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THE ROLE OF CHEMOKINES IN GUILLAIN-BARRÉ SYNDROME

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Abstract

Introduction—Chemokines and their receptors are important mediators of inflammation.

Guillain-Barré syndrome (GBS) is the most common cause of acute paralysis worldwide. Despite current treatments, outcomes are suboptimal. Specific chemokine receptor antagonists have the potential to be efficacious against pathogenic leukocyte trafficking in GBS.

Methods—A 36-year literature review was performed to summarize available data on chemokine expression in GBS and its representative animal model, experimental autoimmune neuritis (EAN).

Results—Although there were a few observational human and animal studies demonstrating chemokine ligand/receptor expression in GBS and EAN, *in vitro* and *in vivo* functional studies using gene knockouts, neutralizing antibodies, or small molecular antagonists were limited. CCL2–CCR2, CCL5–CCR5, and CXCL10–CXCR3 have been most strongly implicated in EAN and GBS pathogenesis, providing targets for molecular blockade.

Conclusions—Preclinical human *in vitro* and *in vivo* EAN studies are needed to evaluate the potential efficacy of chemokine signaling inhibition in GBS.

Keywords

chemokine; chemokine receptor; experimental autoimmune neuritis; Guillain-Barré syndrome; therapy

Guillain-Barré syndrome (GBS) is an autoimmune disorder that affects peripheral nerves and nerve roots; it is characterized by rapidly progressive weakness and variable sensory, autonomic, and cranial nerve deficits, and peaks within 4 weeks of symptom onset. There are several subtypes of GBS, including acute inflammatory demyelinating polyradiculoneuropathy (AIDP, the most common variant in the western hemisphere and industrialized nations),^{1,2} acute motor axonal neuropathy (AMAN), acute motor-sensory axonal neuropathy (AMSAN), Miller-Fisher syndrome (MFS), poly-neuritis cranialis variant, acute pandysautonomia, brachiocervicopharyngeal variant, and rare pure sensory GBS.^{2–4}

GBS has an annual incidence of 1.2–2.3 cases per 100,000 people worldwide,⁵ with estimated health-care costs over \$2 billion per annum (AIDP variant) in the USA. Although full motor recovery is eventually expected in ~60%, over 15% of patients have residual moderate-to-severe weakness 1 year after disease onset, and up to 10% die despite adequate treatment. Many patients have residual sensory deficits and do not return to previous functional levels.⁶ Lifetime GBS recurrence after full motor recovery is <5%. The most common causes of death include respiratory failure, pulmonary embolism, cardiac arrest, and infection.⁶ Current treatments [intravenous immunoglobulin (IVIg) and plasmapheresis] demonstrably hasten recovery in GBS compared with no treatment. However, these treatments are non-specific and are often associated with adverse effects and complications during the acute phase of GBS.⁶

Postinfectious molecular mimicry is commonly cited as relevant to GBS pathogenesis based on the temporal association of disease onset with antecedent gastrointestinal (commonly *Campylobacter jejuni*) or respiratory infection in two-thirds of cases. This association is more prevalent in the axonal variants, particularly AMAN. Unlike the axonal variants, demyelinating forms of GBS such as AIDP are characterized histopathologically by macrophage-induced demyelination associated with intense T-cell and plasma-cell infiltration into peripheral nerves.^{7–9} Because macrophages are the most prevalent leukocyte subpopulation observed in peripheral nerves in GBS and its representative animal model, experimental autoimmune neuritis (EAN),^{1,7,8,10} the involvement of pro-inflammatory mediators responsible for pathogenic leukocyte infiltration into peripheral nerves and nerve roots requires study.

Despite incomplete understanding of the pathogenesis of GBS, there is a consensus that aberrant cellular and humoral immune responses restricted to peripheral nerves and roots contribute to its clinical manifestations. AMAN, AMSAN, and MFS are associated with circulating autoantibodies against specific complex gangliosides and glycolipids (e.g., GM1, GD1b, and GQ1b), whereas AIDP is related primarily to lymphocytic infiltration and macrophage-mediated demyelination.^{3,4,11} Although both IVIg and plasmapheresis theoretically modulate the pathogenic humoral component of this disorder, it is unclear how effective they are against hematogenous mononuclear leukocyte infiltration into peripheral nerve and nerve root endoneurium, the pathological hallmark of AIDP.

