RESEARCH HIGHLIGHT

Muscularis macrophages: trained guardians of enteric neurons

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An intestinal helminth infection induces a tolerogenic phenotype of resident muscularis macrophages protecting the enteric nervous system against subsequent bacterial infections, an effect mediated by eosinophil-derived IL-4 and IL-13. This work further identifies muscularis macrophages as important players protecting the enteric nervous system against harmful events.

The advances made by the macrophage field in recent years have been staggering. Firstly, we have come to appreciate that macrophages adapt functionally and transcriptionally to the tissue niche within which they reside. Secondly, it is now widely accepted that, contrary to previous dogmas, macrophages are largely tissue-resident and self-maintaining within their tissues, and are thus replaced by circulating cells only slowly, or not at all. These advances have set the stage for the notion that tissue-resident macrophages may retain a memory of inflammatory attacks to their environment, in the form of phenotypic or even epigenetic rewiring. An exciting example of such inflammatory priming was recently demonstrated in the lung, where a primary infection conferred antibacterial resistance to a secondary infection via heightened IL-6 production by monocyte-derived alveolar macrophages.[1] These findings are especially relevant in the context of the current ongoing COVID-19 pandemic, where long-term transcriptional, epigenetic and functional rewiring of macrophages following viral infection may lead to hitherto unknown consequences in patients.[2]

Resident macrophages of the muscularis externa or muscularis macrophages (MMs), play a crucial role in the function and survival of enteric neurons. Indeed, depletion of a self-maintaining population of MMs closely associated with enteric neurons leads to extensive neurodegeneration and impaired gastrointestinal motility.[3] Enteric neurons themselves maintain this population via production of CSF-1, and participate in the instruction of MM phenotype following inflammatory insults. We previously demonstrated that activation of cholinergic enteric neurons via vagus nerve stimulation could signal to MMs via the alpha7 nicotinic receptor (α7nAChR), leading to reduced intestinal inflammation and improved clinical recovery following abdominal surgery.[4] Furthermore, Mucida and co-workers recently demonstrated a similar neuron–MM crosstalk in the context of intestinal infection, demonstrating that bacterial infection leads to activation of sympathetic efferents that signal to MMs via β2 adrenergic receptors, resulting in upregulation of Arginase-1 (Arg1) and neuroprotection.[5] Building upon these findings, these authors now demonstrate that parasitic infections also lead to polarization of macrophages towards a protective phenotype preventing neuronal loss, indicating that MMs represent an important target to protect the enteric nervous system (ENS) against enteric infections.[6]

In this study, the authors elegantly demonstrate that not only infection with *Y. pseudotuberculosis*, but also with *S. venezuelensis*, an intestinal helminth, results in upregulation of Arg1 in MMs for up to 12 weeks, a process which protects the ENS from neuronal loss in the event of a subsequent bacterial infection for up to 24 weeks (Fig. 1). Interestingly, the authors were able to demonstrate a common transcriptional response of MMs to several types of intestinal infection, further underscoring the tolerogenic and tissue-protective potential of these cells, which was first described by the same group in 2016.[7] While this study was limited to infection by Gram-negative bacteria and helminths, it will be interesting to investigate whether these findings can be extended to other types of enteric pathogens in future studies.

In a further set of experiments, the authors explore the mechanisms underlying sustained Arg1 expression by MMs induced by a helminth infection. In line with previous findings, infection with *S. venezuelensis* led to an increase in the frequency of Th2 cells in the intestinal lamina propria, eosinophilia and heightened systemic levels of IL-4 and IL-13 (Fig. 1). Interestingly, eosinophil-derived systemic IL-4 and IL-13 are required for the upregulation of Arg1 in MMs, as depletion of eosinophils or eosinophil-derived IL-4 and IL-13 abolished Arg1 upregulation and neuroprotection by MMs. Similarly, $CD4^+$ T cells and eosinophilia induced by IL-5 were also required for this memory axis, as loss of either was sufficient to impair Arg1 upregulation in MMs and abolish neuroprotection. Taken together, these findings illustrate an elaborate, multi-tissue axis that instructs and primes MMs for subsequent enteric infections.

