Boosting the Brain's Ability to Block Inflammation via MicroRNA-132

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The brain-immune axis continues to fascinate. In this issue of *Immunity*, Shaked et al. (2009) describe how miR-132 mediates an anti-inflammatory effect via the targeting of acetylcholinesterase, leading to an increase in the neurotransmitter acetylcholine.

MicroRNAs (miRNAs) are emerging as yet another determining set of regulators of immunity and inflammation. They have roles in B cell development, T cell polarization, and macrophage function on a par with that of cytokines (Sheedy and O'Neill, 2008). miRNAs bind to mRNA targets via sequence complementarity and either repress translation or cause degradation of the mRNA (Ambros, 2004). Overall, therefore, their role is to downregulate responses. This can have an inhibitory effect, if the target is required for the response, or a stimulatory effect, if the target is an inhibitor. Among the best characterized in innate immunity are miR-146a, which targets the signaling molecule Traf6 and thereby limits Toll-like receptor signaling (Taganov et al., 2006), and miR-155, which targets the lipid phosphatase SHIP1 (O'Connell et al., 2009), an important signal for macrophage activation. miR-155-deficient mice have a profound immune phenotype because of enhanced T helper 2 (Th2) cell responses (Rodriguez et al., 2007). The mechanism here appears to be the ability of miR-155 to additionally target the 3′UTR in the mRNA encoding c-Maf. The miR-155-deficient mouse therefore has elevated c-Maf, leading to overproduction of the cytokine IL-4 and the enhanced Th2 cellular response. In this issue of *Immunity*, Shaked et al. (2009) provide evidence that miR-132 has anti-inflammatory effects via the targeting of the mRNA encoding acetylcholinesterase (AChE) (Shaked et al., 2009). This enzyme limits the level of acetylcholine (ACh), an important inhibitor of peripheral inflammation, and one of the better-characterized mediators of the neuroimmune axis (Sternberg, 2006). By targeting the mRNA encoding AChE, an increase in ACh occurs, leading to an anti-inflammatory effect. miR-132 is therefore acting as an inhibitor of an enzyme that blocks an inhibitor of inflammation. The net result is decreased inflammation, with miR-132 acting as an important regulator of the brain-to-body resolution of the inflammatory response.

The study began with a demonstration that treatment of murine splenocytes with the TLR4 ligand LPS led to a downregulation of AChE. A decrease in serum AChE was also observed upon injection of LPS intraperitoneally. The authors explored whether miRNAs might be involved in the downregulation of AChE and found that two miRNAs known to be induced by LPS—miR-132 and the miR-182 subtype miR-182*—had complimentary sites in the 3′UTR of the mRNA for AChE. Mice were then injected with an antisense oligonucleotide that targets miR-132. This treatment led to an increase in AChE protein expression in vivo, indicating that miR-132 is a major systemic regulator of AChE. The opposite approach of overexpressing miR-132 downregulated AChE activity, confirming that miR-132 can regulate AChE expression.

The authors then tested a transgenic mouse that had an AChE gene lacking the miR-132 target sequence. Intestinal explants from the mice had elevated AChE activity and protein expression, in spite of robust amounts of miR-132. The mice also had higher basal amounts of IL-1β and IL6 in their circulation. AChE expression in the intestine from the transgenic mice reached similar amounts as those in the anti-132-treated mice. Furthermore, injection of the mice with LPS led to a hyperthermia, and macrophages from the mice overproduced the cytokines IL-6, IL-12, and TNF when challenged with LPS. The mice also displayed increased leukocyte recruitment in the peritoneum after thioglycolate injection. The excessive inflammatory response in the mice could be overcome by nicotine, which is not metabolized by AChE. This study therefore indicates that AChE is under the control of miR-132. The targeting of AChE by miR-132 leads to an elevation in the anti-inflammatory neuromodulator ACh, the net effect of miR-132 being the suppression of inflammation via inhibition of AChE production, as shown in Figure 1.

This study emphasizes the importance of ACh in the control of inflammation and of miRNA—miR-132—in promoting this anti-inflammatory effect. The mechanism here is the targeting of the ACh-metabolizing enzyme AChE via a decrease in mRNA translation mediated by miR-132, which has a net anti-inflammatory effect via the stabilizing of ACh amounts. This process is likely to be important for the brain's ability to control inflammation via cholinergic signaling. Activation of this "cholinergic" reflex inhibits inflammation, although precisely how the reflex is triggered is not known. Early in an inflammatory response, AChE expression will be high, and this in turn will limit ACh and thereby promote inflammation. From this study, we can infer that miR-132 expression will rise in response to inflammatory stimuli such as LPS. This in turn will inhibit the production of AChE, allowing ACh expression to rise and exert anti-inflammatory effects, in a classic negative-feedback loop. miR-132 expression may in fact be the key promoter of the anti-inflammatory effect of ACh.