Animal models provide tools to better understand the immunopathogenesis of GBS and to evaluate therapies with translational potential. EAN can be induced through immunization of animals (mice, rats, guinea pigs, rabbits, monkeys) with purified whole peripheral nerve myelin or its protein components, myelin protein 2 (P2) or myelin protein zero (P0), emulsified in complete Freund adjuvant.^{12–14} In general, mouse models have contributed significantly to our understanding of human neuroimmunology due to similarities in neural immune responses. A recently characterized mouse model, called severe murine EAN (sm-EAN) is a reliably robust AIDP animal model due to its high induction rates and better correlation of electrophysiological and pathological characteristics with disease severity,¹⁵ as compared with other murine EAN models. In sm-EAN, 8–12-week-old female SJL/J mice are immunized with bovine peripheral nerve myelin (BPNM) emulsified in complete Freund adjuvant, using pertussis toxin and recombinant mouse interleukin-12 (IL-12) as

coadjuvants to induce a severe monophasic demyelinating polyneuritis with associated axonal loss.^{1,15}

Due to the separation of the endoneurium of peripheral nerves and nerve roots from the systemic immune compartment by the blood–nerve barrier (BNB), pathogenic studies have emerged that are dedicated to potential mechanisms of BNB disruption and leukocyte extravasation. Chemokines, leukocyte integrins, cell-adhesion molecules, and matrix metalloproteinases are families of pro-inflammatory molecules involved in the sequential cascade of leukocyte trafficking to sites of inflammation. Chemokines are of particular interest due to their ability to attract selectively leukocyte subsets across chemotactic and haptotactic gradients *in vitro* and *in vivo*.^{6,16–19}

Chemokines are low-molecular-weight (8–14 kDa) cytokines involved in chemotaxis (i.e., “chemotactic cytokines”) across concentration gradients *in vitro* and haptotaxis to sites of inflammation *in vivo*.¹⁹ They are also involved in embryogenesis, normal neuronal–glial interactions, neuronal protection from toxins, and synaptic transmission, reflecting their importance in development and homeostasis in addition to inflammation.⁶ Chemokines are classified into 4 subfamilies based on the organization of 2 positionally conserved cysteine residues near the N-terminus: the CC (β), CXC (α), CX3C (δ), C (γ) subfamilies,^{1,20} all of which signal via G-protein-coupled receptors (GPCRs). Based on this classification system, approximately 50 human and mouse chemokines and 18 chemokine receptors have been identified to date.⁶ Some chemokines bind to a single receptor (e.g., CCL2 to CCR2), whereas some chemokines bind to several receptors (e.g., CCL5 to CCR1, CCR3, CCR5), or several chemokines bind to a single receptor (e.g., CXCL9, CXCL10, and CXCL11 to CXCR3).^{1,6} Despite a large number of publications on chemokines and their receptors in human disease over the last 20 years, relatively little has been published on the association between these molecules and leukocyte infiltration in GBS. Because hematogenous leukocyte infiltration and macrophage-induced demyelination are pathological hallmarks of AIDP, targeting specific chemokine mediators early in the disease process may limit the initial peripheral nerve injury and facilitate recovery. We review published data on chemokines and their receptors in GBS and EAN and discuss the implications of these data on GBS pathogenesis. We highlight opportunities for further studies using specific chemokine receptor antagonists with translational potential to demyelinating GBS variants such as AIDP.

METHODS

Description of Analytic Process

A comprehensive search of publications was performed using the National Center for Biotechnology Information, United States National Library for Medicine PubMed database from 1966 to 2012, using the search terms “chemokines and Guillain–Barré syndrome” or “chemokines and experimental autoimmune neuritis.” Only studies published in English were reviewed. Articles were included if they were observational studies (cohort or case–control), review articles discussing chemokines, or functional (mechanistic) studies looking at chemokines in GBS or EAN. Additional publications were identified by cross-referencing bibliographies. A total of 18 publications were reviewed, including 6 reviews, 4 human

observational, 6 EAN observational (with a study including some mechanistic data), 1 with both human GBS and EAN observational data, and 1 gene knockout study in murine EAN.

RESULTS: ANALYSIS OF EVIDENCE

Question 1: Are Specific Chemokine Ligands/Receptors Expressed in GBS or EAN or both Relative to Healthy/Unaffected Controls?.

Question 2: Is There Evidence that Blockade of Chemokine-Dependent Signaling Is a Plausible Therapeutic Strategy for GBS or EAN?

Evidence—Evidence has emerged that supports the hypothesis that chemokines and their receptors are essential to the pathogenesis of several autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, and inflammatory bowel disease,^{16,17,21} as well as cancer and human immunodeficiency virus (HIV) infection.^{22–26} Several groups have demonstrated that specific chemokines are expressed in both EAN and GBS compared with unaffected or non-inflammatory disease controls.

Human Observational Studies—Human blood, cerebrospinal fluid (CSF), and nerve biopsy specimens have been evaluated to determine chemokine ligand and receptor expression profiles in GBS. Studies have focused on chemokine ligands and receptors considered to be pro-inflammatory rather than homeostatic. Although the direct relationship between chemokines expressed within peripheral nerve endoneurium and the CSF is unknown, CSF chemokine levels may better reflect pathological aberrations in spinal nerve root endoneurium than blood chemokine levels.²⁷ Five human observational studies addressing chemokine expression in GBS were identified. These results are summarized in Table 1.

