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Muramyl dipeptide

Not just another brick in the wall

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The robust expression of microbial pattern recognition receptors such as TLR4 and Nod2 in intestinal stem cells reflects an active communication dynamic between the host and the gut microbiota. A new study reveals that muramyl dipeptide, the bacterial cell wall peptidoglycan motif, activates Nod2 within crypt base columnar Lgr5-positive stem cells and promotes their survival. Apart from the immediate relevance to the growth of organoids for in vitro experiments, the study raises new questions about the molecular mechanisms whereby gut microbes influence intestinal physiology.

"....and from their secret inner crypts
Great Vesta's fillets and her statue brought,
And the undying fire from out her shrines."
The Aeneid by Virgil.

Vesta, Roman goddess of the hearth and personification of the ceremonial flame, represented shelter, safety, and the preservation of life.[1] She was worshipped in a large chamber that housed an eternal flame, and six priestesses known as the Vestal virgins tended to her temple and the fire. A more corporeal and tangible manifestation of persistent, albeit not eternal, life are intestinal stem cells, located deep within the crypts of the inside passage. While their identity and "stemness" has been fiercely debated, the emerging consensus is that the crypt base columnar (CBC) cells that express the Lgr5 protein have the typical attributes of a stem cell.[2] (To borrow an aphorism, we may not be able to adequately define "stemness," but we can recognize it when we see it).

Pointedly, a single fluorescently-sorted CBC cell, embedded in matrigel in the presence of appropriate signaling molecules, can produce in vitro "organoids" that recapitulate intestinal structure and differentiation.[2] Lgr5 is a seven-transmembrane receptor that has close ties with the canonical Wnt signaling pathway, which is central to the formation and maintenance of stem cells. Lgr5 proteins are regulated by Wnt, respond to the secreted Wnt agonists R-spondins, and boost canonical Wnt signals in responding cells.

In a recent study, Nigro et al. explored the effect of the bacterial cell wall component muramyl dipeptide (MDP), and its (host) intracellular receptor Nod2, in the in vitro growth of mouse organoids.[3] Stimulation with MDP or peptidoglycan (Nod2 agonists), but not Tetra-dap (Nod1 agonist), resulted in a 5-fold increase in the number, but not the maximum size, of organoids compared with control untreated samples. MDP treatment promoted greater stem cell survival without affecting the rate of proliferation. Consistent with these observations, organoids derived from Nod2 knockout (KO) mice did not respond to MDP treatment, while those from Nod1 KO mice displayed increases in numbers similar to the parent WT mice. RT-PCR analyses revealed 5-fold higher levels of Nod2 mRNA in Lgr5-positive intestinal stem cells compared with the adjacent Paneth cells, and high level Nod2 expression was independently confirmed by other methods.

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Lgr5-positive stem cells isolated from wild type (WT) mice yielded 1.5-fold more organoids than those isolated from Nod2 KO animals, with only the former responding to MDP stimulation.[3] Addition of Paneth cells (either from WT or from Nod2 KO mice) to WT-stem cells resulted in a >2-fold increase in the number of organoids, compared with stem cells incubated alone, with MDP treatment resulting in an additive stimulation in both cases. This nurturing role of Paneth cells on stem cells, also reported earlier by Sato et al., is likely via provision of niche signals like Wnt and Notch ligands to stem cells.[4] Notably, since the Nod2 status of the PCs did not significantly impact stimulation, these data implicate a direct role for MDP-dependent Nod2 activation within stem cells in supporting their survival.

To explore the relevance of MDP-dependent cytoprotection of stem cells in vivo, the investigators first depleted mice of their normal flora using a cocktail of antibiotics.[3] They then assessed if MDP could protect stem cells from the effects of doxorubicin hydrochloride, a DNA-intercalating agent that damages DNA, and is toxic to intestinal stem cells. Compared with untreated control mice, crypts of MDP-treated animals displayed an enhanced capacity to proliferate within 24 h after doxorubicin treatment, and reached normal levels by 72 h post-insult. Doxorubicin treatment induced greater levels of crypt cell apoptosis in microbiota-depleted Nod2 KO mice, compared with similarly treated WT animals. Further, unlike WT animals, Nod2 KO mice had significantly lower levels of regenerative crypts at 72 h after doxorubicin treatment.

The immediate implication of these studies is that researchers will be coaxing their organoid cultures with a soupçon of MDP. More broadly, Nigro et al. propose that the delayed restitution of damaged epithelium could be an unrecognized etiological component in Crohn's disease cases associated with Nod2 mutations. The broader impact of the gut microbiota on the biology of stem cells, however, is likely more complicated. In a different study, Neal et al. demonstrated that Toll-like receptor 4 (TLR4), the receptor for bacterial lipopolysaccharide influenced stem cell survival in a mouse model.[5] Specifically, they showed high level expression of TLR4 within Lgr5-positive cells in mouse crypts, and observed increased apoptosis and reduced proliferation in LPS-treated crypt organoids from WT mice, but not in similarly treated crypts harvested from TLR4-deficient mice. Their studies suggested a role for the p53-upregulated modulator of apoptosis (PUMA), but not MyD88, in TLR4-dependent inhibition of stem cell proliferation and increased apoptosis.

Curiously, in the experimental setup used by Neal et al. to show TLR4-dependent apoptosis of stem cells, LPS was delivered by injection into the animals, and conventional animals with intact microbiota were used for these studies. In fact, studies by Santaolalla et al. demonstrated that TLR4 overexpression in enterocytes (villin-TLR4) induced proliferation in the intestine, with an expansion of Lgr5-positive cells up along the lengths of the crypt.[6] Given the broad variations in the experimental methods, such differences cannot be reconciled until further studies are performed.

The pronounced expression and activity of pattern recognition receptors such as Nod2 and TLR4 in intestinal stem cells has fundamental implications to normal host physiology, to disease states such as inflammatory bowel disease and cancer, and to recovery and restitution after insult from inflammation or infection. Given the many unanswered questions, this will be an exciting area of research in the years to come. Is the pathway of MDP/Nod2 dependent cytoprotection similar to that observed in other cell types, such as intraepithelial lymphocytes?[7] Does it involve the canonical Nod2 downstream molecules such as Rip2 and NF-κB?[8] How are microbial molecules sensed, and how are the signals integrated within the stem cells? Finally, how do factors such as Paneth cell antimicrobials, integrity of the barrier at the bottom of the crypt, sequestration of receptors such as TLR4, and the balance between Gram-positive and Gram-negative organisms, influence the local crypt microenvironment to influence stem cell function?

Entities bestowed with exalted powers pay a heavy price if they fail to carry out their function. The Vestal virgins were revered and operated with considerable autonomy and enjoyed many privileges. But extinction of the sacred fire, which presaged the destruction of Rome, implied that a Vestal was impure. Such derelict Vestals were flogged, and those that broke their vow of chastity were buried alive. Their partners in crime often faced a worse fate. In the unlikely temples of the inside passage, our microbes, and the receptors that sense them, have an equally disproportionate hold on our lives.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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