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## Spotlight

# ILC3s: Rhythmic Keepers of Gut Integrity at Mealtime

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**Cyclically, during the day, increased permeability of the intestinal epithelial barrier, allowing nutrient uptake, must be compensated for, to achieve increased protection against potentially harmful components. Seillet *et al.* demonstrate that, upon food intake, enteric neuron-derived VIP promotes anticipatory mucosal immunity by inducing ILC3s to produce protective IL-22.**

The intestinal mucosa comes into contact with food cyclically during the day. Therefore, a precise regulatory mechanism allowing nutrient uptake while maintaining barrier integrity against potentially deleterious compounds must exist. Recently, emerging data support the concept that coordinating these two important functions depends on the interaction between the nervous and immune systems. Indeed, the intestine harbors a dense neuronal network (also referred to as a 'second brain'), and is known to contain the largest lymphoid cell compartment in the body. In particular, innate lymphoid cells (ILCs) have a crucial role as sentinels of mucosal tissue homeostasis due to their ability to respond to neuropeptides, alarmins, hormones, and metabolic signals, by rapidly secreting cytokines [1]. Among ILCs, ILC3s represent the most abundant subset in both the human and murine gut and are a major source of IL-22, a cytokine that acts mainly on epithelial cells, ensuring tissue homeostasis and repair [2].

In a recent study, Seillet and colleagues [3] investigated how circadian light–dark cycles and feeding rhythms might regulate ILC3 responses in murine intestinal mucosae. They showed that IL-22 production by ILC3s oscillates during the day, with a higher peak during dark hours and a lower peak during light hours. This rhythmicity appears unique to ILCs localized in the gut, because it was not detectable in mesenteric lymph nodes or lung. Accordingly, RNA-sequencing analysis of ILC3s in the intestine revealed an upregulated expression of genes involved in the regulation of circadian rhythm. Circadian rhythms rely on local and systemic cues to coordinate mammalian physiology, and are genetically encoded by molecular clocks that allow organisms to anticipate, and adapt to, extrinsic environmental changes [4]. By using a mouse model of genetic ablation of the master circadian activator Arntl in IL-7 receptor-expressing cells ($Arnt^{\Delta IL7R}$), Seillet and coworkers demonstrated that intrinsic clock genes could regulate ILC3s [3]. Their data are in agreement with a previous report [5], in which mice with *Arntl* ablation in Rorγt-expressing cells ($Arnt^{\Delta Rorgt}$) also displayed a selective reduction of ILC3 numbers and cytokine production in their intestine relative to controls. In this report, the authors demonstrated, by surgical- and genetically induced ablation of brain rhythmicity, that extrinsic light signals, integrated in the suprachiasmatic nuclei (SCN) of the hypothalamus, controlled intrinsic circadian rhythmicity in ILC3s. Notably, circadian genes, such as *Arntl*, contributed to the expression, in ILC3s, of receptors mediating the homing of these cells to the lamina propria, with consequences for gut homeostasis and defense against pathogens. While this study identified light–dark cycles as major entraining controllers for ILC3s [5], Seillet *et al.* showed that rhythmic production of IL-22 by ILC3s is only partially dependent on clock genes, and identified food intake as the main extrinsic factor responsible for the oscillations noted in the ILC3s response [3]. In particular, they found that the tissue-specific modulation of IL-22 production by ILC3s in the small intestine was dependent on the production of the neuropeptide vasoactive intestinal polypeptide (VIP) by enteric neurons in mice, which increased after feeding during the dark hours, and decreased after fasting. VIP is expressed throughout the nervous system, including intestinal neurons, in response to metabolic cues, such as food intake [6]. Seillet and co-authors showed that ILC3s expressed the VIP receptor gene *Vipr2* and, using confocal microscopy, revealed that ILC3s were localized in close proximity to $VIP^+$ neurons in the small intestine [3]. They further demonstrated that VIP could induce IL-22 production by ILC3s not only *in vitro*, but also *in vivo*; specifically, this was observed upon intraperitoneal VIP injection of mice, or by comparative analysis of ILC3s from wild-type and VIPR2 knockout mice ($Vipr2^{-/-}$). Finally, they showed that the VIP-VIPR2 pathway in ILC3s had an

