3^{ns}	$-50.1 \pm 3.2^{***}$	-36.9 ± 0.8^{ns}	ns	<0.001
Recovery cycle						
RRP (ms)	3.0 ± 0.1	2.7 ± 0.2^{ns}	2.5 ± 0.2^{ns}	2.9 ± 0.1^{ns}	ns	ns
Superexcitability (%)	-23.2 ± 1.0	$-39.3 \pm 2.0^{****}$	-24.2 ± 2.7^{ns}	-21.5 ± 1.3^{ns}	<0.0001	ns
Subexcitability (%)	14.9 ± 0.7	12.6 ± 2.0^{ns}	13.0 ± 1.8^{ns}	12.1 ± 1.0^{ns}	ns	ns

*mean(SEM)

****<0.0001;***<0.001;**<0.01;ns=not significant compared with HC

FIGURE LEGENDS

Figure 1. Excitability profiles in CIDP and MMN patients

Figure Legend: (A and B) marked changes in threshold electrotonus (TE) and recovery cycle (RC) in patients with MMN(CB);(C and D) marked changes in TE without superexcitability change in patients with CIDP (CB);(E and F) CIDP (CB) and MMN (CB) have differing TE and RC profiles; (G and H) CIDP patients without median motor block did not differ from healthy controls in TE and RC parameters.

MMN(red); CIDP(CB)(blue); CIDP(green); Healthy controls(grey)

Figure 2. Motor nerve excitability scatter plots

Figure Legend: Stimulus response curve, demonstrating increase in threshold in CIDP (CB) group with curve shifted to the right (A). Greater threshold changes in threshold electrotonus (TE) both in hyperpolarising and depolarising directions (B and C, respectively). Marked increase in superexcitability in MMN patients not seen in CIDP patients (D).

MMN(red); CIDP(CB)(blue); CIDP(green); Healthy controls(grey)

Figure 3: Patterns of serum binding observed using indirect immunofluorescence with healthy control and CIDP serum on teased nerve fibres.

Teased fibres were triple stained for human IgG (green, left column), neurofascin (red) and caspr (blue) using serum from healthy controls (A,B) or CIDP patients (C-H). Serum from healthy controls did not bind to the teased nerve fibres while serum from some CIDP patients

bound to the node of Ranvier (C,D), the paranode (E, F) or to myelin/Schwann cell (G, H).

Bar=10um