The Critical Role of the Toll Like Receptor 2 / Myeloid Differentiation Primary Response Gene (88) Pathway in Danger Signaling and failure of Myelin Regeneration

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Abstract

Background: Biologic processes, such as myelination, are regulated by a balance of “on” and “off” signals. Regeneration of myelin in multiple sclerosis appears to be primarily blocked by the presence of “off,” or inhibitory, signals. During the process of inflammatory demyelination in MS, molecules are released that are termed “danger signals.” These danger signals alert cells that the local environment is hostile and not suitable for growth. One of the most abundant danger signals in the CNS is low molecular weight hyaluronan (LMWHA). LMWHA is generated following tissue injury by the action of hyaluronidases on native hyaluronan. Hyaluronan is the most abundant glycosaminoglycan in the CNS and its expression is significantly increased in Multiple Sclerosis lesions. LMWHA is a selective agonist for TLR2 and TLR4. In multiple sclerosis, TLR2 expression is significantly upregulated on oligodendrocytes and oligodendrocyte progenitor cells.

Objectives: In this study, our goal is to determine the importance of the TLR2/MyD88 pathway in causing demyelination and axonal injury, and in inhibiting remyelination, through in vivo and in vitro modeling.

Methods: All animals are on a C57/B6 background. The TLR2-/-, MyD88-/- mice have now been backcrossed onto the C57/B6 background >32 generations. MyD88-/- male mice and wild-type strain matched control mice at the age of eight wks were fed ad libitum a rodent diet (SF79, LabDiet) containing 0.2% (w/v) capricose (Sigma-Aldrich) for 6 wks to induce demyelination. Mice were anesthetized and sacrificed, and brains frozen in cold 2- methyl butane on dry ice. Purified oligodendrocyte cultures for the first 144 culture, cells were grown in DMEM with 10% FBS, and DMEM with 0.05% BSA and N2 for the subsequent 4d. Purified OPCs were cultured in DMEM with 0.025% BSA and N2. All oligodendrocyte cultures were assayed for microglial and astrocyte contamination by cell-type specific immunocytochemistry. To induce differentiation, we cultured oligodendrocytes in DMEM supplemented with 10ng/ml CNT, 15ng/ml T3, N2, and 0.025% BSA.

Results: We have previously shown that hyaluronan inhibits oligodendrocyte maturation and prevents myelin regeneration through activation of TLR2 on oligodendrocyte progenitor cells. In this study, we now show that TLR2 loss-of-function promotes remyelination in the cuprizone model of chronic demyelination. Moreover, we also report that other danger signals present in MS lesions converge on the TLR2/MyD88 pathway to prevent normal remyelination.

Conclusions: Targeting the TLR2/MyD88 pathway is proposed for enhancing remyelination in MS.

Conclusions: In this study we provide preliminary data supporting the hypothesis that inhibition of the TLR2/MyD88/RACK1 pathway is a novel target for limiting demyelination and promoting remyelination in MS. We found that inhibition of oligodendrocyte maturation in vitro by hyaluronan is reversed using a neutralizing monoclonal antibody to TLR2. We showed that well-defined pathogen derived TLR2 agonists also function to inhibit oligodendrocyte maturation. In mixed CNS cultures where oligodendrocyte maturation is normally limited, TLR2 or MyD88 null mutations accelerate oligodendrocyte maturation. Similarly, MyD88 or RACK1/4 pharmacologic antagonists also induce oligodendrocyte maturation. In initial experiments designed to study the impact of MyD88 on demyelination and remyelination in vivo, we have made use of two well-accepted animal models, cuprizone and lyssolecithin. In the cuprizone model of demyelination, loss of functional TLR2 or MyD88 resulted in a significant reduction of demyelination after 7 weeks of treatment. Following lyssolecithin mediated demyelination, loss of functional TLR2 reverses the inhibitory effect of hyaluronan on remyelination. Taken together, our data supports the hypothesis that the TLR2/MyD88/RACK1 pathway functions to promote demyelination through inflammatory pathways, and functions to limit remyelination by acting directly as an inhibitor of oligodendrocyte maturation.