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Inflammatory Neuropathies: Pathology, molecular markers and targets for specific therapeutic intervention

Eroboghene E. Ubogu, M.D.

Neuromuscular Immunopathology Research Laboratory, Division of Neuromuscular Disease, Department of Neurology, The University of Alabama at Birmingham, Birmingham, Alabama, United States of America

Abstract

Inflammatory neuropathies encompass groups of heterogeneous disorders characterized by pathogenic immune-mediated hematogenous leukocyte infiltration of peripheral nerves, nerve roots or both, with resultant demyelination or axonal degeneration or both. Inflammatory neuropathies may be divided into three major disease categories: Guillain-Barré syndrome (particularly the acute inflammatory demyelinating polyradiculoneuropathy variant), Chronic inflammatory demyelinating polyradiculoneuropathy and nonsystemic vasculitic neuropathy (or peripheral nerve vasculitis). Despite major advances in molecular biology, pathology and genetics, the pathogenesis of these disorders remains elusive. There is insufficient knowledge on the mechanisms of hematogenous leukocyte trafficking into the peripheral nervous system to guide the development of specific molecular therapies for immune-mediated inflammatory neuropathies compared to disorders such as psoriasis, inflammatory bowel disease, rheumatoid arthritis or multiple sclerosis. The recent isolation and characterization of human endoneurial endothelial cells that form the blood-nerve barrier provides an opportunity to elucidate leukocyte-endothelial cell interactions critical to the pathogenesis of inflammatory neuropathies at the interface between the systemic circulation and peripheral nerve endoneurium. This review discusses our current knowledge of the classic pathological features of inflammatory neuropathies, attempts at molecular classification and genetic determinants, the utilization of in vitro and in vivo animal models to determine pathogenic mechanisms at the interface between the systemic circulation and the peripheral nervous system relevant to these disorders and prospects for future potential molecular pathology biomarkers and targets for specific therapeutic intervention.

Keywords

Blood-nerve barrier; Chronic Inflammatory Demyelinating Polyradiculoneuropathy; Genetic Polymorphisms; Guillain-Barré syndrome; Inflammation; Leukocyte Trafficking; Mechanisms; Pathology; Peripheral nerves; Vasculitic Neuropathies

Address all correspondence to: Eroboghene E. Ubogu, M.D., Professor and Director, Division of Neuromuscular Disease, Department of Neurology, The University of Alabama at Birmingham, 1720 7th Avenue South, Sparks Center 200, Birmingham, AL 35294-0017, United States of America, Telephone Number: +1-205-975-4270, Facsimile Number: +1-205-934-2120, eeubogu@uabmc.edu.

INTRODUCTION

Inflammatory neuropathies can be considered as a group of immune-mediated disorders affecting the peripheral nervous system in which hematogenous leukocytes actively participate in axonal degeneration, demyelination or both, with resultant motor and sensory deficits. Inflammatory neuropathies may be divided into three major clinicopathological subgroups: Guillain-Barre syndrome (GBS), an acute disorder affecting peripheral nerves and nerve roots with maximum severity attained within 4 weeks from disease onset; chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), a chronic disorder affecting peripheral nerves and nerve roots with maximal severity attained after 8 weeks following disease onset, and vasculitic neuropathy, an acute-subacute disorder that commonly affects multiple individual or groups of peripheral nerves sequentially [30,80,37,159,23,27].

GBS may be further classified into variants based on clinical features and electrodiagnostic findings. These variants include acute inflammatory demyelinating polyradiculoneuropathy (AIDP); the most common variant in North America and Europe, acute motor axonal neuropathy (AMAN); the most common variant in China and Japan, acute motor and sensory axonal neuropathy (AMSAN), Miller Fisher syndrome, pharyngeal-cervical-brachial variant, polyneuritis cranialis and acute pandysautonomia [147,159]. CIDP may be classified as being “idiopathic” or associated with/secondary to systemic disorders, and further classified based on the pattern of peripheral nerve or nerve root involvement on electrodiagnostic testing into typical (motor and sensory), pure motor or motor predominant, pure sensory or sensory predominant, focal, multifocal acquired demyelinating sensory and motor (MADSAM) neuropathy, and distal acquired demyelinating sensory (DADS) neuropathy [80]. In AIDP, maximum disease severity is expected within 4 weeks from disease onset, while in CIDP maximum severity is expected after 8 weeks from disease onset. AIDP is typically self-limiting and rarely recurs, while CIDP is rarely monophasic and can be relapsing-remitting, step-wise progressive or steady progressive. Vasculitic neuropathy may be classified based on restricted peripheral nervous system involvement (nonsystemic vasculitic neuropathy [NSVN] or primary peripheral nerve vasculitis) or occurrence with other organ systems (systemic vasculitic neuropathy or secondary peripheral nerve vasculitis)[23,37]. For the purpose of understanding the specific crosstalk between components of the systemic circulation and the peripheral nervous system necessary to elucidate the role of leukocyte-endothelial cell interactions in the pathogenesis of inflammatory neuropathies, this review is restricted to the AIDP variant of GBS, “idiopathic” CIDP and NSVN.

Acute Inflammatory Demyelinating Polyradiculoneuropathy

Clinical features—AIDP is the most common variant of GBS, clinically recognized as an acute progressive disorder affecting peripheral nerve (motor, sensory or both) and nerve roots with maximum severity attained within 4 weeks following symptom onset. The clinical features of AIDP include ascending (or less commonly descending) appendicular and truncal paresis that may progress to paralysis, varying degrees of sensory loss, diminished or loss of myotactic stretch reflexes, respiratory dysfunction, cranial nerve deficits (commonly

bilateral facial paresis) and dysautonomia (urinary retention, constipation, labile blood pressure and heart rate) [30,159].

Electrodiagnostic studies may show evidence of peripheral nerve demyelination such as prolonged distal latencies, reduced conduction velocities, conduction block and temporal dispersion; however within the first week of the disorder, nerve conduction studies may be normal or show prolonged F-wave responses only, suggestive of early proximal nerve or nerve root demyelination. Needle electromyography typically shows reduced recruitment of motor unit action potentials without signs of muscle reinnervation. Cerebrospinal fluid analysis shows elevated protein with a normal white blood cell count, known as albuminocytologic dissociation in 50% of patients by 2 weeks and 90% by 4 weeks, distinguishing immune-mediated polyradiculopathies from infectious causes where pleocytosis is common [30,159]. Several diagnostic criteria exist for clinical and research purposes, as well as to guide institution of therapy [6,34,143].

Neuropathological features and Molecular Pathology—Classic neuropathological features of AIDP are based on observations made on peripheral nerve roots in autopsy cases and sensory nerve (mainly sural) nerve biopsies in affected patients [5,108,14,41,51,47,121]. AIDP is characterized by mononuclear leukocyte infiltration into peripheral nerve and nerve root endoneurium with macrophage-mediated demyelination (Fig 1a). Perivascular collections of lymphocytes within the endoneurium and epineurium have also been described (Fig 1b). Thinly myelinated or frankly demyelinated large and small myelinated axons are classically observed with or without macrophage myelin stripping (Fig 1c). Infiltration of leukocytes within the endoneurium and collections of perivascular leukocytes within the epineurium may be seen (Fig 1d). Endoneurial inflammatory cells predominantly consist of monocytes/macrophages (Fig 1e), followed by T lymphocytes (Fig 1f) and rarer B lymphocytes which may be seen around endoneurial microvessels (Fig 1g) or scattered within the endoneurium. Paucity of inflammatory infiltrates with prominent signs of demyelination is still supportive of AIDP in the right clinical setting, raising the importance of humoral factors in axonal demyelination. Secondary axonal loss with myelin ovoids or debris, indicative of active Wallerian degeneration may be observed in affected nerves (Fig 1c). Subperineal or intraendoneurial edema, suggestive of increased endoneurial interstitial fluid due to inflammation may be present as well.

Membrane bound complement components (e.g. complement activation marker, C3d and membrane attack complex, C5b-9) and immunoglobulin deposition has been described in peripheral nerve biopsies of AIDP patients [40,110,97], however detection of these molecules is neither sensitive nor specific enough to serve as a diagnostic test. Antibody-mediated detection of specific macrophage differentiation markers within the endoneurium has been proposed as a simple tool to differentiate autoimmune from non-inflammatory neuropathies [64], but these are not routinely performed in clinical practice. Similarly, detection of major histocompatibility complex (MHC) Class II on endoneurial endothelial cells, monocytes/macrophages and Schwann cells, tumor necrosis factor-alpha (TNF- α) on both Schwann cell membranes and in myelinated and unmyelinated axons, interleukin 1-beta (IL-1 β) on Schwann cells, endothelial cells and macrophages; interferon-gamma (IFN- γ) on endothelial cells and lymphocytes; intercellular adhesion molecule-1 (ICAM-1) on

endothelial cells and macrophages; and chemokine ('chemotactic cytokine') CCL2 on epineurial and endoneurial vessels, infiltrating cells, Schwann cells and in the endoneurial extracellular matrix and CXCL10 on endothelial cells, have been described in nerves biopsies of patients with AIDP [103,110,66,101].

