COMMENTARY

Anacardic acid, interleukin-33, and the quest for remyelination

Murali Ramanathan[a,b,1]

In PNAS, Ljunggren-Rose et al. (1) present results from a study assessing the effect of anacardic acid, a salicylic acid derivative with a 15- or 17-carbon-containing alkyl chain substituent (i.e., 2-hydroxy 6-alkylbenzoic acid), on remyelination. Anacardic acids are phytochemicals that are found in foods such as cashews and mangoes.

Myelin is the lipid-rich lamellar sheath that provides the electrical insulation around axons of nerve cells. Intact myelin is critical for axonal function because it enables efficient saltatory propagation of the action potential. In the central nervous system (CNS), myelin is formed and actively maintained by oligodendrocytes. Remyelination is the repair and formation of new myelin sheaths in areas where damage has occurred.

Remyelination in Multiple Sclerosis

Multiple sclerosis (MS), a chronic and disabling CNS disease characterized by blood–brain barrier breakdown, inflammation and lesion formation, demyelination, axonal injury, and neurodegeneration, is the most common disorder of myelin. Although several disease-modifying therapies, including oral medications, have become available for treating MS, all of the currently approved drugs are immunomodulatory agents that do not directly address the axonal injury and neurodegeneration that commences early in the course of the disease and contributes to disability and disease progression. There is an unmet need for neuroprotective and neuroreparative therapies for progressive MS.

Myelin loss in MS can occur as a consequence of inflammatory attack on the myelin sheath, oligodendrocyte loss, and loss of trophic support from glia. When demyelination occurs, axons become vulnerable to neurodegeneration. Remyelination of MS lesions is variable but occurs as a result of net myelin production by oligodendrocyte precursor cells (OPC) that undergo proliferation and maturation with some contributions from the oligodendrocytes that have retained functional capacity to produce myelin. When lesions remyelinate, they can be seen as "shadow plaques" in magnetic resonance imaging, but the resultant degree of myelination is generally to levels that are lower compared to normal-appearing white matter.

Remyelination in the CNS is particularly challenging because myelin formation in the CNS is regulated by inhibitory pathways, and OPC differentiation is suppressed by degraded myelin in the pathological milieu. Microglia and macrophage clear myelin debris by phagocytosis and can thereby create an environment favorable for remyelination. Epigenetic changes are also crucial because the orchestrated expression of many distinctive gene products and myelin components is required for repairing the myelin sheath. Overcoming these mechanistic barriers to foster successful remyelination is widely viewed as the critical imperative for developing the next generation of MS therapies.

High-throughput screens have identified a number of molecules spanning a surprisingly diverse range of drug classes that are capable of promoting myelination. Approved drugs with histamine receptor blocker, anticholinergic, dopamine receptor antagonist, and androgen activities have been shown to increase myelin gene expression (2–6). Among the endogenous cytokines and hormones, brain-derived neurotrophic factor, leukemia inhibitory factor, epidermal growth factor, insulin-like growth factor-1, and thyroid hormone have myelinating activity. Opicinumab, a monoclonal antibody that inhibits remyelination-inhibiting leucine rich repeat and immunoglobin-like domain-containing protein 1 (Lingo-1) signaling, and high-dose biotin have been evaluated in randomized clinical trials. Despite the diversity of promising candidate molecules, clinically meaningful success at remyelination has proven elusive in MS so far.

Anacardic Acid, IL-33 and Remyelination

Ljunggren-Rose et al. (1) evaluate anacardic acid in cultured rat OPC in vitro and in experimental allergic encephalomyelitis (EAE) and cuprizone-induced

[a]Department of Pharmaceutical Sciences, The State University of New York at Buffalo, Buffalo, NY 14214; and [b]Department of Neurology, The State University of New York at Buffalo, Buffalo, NY 14214

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[1]Email: murali@buffalo.edu.

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demyelination mouse models that mimic the inflammatory and demyelinating aspects, respectively, of MS pathophysiology. Anacardic acid treatment promotes OPC differentiation and increases expression of myelin basic protein in the in vitro model. In the EAE model, anacardic acid treatment results in a reduction of the EAE clinical score, which assesses the severity of paralysis, compared to the vehicle control treatment. In the cuprizone model for demyelination, treatment with anacardic acid for 3 wk results in greater myelin protein expression, and the axons have more myelination as assessed by the g-ratio, a measure of axon myelin thickness obtained from electron microscopic images.