The first study, conducted by Sainaghi *et al.*, evaluated CSF levels of CCL2, CCL3, CCL4, CCL5, CCL7, CCL11, CCL27, CXCL1, CXCL9, CXCL10, and CXCL12 in 9 patients with GBS. They reported elevated CSF levels of CCL2, CCL7, CCL27, CXCL9, CXCL10, and CXCL12 in affected patients compared with controls.²⁸ Their study was limited by its small sample size and was primarily intended to screen for potentially pathogenic pro-inflammatory molecules for further investigation in larger studies. The second study, conducted by Press *et al.*, evaluated 39 GBS patients whose CSF chemokine levels were compared with 26 patients with non-inflammatory neurological disorders (NIND). Press and colleagues compared levels of chemokines CCL2, CCL4, CCL5, CCL19, CCL21, CXCL10, CXCL12; they demonstrated elevated CSF levels of CCL2 and CXCL10 in GBS compared with controls.²⁷ Notably, CXCL12 was not elevated in this study. The third study, based on sural nerve biopsies by Kieseier *et al.*, compared tissue expression of chemokine receptors CCR1, CCR2, CCR4, CCR5, CXCR3, and the chemokine ligand CXCL10 in 10 patients with GBS to 8 patients with NIND. They reported increased sural nerve CCR1, CCR2, CCR4, CCR5, and CXCR3 expression based on immunohistochemistry and elevated CSF levels of CXCL10 in GBS patients compared with controls.¹⁸

The above study also demonstrated that CXCL10 messenger ribonucleic acid (mRNA) colocalized to peripheral nerve vascular endothelium using *in-situ* hybridization techniques, implying that CXCL10 participated in recruiting T lymphocytes from circulating blood into

peripheral nerves. CCR1 colocalized to endoneurial macrophages. The significance of this was unclear, because CCR1 binds several chemokine ligands.⁶ CCR2 colocalized to mononuclear cells, most likely macrophages and T cells located around blood vessels and perivascular cuffs. Because CCR2 expression is increased on lymphocytes after chronic stimulation by interleukin-2, Kieseier and colleagues suggested that this may reflect chronically activated T cells trafficking into peripheral nerves.¹⁸ CCR4 binds CCL17 and CCL22, and is expressed primarily by T-helper 2 T cells.⁶ However, given that inflammatory demyelination in the nervous system is commonly associated with a T-helper 1 T-cell phenotype, CCR4 expression may reflect anti-inflammatory or regulatory T-cell migration at later stages of GBS.¹⁸ CCR5 colocalized to endoneurial mononuclear cells, most likely actively demyelinating macrophages.¹⁸

Other human observational studies have implicated CCL2 and CCR2 in GBS pathogenesis. Orlikowski *et al.* compared the serum levels of CCL2 in 38 GBS patients with 17 healthy controls. They reported elevated levels of circulating CCL2 in GBS patients, which increased at the time of progression, peaked at time of plateau (maximal severity), and normalized with recovery. The highest CCL2 concentrations were observed in the most disabled patients. Notably, no decrease in circulating CCL2 levels was observed after IVIg treatment or plasmapheresis,²⁹ supporting data that implies IVIg does not exert an effect on circulating leukocyte chemokine receptor expression in GBS.³⁰ The relationship between circulating blood and endoneurial CCL2 levels is unclear. It is provocative to speculate that inflammatory changes at the BNB may be associated with increased nerve root chemokine secretion into the CSF, based on the Press *et al.* observation that CSF CXCL10 and CCL2 levels correlated with increased CSF:plasma albumin ratios in GBS.²⁷ It is also plausible that CCL2 secreted within inflamed peripheral nerves is directly released into the bloodstream via efflux mechanisms at the BNB. Nonetheless, their study supports the notion that CCL2 may be associated with GBS pathogenesis, providing a rationale for further mechanistic studies.

The fifth study, conducted by Kastenbauer *et al.*, compared CSF and serum CX3CL1 (also known as fractalkine) levels of 12 GBS patients versus 14 patients with NIND.³¹ CX3CL1 is a membrane-bound chemokine, expressed mainly by neurons and endothelial cells. It has chemoattractant activity for T cells and monocytes and may facilitate adhesion and activation of leukocytes on the microvascular endothelium.²⁰ They reported increased CSF, but not serum levels of CX3CL1 in GBS patients compared with controls. Despite the limitations of selective chemokine assays, based on these 5 articles, the most consistent evidence supports a role for CCL2/CCR2 and CXCL10/CXCR3 in the immunopathogenesis of AIDP. Further studies, with particular emphasis on endoneurial chemokine ligand/receptor expression using broad mRNA or protein arrays, are needed in untreated patients at early stages of the disease to determine the spectrum of chemokine ligands and receptors expressed in active GBS that may have pathogenic significance.