Downregulation of $\beta 4$ integrin subunit (component of the laminin binding receptor) has been described in Schwann cells associated with inflammatory demyelination in AIDP [107] and endoneurial expression of complement receptor 1 (complement receptor 3b receptor) was described in a subset of sural nerve biopsies from affected patients, implying a pathogenic role of complement-containing complex binding [97]. None of these markers are consistently expressed in peripheral nerve biopsies to allow their routine use as molecular diagnostic markers of AIDP.

In situ cellular infiltrates in AIDP have been shown to express certain proinflammatory molecules by immunohistochemistry such as costimulatory molecule CD80 by endoneurial macrophages, inducible costimulatory (ICOS) on T lymphocytes and ICOS ligand on endoneurial macrophages, nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) was on endoneurial macrophages, inhibitor of kappa B (I- κ B) on endoneurial macrophages and T lymphocytes, interleukin-23 p19 subunit by endoneurial macrophages, Fc γ receptors on endoneurial macrophages, and macrophage differentiation markers MRP14 and 27E10 [64,63,49,3,48,97]. Chemokine receptors CCR1, CCR2 and CCR5 on endoneurial macrophages, and CCR2, CCR4 and CXCR3 on infiltrating T lymphocytes have also been described in AIDP [66,101]. These support the notion that AIDP is an inflammatory neuropathy mediated by subsets of activated macrophages and T lymphocytes within the endoneurium without providing a specific diagnostic molecular pathological marker for clinical purposes.

There are no widely accepted circulating molecular markers of AIDP that aid diagnosis in this disorder. This is in contrast to axonal variants of GBS where serum IgG or IgM antibodies to complex gangliosides such as GM1, GM1b, GD1a, GD1b and GalNAc-GD1a singly or in combination are either pathogenic, serve as diagnostic biomarkers or both [159,58,20], or the Miller Fisher variant where IgG antibodies against GQ1b are present in about 90% of cases [129,70]. Antibodies against myelin glycoproteins, such as myelin protein zero (the most prevalent peripheral nerve myelin protein) have been observed in the serum 20-30% of AIDP patients while antibodies to peripheral nerve myelin 22 have been described in <50% of patients via ELISA or Western blot. These are in contrast to a variable detection of antibodies against the basic peripheral nerve protein P2 in small cohorts [77,52,163]. IgA, IgM and IgG antibodies against peripheral nerve myelin with possible binding to a neutral glycolipid component and complement fixing properties have been described in 50-60% of patients in small series [141,68]. Antibodies against components at the node of Ranvier such as neurofascin, gliomedin and contactin have also been observed in <50% of AIDP patients in a small patient cohort [29]. These frequencies derived from small cohorts of patients are not high enough to support the application of these antibodies as reliable AIDP molecular markers.

Elevated plasma, serum or cerebrospinal cytokine and proinflammatory molecule levels have been described in AIDP, but none of these are routinely performed in clinical practice due to lack of sensitivity or specificity, or a failure to validate proposed biomarkers in different patient cohorts. Such molecules include TNF- α , IL-1 β , interleukin-12 (IL-12), interleukin-2 (IL-2), interleukin-6 (IL-6), neopterin (a by-product of guanosine triphosphate metabolism produced by macrophages in response to lymphocyte activation), osteopontin, ICAM-1, E-selectin, IFN- γ , interleukin-18 (IL-18), matrix metalloproteinase 9 (MMP-9), vascular cell adhesion molecule-1 (VCAM-1), interleukin-1 receptor antagonist (IL-1RA), interleukin-37 (IL-37) $\alpha\beta$ 4 integrin (laminin receptor for Schwann cells and myelin) and vascular endothelial growth factor (VEGF) [96,28,53,93,76,7,44,72,42,117,122]. More recently, elevated plasma levels of interleukin-17 (IL-17) and interleukin-22 (IL-22) have been described in patients with GBS, with reduced levels seen following treatment with intravenous immunoglobulin [73,42]. Elevated CSF Prostaglandin D2 synthase with reduced ratio relative to albumin has been described in AIDP compared to controls; however a prior study demonstrated CSF reduced levels [19,50]. Reduced CSF hypocretin-1 levels have also been described in GBS patients [95].

Several chemokines such as CCL2, CCL7, CCL27, CXCL9, CXCL10, and CXCL12 have been detected at elevated levels in AIDP patients compared to non-inflammatory controls [117]. In a series of GBS patients with elevated CSF CCL2 and CXCL10 levels prior to treatment, levels correlated with the CSF: serum albumin ratio, suggesting a pathogenic role of these chemokines [106]. Increased CSF calcium binding astroglial protein S100 β (which is expressed by Schwann cells) during the acute phase of disease has been shown to correlate with GBS severity scores in patients with AIDP. Elevated phosphorylated neurofilament heavy chain protein (a component of peripheral nerve axons) and tau have also been proposed to differentiate between AMAN and AIDP, or indicate severe secondary axonal involvement in AIDP [144,102].

CSF protein proteomic studies in small cohorts have been conducted to search for potential AIDP disease biomarkers. Several interesting molecules have been detected at elevated levels (vitamin D-binding protein, beta-2 glycoprotein I, complement component C3 isoform, haptoglobin, apolipoprotein A-IV, PRO2044, serine/threonine kinase 10, alpha spectrin II, IgG heavy chain, SNC73 protein, cathepsin D preprotein, serine proteinase inhibitor) while others were detected at reduced levels (cystatin C, transthyretin [monomer and dimer forms], apolipoprotein E, albumin and five of its fragments, fibrinogen, caldesmon 1 isoform, UDP glucose-hexose-1-phosphate uridylyltransferase, heat shock protein 70, amyloidosis patient H1 heart peptide, transferrin) compared to controls [19,55,153,154]. These molecules may provide insights into the pathogenic signaling pathways in AIDP, or may serve as diagnostic biomarkers or measures of disease severity or recovery if confirmed in large population studies.

Pathogenesis

Genetics: Although AIDP is an acquired immune-mediated disorder of the peripheral nervous system that affects about 1 in 100,000 individuals in the general population, occurrence in rare family cohorts suggests a genetic role in disease susceptibility

[145,35,11]. Genetic linkage association studies have been performed in cohorts of affected patients for several decades to further understand its pathogenesis, contributors to disease manifestation or treatment response. There has been particular interest in studying human leukocyte antigens (HLA) class I and II molecules for their integral roles in the innate and adaptive immune responses, as well as other genes involved in lipid presentation, inflammatory responses and tissue injury. There are no genes unequivocally associated with AIDP either due to negative studies or a failure to replicate the positive results from another cohort.

Genes that have been looked at include HLA-A (with a single study showing slight reduction in HLA11 in GBS), HLA-B (with increased isoleucine carriage at position 80 of Bw4 alleles), HLA-C (with increased carriage of alleles with lysine at position 80), HLA-DPB1, HLA-DQA1, HLA-DQB1 (with increased HLA-DQB1*03 in patients with *Clostridium jejuni* infection in a single study, DQ epitopes associated with AIDP but not AMAN in another study and increased DQ*06 in another study; these studies were from disparate populations) and HLA-DRB1 (with 4 out of 13 studies showing some association: increased DR3, increased DRB1*13, increased DRB1 epitopes, HLA-DRB1*14 and DRB1*13 with haplotypes DRB1*14/DQB1*05 and DRB1*13/DQB1*03 conferring susceptibility, and haplotypes DRB1*07/DQB1*02 and DRB1*03/DQB1*02 conferring protection against GBS, and DRB1*0701 increased in GBS patients with preceding infection) [11,61,10,111,90,33].

Other non-HLA genes that have been studied that demonstrate a link to GBS susceptibility, severity, clinical course or other markers of disease activity include TNF- α , alpha 1 antitrypsin, IL-10, Fc Receptor-like 3, mannose binding lectin 2, MMP 9, glucocorticoid receptor, immunoglobulin kappa constant, and Fas (CD95). Other genes that have equivocal associations with GBS include CD1a, CD1e, Fc γ Receptor IIa, Fc γ Receptor IIIa, immunoglobulin heavy constant gamma 1, immunoglobulin heavy constant gamma 2, immunoglobulin heavy constant gamma 3 and toll-like receptor 4. Genes that have shown no linkage association with GBS include CD1d, CD14, Fc γ Receptor IIb, Fc γ Receptor IIIb, and several killer immunoglobulin-like receptors [11]. With significant advances in gene analysis afforded by genotyping to detect single nucleotide polymorphisms and whole exome sequencing, collaborative international prospective gene wide association studies are needed in AIDP to better understand the roles specific genes may have on AIDP susceptibility, severity, response to treatment and clinical outcomes.

Immunology: The pathogenesis of AIDP has remained elusive despite significant research advances over the last 50 years, including initial pathological descriptions. Due to the temporal association of disease onset with antecedent bacterial or viral infections, or tissue injury (such as surgery), molecular mimicry of peripheral nerve antigen(s) with tissue-specific immune attack of peripheral nerves and nerve roots has been hypothesized. This hypothesis is further supported by the ability to experimentally induce demyelinating peripheral neuritis in susceptible animal strains via inoculation with peripheral nerve myelin, or specific myelin protein or peptides. A representative animal model, experimental autoimmune neuritis (EAN) has greatly enhanced our mechanistic knowledge of acute demyelinating peripheral neuritis in vivo. Unfortunately, no novel treatments for AIDP have

been developed despite significant advances in molecular biology and therapeutics over the last 30 years.

