Key role found for gut epithelial cells in the defense against deadly diarrheal infections

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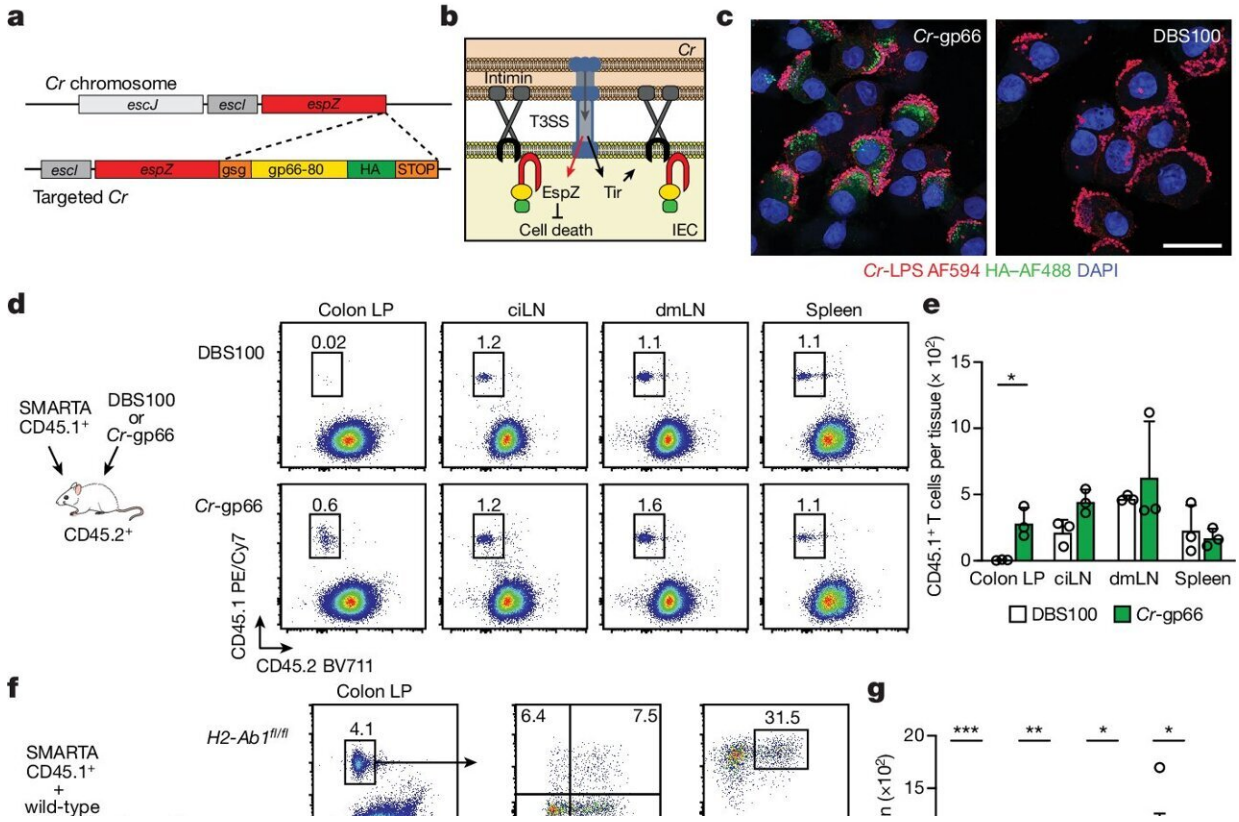

Epithelial MHCII is required for mucosal retention of Cr-specific TH cells, prolonged colonocyte STAT3 activation and crypt protection. Credit: *Nature* (2024). DOI: 10.1038/s41586-024-07288-1

Intestinal epithelial cells line the inner wall of the gut, creating a barrier against dangerous bacteria like enteropathogenic E. coli that seek to

attach to and destroy this barrier. Such pathogens pose significant risks to human health, including infant deaths due to diarrhea, particularly in developing countries.

A study published in the journal *Nature*, led by Carlene Zindl, Ph.D., and C. Garrett Wilson in the research group of Casey Weaver, M.D., at the University of Alabama at Birmingham, brings new understanding to how absorptive intestinal epithelial cells, or IECs, and T cells work together to defend the gut barrier.

They describe a newly identified subset of absorptive IECs that serve as both the major target and a key responder in a mouse model of gut infection induced by the bacteria Citrobacter rodentium.

This research builds on a study published in *Immunity* in 2022 by Zindl and Weaver, which showed that interleukin-22 signaling by innate lymphoid cells in the early stages of C. rodentium infection, and interleukin-22 signaling by CD4 T cells in the late stages of C. rodentium infection, play distinct and indispensable roles in defending the intestinal barrier. Signaling mediated by the cytokine interleukin-22, or IL-22, derived from CD4 T cells was required to protect colonic crypts against invasion of C. rodentium and ensure mouse survival.

This current study explains how prolonged interleukin-22 signaling from CD4 T cells is sustained through interaction with the newly identified subset of absorptive IECs. It further shows that this IEC cell response—in addition to the early IL-22 from innate lymphoid cells and late IL-22 from CD4 T cells—is a newly discovered and key piece of a coordinated interplay between innate and adaptive immune systems and tissue-specific, non-immune cell populations to resist pathogen incursion.