Animal EAN Models—Seven observational studies were identified that evaluated chemokine ligand and receptor expression in EAN. Data from these studies are summarized in Table 2. Abbas *et al.* demonstrated suppression of the clinical signs of Lewis rat EAN by rolipram, a phosphodiesterase type 4 inhibitor associated with dose-dependent

downregulation of CCL2, CCL3, and CXCL2, along with interferon- γ and tissue necrosis factor (TNF)- α .³² The second study, by Zou *et al.*, compared CCL2, CCL3, and CXCL2 sciatic nerve expression in 20 Lewis rats with EAN to 20 unaffected controls. They independently corroborated the importance of CCL2, CCL3, and CXCL2 in this EAN model, reporting elevated levels in affected rats. In particular, maximum CCL2 expression occurred on day 7 post-immunization (PI) (preceding maximum disease severity), maximum CCL3 expression on day 14 PI (concurrent with maximum disease severity), and maximum CXCL2 expression on day 21 PI (during recovery).³³

The third study, by Fujioka *et al.*, examined CCL2, CCL3, CCL5, and CXCL10 mRNA expression in the cauda equina of 28 EAN-affected Lewis rats. This study did not utilize controls but compared expression profiles at different stages during EAN to levels prior to disease induction. They found elevated levels of CCL2, CCL3, CCL5, and CXCL10 during EAN compared with baseline, with maximum CXCL10 and CCL3 expression occurring on day 13 PI (preceding maximum disease severity), and maximum CCL2 and CCL5 expression occurring on day 17 PI (concurrent with maximum disease severity). Only CCL2 levels returned to normal levels during recovery, with the other chemokines remaining elevated.³⁴ The fourth study, performed by Kieseier *et al.* on 20 EAN-affected Lewis rats, examined the sequential expression of CCL2, CCL3, CCL4, CCL5, and CXCL10 in EAN-affected sciatic nerves. They reported an increase in CCL3 and CCL4 mRNA expression preceding maximum disease severity, with increased mRNA expression of CCL2, CCL5, and CXCL10 at maximum clinical disease. During recovery, CCL3 mRNA levels were found to return to normal, whereas CCL4 mRNA levels remained slightly elevated.³⁵

The findings of these studies support a regulated expression of specific pro-inflammatory chemokines during the clinical course of Lewis rat EAN. The differences in chemokine ligand expression at different stages in these EAN models may reflect differences in gender, age, source, and immunization regimen among these cohorts of Lewis rats, as shown in Table 2, or inherent variability in the EAN disease process. Nonetheless, these studies imply a temporal relationship between specific chemokine expression and disease stages in Lewis rat EAN, suggesting direct roles in hematogenous leukocyte trafficking into peripheral nerves during its disease course.³⁵

An independent study further supported a pathogenic role for CCL2 in EAN. Orlikowski *et al.* examined sciatic nerve CCL2 and CCR2 mRNA expression levels in 20 EAN-affected Lewis rats and 10 unaffected controls. They reported that the number of CCR2-positive mononuclear cells infiltrating the sciatic nerve endoneurium paralleled the EAN time course. The highest number of CCR2-positive cells was associated with peak clinical weakness,²⁹ supporting data from Fujioka *et al.* and Kieseier *et al.* Changes in CCL2 concentration may also regulate the type of immune cell infiltration into peripheral nerves during the time course of EAN, favoring monocytes and pro-inflammatory T-helper 1 cells when the concentration is high (during disease progression and peak severity), and anti-inflammatory T-helper 2 T cells when the concentration is low (recovery phase).³⁶

In addition, a Lewis rat EAN study by Luongo *et al.* compared CX3CL1/CX3CR1 sciatic nerve levels in 6–10 affected and unaffected control rats; they demonstrated increased

relative expression of CX3CL1/CX3CR1 in EAN. They also found that allodynia in EAN was associated with microglial activation and increased expression of CX3CL1/CX3CR1 in the dorsal horn of the spinal cord,³⁷ suggesting a role for CX3CL1/CX3CR1 signaling in the pathogenesis of chronic pain in GBS. These results were congruent with human observational studies, which reported increased CSF CX3CL1 levels in GBS patients compared with controls.³¹ However, the study by Duan *et al.* on 19 EAN-affected Lewis rats failed to demonstrate increased CX3CL1 expression in their cohort.²⁰ These conflicting findings suggest some ambiguity in the role of CX3CL1 in EAN, providing an avenue for additional studies.

Guided by data from the Lewis rat EAN model, a study by Xia *et al.* compared CCL2/CCR2, CXCL10/CXCR3, and CCL5/CCR1, CCR5 expression in the sciatic nerves of 10 sm-EAN-affected mice versus 10 unaffected controls by quantitative real-time polymerase chain reaction. We reported significantly increased levels of chemokines CCL2, CCL5, and CXCL10 and receptors CCR1, CCR2, CCR5, and CXCR3 in affected mice compared with controls at expected peak clinical severity.¹ Using immunohistochemical techniques, we demonstrated that CCL2 primarily colocalized to Schwann cells, CCL5 to axons, and CXCL10 to endoneurial endothelial cells. CXCR3 was expressed by infiltrating T cells, whereas CCR2, CCR1, and CCR5 were expressed by endoneurial macrophages and a subset of infiltrating T cells.¹ These data suggest that CXCL10/CXCR3 may be required for T-cell migration across the BNB and CCL2/CCR2 may be required for macrophage-dependent demyelination or Schwann-cell injury, whereas CCL5 interacting with either CCR1 or CCR5 may be required for axonal degeneration in this EAN model that closely resembles untreated AIDP pathologically.

