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INSIGHTS

Beyond antiviral: Interferon induced by bacteria maintains tolerance in the gut

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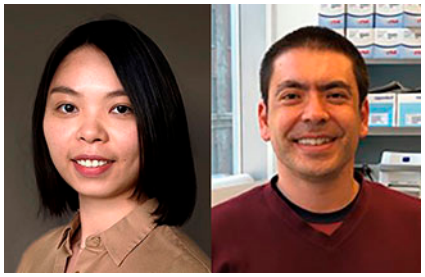
Type I interferons are best known for their antiviral role. Here, Ayala et al. (https://doi.org/10.1084/jem.20230063) reveal that commensal bacteria elicit tonic type I interferons to prime dendritic cells and induce regulatory T cells that maintain a tolerogenic intestinal milieu.

Type I interferons (IFNs) are well known for directly activating antiviral responses in infected and neighboring cells, inducing over 300 IFN-stimulated genes (ISGs) that inhibit viral replication. Comprising 14 subtypes of IFN-α, alongside IFN-β, IFN-ε, IFN-κ, IFN-ω, IFN-δ, IFN-ζ, and IFN-τ, these cytokines are rapidly produced in response to recognition of viral nucleic acid by innate immune sensors, signaling through the IFN α/β receptor complex IFNAR1 and IFNAR2. Type I IFNs are also triggered during bacterial infections, which can have context-specific outcomes (Boxx and Cheng, 2016). In addition to an inducible response to an infection, tonic type I IFN levels that promote antiviral immunity across organs are maintained by the presence of commensal bacteria that are part of the gut microbiota (Abt et al., 2012; Ichinohe et al., 2011; Steed et al., 2017).

Beyond innate immune resistance to pathogens, a growing list of complex multicellular functions are attributed to type I IFNs, including fortifying barrier tissue (Neil et al., 2019), promoting adaptive immunity through antigen presentation by dendritic cells (DCs) (Simmons et al., 2012), and suppressing unwanted pro-inflammatory T cell activity by mediating the expansion of Foxp3$^+$ regulatory T cells (Tregs) (Lee et al., 2012). Despite these insights, the regulation of tonic type I IFN signaling and how it contributes to tissue homeostasis is less characterized compared with its role in host defense.

Intestinal colonization by commensal bacteria, including *Bacteroides fragilis*, primes immunosuppressive DCs that induce IL-10–producing Tregs (Chu et al., 2016). Such regulatory networks are essential for preventing excessive immune reactions in the microbially rich environment of the gut, which underlies diseases such as inflammatory bowel disease (IBD). Given the evidence that microbiota contribute to tonic type I IFN signaling (Schaupp et al., 2020), Ayala et al. (2023) sought to determine whether IFNs mediate the maintenance of Tregs downstream of intestinal colonization by commensal bacteria such as *B. fragilis*. First, by analyzing germ-free mice that lack a gut microbiota, they confirmed that basal IFNβ and ISG production were dependent on commensal bacteria. Remarkably, IFNβ production was restored through colonization with *B. fragilis* alone.

To determine whether type I IFN induction is specific to *B. fragilis*, the authors compared the levels of IFNβ secretion in DCs cultured with seven common intestinal bacteria. Intriguingly, all three *Bacteroides* species, which are Gram-negative bacteria, induced substantial IFN signaling, while the Gram-positive species did not. Further characterization revealed that innate immune sensors NOD2 and TLR4, which are activated by microbially derived peptidoglycan muropeptides and lipopolysaccharide (LPS), respectively, are both essential



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for eliciting type I IFN in response to *B. fragilis* in DCs. This finding may explain why the Gram-positive strains, which lack LPS, failed to induce an IFN response. Notably, IFNβ production remained intact in DCs lacking TLR2, an innate immune sensor that responds to *B. fragilis*–derived polysaccharide A (Round et al., 2011), indicating that tonic type I IFN responses involve a distinct mechanism from those previously associated with this bacterium.