The long-lasting protection induced by intestinal helminth infection observed by the authors raises the question of how eosinophils, that have a relatively short lifespan within tissues, are able to induce prolonged changes in MM phenotype. In line with recent studies, the authors report changes in the bone-marrow progenitor landscape, which may be sufficient to sustain long-term eosinophilia. The notion that a helminth infection may 'instruct' the bone marrow, skewing progenitors toward eosinophilic lineage via epigenetic modulation is interesting, yet requires further experimental exploration and validation beyond the data generated in this study. The role of the local, inflammatory microenvironment however appears to be essential. Indeed, transplantation of eosinophils from the duodenum, which is the primary site of helminth infection, but not from the ileum, of a helminth-infected donor was able to confer neuroprotection to a

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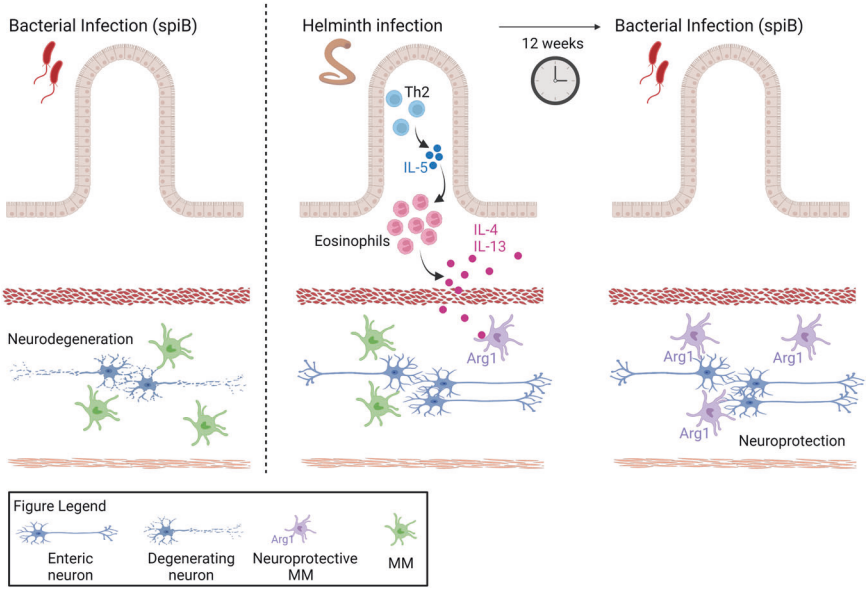

Fig. 1 Intestinal helminth infections instruct muscularis macrophages (MMs) to adopt a tolerogenic phenotype, leading to neuroprotection in a subsequent bacterial infection. Enteric infection with *Salmonella typhimurium* (spiB) leads to loss of enteric neurons. However, a prior infection with *S. venezuelensis* protects enteric neurons during a subsequent bacterial infection for up to 24 weeks via upregulation of Arg1 in MMs, which was observed for up to 12 weeks after helminth infection. Th2 cells induced by helminth infection produce IL-5, leading to eosinophilia and increase in systemic IL-4 and IL-13, which in turn upregulate Arg1 expression by MMs. Created with Biorender.com.

host. Conversely, while IL-5 overexpression alone did induce eosinophilia, it was not sufficient for Arg1 upregulation in MMs. These findings highlight the necessity of priming of eosinophils by the local environment for the establishment of the tolerogenic phenotype of distant MMs, while leaving some open questions regarding which mediators may be involved in this process.

An interesting aspect of this study is that the authors are able to relate their findings to a physiological, non-laboratory setting. When assessing the intestine of mice bought from a conventional store and not housed in a pathogen-free environment, the authors report a similar protective profile, including heightened systemic IL-4 and IL-13, eosinophilia, upregulation of Arg1 in MMs and neuroprotection in the event of a bacterial infection. These findings raise the intriguing hypothesis that free-living animals may maintain tissue tolerance and neuroprotection thanks to the constant exposure to pathogens colonizing the gastrointestinal tract, although further studies are required to truly understand the effect of repeated pathogen exposure on immune cells, including MMs.

These findings shed light on the mechanisms underlying the establishment of tissue tolerance to pathogens, and highlight the crucial role that macrophages play in safeguarding homeostasis in the event of disease. Future studies should focus on elucidating the long-term effects of intestinal infection on tissue tolerance and MM phenotype. Taken together, these findings set MMs at center stage for the protection of enteric neurons, and highlight the need for further exploration of the mechanisms involved in their instruction in order to harness the neuroprotective potential of these cells in the context of gastrointestinal disease.

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ADDITIONAL INFORMATION

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