Several excellent reviews on the immunopathogenesis of GBS have been published, highlighting the complexities of systemic immune activation with T lymphocyte activation with polarization towards CD4⁺ T helper 1 (Th1) and T helper 17 (Th17) phenotypes, with reduction on T helper 2 (Th2) and possible alterations in CD25⁺ FoxP3⁺ regulatory T lymphocytes, polyclonal B-cell maturation and immunoglobulin synthesis within primary and secondary lymphoid organs, complement-mediated lysis, monocyte/macrophage and lymphocyte-mediated demyelination via cytokines, as well as complement- and antibody-dependent cellular cytotoxicity, Schwann cell roles in potentiating the local innate and adaptive immune response as well as in terminating inflammation by inducing T lymphocyte apoptosis [26,65,86,114,137,159,161]. Hematogenous leukocyte trafficking and immunoglobulin transport across the blood-nerve barrier (BNB; formed by tight junction forming microvascular endothelium within peripheral nerve and nerve root endoneurium) are commonly depicted; however, very little is known about pathologic leukocyte-BNB endothelial interactions and BNB permeability changes that occur in AIDP. Most commentaries give the impression that these are relatively passive processes that inevitably occur following systemic immune activation.

Active hematogenous mononuclear leukocyte trafficking across the BNB endothelium has been pathologically observed in AIDP and EAN (Figs 1h-j), including some phenotypic characterization performed on teased endoneurial microvessels suggesting dynamic trafficking of macrophages in and out of the endoneurium [36]. In contrast to endoneurial macrophages, resident T lymphocytes are not usually observed within peripheral nerve endoneurium. Increased leukocyte trafficking and the presence of immunoglobulin complexes within peripheral nerve endoneurium have been interpreted to reflect a breakdown in the BNB, supported by ultrastructural loss of electron-dense intercellular tight junctions between endoneurial endothelial cells in peripheral nerve biopsies from AIDP patients. Persistent breakdown or loss of BNB function would have devastating effects to peripheral nerve function due to the influx of polar and nonpolar solutes and macromolecules of varying sizes, water and xenobiotics from the bloodstream. This will significantly affect the internal endoneurial homeostasis (particularly ionic concentrations) crucially needed to maintain axonal resting potential and facilitate signal transduction.

Based on knowledge derived from other microvascular barriers, leukocyte trafficking into tissues is a coordinated, sequential multi-step process ("the multi-step paradigm") which involves leukocyte rolling on activated endothelium mediated by selectins expressed on the endothelium and their counterligands (such as P-selectin glycoprotein ligand-1 and Sialyl Lewis x) expressed on leukocytes, leukocyte arrest which is mediated by specific chemokines expressed on the endothelium bound by glycosaminoglycans binding to chemokine receptors expressed on specific leukocyte subsets, leukocyte integrin activation resulting in firm adhesion to the endothelium via cell adhesion molecules such as ICAM-1 and VCAM-1, cytoskeletal modification and transmigration (diapedesis across the vascular endothelium) in response to haptotactic signals such as chemokines (via binding to a specific G-protein coupled chemokine receptors) and extravasation across the endothelial basement

membrane via secretion of matrix metalloproteinases such as MMP2 and MMP9. Once within the tissue, additional chemotactic factors (e.g. chemokines, complement, cytokines) may further activate and drive leukocyte migration towards specific targets [78].

The interaction between the systemic and local immune response in AIDP occurs at the BNB. Functional studies of leukocyte-BNB endothelial interactions required to deduce potential mechanisms of pathologic leukocyte trafficking that may be amenable to pharmacologic blockade prior to transmigration. Such studies may be performed using an in vitro model of the human blood-nerve barrier or EAN models guided by observational studies from AIDP patient biopsies [132,133]. The relative paucity of patient nerves for research, the predilection to study clinically biopsied sural nerves which may be relatively spared early in AIDP or less severely affected than motor nerves or nerve roots that are rarely biopsied premortem [41] and the incomplete in situ molecular characterization of inflammation which can be patchy provide some obstacles to designing pathologically relevant functional studies with translational potential.

Mechanisms/Research

Leukocyte Trafficking: Human data (in vitro): The recent isolation of primary endoneurial endothelial cells that form the BNB from human sciatic nerves has provided an opportunity to decipher the effects of physiological cytokine stimulus on endothelial cells and study mechanisms relevant to pathologic AIDP leukocyte trafficking in vitro [157,134]. Although it is undetermined precisely how BNB endothelium becomes activated in vivo, exogenous administration of TNF- α and IFN- γ at concentrations within the range measured in vivo during systemic illness resulted in increased expression of E-selectin, P-selectin, ICAM-1, VCAM-1, and the alternatively spliced pro-adhesive fibronectin variant called type III connecting segment (which serves as a counterligand for α 4 integrin) in a time-dependent manner [156]. These data are consistent with microvascular endothelium primed for leukocyte trafficking.

Physiological cytokine stimulus also resulted in de novo expression of proinflammatory chemokines CCL2, CXCL9, CXCL11, and CCL20, with >2-fold increased expression of CXCL2-3, CXCL8, and CXCL10 relative to basal levels. Less than 2-fold increases in CCL4, CCL5, CCL23, CXCL5, and CXCL7 were observed. CCL26 was reduced to 0.9 times its basal level. There was no statistically significant change in CCL22 and CCL24 levels. These observations suggest potential early activation of the innate (i.e., neutrophil dependent: CXCL2-3, CXCL8 interacting with chemokine receptors CXCR1 and CXCR2; monocyte-dependent: CCL2 interacting with CCR2) and adaptive (i.e., T-cell dependent: CXCL9-11 interacting with CXCR3 on CD4⁺ Th1 cells, CCL20 interacting with CCR6 on CD4⁺ Th17 cells and CCL27 interacting with CCR10 on activated T cells) immune signaling pathways at the human BNB. It is important to note that physiological cytokine stimulus did not alter BNB transendothelial electrical resistance in vitro relative to untreated conditions [156].

The study above also evaluated mediators of untreated AIDP patient peripheral blood mononuclear leukocyte trafficking across a cytokine treated in vitro model of the human BNB under hydrodynamic forces designed to mimic in vivo capillary hemodynamics. This

study demonstrated that leukocyte trafficking was more dependent on BNB endothelial cytokine-mediated activation than the leukocyte activation state as a consequence of AIDP. Using function neutralizing monoclonal antibodies, leukocyte trafficking above basal levels was mediated by α M integrin-ICAM-1 interactions, with monocytes being the most prevalent adherent leukocyte subpopulation, as expected in AIDP [156]. Importantly, this study demonstrated the multi-step paradigm for leukocyte trafficking at the BNB in vitro, consistent with leukocyte trafficking in other microvascular endothelial cells, providing potential targets to abrogate pathologic leukocyte entry into the endoneurium in AIDP (Supplementary video 1).

Leukocyte trafficking: Animal model data: Functional studies relevant to pathogenic leukocyte trafficking have been performed in EAN using function neutralizing antibodies, small molecular antagonists or gene knockouts. Ideally, the choice of potential targets should be driven by observational data from AIDP, as these models are inherently different from AIDP with respect to disease induction typically requiring aggressive protocols to facilitate demyelinating polyneuritis, although essential features are recapitulated. It is important to recognize that hematogenously derived macrophages are more prevalent in sciatic nerves of EAN mice than endoneurial macrophages [127,92], supporting human observational data showing active trafficking across the BNB and attesting to the pathogenic importance of monocyte and T cell trafficking into the endoneurium [36]. A single study demonstrated a potential role for leukocyte function antigen-1 (α L integrin; a counterligand for ICAM-1) in the induction phase of a Lewis rat EAN model, while another study demonstrated a potential role for CCL3 and partial role for CCL2 in pathogenic leukocyte trafficking in Lewis rat EAN [4,162].

CCR2 gene deletion and pharmacologic blockade following observed clinical signs was associated with disease resistance and rapid near complete recovery respectively, associated with reduced peripheral nerve demyelination and inflammation in a severe murine EAN model induced by bovine peripheral nerve myelin [158]. Supported by observational studies showing increased CCR2 positive mononuclear cells in AIDP nerve biopsies and increased CCL2 and CCR2 in EAN mice sciatic nerves [22,101,148,66], CCL2-CCR2-mediated leukocyte trafficking is proposed to drive pathogenic monocyte and a subset of T lymphocyte trafficking across the BNB.

CCR5 gene deletion failed to protect mice from a less severe form of EAN induced by myelin protein zero peptide 180-199, with compensatory increase in CCL4 and CXCL10 hypothesized to drive pathogenic leukocytes into the peripheral nerves in knockout mice [31]. CCR5 positive macrophages have been observed in AIDP nerve biopsies and EAN mice sciatic nerves, associated with high levels of CCL5 [66,148]. Taking the above EAN study into account, it could be argued that CCR5 is not required for pathogenic leukocyte trafficking across the BNB, implying that expression of chemokine ligand and receptor within tissue does not directly equate to transmigration. Redundancy and promiscuity of chemokine ligand-receptor interactions cannot be ignored [131]. Furthermore, compensatory changes in germline knockouts imply the need to evaluate the effect of conditional gene knockout, function neutralizing antibody or small molecular antagonist prior to concluding mechanistic irrelevance to leukocyte trafficking in AIDP [22].