Ayala et al. (2023) then utilized *B. fragilis* as a model commensal bacterium to thoroughly investigate how type I IFNs mediate immune tolerance. DCs cultured in the presence of *B. fragilis* produced IL-10 and IL-27. However, the production of IL-10 and IL-27 was reduced in IFNAR1-deficient DCs that lack the type I IFN receptor subunit. IL-10 can be produced by Tregs and also act on Tregs to reinforce anti-inflammatory reactions, and IL-27 is a cytokine that can

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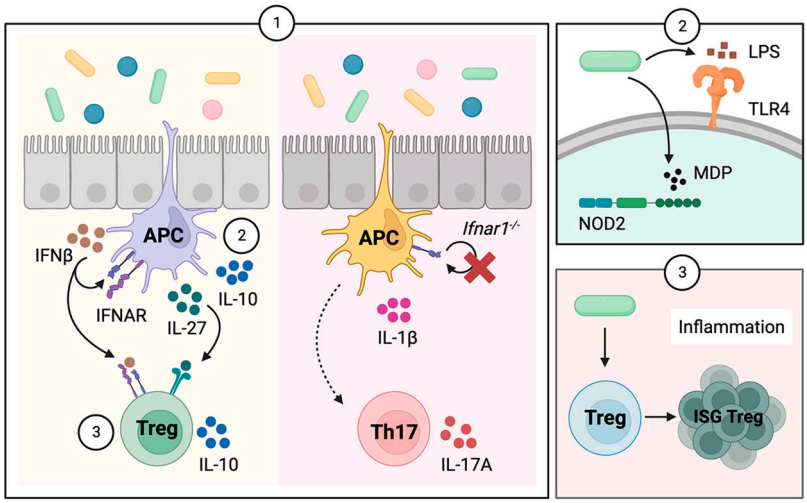

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Commensal bacteria–induced tonic type I IFN creates a tolerogenic intestinal milieu. Beyond its well-documented antiviral roles, Ayala et al. (2023) revealed that commensal bacteria, such as *B. fragilis*, elicit a subtle yet essential type I IFN response dominated by IFNβ, termed the tonic IFN response, in the intestine. Following the sampling of bacterial molecules from the intestinal lumen, APCs, including DCs and macrophages, undergo priming and initiate IFN signaling in an autocrine and paracrine manner. Primed APCs release immunomodulatory cytokines, including IL-27, targeting Tregs to induce IL-10 production and thereby sustain immune tolerance. Both IFNAR and IL-27RA signaling in Tregs are crucial for their induction of immunosuppressive activity in response to commensal bacteria. Impairment in the IFN pathway disrupts the Treg/Th17 balance, leading to APCs skewing toward pro-inflammatory cytokine production, such as IL-1β. Consequently, the expansion of IL-10–producing Tregs is diminished, while an increased population of IL-17A–producing Th17 cells emerges (1). The activation of the type I IFN response in APCs is contingent upon the sensing of bacterial components mediated by NOD2 and TLR4 (2). Commensal colonization induces an IFN gene signature in Tregs during intestinal inflammation (3). MDP, muramyl dipeptide.

target Tregs to mediate immune tolerance (Hall et al., 2012). To delineate the role of IL-27, the authors co-cultured DCs with CD4$^+$ T cells obtained from mice with or without the receptor for this cytokine, IL-27RA. The addition of *B. fragilis* to this co-culture model resulted in expansion of IL-10–producing Tregs, which was blunted when either the DCs lacked IFNAR1 or CD4$^+$ T cells lacked IL-27RA. These findings support a model in which commensal bacteria promote the production of IL-27 by DCs in a type I IFN–dependent manner, which in turn, signals via IL27RA in Tregs to orchestrate immune tolerance.