Where does the Ljunggren-Rose et al. (1) study with anacardic acid fit in mechanistically, and what are its implications for remyelination therapy for MS? Notably, these results build a trail from anacardic acid to remyelination that involves a promising player: interleukin-33 (IL-33). IL-33 is a cytokine, and it has proven difficult to turn cytokines into useful drugs. However, because IL-33 is inducible, drug discovery efforts might be directed at finding suitable small-molecule inducers of IL-33 for remyelination. Clearly, this therapeutic approach will warrant further study. This group has previously shown that treatment with poly-IC, a toll-like receptor 3 agonist, increased IL-33 expression in areas of experimental demyelination and promoted remyelination (7). IL-33 has been found to be effective in reducing injury in both inflammatory and in traumatic models of CNS injury (7–11).

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IL-33 is an interesting member of the IL-1 superfamily that is expressed in the periphery and the CNS. In the periphery, it is produced by many cell types, including macrophages, dendritic cells, and endothelial cells, among others. IL-33 is viewed as an "alarmin" whose release occurs upon tissue injury and facilitates the recruitment of immune response (8). The IL-33 receptor ST2 is expressed on Th2 cells and on group 2 innate lymphoid cells that produce Th2 cytokines. It is therefore unsurprising that IL-33 is effective at driving Th2 responses. IL-33 exposure promotes the M2 phenotype polarization of macrophages, which increases their greater phagocytic capacity. High levels of IL-33 are found in white matter, but it is expressed throughout the CNS by oligodendrocytes and gray matter astrocytes (8). In IL-33 knockout mice, recruitment of phagocytic myeloid cells to injured CNS areas is diminished, and recovery is impaired (8). In the pathophysiological milieu of the MS brain, increased macrophage phagocytosis activity stimulated by IL-33 could enhance clearance of myelin debris that inhibits OPC differentiation (12, 13). The effectiveness of IL-33 at driving Th2 responses may inhibit pathophysiological inflammatory processes in MS. Overall, the expression patterns and biological effects of IL-33 give it strong bona fides for mediating interactions between endothelial, immune, and oligodendrocytes and promoting remyelination in MS. Ljunggren-Rose et al. (1) investigate IL-33 in the corpus callosum of cuprizone-treated mice and the demyelinated areas of EAE mice using histochemical methods and find increased IL-33 expression in the anacardic acid-treated group compared to vehicle-treated controls. The IL-33 expression colocalizes with oligodendrocytes markers rather than astrocyte markers. A potential weakness is that this study does not measure IL-33 in the systemic circulation of animals treated with anacardic acid.

As a pharmacological agent, anacardic acid has a surprisingly broad range of effects and is therefore more likely to behave like a shotgun rather than as a targeted silver bullet. Anacardic acid with 15-carbon-long unsaturated side chains are bacteriocidal to Gram-positive bacteria such as antibiotic-resistant *Staphylococcus aureus* and *Streptococcus mutans* but are not active against Gram-negative bacteria (14). Anacardic acids also inhibit the p300/CREB-binding protein family of nuclear histone acetyl transferase enzymes that modify histones and can modulate patterns of gene expression. Anacardic acid exposure is cytotoxic to several human cancer-derived cell lines. The histone acetyl transferase and cytotoxic activities of anacardic acid are relatively independent of the saturation of the chain (14, 15). Anacardic acid has antioxidant activity because it inhibits enzymes such as xanthine oxidase that generate reactive oxygen species and chelates transition metal ions like Fe^{2+} and Cu^{2+}. Anacardic acid has antiinflammatory effects and blocks the nuclear factor kappa B pathway activation (16). Ljunggren-Rose et al. (1) use anacardic acid with a saturated side chain in their report.

Given the promising results, there is now rationale for conducting structure–activity studies with different chemical variants of anacardic acid to identify more-potent compounds. For example, it will be important to compare the neuroprotective potential of anacardic acid with unsaturated alkyl side chains and saturated side chains given that anacardic acid with unsaturated side chain is present at higher levels in natural products. Moreover, anacardic acid has an allergic propensity that needs to be addressed during the course of drug development. It is also not clear what other side effects and unexpected "off-target" effects might occur when anacardic acid is used as a drug long term.

Nuts are considered a heart-healthy food, and the American Heart Association recommends consumption of about five servings of unsalted nuts per week (17). The results with anacardic acid are promising, but it is premature to advocate increased consumption of cashews, mangoes, and anacardic acid-containing products for the clinical goal of promoting remyelination or managing disease progression in MS patients. It is not quite time to go nuts about cashews for treating MS just yet!

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