Although CXCR7 has not been demonstrated on leukocytes in AIDP nerve biopsies, a single study using small molecular antagonists described a role for CXCR7-CXCL12 signaling in pathogenic leukocyte trafficking in a less severe murine EAN model induced by myelin protein zero peptide 106-125. Interestingly in this model, CXCR4 blockade resulted in upregulation of ICAM-1 and VCAM-1 on endoneurial endothelial cells coupled with increased proinflammatory cytokine expression in the serum as well as regional lymph nodes and spleen, resulting in increased infiltration of CD4+ T lymphocytes and macrophages into the sciatic nerves [18]. Several mediators of the multi-step paradigm of leukocyte trafficking (e.g. rolling, firm arrest, transmigration of specific leukocyte subsets) at the BNB remain to be elucidated using animal models or functional in vitro assays.

Serum factors: Human data (in vitro): There are currently no studies that look at the permeability of soluble serum factors such as complement, cytokines and immunoglobulin from patients with AIDP at the human BNB in vitro. A single study using a transwell BNB model developed using bovine endoneurial microvascular cells without incorporation of flow showed that a reduction in transendothelial electrical resistance and increased clearance of [carboxyl-(14)C]-inulin with or without complement induced by GBS sera, with the observation that sera containing GM1 antibodies were more potent [60]. It is unclear how many patients had AMAN or AIDP in this study, as GM1 antibodies are not usually detected in AIDP patients. Furthermore, the xenotypic effect of human antibody on bovine endothelial cells and lack of direct permeability data for specific serum components limits the applicability of such assays.

Serum factors: animal models: Several animal studies have been performed with injection of GBS patient sera into peripheral nerves to determine effects on inflammatory demyelination. Several studies have demonstrated evidence of demyelination with intraneural serum injection (by-passing the restrictive BNB and perineurial interfaces) at higher rates than or similar to control sera in rat sciatic nerves [116,43,16,100]. Intraperitoneal administration of GBS patient sera demonstrated mixed results with abnormal clinical and electrophysiological signs without morphological changes in the sciatic nerve observed in mice and no significant clinical, electrophysiological or pathological effect of sera or monoclonal mouse anti-ganglioside antibodies in a mild adoptive transfer rat EAN model [138,39].

In another study, 125I-labelled immunoglobulin administered intraperitoneally into rats with mild adoptive EAN was detected in spinal nerve roots (encompassing ventral and dorsal nerve roots, as well as dorsal root ganglia that contain fenestrated capillaries) prior to infiltration by polymorphonuclear neutrophils, T lymphocytes, macrophages and erythrocytes with resultant demyelination and axonal degeneration [38]. The mechanisms by which these soluble factors may gain access to peripheral nerve and nerve root endoneurium from the circulation (e.g. translocation or downregulation in adherens or tight junction proteins or their adaptor proteins linking them to the cytoskeleton, increased receptor-mediated, clathrin-dependent or lipid raft/caveolae mediated transcytosis) remain to be determined. The xenotypic effects of human sera in the rat EAN models needs to be considered, as well as the relative impermeability of the sciatic nerves that are commonly

infiltrated by hematogenous leukocytes in rat EAN models following intraperitoneal injection.

Future directions: Despite significant advances in understanding the pathogenesis of AIDP guided by observations made in situ on peripheral nerve biopsies, on isolated leukocytes, sera, cerebrospinal fluid or in a representative animal model, EAN, significant gaps in our knowledge exist. High throughput screening methods applied to peripheral nerves may provide pathogenic clues that may be amenable to therapeutic intervention or serve reliable disease biomarkers. A fundamental question that has not been addressed is the relationship between proteins detected in serum and cerebrospinal fluid with proteins within the endoneurium of affected patients with AIDP. Deciphering this relationship will involve a more detailed understanding of influx and efflux dynamics at the BNB and perineurial interfaces, as well as the permeability characteristics of the vasa nervosum of spinal roots with cerebrospinal fluid. Modulating pathogenic leukocyte trafficking by specifically targeting the molecular determinants of the multistep paradigm and BNB permeability by perturbing specific molecular transport mechanisms early in AIDP provide avenues for therapeutic intervention. Such drugs may be administered systemically without need to overcome potential BNB permeability restrictions inherent while targeting signaling pathways within the peripheral nerve or nerve root endoneurium in AIDP.

Chronic inflammatory demyelinating polyradiculoneuropathy

Clinical features—CIDP is a clinically heterogeneous disorder affecting peripheral nerve (motor, sensory or both) and nerve roots with maximum severity attained after 8 weeks of following symptom onset. CIDP may account for about 14% of chronic disability in adults above the age of 65. The clinical course may be described as relapsing-remitting, steady progressive or step-wise progressive. CIDP is commonly considered to be a chronic form of GBS or the peripheral nervous system equivalent of multiple sclerosis. However, those conceptions may be overly simplistic. The clinical features of CIDP are similar to AIDP with respiratory dysfunction, cranial nerve deficits and dysautonomia less commonly observed [80,27,26,21].

Electrodiagnostic studies commonly show some evidence of peripheral nerve demyelination such as prolonged distal latencies, reduced conduction velocities, conduction block and temporal dispersion in at least two motor nerves; however with disease restricted to the nerve roots prolonged F-wave responses may be the only evidence of dysfunction. Needle electromyography typically shows reduced recruitment of motor unit action potentials with signs of muscle reinnervation. Cerebrospinal fluid analysis typically shows albuminocytologic dissociation as observed in AIDP. Clinical or radiological evidence of peripheral nerve or nerve root hypertrophy have been described. Several diagnostic criteria exist for clinical and research purposes. Consensus statements have been published by consortia of experts to aid clinicians diagnose patients early and institute therapy [26,80,56].

Neuropathological features and Molecular Pathology—Classic neuropathological features of CIDP are based on observations made on predominantly on sural nerve and rarely posterior nerve root biopsies in affected patients [13,121,69,80]. Similar to AIDP,

CIDP is characterized by mononuclear cell infiltration of predominantly monocytes/macrophages and less commonly T lymphocytes into peripheral nerve and nerve root endoneurium with macrophage-mediated demyelination (Figs 2a-d). Perivascular collections of macrophages and lymphocytes within the endoneurium, and macrophage clustering have also been described (Fig 2c-d). However, there may be a paucity of inflammatory infiltrates with prominent evidence of demyelination with or without remyelination. Thinly myelinated or frankly demyelinated large and small myelinated axons are classically observed with or without macrophage myelin stripping (Figs 2b-d). Onion bulb formation, indicative of repetitive demyelination and remyelination may be observed in more chronic cases (Figs 2e-f). Secondary axonal loss with myelin ovoids or debris, indicative of active Wallerian degeneration or significantly reduced axonal density without significant reinnervation may be seen. Subperineal or intraendoneurial edema, and endoneurial microvessel basement membrane thickening or reduplication of the basal laminae have also been observed (Fig 2g) without being specific for CIDP.

Similar to AIDP, there are no molecular pathologic markers deemed specific or sensitive enough to diagnose CIDP on nerve biopsy. Detection of complement, including membrane attack complex (C5b-9) and immunoglobulins within the endoneurium of CIDP affected nerves differs from AIDP. A single study of 9 patients failed to demonstrate B-lymphocyte infiltration or complement and immunoglobulin deposits, while another study of 105 patients demonstrated C3d in 30% of patients without B-lymphocyte infiltration or immunoglobulin [82,115]. Increased expression of HLA-DR on monocytes/macrophages, Schwann cells, in capillary endothelial cells and within the perineurium in areas of active demyelination or remyelination in CIDP compared to basal expression on endothelial cells, very occasional mononuclear cells and sparsely within the perineurium in controls has been described [82,88,104].

In situ hybridization studies have shown TNF- α , IFN- γ and IL-2 mRNA expression in CIDP sural nerves in patients with active disease, localizing to the innermost layer of the perineurium, epineurial and endoneurial blood vessels and infiltrating inflammatory cells, with further widespread TNF- α mRNA within endoneurium in a pattern suggestive of Schwann cell expression. IL-1, IL-6 and TNF- α have also been detected by immunohistochemistry at higher levels than in nerves from chronic axonal neuropathies. Schwann cells were shown to consistently express adhesion/T-cell stimulatory molecule CD58 (LFA-3) in CIDP, with high CD74 (associates with MHC Class II serving as a chaperone for antigen presentation) in both CIDP and control nerves. ICAM-1 expression on CIDP and healthy control endoneurial microvessels was also observed consistently [81,74,140].

Interestingly, E-selectin expression was observed on epineurial vessels in a subset of CIDP sural nerves with Sialyl Lewis x positive cells adherent to their lumens. E-selectin was not expressed on endoneurial microvessels as would be expected to facilitate leukocyte rolling prior to adhesion and transmigration into the endoneurium [98]. As described with AIDP, increased expression of chemokine CXCL10 has been observed in CIDP nerve biopsies [66]. Downregulation of tight junction protein claudin-5 and altered localization of zona occludens-1 on endoneurial microvessels has been described to suggest BNB dysfunction in

CIDP [59]. MMP-2 has also been described in sural nerve biopsies of affected CIDP patients. MMP-9 has been suggested a potential biomarker to distinguish CIDP from non-inflammatory neuropathies by immunohistochemistry [71,125]; however, this is not specific to this disorder.