Might this process be amenable to therapeutic manipulation? Several

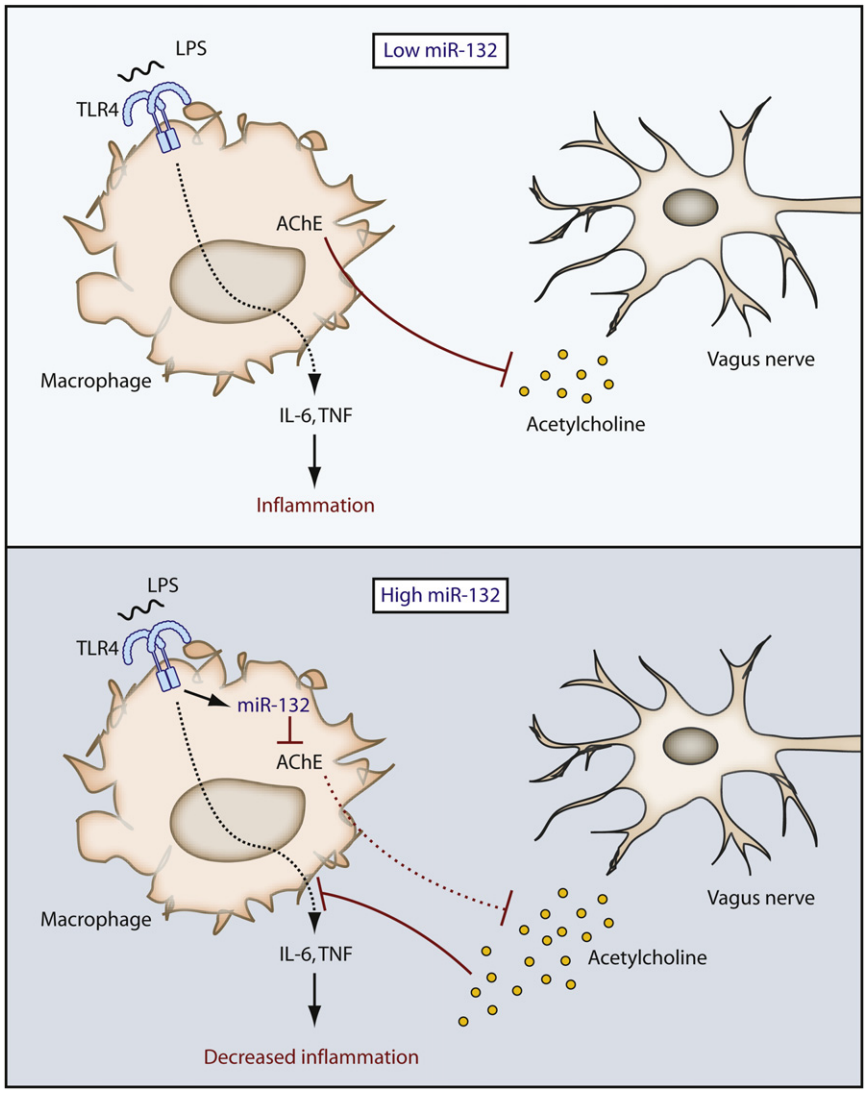

Figure 1. Targeting of Acetylcholinesterase by miR-132 Limits Inflammation
Top: Stimuli such as LPS promote inflammation via the induction of cytokines such as IL-6 and TNF via TLR signaling. Acetylcholine (ACh) from the vagus nerve can block this response. The enzyme acetylcholinesterase (AChE) controls the amount of ACh, allowing inflammation to proceed. Bottom: LPS also induces miR-132, which targets the mRNA encoding AChE, leading to decreased production of the enzyme. This in turn leads to an elevation in ACh, which has an anti-inflammatory effect.

approaches are currently being investigated that might modulate miRNA amounts or affect their targets. If the effect of miR-132 on AChE were to be blocked, a decrease in ACh would be expected, which would have a proinflammatory effect. This could be useful in vaccine adjuvancy in infectious diseases or in cancer. On the other hand, if miR-132 were to be enhanced, this would lead to an increase in ACh, which in turn would have an anti-inflammatory effect. The observation of the role of miR-132 here adds to a growing and important literature on the control of immunity and inflammation by miRNAs. Further analyses are required to determine the importance of miR-132 in human inflammatory diseases and whether this neuroimmune axis might lend itself to therapeutic targeting.

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