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That Wasn't a Complement—Too Much C3 in Demyelinating Disease

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Abstract

Multiple sclerosis is a chronic inflammatory disease characterized by demyelination in the central nervous system. In this issue of *Immunity*, Werneberg et al. report a striking loss of synapses driven by excessive microglial pruning early in demyelinating disease, which can be rescued by inhibiting the complement component C3.

Multiple sclerosis (MS) is a chronic inflammatory disease in which peripheral immune cells attack central nervous system (CNS) myelin, the fatty sheath that enwraps axons and facilitates rapid neuronal signaling. The resulting demyelination is accompanied by a host of debilitating neurological symptoms, including loss of visual acuity, limb weakness, cognitive impair, and ataxia (Dutta et al., 2011; Reich et al., 2018). In addition to a loss of myelin and oligodendrocytes (the myelin-producing cells of the CNS), postmortem tissue from MS patients also exhibit signs of pathology shared by other neurodegenerative diseases, such as decreased synapse density and the presence of inflammatory microglia (Dutta et al., 2011; Jüurgens et al., 2016).

In this issue of *Immunity*, Werneburg et al. (2020) investigated the link between synapse loss and microglial reactivity in demyelinating disease. Consistent with previous studies, they found a significant reduction in synapse density in the visual thalamus of MS patients, as well as in a marmoset model and two mouse models of demyelination. They also observed increased deposition of the complement component C3 on presynaptic puncta and increased localization of these synaptic puncta within microglial lysosomes, indicating that excessive microglial pruning may be driving the decrease in synapse density. To functionally test the relationship between C3 upregulation and synapse loss, the authors developed a targeted approach to interfere with C3 activity by virally overexpressing the murine protein Crry, a membrane bound inhibitor of C3, in retinal neurons projecting to the thalamus. Crry overexpression decreased microglial engulfment of presynaptic terminals and attenuated synapse loss in a mouse model of demyelination (Figure 1). Furthermore, mice that received Crry showed significantly less visual impairment following demyelination than mice that received a control virus. These results strongly suggest that the observed synapse loss is

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driven by microglia and that preventing microglial pruning through C3 inhibition may improve functional outcomes in demyelinating disease.

The authors' use of multiple demyelination models in primates and rodents, combined with validation in postmortem MS tissue, provides robust evidence that synapse loss is occurring in demyelinating disease. Given the multifaceted nature of MS symptomology, it is very possible that synaptic dysfunction underlies at least a subset of these symptoms. Intriguingly, the C3 inhibitor Crry exerted a protective effect on synapses without noticeably altering the extent of demyelination, neurodegeneration, or peripheral immune cell infiltration, thus highlighting a dissociation between synapse loss and myelin loss. Indeed, in the mouse model of experimental autoimmune encephalitis (EAE), the decrease in synapse density observed at the onset of EAE does not appear to progress further in later stages, despite progression of demyelination and axon degeneration. Importantly, Crry was able to attenuate the loss of visual acuity at both the onset and peak of EAE without a quantifiable change in demyelination. These results imply that gene therapy or small molecules targeting the complement pathway may be combined with remyelinating therapies to achieve additive effects on clinical outcomes.

One striking observation made in this study is the early onset of synapse loss, which in the mouse models seems to precede overt demyelination and neurodegeneration. Both mouse models—EAE induced by MOG-peptide immunization and oligodendrocyte diphtheria toxin A (DTA)—induce significant inflammatory responses in the brain, as evidenced by increased peripheral immune cell infiltration and altered microglial morphology. This early inflammatory response may trigger complement deposition and microglia phagocytic behavior prior to the onset of demyelination. However, it should be noted that subtle changes in myelin are likely already present at the time when synapses are being pruned. Indeed, the EAE and DTA models, by their very design, presuppose that any inflammation should be a response to changes in myelin and/or oligodendrocytes, even if they are not detectable at the level of myelin thickness. In the DTA model, diphtheria toxin is specifically expressed in oligodendrocytes to cause primary demyelination; in the EAE model, MOG-peptide immunization targets myelin and oligodendrocytes, which are the sole cell type in the brain to express MOG. This interpretation is also supported by findings from other models of myelin dysfunction. For example, mice lacking 50% of the myelin protein CNP form normal-appearing myelin but exhibit chronic low-grade neuroinflammation that eventually triggers neurodegeneration (Janova et al., 2018). Nevertheless, the data from the present study suggest that targeting microglia-mediated synapse loss may be beneficial regardless of whether the initiation of synapse loss is directly triggered by demyelination.