In situ leukocyte infiltrates in CIDP nerve have shown a relative higher expression of $\gamma\delta$ -T cells (in 70% of patients, suggestive of immune response to non-protein antigens) in addition to the more prevalent $\alpha\beta$ -T cells, compared to AIDP and controls consisting of axonal neuropathies. Clonally expanded T lymphocytes based in a dominant T cell receptor variable beta region utilization are not seen in CIDP sural nerves [62,146,12]. CD73+ (a marker of lymphocyte differentiation) CD4+ and CD8+ T lymphocytes have been detected in CIDP (as well as AIDP) in higher levels than controls [87]. Macrophage differentiation markers MRP8 and 25F9 have been observed on endoneurial macrophages in CIDP at higher levels than controls [64]. CD80 and NF- κ B expression have been described on endoneurial macrophages in CIDP [63]. Endoneurial CD11c+ CD14+CD16+ macrophages were higher but not perineurial CD11c+ CD83– CD14– CD16– immature myeloid dendritic cells in CIDP patients compared to controls. In that study, elevated levels of CD11c+ myeloid dendritic cells were observed in the CSF. However, another study failed to detect any dendritic cells in sural nerve biopsies of affected patients [105,140]. As seen in AIDP, CCR1 and CCR5 expression on endoneurial macrophages, with CCR2, CCR4 and CXCR3 expression on infiltrating T lymphocytes has been observed, as well as ICOS expression by T lymphocytes, and ICOS-L expression by macrophages in CIDP [66,49].

Antibodies against peripheral nerve myelin proteins or node of Ranvier components are too infrequently detected in the sera of CIDP patients to be considered pathogenic or molecular markers of disease. IgG or IgM antibodies against myelin protein zero (0-30%), myelin basic protein P2 (11-35%), peripheral nerve myelin 22 (0-50%) and connexin 32 and myelin basic protein in about 2.5% of patients with CIDP with frequencies not higher than healthy or non-inflammatory neuropathy controls in about 50% of studies. IgM or IgG antibodies against nodal components neurofascin 155 (0-25%), neurofascin 186 (0-2.5%) and contactin-1 (2-7%) have also been described with a similar frequency of studies showing no difference to controls as seen with myelin proteins. Antibodies against complex gangliosides, chondroitin sulfate C or sulfatide are rarely detected in CIDP patient sera or cerebrospinal fluid, with frequencies that are not significantly different from control patients with other neuropathies or household contacts of affected patients [80].

Elevated plasma, serum or cerebrospinal cytokine and proinflammatory molecule levels have also been described in CIDP. Elevated plasma IL-17 and IL-12, as well as CSF IL-17, IL-5, IL-6, growth arrest specific 6, MMP-2, high cathepsin B with low cystatin C levels have been described. Increased levels of CSF CCL2, CCL7, CCL27, CXCL9, CXCL10, CXCL12, ICAM-1, VCAM1 and VEGF have been described in CIDP as seen with AIDP [84,117,118,94]. Elevated levels of CCL3, CCL19 (correlated with the CSF: plasma albumin ratio), stem cell factor (SCF) and hepatocyte growth factor (HGF) compared to GBS and other non-inflammatory controls have also been described [106]. Furthermore, normal CSF index levels of prealbumin, low fibrinogen, and high levels of haptoglobin has been suggested as a characteristic pattern of CIDP [160].

In a single study, markedly elevated IFN- γ + IL-4- CD4+ T cell percentages in CSF were observed in CIDP patients with a significant increase of intracellular IFN- γ /IL-4 ratio in the absence of pleocytosis suggestive evidence of Th1 shift over Th2 phenotype in this disorder. Marked upregulation of Th1 cytokines, IL-17, and downregulation of Th2 cytokines, together with infiltration of IFN- γ -producing CD4+ T cells in the cerebrospinal fluid were proposed as useful diagnostic markers for CIDP [84]. In addition to higher in vitro mononuclear production of IL-10, IL-17 and IFN- γ in CIDP patients, elevated expression of pSTAT1, T-bet, and pSTAT3 has been proposed as markers of disease activity [75]. Despite some evidence to suggest alterations with treatment and treatment responses, none of these are routinely performed in clinical practice due to significant variability and lack of validation between different patient cohorts.

Nerve biopsy or CSF proteomic studies have been conducted to look for potential pathogenic markers or biomarkers of CIDP disease activity. Elevated proteins include tachykinin precursor 1, stearoyl-co-enzyme A desaturase, HLA-DQB1, CD69, macrophage scavenger receptor 1, PDZ and LIM domain 5, CXCL9, CCR2, mast cell carboxypeptidase 3, allograft inflammatory factor-1 (AIF-1), two transferrin isoforms, alpha-1 acid glycoprotein 1 precursor, apolipoprotein A IV, two haptoglobin isoforms, transthyretin, retinol binding protein and two isoforms of proapolipoprotein. Reduced levels of integrin β 8 compared to controls have also been described [130,126]. The clinical heterogeneity of CIDP provides a challenge to finding potential biomarkers in CSF, as well as the undetermined relationship between the composition of nerve root endoneurial interstitial fluid and CSF in this disorder.

Pathogenesis

Genetics: Similar to AIDP, genetic studies have been performed to look for genetic susceptibility factors that may aid elucidate CIDP pathogenesis or provide insights into therapeutic response. Increased frequency of HLA-Aw30, HLA-B8 and HLA-Dw3 in CIDP has been described in three studies. A strong association of HLA-Cw7 and slight association of HLA-B7 have been described in a single study. A higher HLA-DR2 gene frequency has been described in female CIDP patients in addition to a study that found increased HLA-DR2 in CIDP as a whole [11]. A more recent report from a specific geographical region showed an association of CIDP with HLA-DRB1*13 [91]. However, a prior single study from another geographical region demonstrated no HLA associations with CIDP [139].

Several non-HLA genes have been studied with links to CIDP. These include alpha-1 antitrypsin, Fc γ RIIb, and T-cell specific adaptor protein. Contactin 2 was shown to have a link with response to IVIg in CIDP in one study, while another study showed no linkage association with this disorder. Genes studied without associations to CIDP include CD1a, CD1e, immunoglobulin heavy constant gamma 1, immunoglobulin heavy constant gamma 2, and immunoglobulin heavy constant gamma 3 [11]. Large population prospective gene wide association studies are needed in well-defined cohorts of CIDP patients to better understand the roles specific genes may have disease susceptibility (including different subtypes), severity, clinical progression and response to immune suppressant treatment.

Immunology: The immunopathogenesis of CIDP has yet to be elucidated. Based on observational data from human nerves and observations in animal models of chronic peripheral nerve inflammation, the combined effect of defects in immune tolerance with persistent activation of the immune system with resultant cell and humoral-mediated inflammation has been proposed. Unlike AIDP, antecedent infections or trauma rarely precipitate CIDP, reducing the likelihood that molecular mimicry serves as a trigger to initiate aberrant tissue-specific pathogenic immune responses. The co-existence of CIDP or a CIDP-like disorder with other systemic autoimmune and dysimmune disorders, induction by certain immune modulatory drugs and response to immune suppressant or immune modulatory treatments provides indirect evidence that CIDP occurs in the setting of a dysregulated immune system [27].

Several excellent reviews on the immunopathogenesis of CIDP highlighting the complex interactions proposed to induce systemic activation with compromised immune tolerance or dysfunction in regulatory leukocytes such as CD4⁺ CD25⁺ FoxP3⁺ T lymphocytes, altered cytokine profiles towards a Th1 (and more recently Th17) phenotype, possible B-cell maturation and polyclonal antibody synthesis, monocyte/macrophage-mediated demyelination (direct and indirect), roles of T-lymphocytes in maintenance of endoneurial inflammation via cytokine secretion, as well as Schwann cell roles in potentiating the local innate and adaptive immune response with an inability to persistently terminate local inflammation by inducing T lymphocyte apoptosis have been published [80,26,65].

As described with AIDP, the mechanisms by which hematogenous leukocytes and possibly immunoglobulins gain access to the endoneurium (crucial events necessary for the interaction between the systemic immune compartment and peripheral nerves and nerve roots) are largely unknown. Another puzzling issue is the relationship between perivascular accumulation of macrophages or T cells at epineurial macrovessels and the observed endoneurial pathology. Challenges that have to be overcome to decipher the molecular mechanisms relevant to CIDP via functional assays including modeling chronic leukocyte trafficking at the human BNB in vitro, modeling BNB permeability to immunoglobulins and other soluble humoral factors under flow conditions in vitro and studying BNB-dependent signaling pathways suggested by human in situ data in reliable animal models that recapitulate essential pathological features of CIDP.

