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INSIGHTS

IL-23 to see: Gut DCs shine bright in inductive sites

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The cytokine IL-23 plays important roles in intestinal barrier protection and integrity, but is also linked to chronic inflammation. In this issue of *JEM*, Ohara et al. (https://doi.org/10.1084/jem.20230923) provide clarity on the much-debated question of which cells produce IL-23.

IL-23 is a critical cytokine for intestinal barrier integrity, inducing the production of IL-22 from innate and adaptive immune cells (Aychek et al., 2015). IL-22 plays crucial roles in intestinal epithelial cell regeneration, intestinal barrier protection, and antimicrobial defense and dysbiosis (Keir et al., 2020). The importance of IL-23 in IL-22 induction is exemplified by the equal inability of IL-22– and IL-23–deficient mice to control infection caused by attaching and effacing bacterial pathogens (Zheng et al., 2008). Conversely, dysregulated IL-23 expression is linked to the development of intestinal inflammation in mice and humans (Neurath, 2019). Therefore, it would be important to identify exactly which cells produce IL-23 and to explore how its expression is controlled. However, these topics have been contentious. In particular, although it is acknowledged that IL-23 is produced by CD11c- and CX3CR1-expressing mononuclear phagocytes (MNPs) at steady state and in response to extracellular bacteria (Longman et al., 2014; Aychek et al., 2015), there is conflicting information on whether these cells are conventional dendritic cells (cDCs) or monocytes/macrophages.

Intestinal MNPs comprise macrophages, monocytes, and cDCs, of which the latter are further divided into subsets based on their CD103 and CD11b expression (Joeris et al., 2017). $CD103^+CD11b^+$ cDC2s are the most abundant cDCs in the small intestine, but they are largely absent from the colon, where $CD103^+CD11b^-$ cDC1s and $CD103^-CD11b^+$ cDC2s are represented in similar numbers. $CD103^-CD11b^-$ cDCs are rare in both effector sites and, consequentially, quite understudied. Several studies have suggested that $CD103^+CD11b^+$ cDC2s were the main producers of IL-23 in response to flagellin administration or *Citrobacter rodentium* infection (Kinnebrew et al., 2012; Satpathy et al., 2013). However, this conclusion has been challenged by the finding that mice carrying the toxic subunit of diphtheria toxin under the control of the human langerin promoter (*huCD207*.DTA) had no defect in the IL-23–dependent IL-22 responses to these stimuli, despite lacking small intestinal $CD103^+CD11b^+$ cDC2s (Welty et al., 2013). Instead, other studies have reported that $CD11c^+$MHC-II^+ monocytes and macrophages were the primary sources of IL-23 in chronic intestinal inflammation models (Arnold et al., 2016; Bernshtein et al., 2019). The confusion about the identity of IL-23–producing cells may reflect context-dependent IL-23 sources, as well as the difficulties in discriminating between monocytes/macrophages and DCs using genetic targeting systems due to substantial overlap in the markers expressed by monocytes, macrophages, and cDC2s.

In this issue in *JEM*, Ohara et al. (2024) have attempted to resolve these issues using a novel *Il23a*-venus reporter mouse. In this way, they showed that IL-23 production was largely confined to a small population of $CD103^-CD11b^-$ cDCs in isolated lymphoid follicles, cryptopatches, and mesenteric lymph nodes (Peyer's patches were not assessed) at steady state and in response to flagellin administration or *C. rodentium* infection. This IL-23 expression presumably supports IL-22 production by local innate lymphoid cells and T helper 17 (Th17) cells. Importantly, although some IL-23 production was also seen from $CD103^-CD11b^+$ and $CD103^+CD11b^+$ cDC2s in the intestine, no IL-23 was seen in cDCs from other tissues. Further investigations showed that the IL-23 expressing $CD103^-CD11b^-$ cDCs in the intestine were dependent on Notch2 signaling for their development and were highly enriched for expression of EpCAM and the DC-specific marker DCIR2. In addition, they did not express classical macrophage markers and were derived from pre-cDCs in vivo, indicating clearly that cDCs are the main producers of IL-23 in the steady-state immune system and in response to inflammatory stimuli in the intestine. Although a defining characteristic of cDCs is their ability to migrate from effector sites, such as the small and large intestinal lamina propria to draining lymph nodes, it remains unclear whether the IL-23–producing cDCs found in the mesenteric lymph nodes have migrated there from the mucosa or gut-associated lymphoid tissue (GALT), nor is it known where they might first acquire the ability to produce the cytokine.

