Structural homology of myelin basic protein and muscarinic acetylcholine receptor: Significance in the pathogenesis of complex regional pain syndrome

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Abstract
Complex regional pain syndrome is an extremely painful condition that develops after trauma to a limb. Complex regional pain syndrome exhibits autoimmune features in part mediated by autoantibodies against muscarinic-2 acetylcholine (M2) receptor. The mechanisms underlying the M2 receptor involvement in complex regional pain syndrome remain obscure. Based on our recent work demonstrating that limb nerve trauma releases a potent proalgesic, immunodominant myelin basic protein fragment, our present sequence database analyses reveal an unexpected and previously undescribed structural homology of the proalgesic myelin basic protein fragment with the M2 receptor. As both complex regional pain syndrome and the proalgesic myelin basic protein activity are prevalent in females, this myelin basic protein/M2 homology presents an inviting hypothesis explaining the mechanisms of autoimmune pathogenesis and sexual dimorphism that underlies vulnerability toward developing complex regional pain syndrome and other pain states with neuropathic features. This hypothesis may aid in the development of novel diagnostic and therapeutic strategies to chronic pain.

Keywords
Acetylcholine muscarinic receptor, autoimmunity, complex regional pain syndrome, myelin basic protein, pain

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Complex regional pain syndrome (CRPS) is an exceedingly painful and refractory condition that develops after trauma to a limb.¹-³ Patients with CRPS present with homotopic sensory, autonomic, motor, skin and bone changes (e.g., limbs hot or cold, swollen or thin, red or blue, with scaling or clammy skin, and localized osteoporosis) and, most importantly, pain. The classic expression of this syndrome was described first by Weir Mitchell in 1864⁴ in battlefield patients suffering soft tissue injury and fractures. In patients so afflicted, the characteristic pain phenotype was a burning dysesthesia and the intolerability of low-threshold mechanical stimulation, such as even a slight air movement on their skin (tactile allodynia). While mechanisms underlying this syndrome have been at best controversial, recent work has provided novel insights into the unifying role of adaptive immunity. Here, we describe an unexpected structural homology between an immunogenic peptide formed due to nerve injury and a structurally homologous epitope present on nociceptive afferents.

CRPS as an autoantibody-mediated syndrome
While CRPS has been long thought to arise as a result of an inflammatory process, more current work has it to be deemed an autoimmune condition.¹-³,⁵-¹²

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Several observations lend strong support to this assertion. (i) The passive transfer of serum immunoglobulin G (IgG) antibodies from a CRPS patient, but not a normal patient, elicits CRPS-like pathology in mice.5,6 (ii) Intravenous infusion of immunoglobulin reduced pain in patients with long-standing CRPS.1–3,7 (iii) In a murine tibial fracture and cast immobilization model of CRPS, B-cell depletion in mice treated with CD20-neutralizing antibody or genetically lacking the ability to produce IgM (μMT mice) attenuated pain, which resumed after injection of serum IgM autoantibodies from wild-type to μMT-fracture mice.9,10 Against this assertion serves the evidence from the multicenter randomized blinded placebo-controlled trial that 0.5 g/kg intravenous immunoglobulin immunotherapy produced no benefit in CRPS compared with placebo-treated patients.8

Nerve injury after limb trauma has been considered as a contributing factor of CRPS,1–3 and limb fracture has been suggested to produce neoantigens in the skin, nerve, and cord, which trigger B cells to secrete IgM antibodies that bind those antigens and initiate a nociceptive antibody response.11,12 One such target which may contribute to the pathogenesis of CRPS is myelin basic protein (MBP).

**MBP is a pivotal autoantigen in nerve injury pain states**

MBP, a major component of the myelin sheath, is an intrinsically disordered, cationic protein, interacting with polyanionic cellular partners.13–15 The centrally located 68-100 region of MBP (MBP68-100: residues are numbered according to the GenBank AAH08749 human MBP sequence, Table 1) includes a functionally important and strictly conserved α-helix. In the course of several autoimmune demyelinating conditions, including multiple sclerosis and Guillain-Barré syndrome, this major immunodominant epitope region is liberated by proteases of the cathepsin and metalloprotease families13–15 and functions as a highly immunogenic autoantigen. In rodents, we find the same peptides within the MBP68-100 region are released in response to physical peripheral nerve trauma.16–18

Injection of peptides encoding the MBP68-100 region into an intact sciatic nerve is sufficient to produce a T-cell-dependent tactile allodynia lasting for several weeks; whereas, the control and scrambled peptides are inert.17–19 The MBP68-100 peptides are released in the nerve preceding morphological signs of demyelination, at day 1 after sciatic nerve chronic constriction injury (CCI),16 suggesting release through a localized and precise proteolytic event. The IgM autoantibodies contribute to pain in a mouse model of CRPS9,10 and serum IgM autoantibodies against the algesic MBP epitopes persist in CCI allodynia.20 These data led us to suggest that MBP68-100 mediates tactile allodynia in the absence of overt neuropathological findings and contributes to autoimmune pathogenesis of neuropathic pain phenotypes mediated by myelinated A-afferent fibers.17,21

**Table 1.** Sequence alignment of the proalgesic, immunodominant 68-100 region of MBP and the intracellular 291-320 region of muscarinic acetylcholine M2 receptor.

| Myelin basic protein | Mouse | 68-AHYGSLPQKSHGR-TQDENPV-VHFFKNIVTPRT-P-100 |
|----------------------|-------|---------------------------------------------|
| Human                |       | THYGSLPQKSHGR-TQDENPV-VHFFKNIVTPRTP        |
| Muscarinic 2 receptor| Human | 291-AVASNRDDEI-TQDENVTSTLGHSDKDENS-320      |
| Mouse               |       | AVASNMRDDEI-TQDENVTSTLGHSDKDDNS            |

MBP: myelin basic protein.