Evidence from rodent EAN studies most consistently suggests pathogenic roles for CCL2, CCL3, CCL5, and CXCL10 in acute immune-mediated peripheral nerve inflammation. Taking into account inter- and intraspecies differences, as well as variability in EAN induction methods, these studies support a clear association between pathogenic chemokine expression and disease course, implying direct roles during inflammatory mononuclear leukocyte infiltration, demyelination, and axonal degeneration in EAN and possibly GBS.^{1,35} The putative role of chemokines and their receptors in the pathogenesis of GBS based on human observational and EAN studies is depicted in Figure 1.

Functional Studies—Compared with observational studies, functional studies of chemokine-mediated signaling in peripheral nerve inflammation are very limited, despite the availability of gene knockouts, small molecular antagonists, and function-neutralizing antibodies for research use. A single gene knockout study was identified that evaluated the effect of CCR5 disruption in a mild mouse EAN model.²⁰ Another study looked at the effect of chemokine ligand antibody blockade on the immunopathogenesis of Lewis rat EAN.³³ Data from these studies are summarized in Table 3.

Duan *et al.* compared the clinical response to EAN induction and the profile of cauda equina-infiltrating cells in 9 CCR5 knockout and 10 CCR5 wild-type C57BL/6 mice. They found that CCR5 knockout did not protect mice from P0 peptide 180–199-induced EAN, based on clinical course or severity of EAN, levels of spleen mononuclear cell response, or

profile of infiltrating macrophages and T cells into the cauda equina.²⁰ They attributed these findings to a compensatory increase in CCL4 and CXCL10 in knockout mice compared with wild-type controls.²⁰ It is also possible that other chemokine-dependent signaling pathways (e.g., CCL2/CCR2, CXCL10/CXCR3) were necessary for EAN induction and progression. Another potential explanation is redundancy in CCR5-mediated signaling pathways *in vivo*, as CCL5 can also signal through CCR1 and CCR3. Therefore, the Duan *et al.* study does not completely exclude a role for CCL5 in EAN pathogenesis.^{1,6}

Zou *et al.* investigated the effect of neutralizing antibodies against CCL2, CCL3, and CXCL2 on the clinical course of Lewis rat EAN, guided by their observational studies. An anti-CCL3 antibody was found to suppress the clinical signs of EAN from onset to recovery, associated with significantly reduced macrophage and T-cell infiltration into the sciatic nerve and reduced sciatic nerve demyelination and inflammation. An anti-CCL2 antibody treatment delayed onset of EAN by about 4 days and inhibited clinical signs of EAN (although less markedly than the anti-CCL3 antibody), but there was no significant effect on sciatic nerve demyelination or inflammation compared with controls. An anti-CXCL2 antibody failed to delay disease onset or suppress clinical signs of EAN or sciatic nerve demyelination or inflammation. Only the anti-CCL3 antibody resulted in a decreased number of CCL3-positive cells; no decrease in CCL2 or CXCL2 was observed in EAN rats treated with anti-CCL2 or anti-CXCL2 antibody, respectively.³³ The efficacy of neutralizing antibodies against chemokines would depend on the site of chemokine secretion and its binding efficacy; these are important factors to be considered for effective modulation of ligand/receptor signaling. A therapeutic challenge would arise if the pathogenic chemokine is secreted within the endoneurium, as the antibody would need appreciable BNB permeability and endoneurial retention to be efficacious.

Conclusions—Compared with over 12,000 publications on chemokines in human disease and over 700 publications on chemokines in multiple sclerosis, the chemokine biology of GBS is understudied. Human observational studies have most consistently reported increased levels of CCL2 and CXCL10 in patients with demyelinating forms of GBS compared with healthy controls or patients with NIND. Rodent EAN models, for the most part, demonstrate increased expression of these chemokine ligands and their receptors that are temporally associated with disease course and are maximal at or around peak disease severity. *In vitro* functional studies of chemokine ligand/receptor signaling in GBS do not exist, and the few EAN studies are inconclusive due to model limitations and problems with experimental design.

Recommendations—Although specific chemokine ligand/receptor pairs have been implicated strongly in EAN and GBS pathogenesis based on observational studies, more work is required to confirm chemokine ligand/receptor expression in the peripheral nerves of affected patients. Collaborative efforts are needed to perform expression profile studies in the nerves of large cohorts of well-characterized, similarly affected patients during the GBS disease course prior to immunomodulatory treatment. Functional studies using *in vitro* models of GBS leukocyte–BNB trafficking,³⁸ as well as germ-line or conditional chemokine ligand/receptor gene knockouts, are needed to decipher a potential role for these molecules

in EAN. Preclinical studies should be performed to look at the effect of small molecular antagonists and monoclonal antibodies against specific chemokine receptors during the effector phase after disease onset in reliable EAN models, mimicking early-stage clinical trials in GBS. These approaches should improve our understanding of the immunopathogenesis of demyelinating forms of GBS and the contributory role of pathogenic leukocyte infiltration; they can foster the development of targeted molecular therapies for peripheral nerve inflammation.

Question 3: What Is Required to Translate Chemokine Receptor Antagonism toward Efficacious Molecular Therapies for GBS?