The rationale for investigating complement deposition on synapses came from previous work in neurodevelopment, when synapses destined to be eliminated were tagged by complement components C1q and C3. Interestingly, unlike in development in which both C1q and C3 localize to synapses (Schafer et al., 2012; Stevens et al., 2007), only C3 appears to be consistently present on synapses in demyelinating disease. Thus, it seems that aberrant pruning in demyelination is driven by activation of the alternative, but not classical, complement cascade. An interesting future direction will be to determine how these distinct

complement cascades get activated and whether there are differential consequences for synapse pruning.

Given the potential relevance to MS and other neurodegenerative diseases involving synapse pathology, it will be important to elucidate the mechanisms by which the C3 inhibitor Crry exerts its protective effects on synapses. The concomitant reduction in C3 deposition, decrease in microglial synapse engulfment, and rescue of synapse density support the idea that Crry protects synapses by inhibiting microglial synapse pruning. It is nevertheless possible that Crry could have additional effects on neuronal or synaptic function independent of regulating microglial phagocytosis. To ascertain the role of microglia in this pathway, follow-up experiments could be done with microglial-specific C3 receptor (C3R) knockout mice in a demyelination model that does not require C3R to progress (e.g., cuprizone demyelination).

There are also outstanding questions related to the mechanism of Crry at the molecular level. Although the viral strategy employed by authors was designed to specifically target C3-tagged synapses, it seems plausible that Crry may alter the overall inflammatory response of microglia. In line with this interpretation, synapses present in the visual thalamus that did not receive Crry were also less engulfed by microglia. Another possible explanation, as discussed by authors, is that virally transduced synaptic terminals could be shedding Crry (virus or protein) and therefore conferring protection to synapses originating from non-Crry expressing neurons nearby. However, given that the endogenous Crry protein is membrane bound (Turnberg and Botto, 2003), this scenario would imply a different mode of action for the overexpressed form of Crry. An additional consideration of therapeutic relevance is that Crry does not have a genetic human homolog but combines the activities of the human proteins DAF and MCP, which inhibit C3 and C5 (Kim et al., 1995). More work is needed to identify the relevant aspects of Crry function and develop a targeted strategy to achieve the same effects in humans.

In summary, Werneburg et al. (2020) identify synapse loss as a common pathological hallmark across multiple models of demyelination and in MS patients. They further show that this synapse loss is accompanied by increased localization of synapses in microglia and develop a strategy to locally inhibit C3 deposition on synaptic terminals, which reduces the extent of microglial synapse engulfment. Critically, C3 inhibition attenuates both synapse loss and the reduction in visual acuity in mice that underwent demyelination, therefore demonstrating the functional significance of C3-related synapse loss. These results not only expand our understanding of pathology in demyelinating disease but also introduce a new way to prevent functionally relevant synapse loss that could be applicable to a wide range of neurodegenerative diseases.

