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A Breath of Fresh AIRE Surrounds Paneth Cells and Defensins

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In the last several years, the understanding of gastrointestinal (GI) Paneth cells (PCs) during homeostatic and diseased conditions has remarkably expanded. First, PCs occupy a unique and privileged location within the intestinal stem cells (ISCs) niche, where they play a crucial role in regulating ISC function. PCs are derived from Lgr5+ ISCs but, unlike cells of other epithelial lineages, they remain exclusively at the base of the intestinal crypts. In this position, PCs intimately intermingle with its descendent ISCs where they are believed to provide the canonical Wnt signal required to drive self-renewal and differentiation.¹ Second, as key players in host defense and mucosal immunity, PCs secrete numerous anti-microbial peptides including α -defensins and lysozymes that shield ISCs and early progenitor cells from close contact with potentially detrimental microbial flora.² Given these responsibilities in the GI tract, it is not surprising that disturbances in the development and function of PCs are implicated in the pathogenesis of multiple GI diseases, including disorders of immune dysregulation. In a cohort of Crohn's disease patients, the production of α -defensins 5 (HD5) and 6 (HD6) have been found to be attenuated in the ileum during intervals of disease activity. This selective decline in PC function results in concurrent decline of antimicrobial function and alterations in the luminal microflora.³ In humans and mice, antimicrobial responses of PCs also depend on NOD2, a pattern recognition receptor that recognizes bacterial peptidoglycans, as well as Toll-like receptors, which signal via an MyD88-dependent pathway.⁴ Moreover, recent studies suggest that endoplasmic reticular stress and induction of autophagy limited to PCs results in transmural ileitis.⁵ Not only do these findings highlight the role of PCs in healthy individuals and subsets of IBD patients, they also suggest that PCs also contribute to the pathogenesis of other immune mediated GI disorders.

In this issue of *Gastroenterology*, Dobeš et al have identified the role of PCs in the complex GI phenotypes of patients with autoimmune polyendocrinopathy–candidiasis–ectodermal

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Conflicts of interest

The authors disclose no conflicts.

dystrophy (APECED).^{6,7} This monogenetic, autosomal-recessive disorder stems from loss-of-function mutations of the autoimmune regulator (*AIRE*) gene.^{7,8} *AIRE* is a transcription factor expressed in medullary thymic epithelial cells, where it augments the expression of a large repertoire of antigens normally expressed by other cell types, including those in the gut.^{9,10} T-cell precursors that react to these self-antigens normally undergo apoptotic death in the thymus, ensuring a mechanism of central tolerance that diminishes chances of autoimmune responses.¹¹ In individuals with *AIRE* mutations, the ability of mTECs to express endogenous antigens is impaired, resulting in the survival of T cells with specificity toward self-antigens and therefore an enhanced risk of a complex autoimmune response.¹² Tryptophan hydroxylase (TPH) and histidine decarboxylase (HDC) are proteins normally expressed in enteroendocrine cells (EECs) whose expression in the thymus is *AIRE* dependent.^{13,14} In a select group of APECED patients, circulating antibodies to TPH and HDC are detectable, implying the presence of self-reactive adaptive immunity (including autoreactive T cells) that orchestrates the loss of EEC.¹⁵ These APECED patients with defects in *AIRE*-dependent expression of EEC-specific antigens can present with diverse GI symptoms that range from severe constipation to debilitating diarrhea.^{14,16}

However, the complex GI pathophysiology in APECED is explained incompletely by the absence of EECs, and the involvement of adaptive immune cells is not well-established. Nonimmune disorders of EEC maturation, differentiation, and function are associated with severe malabsorptive diarrhea. Germline mutations of *NEUROGENIN-3*—a master transcription factor that drives endocrine cell fate determination—leads to failed development of EECs and β -cells and resultant symptoms of severe diarrhea.¹⁷ Similarly, patients with mutations of *PCSK1*, a gene that encodes the proprotein convertase-1 enzyme that activate GI hormones, also present with unequivocal symptoms of diarrhea despite detectable EECs.¹⁸ The GI phenotype of these 2 disorders demonstrates clearly that a global loss of gut hormones results in the clinical symptom of pure, generalized, malabsorptive diarrhea. In APECED, however, diarrhea can alternate with severe constipation in the absence of EECs, confounding the prediction that loss of EECs lead to diarrhea. Despite the clear association between loss of enteric hormones and GI symptoms in other diseases, establishment of similar, clearcut associations between loss of EECs and GI symptoms in APECED cases have remained elusive.