Evidence—Inhibition of pathogenic leukocyte trafficking is a plausible target for treating nervous system inflammation, as evidenced by the efficacy of natalizumab (humanized anti- α 4-integrin antibody) in relapsing–remitting multiple sclerosis.^{39–41} Our recently published *in vitro* study using peripheral blood mononuclear leukocytes from untreated AIDP patients supports a role for anti- α _M-integrin blockade in modulating pathogenic leukocyte trafficking at the BNB under flow conditions.⁴² Inhibition of TNF- α -dependent pro-inflammatory signaling pathways with monoclonal antibodies has proven efficacious in rheumatoid arthritis.^{43,44}

Over 50% of known drugs currently target GPCRs, and antagonists against chemokine receptors CXCR4 (plerixafor) and CCR5 (maraviroc) have been approved for clinical use in hematopoietic stem-cell mobilization for non-Hodgkin lymphoma and multiple myeloma, and to treat adults with CCR5-tropic HIV infection, respectively.^{45–47} Several chemokine receptor antagonists are currently being studied at various clinical trial phases as potential new treatments for tissue-specific inflammatory disorders such as inflammatory bowel disease.⁶

Unfortunately, despite advances in molecular biology and drug development over the last 25 years, no significant pharmacological advances have been made in treating GBS. Ongoing work suggests an important role for CCR2 in sm-EAN induction and disease progression using gene knockouts (E.E. Ubogu, unpublished observations, 2012). Interestingly, a proprietary CXCR3 antagonist (AMG 487; Amgen) induced rapid recovery in sm-EAN when it was administered days after disease onset in a small cohort of affected SJL/J mice [E.E. Ubogu, unpublished observations, 2012 (Figs. 2 and 3)]. These promising ongoing studies suggest that chemokine receptor blockade may be more efficacious than IVIg in murine models, providing impetus for further studies that could translate toward new therapies for GBS.

To translate observational and mechanistic studies into plausible treatments for GBS, the unique anatomy and physiology of peripheral nerves and potential sites for therapeutic modulation (i.e., systemic, intrathecal, intraneural) need to be considered. Myelinated axons within the peripheral nerve and nerve root endoneurium are the principal targets for infiltrating leukocytes in AIDP. Chemokines may colocalize to BNB endothelial cells, as demonstrated *in vitro* and *in vivo* after pro-inflammatory cytokine exposure,^{1,42} or with Schwann cells, axons, or infiltrating leukocytes within the endoneurium. Efficacious

strategies could deplete activated leukocytes from the blood circulation, inhibit leukocyte–BNB trafficking, or prevent leukocyte migration to the axon within the endoneurium.

Based on currently available therapeutic strategies, potential chemokine targets include: inhibition of chemokine ligand synthesis and expression (e.g., small interfering RNA, small hairpin RNA sequences); competitive chemokine receptor inhibition with soluble chemokines, modified non-signaling chemokines (e.g., N-terminal modification), or inhibitors (e.g., monoclonal antibodies); depletion of chemokine receptor–specific effector cells (e.g., CXCR3⁺ T cells, CCR2⁺ monocytes); and competitive inhibition of chemokine-dependent second messenger signaling pathways.⁶ Strategies that modulate the interaction between chemokine receptor–expressing activated mononuclear leukocytes and expressed chemokines at the BNB should be considered, as these drugs could be administered systemically, without structural modification required to increase BNB permeability or enhance retention within the inflamed endoneurium.

Several issues inherent to chemokine biology must also be taken into account when designing chemokine receptor inhibitors. Chemokines may be upregulated non-specifically after tissue injury, so inappropriate blockade may lead to adverse systemic effects. Redundancy in chemokine receptor/ligand signaling, as well as the large surface of interaction between the chemokine receptor and ligand, provides a challenge. The possibility also exists for cross-reaction between chemokine ligand/receptor antagonists designed to block specific GPCRs with other members of the same family, or with unrelated GPCRs.⁶ It is also unknown whether specific chemokine ligand and receptors participate in peripheral nerve recovery after immune-mediated demyelination. The dose–effect relationship between specific cytokines and chemokines in GBS needs to be considered, as chemokine-dependent signaling has a characteristic inverted “U-shaped” concentration–effect curve. The width of the drug’s therapeutic window for targeted inhibition of leukocyte trafficking is also an important consideration, as suggested by our *in vitro* integrin inhibition studies.⁴² For example, anti-TNF therapy for rheumatoid arthritis has been shown to worsen multiple sclerosis^{48,49} and has been associated with induction of demyelinating disease of the CNS, including optic neuritis.^{50,51} These considerations emphasize the need to perform studies in model systems that closely resemble essential features of GBS prior to clinical trials.^{6,52}

CONCLUSIONS

GBS, the most common cause of acute paralysis, is a major health-care burden worldwide. Outcomes are suboptimal despite the currently available therapies. AIDP, its most common variant, is characterized by hematogenous leukocyte infiltration into peripheral nerves and nerve roots. Targeted blockade of pathogenic leukocyte infiltration by modulating specific or combined chemokine receptor–mediated signaling pathways may be a novel approach to treating GBS, particularly AIDP. Chemokine receptor inhibitors have emerged as a new class of drugs in cancer and HIV treatment. Several clinical trials are ongoing in inflammatory disorders. Human GBS and rodent EAN observational studies suggest that CCR2, CXCR3, and CCR1 or CCR5 are potential therapeutic targets that require further preclinical testing.