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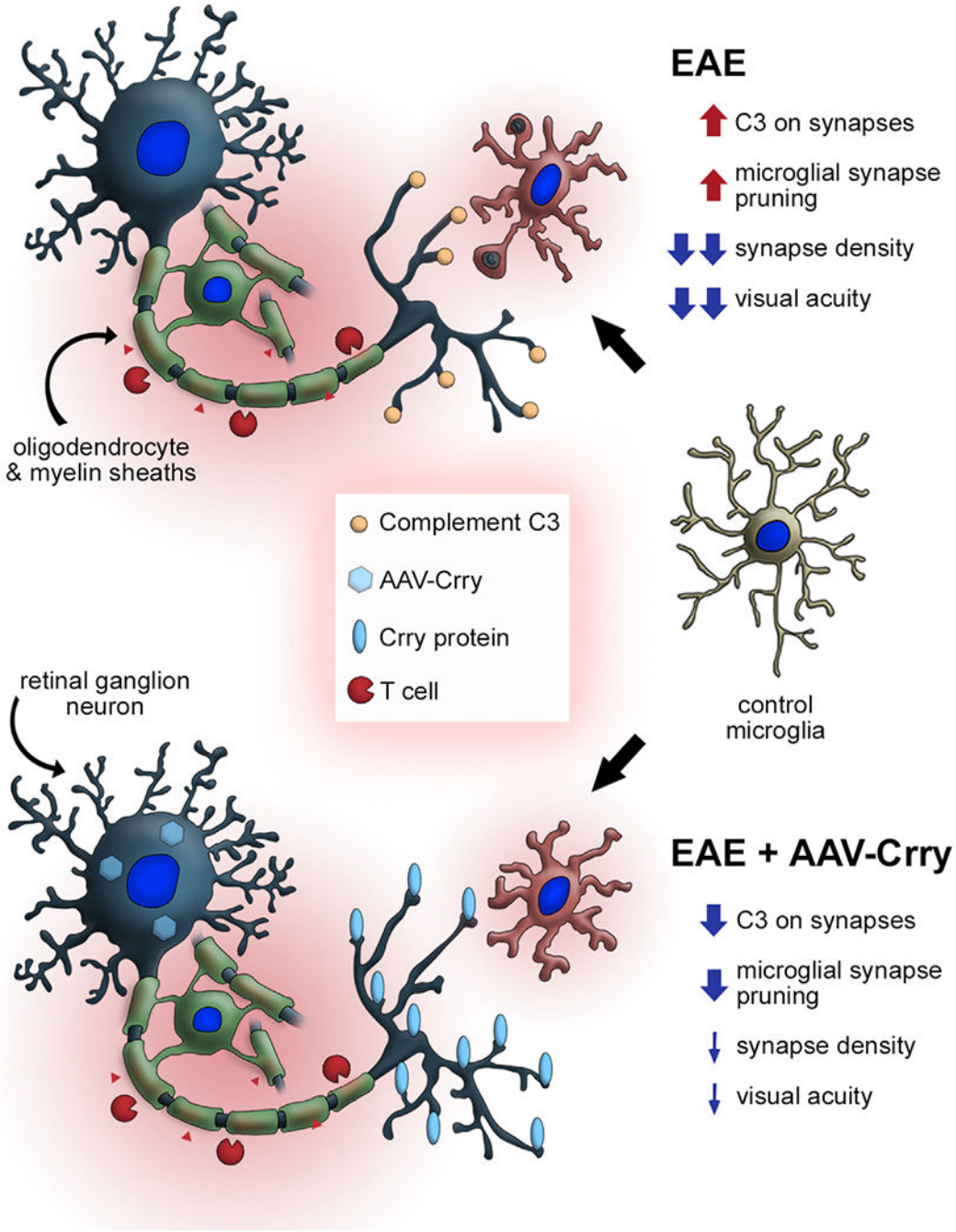

Figure 1. Viral Overexpression of the Complement C3 Inhibitor Crry Protects against Microglia-Mediated Synapse Loss in a Mouse Model of Demyelinating Disease

In experimental autoimmune encephalomyelitis (EAE), T cells attack the myelin sheath, leading to reactive gliosis and demyelination. Werneburg et al. (2020) show that in the early stages of EAE progression, the complement component C3 is highly localized to presynaptic terminals of retinal ganglion neurons in the visual thalamus. The upregulation of C3 is accompanied by increased microglial synapse pruning, decreased synapse density, and impaired visual acuity. Viral overexpression of the C3 inhibitor Crry in retinal ganglion neurons reduces C3 deposition on their synaptic terminals in the thalamus, decreases

microglial synapse pruning, and partially rescues EAE-induced loss of synapses and visual acuity. Thus, complement C3 inhibition may be a viable therapeutic strategy in demyelinating disease.