The study by Dobes et al uncovers a new and clarifying piece to the puzzle: immune-mediated destruction of PCs and its consequences for the composition of luminal microbiota. In a normal thymus, *AIRE* drives expression of the α -defensin proteins, DEFA5 and DEFA6 by mTECs, allowing negative selection of T-cell precursors that react to these antigens, thereby protecting PCs from immune attack. In contrast, the authors predicted that APECED patients would harbor adaptive immunity to these proteins, and hence self-reactive targeting of PCs. Indeed, the authors demonstrated the presence of reactive peripheral T cells as well as antibodies to DEFA5 in a group of patients. And, a subset of patients with anti-DEFA5 antibodies displayed depletion or total absence of PCs.

This interpretation was mechanistically validated in the murine model of APECED (*Aire*^{-/-}). When either *Aire*^{-/-} or control mice were injected with cryptidin (the murine homologue of α -defensin), CD4⁺ and CD8⁺ T cells with reactivity to cryptidin were isolated

from the mesenteric lymph nodes of only Aire^{-/-} mice. Moreover, CD8⁺ T cells isolated from the lamina propria of Aire^{-/-} mice induced apoptosis of PCs in an in vitro co-culture setting. Adoptive transfer of T cells from cryptidin-immunized Aire^{-/-} mice into nude mice resulted in an abundance of activated CD8⁺ T cells in the mesenteric lymph nodes and lamina propria of the recipient nude mice with simultaneous decreases noted in the number of PCs. Taken together, findings from human and murine studies suggest that AIRE deficiency is associated with self-reactive antibodies and proliferation of T cells that recognize α -defensins and lead to either a reduction or a complete loss of PCs.

As discussed, a decrease in luminal α -defensins has been associated with changes in the GI microbial flora. The investigators assessed changes in the luminal microbiome of AIRE-deficient humans and mice with diminished numbers of PCs. Although methodologically limited, this evaluation revealed an expansion of segmented filamentous bacteria (SFB) in AIRE-deficient humans and mice. SFB is associated with enhancement of T helper 17 subset of CD4⁺ T cells, and the authors observed increased production of interleukin (IL)-17 and IL-23R expression in Aire^{-/-} mice, and correlated with SFB abundance. In APECED patients, the clinical pathobiology features an additional autoimmune feature—neutralizing antibodies to IL-17—which attenuate the immune function required to control mucosal candida infection, a defining characteristics of APECED.¹⁹ This feature was not tested in Aire^{-/-} mice, and makes uncertain the connection of SFB expansion and IL-17 response to the APECED disease phenotype.

Although APECED is an immune-mediated disorder, unlike many autoimmune disorders affecting the GI system, there is a lack of robust inflammatory process in the mucosa or epithelial apoptosis. For instance, celiac disease and autoimmune enteropathies such as immunodysregulation polyendocrinopathy enteropathy X-linked syndrome, are characterized by villous atrophy, crypt hyperplasia, and stark inflammatory infiltrates.^{20,21} In these cases, it is apparent that a flagrant inflammatory process mediates global loss of GI epithelial function, yielding combined secretory and malabsorptive diarrhea. The lack of such inflammation or structural changes in the context of dramatic symptoms emphasize the subtlety of mucosal injury in APECED, and also elicits the question of whether nonepithelial components are impaired. For instance, interstitial cells of Cajal or submucosal neuronal and glial cells may be targets of the dysregulated immune response, leading to impaired luminal function and resultant constipation. Abnormal function of these cells may override the symptoms of diarrhea caused by loss of PCs and EECs. To substantiate the involvement of other cell types in GI pathology of APECED, these cell-specific antigens recognized by T cells and autoantibodies need to be identified.

In conclusion, the study from Dobes et al presents the novel observation of immune destruction of PCs, and its role via downstream microbiome disturbance and parallel EEC impairment in the complex functional gut phenotype of human APECED. However, provocative findings yield provocative questions. Although T cells are present in the GI mucosa of AIRE-deficient mice and humans, it is unknown whether they interact directly with PCs or EECs via synaptic contact, or through soluble mediators such as cytokines and chemokines. In addition, the mechanism underlying the disappearances of PC and EECs need to be elucidated, because it is unknown whether the absence of these cells is a

consequence of apoptosis or impaired differentiation of their progenitor cells from Lgr5⁺ ISCs. The data presented by Dobes et al give us an insight into the complex interaction of the mucosal immune system and fluctuations in the microbiome, and remind us of the challenges and limitations of using murine models in investigation human disease mechanisms.

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