Mechanistic studies using *in vitro* leukocyte trafficking-BNB assays and *in vivo* EAN studies are needed to stimulate the translational process of chemokine receptor antagonists and other specific anti-inflammatory drugs in GBS. Mouse models provide the opportunity to ascertain the effect of chemokine receptor disruption or blockade on clinical severity, electrophysiological and histopathological parameters, and the systemic immune response. These models also provide an avenue to deduce any toxic effects of these drugs *in vivo*. These studies would supplement human *in vitro* studies of the role of specific chemokine ligand–receptor pairs on pathological leukocyte trafficking that may prove relevant to GBS immunopathogenesis and the efficacious treatment of demyelinating forms of GBS. Collaborative efforts between scientists in academia and industry are needed to test proprietary chemokine receptor antagonists using human leukocyte trafficking-BNB assays and murine EAN models. These measures should generate essential *in vitro* mechanistic and *in vivo* proof-of-principle data required for early-stage clinical trials in GBS patients.

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Abbreviations

AIDP	autoimmune inflammatory demyelinating polyradiculoneuropathy
AMAN	acute motor axonal neuropathy
AMSAN	acute motor–sensory axonal neuropathy
BNB	blood–nerve barrier
BPNM	bovine peripheral nerve myelin
CSF	cerebrospinal fluid
dCMAP	distal compound motor action potential
GBS	Guillain–Barré syndrome
GPCRs	G-protein-coupled receptors
HIV	human immunodeficiency virus
IL-12	interleukin-12
IVIg	intravenous immunoglobulin
MFS	Miller–Fisher syndrome
mRNA	messenger ribonucleic acid
NIND	non-inflammatory neurological disorders
NMSS	neuromuscular severity scale
P0	myelin protein zero

P2	myelin protein 2
PI	post-immunization
sm-EAN	severe murine experimental autoimmune neuritis
TNF	tissue necrosis factor

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FIGURE 1.

Chemokine ligand and receptor expression and possible roles in GBS. Based on human observational studies and data obtained from EAN models, it is hypothesized that CCR2⁺ monocytes cross the BNB via interaction with Schwann-cell–secreted CCL2 expressed by the BNB endothelium. These monocytes differentiate into endoneurial macrophages that migrate to CCL2-expressing Schwann cells to induce demyelination. CCR1/CCR5⁺ macrophages may migrate to axons expressing CCL5 to induce axonal degeneration. CXCR3⁺ T cells may migrate across the BNB via interactions with BNB endothelium CXCL10 and directly contribute to nerve injury via cytokine secretion or expression of pro-inflammatory chemokines that further attract hematogenous monocytes and lymphocytes, enhancing the inflammatory process. The relationship between chemokines expressed within the endoneurium of peripheral nerves and nerve roots and chemokines expressed in CSF is undetermined.

FIGURE 2.

Effect of CXCR3 antagonism on the behavioral manifestations of sm-EAN. The severity of EAN was assessed in age-matched female SJL/J mice using the 6-point neuromuscular severity scale (NMSS) after disease induction with purified BPNM. Maximum disease severity is expected between days 26–32 PI in this model. After disease onset, equally affected mice were treated with 5 mg/kg AMG 487 (CXCR3 antagonist) in 20% cyclodextrin, 400 mg/kg human intravenous immunoglobulin (hIVIg), or vehicle (20% cyclodextrin) via intraperitoneal injection once daily for 5 days from day 13 PI (black arrow). AMG 487 dramatically reversed disease progression to near baseline levels after 2 days, which persisted for the next 15 days. NMSS scale: 0—no weakness; 1—tail weakness; 2—mild-to-moderate fore- or hind-limb weakness; 3—severe fore- or hind-limb weakness; 4—mild-to-moderate tetraparesis; 5—severe tetraparesis. $N = 3$ per experimental group. Error bars indicate standard errors of the mean.

FIGURE 3.

Effect of CXCR3 antagonism on sm-EAN motor nerve electrophysiology. Bilateral sciatic and dorsal caudal tail nerve electrophysiological studies were performed on anesthetized mice 30 days after EAN induction. Mice were treated with AMG 487 (CXCR3 antagonist), vehicle (negative control, 20% cyclodextran), and human intravenous immunoglobulin (hIVIg; positive control) on days 13–17. Distal compound motor action potential (dCMAP) amplitudes, conduction velocities, and total dCMAP waveform duration (d-Duration) were recorded. No statistically significant differences were observed in mean dCMAP amplitudes after AMG 487 treatment with either nerve (**A**, **B**), whereas mean conduction velocities were higher in both nerves, relative to vehicle and hIVIg (**C**, **D**). Total mean d-Durations were lower after AMG 487 treatment in both nerves (**E**, **F**). * $P < 0.05$ relative to vehicle; # $P < 0.05$ relative to hIVIg; N.S., not significant relative to vehicle (**A–D**, **F**) or hIVIg (**E**). $N = 6$ nerves per experimental group. Error bars indicate standard errors of the mean.