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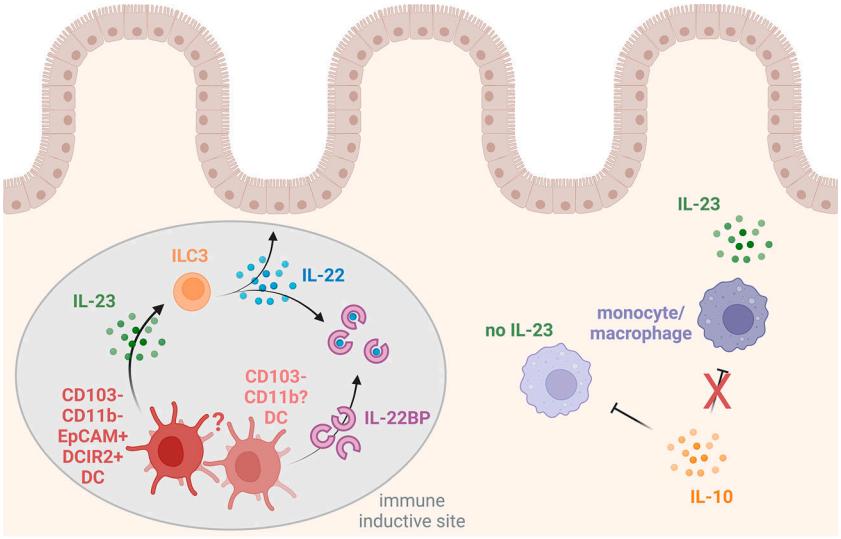

IL-23 expression by intestinal mononuclear phagocytes. Using their novel *Il23a*-venus reporter mouse strain, Ohara et al. (2024) show that CD103⁻CD11b⁻EpCAM⁺DCIR2⁺ cDCs located in intestinal immune inductive sites are the main producers of IL-23 at steady state and in response to bacterial infection. A putatively overlapping subset of DCs in the same location expresses IL-22BP, but direct comparison using the same markers will be needed to draw further conclusions. In the absence of IL-10 signaling, monocytes and macrophages can also produce IL-23, driving chronic inflammation of the intestines.

The identification of CD103⁻CD11b⁻ cDCs as the major producers of IL-23 helps to reconcile several points of confusion in the current literature. First, the finding that CD103⁻CD11b⁻ IL23-producing cDCs were Notch2 dependent may explain the discrepancy between previous studies that used different approaches to examine the role of CD103⁺CD11b⁺ cDC2s as sources of IL-23. Thus, while depletion of these cells using DC-specific Notch2 deficiency was associated with reduced IL-23 production in response to *C. rodentium* infection (Satpathy et al., 2013), no effects on IL-23–dependent IL-22 production were seen when CD103⁺CD11b⁺ cDC2s were selectively depleted in *huCD207*.DTA mice in which Notch2 signaling remains intact (Welty et al., 2013). In addition, the finding that CD103⁻CD11b⁻ cDCs were the main producers of IL-23 is consistent with previous work showing that CD103⁻CD11b⁻ cDCs in steady-state intestinal lymph were the strongest drivers of Th17 responses among intestinal cDCs ex vivo and also expressed the highest amounts of *Il23a* mRNA, suggesting that IL-23 expression might indeed be a defining functional feature of this minor population (Cerovic et al., 2013). The fact that CD103⁻CD11b⁻ DCs found in intestinal lymph are absent in *RORγt*-deficient mice (Cerovic et al., 2013), which lack GALT, further supports their presence in immune inductive sites as reported by Ohara and colleagues. Together, these findings indicate that a bona fide CD103⁻CD11b⁻ cDC subset with unique functions is present in immune inductive sites of the intestine, something that has been debated due to their low numbers and heterogenous surface marker expression.

One point that the work of Ohara et al. (2024) does not resolve fully is that monocytes and/or macrophages were reported previously to produce IL-23 in experimental colitis and in *Helicobacter hepaticus*–induced chronic intestinal inflammation (Arnold et al., 2016; Bernshtein et al., 2019). However, it is important to note that these earlier studies involved deletion or blockade of IL-10 receptor-mediated signaling, a process that is known to potentiate IL-23 production by cells of the monocyte/macrophage lineage (Zigmond et al., 2014). Thus, release from IL-10 control may allow myeloid cells other than CD103⁻CD11b⁻ cDCs to produce IL-23. Given the genetic associations between the IL-10– and IL-23–signaling pathways and human inflammatory bowel disease (reviewed in Uhlig and Powrie [2018]), it would be important now to use the sensitive *Il23a*-venus reporter approach to examine the full range of cells capable of producing IL-23 in the context of IL-10 receptor deficiency.

The work of Ohara et al. (2024) also provides potentially novel insights into how IL-23–dependent production of IL-22 might be regulated. Although essential for protecting the intestinal barrier during infection and in homeostasis, in excess, IL-22 can cause inflammation and promote tumor formation (reviewed in Keir et al. [2020]). Interestingly, expression of the soluble IL-22 binding protein (IL-22BP) that quenches excess IL-22 levels was recently also assigned to a population of CD103⁻LysM⁺Plet1⁺CD64⁻ cDCs found in immune inductive sites (Guendel et al., 2020). Intriguingly, this DC subset also contained the highest message for *Il23a*, suggesting that there may be a feedback loop that allows the same cell both to promote and inhibit IL-22 production. Again, the *Il23a*-venus reporter mice will be a useful tool to interrogate this issue and to facilitate the identification of signals driving the expression of each mediator.

Taken together, the study by Ohara et al. (2024) shines new light on a minor and poorly understood subset of intestinal cDCs in immune inductive sites that is responsible for the bulk of IL-23 production at steady state and during bacterial infection. The addition of *Il23a*-venus reporter mice to the immunologist's toolbox should be invaluable in increasing our understanding of IL-23 expression in the context of chronic inflammation in the future.

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