The cholinergic muscarinic-2 (M2) receptor is a G protein-coupled receptor encoded by the CHRM2 gene in humans. The M2 receptor has been found to be expressed in small primary nociceptive afferents, satellite cells and dorsal root ganglia (DRGs).22,23 The M2 receptor elicits an inhibitory role on primary nociceptive afferents, and its levels are upregulated after peripheral nerve axotomy.24 Serum autoantibodies directed against M2 receptor have been detected in a subset of CRPS patients.11

In our searches in the sequence databases, we unexpectedly observed a sequence homology between the algesic MBP fragments and the intracellular 291-320 region of M2 receptor (Table 1). Here, we suggest that the cryptic MBP68-100 epitopes comprising the TQDENPV sequence are released from the intact MBP protein as a result of minor nerve/myelin injury associated with limb trauma (Figure 1). These anti-MBP autoantibodies would cross-react with the intracellular (TQDENTV) residues of M2 receptor. The resulting autoantibody binding would interfere in the physiological balance of acetylcholine and the M2 receptor and its inhibitory action on primary afferents.
This endogenous process of molecular mimicry would contribute to cross-activation of autoreactive T and/or B cells. In addition, the nerve injury-released MBP68-100 residues may interfere with the M2 receptor binding to interactors and downstream signaling cascades in an autoantibody-independent manner. This MBP68-100-mediated interference with the inhibitory action of the M2 receptor would contribute to sustained sensitization of afferents and the development of persistent pain states (Figure 1). As the MBP68-100 residues are conserved in human and rodents (Table 1), the proposed mechanism likely relates to clinical pain and preclinical pain-like behaviors.

Our model does not exclude a possibility of the anti-M2 receptor autoantibody production and interaction with the M2 receptor on the afferent. Whether and how anti-M2 receptor or anti-MBP autoantibodies contribute to pain remain obscure. Insofar, neural-targeted autoantibodies are thought to stimulate nociceptive signaling by fixing complement, interfering with the physiological activity of an antigen or via the immunoglobulin Fc-receptor activation on afferents.\textsuperscript{25–31} Despite the systemic circulation of the autoantibodies, CRPS presentation is highly localized.

CRPS is defined by a region-confined course or injury-triggered, regionally restricted autoimmune-mediated autoimmune disorder with minimally destructive course.\textsuperscript{2} Similarly, in the CCI mononeuropathy model, unilateral tactile allodynia despite the systemic circulation of the autoantibody against the algesic MBP\textsuperscript{20} may imply a focal afferent (e.g., nodal/paranodal\textsuperscript{32} or DRG soma) site of the autoantibody action in pain. It remains possible that a serum autoantibody represents a byproduct rather than a pathogenic factor in nociceptive circuits.

Spontaneous onset CRPS, without evidence for a precipitating noxious event, occurs in 3\% to 11\% of cases. CRPS may also be triggered by a noxious event remote from the affected limb, such as spine surgery or stroke.\textsuperscript{33,34} Clinical presentation of CRPS is similar, irrespective of the presence or the source of a noxious event.\textsuperscript{34} Thus, localized proteolytic release of MBP68-100 observed in the absence of overt pathology,\textsuperscript{17} and its robust proalgesic action in an intact nerve,\textsuperscript{17–19} imply the potential role of MBP68-100 in both spontaneous and remote onset CRPS.

Sexual dimorphism of painful and autoimmune disorders

Like most known autoimmune disorders,\textsuperscript{35,36} CRPS is disproportionally more prevalent in females (ratio to males is 4:1). Autoantibodies against muscarinic receptor and MBP have been linked to painful, autoimmune, female-prevalent disorders, Sjögren’s syndrome and multiple sclerosis, respectively. Importantly, recent findings of our group suggest that autoantibody production against the proalgesic MBP peptides is prevalent in female, relative to male, rodents post-CCI.\textsuperscript{37} Seminal findings demonstrate female-selective involvement of

\textbf{Figure 1.} MBP/\textit{acetylcholine receptor homology model of pain (a hypothesis diagram).} In peripheral nerve, the muscarinic \textit{acetylcholine} M2 receptor signaling is inhibitory on primary nociceptive afferents. Following nerve injury, degradation of myelin basic protein (MBP) causes the release of the cryptic epitope comprising TQDENPV sequence (1) and generation of the reactive immunoglobulin (e.g., IgM) autoantibody (2). The antibody cross-reacts with the intracellular TQDENTV sequence of the M2 receptor (3). In addition, the MBP68-100/TQDENPV directly interferes with the intracellular M2 receptor/TQDENTV interactors and downstream signaling cascades. The resulting interference with the inhibitory M2 receptor signaling (4) leads to pain facilitation (5). MBP: myelin basic protein.
the adaptive T-cell-dependent tactile allodynia mechanisms in mice with peripheral nerve injury. While this latter work did not implicate specific antigens, MBP is a likely candidate. The T-cell-dependent proalgesic MBP action, prevalent in females, likely contributes to the sexual dimorphism phenomena of neuropathic pain.

In conclusion, high sequence homology between muscarinic-2 receptor and the algesic MBP region released after nerve trauma may contribute to the pathogenesis of pain associated with CRPS and potentially other painful conditions. Accordingly, we suggest that therapeutic approaches directed at the pro-nociceptive MBP sequence, such as the altered mutant ligand, may prove useful as non-narcotic analgesics in patients with CRPS.

Declaration of Conflicting Interests
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