Mechanisms/Research

Leukocyte Trafficking: Human data (in vitro): Despite the recent isolation and characterization of primary human endoneurial endothelial cells that form the BNB and the description of flow-dependent leukocyte trafficking assays under flow [133,156,157], there are currently no published studies looking at the trafficking of CIDP patient-derived mononuclear leukocytes at the BNB. Studies looking at the functional role of specific integrins and cellular adhesion molecules are ongoing on our laboratory using leukocytes from untreated patients that meet clinical and electrophysiological criteria for CIDP.

Leukocyte Trafficking: Animal models: There are currently several animal models designed to mimic typical CIDP. The chronic relapsing or biphasic experimental

autoimmune neuritis, induced in susceptible strains of guinea pigs, rabbits and rats by inoculation with exogenous bovine peripheral nerve myelin or myelin peptides with chronic immune activation facilitated by adjuvants or drugs have been described with variable rates of disease recurrence [128,83,57,86,17]. More recently a murine model of severe chronic demyelinating neuritis with axonal loss akin to progressive CIDP, spontaneous autoimmune peripheral polyneuropathy (SAPP), has been described in non-obese diabetic mice (a mouse strain genetically predisposed to autoimmunity) deficient in CD86, ICAM-1 and autoimmune regulator with cellular and humoral immune responses directed against myelin protein zero associated with altered antigen specific T lymphocyte costimulation or deficient generation of antigen specific regulatory T lymphocytes [124].

Peripheral nerve infiltrates associated with demyelination in SAPP include monocytes/macrophages, dendritic cells, T lymphocytes and B lymphocytes, with monocytes/macrophages being the most prevalent cells seen in sciatic nerves, similar to CIDP [119,135]. There are currently no functional studies evaluating mechanisms of pathogenic leukocyte entry into peripheral nerves in these models. There is some in situ sciatic nerve evidence for marked increased expression of pro-inflammatory cytokines TNF- α , IFN- γ and IFN- γ R, and chemokines CCL5 and CXCL10, with modest increase in CCL2, CCL3, CCL4, and CXCL16 at expected peak SAPP severity in CD86 deficient NOD mice. Interestingly, increase in IL-17 with a decrease in IL-10 were observed during the preclinical phase of the disorder, implying roles of enhanced Th1 and Th17 cytokine production with reduced Th2 signaling in the pathogenesis of chronic peripheral neuritis in this model, as suggested in CIDP [67]. The SAPP model provides an essential tool to determine the molecular signaling pathways relevant to persistent pathologic leukocyte entry into peripheral nerve endoneurium during chronic demyelinating neuritis. Ongoing studies in our laboratory are focused on integrin-dependent leukocyte trafficking using small peptide antagonists or function neutralizing monoclonal antibodies in SAPP.

Serum factors: Human data (in vitro): The effect of heat inactivated sera from different CIDP subtypes on BNB permeability has been recently published, showing relative reduction in tight junction protein claudin 5 levels by western blot and reduced transendothelial electrical resistance of immortalized peripheral nerve microvascular endothelial cell layers in typical CIDP patients, associated with some electrophysiological measures of demyelination and clinical measures of severity [123]. Sera were included with culture medium during seeding and measurements were made within 48 hours of cell culture, calling to question whether tight junctions with maximal TEER were achieved prior to CIDP patient sera exposure, as would be expected in vivo. The distribution of claudin-5 on these endothelial cells following patient sera exposure is not shown to ascertain whether there are similarities to the in situ observations previously published in CIDP [59].

Serum factors: Animal models: The effect of injecting sera from CIDP patients into animals has been described. Conflicting data exist on the ability of sera from patients with active disease to induce demyelination in rodent peripheral nerves. A study demonstrated no evidence for increased demyelination in rat sciatic nerves compared to controls with other neuropathies following intraneural injection. This is in contrast to a study that showed sera

or purified IgG from CIDP patients producing marked conduction block and demyelination via intraneural injection or following adoptive transfer of activated T lymphocytes that was not observed with sera or IgG from healthy individuals or patients with other neuropathies or multiple sclerosis. Intraneural injection of sera from a subset of CIDP patients with IgG antibodies against myelin protein zero produced conduction block and demyelination in experimental animals based on a single series [85,152,151].

The pathogenic relevance of these antibodies in CIDP is debatable due to the low antibody prevalence rates in larger cohorts. In situ studies do not categorically demonstrate immunoglobulin complex deposition in CIDP. As described with AIDP, the xenotypic effects of human sera in rodent peripheral nerves needs to be taken into consideration. In SAPP, a model of severe chronic demyelinating neuritis with intense mononuclear cell infiltration, intact electron dense tight junctions are commonly observed between endoneurial endothelial cells within the chronic inflammatory milieu (Fig 2h-i), implying a need to elucidate the role(s) of active influx and efflux transport mechanisms for small and large polar molecules and other biologically active substances at the BNB during chronic peripheral inflammation rather than assume passive paracellular entry of serum humoral factors into the endoneurium.

Future directions/treatments: As discussed with AIDP, significant gaps exist in our understanding of the immunopathogenesis of CIDP, partly due to the heterogenous nature of this disorder. Collaborative high throughput screening methods applied to peripheral nerves, isolated leukocytes, serum and cerebrospinal fluids of patients with similar CIDP phenotypes should aid with the identification of molecular biomarkers or signaling pathways of pathogenic significance. Targeting specific mechanisms of dysregulated immune tolerance or systemic immune activation, persistent leukocyte trafficking across the BNB and ineffective axonal regeneration and myelination guided by human in situ observations in CIDP should lead towards more effective therapies for CIDP with the ultimate goal to prevent relapses and secondary axonal degeneration. Systemic drug administration should be effective in targeting the systemic immune compartment and persistent leukocyte trafficking across the BNB, while drugs with adequate BNB permeability or the utilization of cell-based strategies to deliver biologically active molecules into inflamed nerves [149] may be needed to prevent inflammatory demyelination and axonal degeneration, or enhance remyelination and axonal regeneration.

Nonsystemic Vasculitic Neuropathy

Clinical features—Nonsystemic vasculitic neuropathy (NSVN) or primary vasculitic neuropathy may present with acute-to-subacute painful or painless sensory or motor deficits that may involve individual nerves in a stepwise manner causing multiple mononeuropathies that are typically asymmetrical. In some patients, peripheral nerve involvement could be more confluent, symmetrical or chronic and slowly progressive rather than stepwise. Compared to systemic vasculitic neuropathies, clinical progression may be slower with milder and less common systemic symptoms or abnormal laboratory markers of inflammation [23,37]. There are currently published consensus criteria used to diagnose and

classify vasculitic neuropathies based on size of vessels affected, observed histopathology and associated disorders [24,54].

Electrodiagnostic studies may show evidence of acute-to-subacute sensory and motor axonal loss that is patterned to localize to individual nerves commonly in a multifocal distribution with fascicular variability. Ongoing denervation and varying degrees of reinnervation changes may be seen in more chronic cases. An asymmetrical or non-length-dependent axonal neuropathy pattern may be observed; however a near symmetrical length-dependent pattern may occur in chronic untreated or more fulminant cases [37,23].

Neuropathological features and Molecular Pathology—NSVN is considered a disorder in which blood vessels restricted to the peripheral nervous system are infiltrated and damaged by inflammatory cells with resultant endoneurial ischemia and consequential axonal injury. Epineurial vessel involvement is more commonly seen (Fig 3a-c), with rarer cases demonstrating both epineurial and endoneurial vessel involvement (Fig 3d). Inflammatory cell accumulation within blood vessel walls with evidence of fibrinoid necrosis, endothelial disruption, fragmentation of internal elastic lamina, loss or fragmentation of smooth muscle cells in the media, acute thrombosis, vascular or perivascular hemorrhage or leukocytoclasia provide evidence of acute vasculitis (Fig 3a-e). Inflammatory cell infiltration in NSVN predominantly consists of T lymphocytes (Fig 3e). Intramural iron deposition may provide evidence of older hemorrhages that occurred to peripheral nerve blood vessels secondary to inflammation. Evidence of axonal changes consistent with multifocal patterns of acute ischemia (Wallerian degeneration, myelin debris, inter- and intrafascicular variability) may be seen (Fig 3f). Intramural inflammation with intimal hyperplasia, media, adventitia or pre-adventitia fibrosis or thrombosis with recanalization provides evidence of chronic vascular injury with some repair. In many cases, nonspecific axon loss that may be focally accentuated with or without regeneration may be seen indicative of chronic ischemia secondary to vasculitic neuropathy [23,37]. This observation provides rationale for serial sections and immunohistochemical evaluation for peripheral nerve inflammation. There is some evidence demonstrating increased diagnostic yield of NSVN by performing muscle and cutaneous punch biopsies in affected patients [142,136] (Fig 3g-h).

As is the case with other inflammatory neuropathies discussed, there are currently no diagnostic biomarkers for NSVN. Many of the studies looking for molecular biomarkers or specific pathologic features include patients with both systemic and nonsystemic vasculitic neuropathy. Vascular deposition of complement, immunoglobulin such as IgM or fibrinogen by direct immunofluorescence may be seen as supportive evidence of vasculitic neuropathy. Strong expression of HLA Class I and class II (HLA DR) on affected vascular endothelial cells has been described, typically associated with prominent CD4+ and fewer CD8+ T-lymphocytes and CD68+ macrophages. CD22+ B-lymphocytes and CD16+ Natural Killer cells are less commonly observed in vasculitic neuropathy than T-lymphocytes and macrophages. T lymphocyte infiltrates in vasculitic neuropathy are heterogenous based on T cell receptor V β utilization, as seen in CIDP. CD58 and CD86 expression on vascular endothelial cells has been described, with the former also expressed by Schwann cells [25,32,12,140].