Given that most cell types bear the type I IFN receptor complex, the authors subsequently elucidated the necessity of IFNAR1 in DCs and Tregs during *B. fragilis*–induced immune tolerance in vivo in mice. In line with previous observations, the authors confirmed that Tregs in the colon are reduced in either IFNAR1-deficient mice or mice treated with antibiotics (to reduce commensal bacteria). Also, inoculating mice with *B. fragilis* boosted the proportion of Tregs that produce IL-10. This induction of IL-10–producing Tregs by *B. fragilis* was entirely abrogated in mice lacking IFNAR1 in Tregs, highlighting the critical cell type–specific role of IFN signaling in Tregs. Surprisingly, knocking out IFNAR1 from DCs did not prevent *B. fragilis* from inducing IL-10–producing Tregs in the colon. This discrepancy from the co-culture model may be due to the compensatory effects of other antigen-presenting cells (APCs) with intact IFNAR1. Interestingly, the authors found that type I IFN signaling in DCs has a major role in controlling IL-17A–producing CD4$^+$ T cells (Th17 cells) in this same setting. Mice with IFNAR1-deficient DCs displayed an increased frequency of IL-17A–producing CD4$^+$ T cells in the colon in response to *B. fragilis*, likely explained by skewing of DCs towards a pro-inflammatory fate that includes enhanced IL-1β production in the presence of bacteria. Thus, type I IFN signaling in both Tregs and DCs regulates the Treg/Th17 balance in response to commensal bacteria.

IL-10 production and Treg/Th17 balance are important determinants of susceptibility to colitis, motivating the authors to examine the molecular profile of T cells in a mouse model of chemically induced colitis. Consistent with their data showing that IFNAR1 on Tregs is essential for IL-10 production in response to bacteria, lymph nodes of mice colonized with *B. fragilis* alone exhibited a larger population of Tregs with heightened expression of ISGs compared with germ-free mice. In addition to tying their results back to their initial observations showing that *B. fragilis* is sufficient for supporting tonic type I IFN levels, this finding is notable because recent studies suggest that similar "ISG Tregs" restrain inflammation in humans (Sjaastad et al., 2022, *Preprint*).

When taken together, this study unveils a novel mechanism by which type I IFNs shape a tolerogenic intestinal environment. In contrast to the pro-inflammatory effects induced by viral infections, IFN signaling in response to commensal bacterial colonization exhibits anti-inflammatory features. Upon sensing bacterial components, IFNAR1-bearing APCs including DCs secrete tonic IFNβ and IL-27 to induce IL-10 production by Tregs (see figure). This anti-inflammatory role of type I IFNs in response to commensal bacteria raises intriguing questions about how these responses are precisely coordinated in reaction to distinct microbial signals in different contexts. A successful antimicrobial IFN response to a life-threatening viral or bacterial pathogen would need to override the immunosuppressive mechanism described in this study. The seemingly contradictory effects of type I IFN may be influenced by factors such as the timing, duration, and magnitude of IFN production or the specific type of host cells that are activated. In addition to bacterial cell wall components, as seen in this study, DNA from commensal bacteria activate the cGAS-STING nucleic acid sensing pathway to sustain tonic type I IFN levels (Erttmann et al., 2022). The combination of innate immune sensors that are activated by the microbiota, and modifications of microbial ligands that activate these sensors, may be important sources of interindividual variability, given that the composition of intestinal microbial communities is individual-specific and can include bacteria that straddle the line between commensal and opportunistic pathogen. Notably, Ayala et al. (2023) observed a higher degree of IFN response evoked by pathogens compared with commensal bacterial strains in this study.

Moreover, different subtypes of IFN may contribute to varied immune outcomes.

Although all type I IFN cytokines signal through IFNAR1 and IFNAR2 complex, IFNβ has been shown to activate an additional non-canonical signaling pathway through the IFNAR1 subunit independent of IFNAR2 (de Weerd et al., 2013). Interestingly, *B. fragilis* preferentially induced IFNβ production in DCs rather than IFNα. In addition to elucidating how different magnitudes of IFN production divergently impact downstream effects, it would be worth examining the significance of this preferential production of IFNβ.

Finally, this study shows that one of the innate immune sensors required for IFN-induced Tregs is NOD2. Loss-of-function variants of *NOD2* are associated with IBD susceptibility and can contribute to colitis by impairing sensing of anti-inflammatory bacterial products (Jang et al., 2023). Is it possible that tonic type I IFN levels depend on the combination of our genetic makeup and gut microbiota composition? IBD and other complex immune-mediated disorders have long been considered the result of a confluence of genetic and environmental factors. Controlling homeostatic type IFN responses, and perhaps ISG Tregs, may be an attractive strategy for suppressing unwanted inflammatory reactions that underlie a range of disease conditions.

Acknowledgments

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