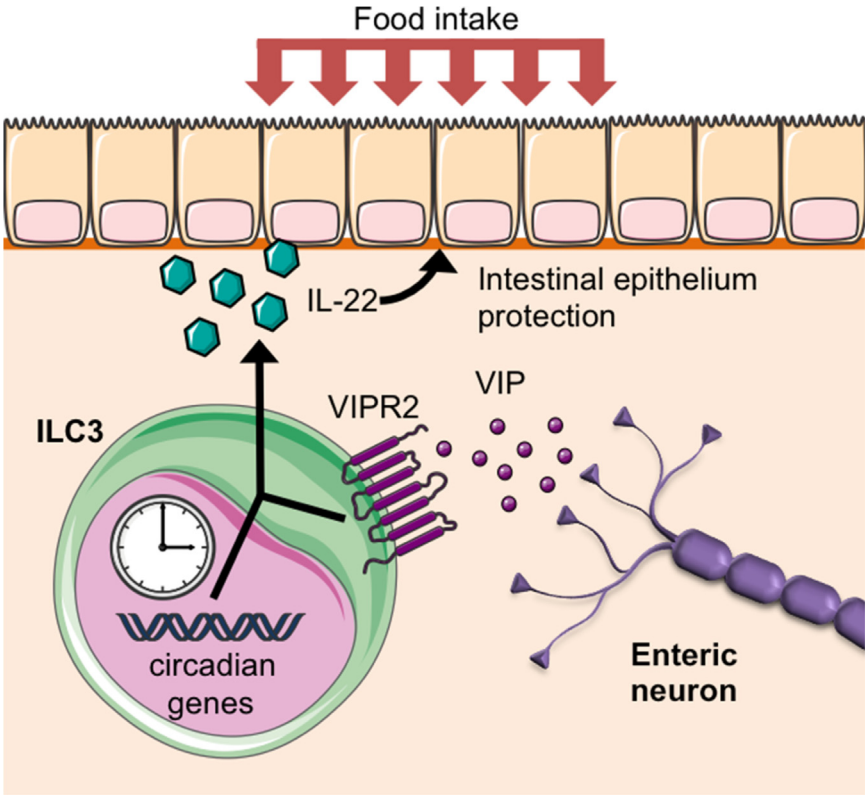


Figure 1. Vasoactive Intestinal Polypeptide (VIP) Controls Interleukin (IL)-22 Production by Group 3 Innate Lymphoid Cells (ILC3s) in the Murine Gut. Upon food intake, enteric neurons release the neuropeptide VIP, which acts on ILC3s present in the intestinal mucosa. VIP-mediated signaling, together with circadian expression of clock genes, can regulate the secretion of IL-22, protecting epithelial barrier integrity in the mouse gut. Abbreviation: VIPR2, VIP receptor 2.

important role in protecting the gut epithelium during inflammation in a mouse model of dextran sodium sulfate (DSS)-induced colitis [3].

Relevant to these findings, a recent article by Talbot and colleagues also investigated the VIP-dependent regulation of ILC3 function in the intestine [7]. These authors showed that ILC3s localized near VIPergic neurons in the mouse intestine, expressed VIPR2, and were sensitive to VIP-mediated regulation. However, in sharp contrast to the data provided by Seillet and colleagues [3], this later study showed that VIP could negatively regulate IL-22 production by ILC3s upon feeding in mice [7]. This discrepancy could reflect different experimental protocols used to alternate feeding and fasting in mice and, more importantly, the different mouse models used. Indeed, Talbot and coworkers used mice with a conditional deletion of *Vipr2* in RORγt-expressing cells [*Rorc(t)*$^{cre}$ *Vipr2*$^{fl/fl}$] [7], while Seillet et al used complete *Vipr2* knockout (*Vipr2*$^{-/-}$) mice [3]. This difference is crucial because VIP is expressed not only by intestinal neurons, but also by neurons of the SCN, and contributes to the coordination of circadian oscillations to metabolic signals [8]. Therefore, it remains to be determined whether VIP$^{+}$ central neurons may act in synergy, or in opposition to VIP$^{+}$ gut neurons in the regulation of ILC3s responses and gut integrity. Moreover, in the two studies, different markers have been used to identify ILC3s. Of note, Group 3 ILCs include classical ILC3s, which may or may not express natural cytotoxicity receptors (NCR) [9], and lymphoid tissue-inducer (LTi) cells. These subsets are developmentally and functionally distinct, although they both express CD90 and CD127, and produce IL-22. In this context, Talbot and colleagues analyzed CCR6$^{+}$ ILC3s residing in tertiary lymphoid structures (cryptopatches and isolated lymphoid follicles), which might in fact represent LTis [7]. It would be interesting to directly compare the effect of VIP on NCR$^{+}$ or NC3$^{-}$ ILC3s and LTis. Indeed, it is possible that, due to their distinct localization in the intestinal mucosa, opposite VIP-dependent regulation of IL-22 production by different ILC3 subsets might spatially affect epithelial barrier permeability and integrity.

Talbot, Littman, and colleagues proposed a working model in which, upon feeding, VIP would reduce ILC3-dependent IL-22 production, allowing nutrient acquisition at the expenses of mucosal antimicrobial and barrier function. By contrast, Seillet, Belz, and coworkers proposed that, upon food intake, VIP promotes anticipatory mucosal immunity by inducing the production of protective IL-22 by ILC3s (Figure 1). This 'anticipatory immunity' model is also supported by previous findings on ILC2s, showing that VIP stimulates IL-5 production in response to feeding, leading to the accumulation of protective eosinophils in the lung and small intestine [10].

Further investigations are clearly needed to better elucidate how imprinted circadian regulation is integrated with local neuronal signals acting on ILC3s in the intestinal

mucosa. This may help not only to understand how our lifestyle is linked to gut homeostasis and inflammation, but also to develop novel therapeutic strategies that target the neuroimmune axis in pathological intestinal conditions, such as Crohn's disease and cancer.

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