Increased sural nerve expression of IL-1 β , IL-6, and TNF- α has been described in vasculitic neuropathy, with immunoreactivity directly correlated with the degree of axonal degeneration, endoneurial macrophages and epineurial T cells and neuropathic pain [74]. Expression of MMP-9, was increased in perivascular inflammatory infiltrate in nerve tissues of vasculitic neuropathy patients, with variable MMP-2 expression that may be expressed by stromal perineurial and epineurial cells [71]. As seen with AIDP and CIDP, ICOS and ICOS-L mRNA was found to be significantly upregulated in samples from patients with vasculitic neuropathy. Similarly, high expression of AIF-1 on vascular smooth muscle cells has been described in vasculitic neuropathy [49,15].

Distinct expression of hypoxia-inducible factor (HIF) 1 α in the nuclei of vascular endothelial cells may aid diagnosis especially in biopsies without definitive evidence for inflammation. Similarly, variable focal expression of HIFs HIF-1 α , HIF-1 β , HIF-2 α , as well as VEGF, VEGF-R and erythropoietin-receptor was seen on endoneurial microvessels in a small percentage of nerve biopsies from patients with vasculitic neuropathy at higher rates than control biopsies [99,109]. In situ expression of nerve growth factor by Schwann cells, infiltrating macrophages, T cells and perivascular cells has been described in vasculitic neuropathy. In addition to nerve growth factor, glial cell line-derived neurotrophic factor and IL-6 mRNA expression were increased in peripheral nerves of a cohort with necrotizing vasculitic neuropathy with a reduction in ciliary neurotrophic factor [89,150]. However, these markers are not sensitive or specific enough for use in clinical practice.

Elevated plasma VEGF has been described in vasculitic neuropathy in a small cohort supporting in situ nerve histochemical data. CSF analyses demonstrated increased IL-6, IL-8 and IL-10 in a cohort of vasculitic neuropathy patients prior to treatment, in support of the Th2 cytokine shift in vasculitic neuropathy in contrast to the Th1/ Th17 shift in AIDP and CIDP [79,84]. DNA microarray analysis of nerve biopsy specimens from vasculitic neuropathy patients showed increased gene expression of immunoglobulin lambda joining 3, immunoglobulin heavy constant gamma 3, immunoglobulin kappa constant, immunoglobulin lambda locus, CXCL9, CCR2 and CX3CR1, with reduced gene expression of Krüppel-Like Transcription Factors KLF2, KLF4 and the nuclear orphan receptor NR4A1 (involved in endothelial activation). AIF-1 expression was also upregulated based on a DNA microarray compared to normal nerves, consistent with immunohistochemistry data [126].

Pathogenesis

Genetics: Unlike AIDP and CIDP, there are currently no published genetic studies to look for genetic susceptibility factors that may aid elucidate the pathogenesis of NSVN or provide insights into disease susceptibility or therapeutic response. Collaborative large population prospective gene wide association studies are needed in NSVN to better understand the roles specific genes may have in disease susceptibility, severity, clinical progression and response to immune suppressant treatment.

Immunology: The immunopathogenesis of NSVN has not been elucidated. The prevalence of vasculitic neuropathy with systemic autoimmune disorders and observational data from affected patients provides significant evidence that aberrant immune function plays a

significant role in the pathogenesis of NSVN. The endothelial antigen(s) against which the immune mediated attack is directed to remains elusive. The determinants of hematogenous leukocyte infiltration of predominantly epineurial or less commonly endoneurial vessels in vasculitic neuropathy are unknown.

A single study of 12 patients with vasculitic neuropathy suggested the activation of receptor for advanced glycation endproducts (RAGE) pathway as critical based on expression of the receptor, RAGE ligand N(ϵ)-(carboxymethyl)lysine, NF- κ B, and IL-6 localized to infiltrating mononuclear cells (CD4+ and CD8+ T lymphocytes and CD68+ macrophages), epineurial and endoneurial vessels and the perineurium [45]. Increased advanced glycation endproducts and RAGE have been described in dermal microvessels and T cells of patients with NSVN; however patients with diabetic neuropathy had similar expression to NSVN patients relative to healthy controls [8], suggesting that this may be a marker of vasculopathy. Another study designed to deduce whether apoptosis played a role in endothelial cell injury in 19 patients with vasculitic neuropathy by immunohistochemistry and terminal deoxynucleotidyl transferase-mediated nick end labeling demonstrated expression of granzyme A, granzyme B and T-cell restricted intracellular antigen, as well as Fas and FasL on epineurial perivascular mononuclear cells by immunohistochemistry, implying that T-lymphocyte and macrophage apoptosis may occur during a phase of peripheral nerve recovery in this disorder [46].

Mechanisms/Research: Advancements in understanding pathogenic mechanisms in NSVN have been hampered by the lack of in vitro human models or animal models of the disease. Evolutionally conserved phenotypic and functional differences exist between macrovascular and microvascular endothelial cells from the same tissue, as well as endothelial cells from different tissues and species [1,2,155]. Accumulation of T-lymphocytes and macrophages, as well as immune complex and complement deposition observed in epineurial vessels associated with transmural vasonecrosis suggest immune attack against endothelial or vascular smooth muscle cells (which are lacking in endoneurial microvessels, which are surrounded by and share the same basement membrane with pericytes) or both. An activated and dysfunctional tissue specific CD4+ Th2 dependent cell-mediated immune response against peripheral nerve vessels is inferred based on in situ observations of affected nerves.

Anti-endothelial cell antibodies, a heterogeneous family of antibodies reacting to endothelial cell antigens such as lamin A/C, vimentin, α -enolase, far upstream binding protein 2 and protein disulfide-isomerase A3 precursor in patients with anti-neutrophil cytoplasmic antibody-associated vasculitides or vinculin, lamin A/C, voltage-dependent anion-selective channel protein 2, and annexin V in patients with giant cell arteritis based on mass spectroscopy-based proteomic approaches, have not been tested in NSVN [9,112,113]. It is provocative to speculate that an aberrant humoral autoimmune response to restricted epineurial endothelial or vascular smooth muscle antigens is relevant to disease pathogenesis. The pathogenic role of anti-endothelial cell antibodies or antibodies reactive to components of vascular smooth muscle wall cells in systemic vasculitides currently remains controversial. Active and passive immunity animal models of anti-endothelial cell antibody disease have been described [9], but these have not been studied in significant depth.

An attempt to induce peripheral nerve ischemia in rat sciatic nerves by stripping extrinsic epineurial macrovessels (vasa nervosum) to simulate vasculitic neuropathy did not show significant pathological findings apart from some peripheral watershed infarcts [120]. Chronic persistent occlusion of epineurial arterioles that may occur as a consequence of vasculitis could provide a means to perform mechanistic studies of pathogenic endoneurial ischemia and reparative process that may be relevant to NSVN.

Future directions/treatments: Despite the relative availability of affected NSVN peripheral nerves in clinical practice compared to disorders like AIDP and CIDP, very little is known about the mediators or mechanisms of pathogenic leukocyte-endothelial-vascular smooth muscle interactions, mechanisms underlying axonal degeneration and Schwann cell death following endoneurial ischemia as well as mechanisms of repair in NSVN. Data is also lacking on genetic risk factors, as well as triggers or activators of the immune system in this disorder. Collaborative gene wide association studies and high throughput screening assays of peripheral nerves, leukocytes and blood should advance our knowledge of the pathogenesis of NSVN with the goal to develop targeted immune therapies with improved outcomes and fewer adverse effects for this disorder.