Table 1

Study design characteristics and data summary of human observational studies on chemokine expression in GBS.

Investigators (publication year)	Study type	Numbers studied	Demographics	Chemokine ligand/receptor examined	Chemokine ligand/receptor overexpressed
Sainaghi <i>et al.</i> ²⁸ (2010)	Human; case—control; CSF	9 GBS, 8 CIDP; 8 DP, 7 controls	Age/gender not reported	CCL2, CCL3, CCL4, CCL5, CCL7, CCL11, CCL27, CXCL1, CXCL9, CXCL10, CXCL12	CCL2, CCL7, CCL27, CXCL9, CXCL10, CXCL12
Press <i>et al.</i> ²⁷ (2003)	Human; case—control; CSF	39 GBS, 23 CIDP, 26 NIND	Mean age 56 years, 95% male	CCL2, CCL4, CCL5, CCL19, CCL21, CXCL10, CXCL12	CCL2, CXCL10
Kieseier <i>et al.</i> ¹⁰ (2002)	Human; case—control; sural nerve biopsy	10 GBS, 10 CIDP, 8 NIND	Mean age 48 years (range 19–69 years), 70% male	CCR1, CCR2, CCR4, CCR5, CXCR3, CXCL10	CCR1, CCR2, CCR4, CCR5, CXCR3, CXCL10
Orlikowski <i>et al.</i> ²⁹ (2002)	Human; case—control; serum	38 GBS, 17 healthy	Age/gender not reported	CCL2, CCR2	CCL2, CCR2
Kastenbauer ³¹ (2003)	Human; case—control; CSF; serum	12 GBS, 10 Bell palsy, 15 MS, 14 viral meningitis, 14 bacterial meningitis, 8 bacterial meningitis (follow-up), 14 NIND	Mean age 60 years (range 34–84), 36% male	CX3CL1	CX3CL1

GBS, Guillain-Barré syndrome; CSF, cerebrospinal fluid; CIDP, chronic inflammatory demyelinating polyradiculoneuropathy; DP, diabetic polyneuropathy; NIND, non-inflammatory neurological disorder; MS, multiple sclerosis.

Table 2

Study design characteristics and data summary on chemokine expression in EAN.

Investigators (publication year)	Study type	Numbers studied	Model feature	Chemokine ligand/receptor examined	Chemokine ligand/receptor overexpressed
Xia <i>et al.</i> ¹ (2010)	EAN; sciatic nerve	10 sm-EAN, 10 controls, 4 IL-12 controls	8–12 weeks old, female mice	CCR1, CCR2, CCR5, CXCR3, CCL2, CCL5, CXCL10	CCL2/CCR2, CXCL10/CXCR3, CCL5/CCR1, CCR5
Orlikowski <i>et al.</i> ²⁹ (2003)	EAN; sciatic nerve	20 EAN, 10 controls	5–6 weeks old, female rats	CCL2, CCR2	CCL2, CCR2
Kieseier <i>et al.</i> ³⁵ (2000)	EAN; sciatic nerve	20 EAN	7–8 weeks old, female rats	CCL2, CCL3, CCL4, CCL5, CXCL10	CCL2, CCL3, CCL4, CCL5, CXCL10
Luongo <i>et al.</i> ³⁷ (2008)	EAN; dorsal horns	6–10 EAN, 6–10 controls	Female rats, age not reported	CX3CL1/CX3CR1	CX3CL1/CX3CR1
Abbas <i>et al.</i> ³² (2000)	EAN; sciatic nerve	2–4 EAN	6–8 weeks old, male rats	CCL2, CCL3, CXCL2	Not applicable
Zou <i>et al.</i> ³³ (1999)	EAN; sciatic nerve	20 EAN, 20 control	5–6 weeks old, male rats	CCL2, CCL3, CXCL2	CCL2, CCL3, CXCL2
Fujioka <i>et al.</i> ³⁴ (1999)	EAN; cauda equina	28 EAN	7 weeks old, female rats	CCL2, CCL3, CCL5, CXCL10	CCL2, CCL3, CCL5, CXCL10

Table 3

Study design characteristics and data summary of chemokine ligand/receptor functional studies in EAN.

Investigators (publication year)	Study type	Interventional cohorts	Chemokine ligand/receptor examined (outcomes)
Duan <i>et al.</i> ²⁰ (2004)	CCR5 gene knockout	9 CCR5 ^{-/-} ; 10 CCR5 ^{+/+} C57BL/6 mice	CCR5 (no effect)
Zou <i>et al.</i> ³³ (1999)	Neutralizing antibody	10 anti-CCL2 antibody, 10 anti-CCL3 antibody, 10 anti-CXCL2 antibody, 10 control Lewis rats	Anti-CCL3 (effect), anti-CCL2 (partial effect), anti-CXCL2 (no effect)