CONCLUSIONS

Inflammatory neuropathies associated with pathogenic hematogenous leukocyte infiltration into peripheral nerves are groups of disorders with undetermined pathogenesis that lack reliable biomarkers and currently have nonspecific therapies. Molecules implicated in the pathogenesis of these disorders are shown in Table 1. Significant progress has been made in understanding the pathogenesis of AIDP and CIDP, with less progress made understanding NSVN pathogenesis, as shown in Fig 4. The molecular determinants and mechanisms of pathogenic leukocyte infiltration into peripheral nerves are being elucidated in AIDP and CIDP with the use of in vitro human blood-nerve barrier assays and representative animal models. Work is significantly needed to better characterize transmural inflammation in NSVN and develop experimental models to evaluate hypothesized mechanisms guided by in situ observations. Collaborative gene wide association studies and the use of high throughput genomic and proteomic studies in addition to functional assays should aid expand our knowledge in the quest to elucidate key pathogenic signaling pathways that may provide molecular targets for specific drug therapy design or reliably serve as disease biomarkers in these groups of disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1. Histopathological features of AIDP

Digital photomicrographs of a sural nerve biopsy from a patient with AIDP show classic pathological features. An axial frozen thick section demonstrates endoneurial mononuclear leukocyte infiltrates emerging from endoneurial microvessels (a; white arrows; Periodic Acid Schiff stain). Perivascular accumulation of predominantly lymphocytes is seen associated with an endoneurial microvessel in this longitudinal frozen section (b; white arrow; Hematoxylin and Eosin stain). Numerous thinly myelinated large and small diameter axons are diffusely seen in this plastic embedded axial semi-thin section, with myelin debris indicative of active Wallerian degeneration (c; black arrow; Toluidine Blue/ Basic Fuchsin stain). Indirect immunohistochemistry performed on frozen axial thick sections counterstained with Hematoxylin (d-g) shows mononuclear leukocyte (CD45+) infiltration within the endoneurium (d; black arrows) with some perivascular leukocyte accumulation within the epineurium (d; red arrow). Mononuclear leukocyte infiltration across the BNB into the endoneurium predominantly consists of CD68+ monocytes/macrophages (e; black arrows) with clusters of CD3+ T lymphocytes seen in some sections (f; black arrows). A rare endoneurial perivascular accumulation of CD20+ B lymphocytes (g; black arrows) is shown. Hematogenous leukocyte trafficking at the BNB is shown in AIDP (h; black arrows; Periodic Acid Schiff-stained longitudinal frozen thick section and i; black arrows; Toluidine Blue/ Basic Fuchsin-stained axial plastic embedded semi-thin section) and in the sciatic nerve of a severe murine EAN mouse (j; white arrows; direct immunohistochemistry of frozen axial thick section with green representing endothelial cells stained with fluorescein-conjugated Ulex Europaeus agglutinin and blue representing nuclei stained with 4',6-diamidino-2-phenylindole [DAPI]). Magnification bars: a and d = 200 μ m; b, c and g = 50 μ m; d = 5 μ m; e = 50 μ m; f = 75 μ m, g, h and i = 20 μ m, j = 10 μ m.

Fig 2. Histopathological features of CIDP

Digital photomicrographs of Toluidine Blue/ Basic Fuchsin stained plastic-embedded axial semi-thin sections from patients with CIDP show intense endoneurial mononuclear cell infiltrates with severe axonal loss (a). Reduced axonal density with a moderate number of thinly myelinated large and small diameter axons is a common feature of CIDP (b).

Endoneurial accumulation of mononuclear leukocytes (c) may be seen. Indirect immunohistochemistry of frozen longitudinal thick sections with counterstained with Hematoxylin shows endoneurial CD68+ monocyte/macrophages (d; black arrows) closely associated with large diameter myelinated axons (white asterisks) suggestive of focal demyelination. Onion-bulb formation, indicative of repetitive demyelination and remyelination in chronic CIDP cases is shown (e and f), with endoneurial microvessel basement membrane thickening (g; black arrows) associated with reduced axonal density and increased endoneurial edema. A digital electron ultramicrograph of a sciatic nerve endoneurial microvessel within the inflammatory milieu of a 40 week old female CD86 deficient non-obese diabetic mouse with SAPP for 18 weeks shows intact electron-dense intercellular tight junctions (h; black arrows) with a cluster of pinocytic vesicles (PV), with an endoneurial microvessel intercellular tight junction from an age matched control mouse for comparison (i; black arrow). L indicates the vessel lumen and BM the basement membrane (h and i). Magnification bars: a and d= 50 µm; b = 100 µm; c = 10 µm; e and f = 5 µm; g = 25 µm; h and i = 200 nm

Fig 3. Histopathological features of NSVN

Digital photomicrographs of sural nerve biopsies from patients with NSVN show classic histopathological features. Axial paraffin-embedded Hematoxylin and Eosin-stained thick sections demonstrate lymphocytic and macrophagic infiltration of an epineurial medium diameter vessel (a) with higher magnification images demonstrating epineurial vasculitis with transmurial vasonecrosis with luminal occlusion (b; white arrow and c; black arrow, white asterisk indicates expected location of lumen). A longitudinal paraffin-embedded Hematoxylin and Eosin-stained thick section depicts the less commonly observed endoneurial microvasculitis (d; black arrow). Indirect immunohistochemistry of frozen axial thick sections counterstained with Hematoxylin demonstrates predominantly CD3+ T lymphocyte infiltration of the media and adventitia of an epineurial medium diameter vessel (e, black arrow) without involvement of an adjacent epineurial small diameter vessel (e, red arrow). In acute and severe cases, marked endoneurial ischemia occurs with active Wallerian degeneration with diffuse myelin debris and ovoids, as demonstrated in the Toluidine Blue/ Basic Fuchsin-stained, plastic-embedded semi-thin section (f; black arrows). Endomysial microvasculitis with vasonecrosis and luminal occlusion (g; black arrow) with focal inflammatory cell infiltration into muscle fibers and the surrounding endomysium associated with myonecrosis (h) are demonstrated in Hematoxylin and Eosin-stained frozen axial thick sections of a quadriceps muscle biopsy of a patient with NSVN. Magnification bars: a, d, e, f, g = 100 μ m; b = 50 μ m; c and h = 200 μ m

Fig 4. Hypothesized pathogenic mechanisms relevant to inflammatory neuropathies
Current hypotheses relevant to the immunopathogenesis of inflammatory neuropathies, AIDP, CIDP and NSVN, are shown expanding on potentially relevant mechanisms at the interface between the systemic immune compartment and peripheral nerves at the endoneurial (BNB: AIDP, CIDP and less commonly NSVN) and epineurial (NSVN) vessels.

Table 1

Molecules implicated in the pathogenesis of inflammatory neuropathies

	AIDP	CIDP	NSVN
Peripheral Nerve	C3d, C5b-C9, immunoglobulin, MHC Class II, TNF- α , IL-1 β , IFN- γ , MMP-9, ICAM-1, CCL2, CXCL10, β4 integrin , CR1, CD80, ICOS, ICOS-L, NF- κ B, I- κ B, IL-23p19, Fc γ , macrophage differentiation markers (MRP14, 27E10), CCR1, CCR2, CCR4, CCR5, CXCR3	HLA-DR, TNF- α , IFN- γ , IL-1, IL-2, IL-6, CD58, CD74, ICAM-1, E-selectin, CXCL10, Claudin-5 , MMP-2, MMP-9, γ δ -T cells, CD73, Macrophage differentiation markers (MRP8, 25F9), CD80, NF- κ B, ICOS, ICOS-L, CCR1, CCR2, CCR4, CCR5, CXCR3	Complement, immunoglobulin, fibrinogen, HLA class I and II, CD58, CD86, IL1- β , IL-6, TNF- α , MMP-2, MMP-9, ICOS, ICOS-L, AIF-1, HIF-1 α , HIF-1 β , HIF-2 α , VEGF, VEGF-R, NGF, erythropoietin-receptor, GDNF, CNTF , NF- κ B, RAGE, RAGE ligand N(ϵ)-(carboxymethyl) lysine, granzyme A, granzyme B and T-cell restricted intracellular antigen, Fas, FasL
Cerebrospinal fluid/ Plasma/ Serum	TNF- α , IL-1 β , IFN- γ , IL-1RA, IL-2, IL-6, IL-12, IL-17, IL-18, IL-22, IL-37, neopterin, osteopontin, ICAM-1, VCAM-1, E-selectin, α 6 β 4 integrin, VEGF, hypocretin-1 , CCL2, CCL7, CCL27, CXCL9, CXCL10, CXCL12, S100 β , phosphorylated neurofilament heavy chain protein and tau	IL-5, IL-6, IL-12, IL-17, growth arrest specific 6, MMP-2, cathepsin B, cystatin C , CCL2, CCL3, CCL7, CCL19, CCL27, CXCL9, CXCL10, CXCL12, ICAM-1, VCAM1, VEGF, SCF, HGF, IFN- γ + IL-4- CD4+ T cells, intracellular IFN- γ /IL-4 ratio	VEGF, IL-6, IL-8, IL-10
Cerebrospinal fluid/ Nerve Microarray or Proteomics	vitamin D-binding protein, beta-2 glycoprotein I, complement component C3 isoform, haptoglobin, apolipoprotein A-IV, PRO2044, serine/threonine kinase 10, alpha spectrin II, IgG heavy chain, SNC73 protein, cathepsin D preprotein, serine proteinase inhibitor, cystatin C , transthyretin , albumin , apolipoprotein E , caldesmon 1 isoform , UDP glucose-hexose-1-phosphate uridylyltransferase , HSP70 , amyloidosis patient HI heart peptide , transferrin	tachykinin precursor 1, CD69, stearoyl-co-enzyme A, CXCL9, desaturase, HLA-DQB1, AIF-1, macrophage scavenger receptor 1, PDZ and LIM domain 5, CCR2, mast cell carboxypeptidase 3, alpha-1 acid glycoprotein 1 precursor transferrin, apolipoprotein A IV, haptoglobin, transthyretin, retinol binding protein, pro-apolipoprotein, integrin β8	AIF-1, immunoglobulin lambda joining 3, immunoglobulin heavy constant gamma 3, immunoglobulin kappa constant, immunoglobulin lambda locus, CXCL9, CCR2 and CX3CR1, Krüppel-Like Transcription Factors KLF2 , KLF4 , nuclear orphan receptor NR4A1

Bold indicates reduced levels compared to unaffected patients or healthy controls