"Here we identify a sub-lineage of absorptive IECs that is regionally

restricted to the mid-distal colon and is targeted by C. rodentium, which explains the pathogen's preference for this tissue niche," said Zindl, an assistant professor in the UAB Department of Pathology.

"We find that IL-22 signaling triggers a unique gene-expression program in this population, distinct from that of conventional absorptive IECs that populate the proximal large and small intestines. We further identify a key role for antigen presentation by IECs to elicit optimal IL-22 signaling from T cells."

In both mice and humans, the colons have surface IECs that face the lumen of the colon and line the mouths of a multitude of colonic crypts—approximately 9.9 million of them in humans. These crypts have tiny indentations shaped like thick-walled test tubes; at the bottom of each crypt are stem cells that continuously produce new IECs, creating an escalator of maturing cells from the crypt base toward the lumen. IECs need constant replacement since they live only two to six days.

C. rodentium attaches to the mid-distal region of the mouse colon, initiating a rapid elongation of the colonic crypts—a hallmark of the infection—that is believed to protect the intestinal stem cells by distancing them from the pathogen at the lumen surface and speeding the shedding of pathogen-laden IECs.

In the study, single-cell RNA sequencing of IECs from the mid-distal colon in uninfected mice was used to discover the two lineage subsets of absorptive IECs, as identified by differential gene expression profiles for 50 different genes. The two subsets were labeled as distal colonocytes, or DCCs, and proximal colonocytes, or PCCs. Each subset was distinguished by specific cell markers—Ly6g for DCCs and Fabp2 for PCCs.

Furthermore, single-cell RNA sequence analysis of IECs from the mid-

distal colons of naïve versus C. rodentium-infected mice showed that the DCCs, not the PCCs, were the dominant responder to C. rodentium infection. Progenitors of DCCs underwent considerable expansion, and the DCC lineage underwent rapid maturation and an altered cell fate to become hyperactive pathogen-induced colonocytes that contributed to host defense, including IL-22 and interferon-gamma–inducible host defense genes.

Using the distinct cell markers, Zindl and colleagues found that DCCs were exclusively found in the distal colon and were interspersed with PCCs in the middle colon. In contrast, PCCs dominated in the proximal colon and the terminal ileum of the small intestine. This was the basis to name the two lineages as distal or proximal colonocytes.

The researchers further found that C. rodentium preferentially bound to DCCs, not PCCs, at the luminal surface of the colon, explaining why colonization by the pathogen is restricted to the mid-distal colon of mice.

The researchers also found a stratification of host defense responses to IL-22 between the two subsets. Many genes that Zindl and Weaver had previously reported as dependent upon T cell-derived IL-22 were upregulated in the DCCs, including antimicrobial peptides and neutrophil-recruiting chemokines. In contrast, upregulation of the IL-22–inducible lectin family antimicrobial peptide genes was specific to PCCs.

Besides activating DCCs for enhanced anti-bacterial defense, delivery of IL-22 to DCCs by CD4 T cells is required to accelerate the removal and replacement of C. rodentium-laden colonocytes at the luminal surface to speed pathogen clearance. Weaver says that this represents a novel mechanism by which IL-22 signaling into DCCs can counteract the effector mechanisms deployed by C. rodentium to retain the infected DCCs to which they are bound.

In a classic adaptive immune response, T cells are activated by dendritic cells that capture pathogens at the site of infection, such as the colon, and then migrate to lymph nodes to present the pathogen antigens to T cells via major histocompatibility molecules, or MHC, on the surface of the dendritic cell. This presentation occurs through direct cell-to-cell contact.

C. rodentium anchors itself to host IECs and injects its own proteins inside IECs to help them survive longer. It was known that IECs express MHC class II molecules during the later stages of infection when CD4 T cells arrive at the mid-distal colon, but it was unclear whether C. rodentium antigens could be presented by IECs to directly recruit CD4 T-cell help.

To test this, Weaver's group engineered a new C. rodentium bacteria that carries a molecule which these specific T cells can recognize. They found that MHC class II-dependent antigen presentation of C. rodentium antigens by colonic IECs was indeed required to retain T-helper 17 (Th17) and T-helper 22 (Th22) cells and elicit IL-22–dependent help to defend against C. rodentium.

"Our findings address the longstanding conundrum of why C. rodentium colonization is regionally restricted, and they reveal a basis for the indispensable role of T cells in protection of the intestinal barrier—namely, that T cells are activated locally via non-classical antigen presentation by the intestinal epithelium to sustain high amplitude IL-22 signaling to IECs as they undergo developmental shifts in response to infection," Wilson said.

"Our identification of a specific absorptive cell type that is targeted by C. rodentium should facilitate comparative studies to define the basis for species-specific and cell-specific tropism of attachment and effacement enteropathogens."

"In addition to providing new insights into the importance of epithelial cell expression of the MHC class II antigen processing-and-presenting machinery for host defense, our findings open a window into coordination of immune interactions by which attachment and effacement enteropathogens are resisted and identify a new absorptive enterocyte subset with which T cells can dialogue," Weaver said.

More information: Carlene L. Zindl et al, Distal colonocytes targeted by C. rodentium recruit T-cell help for barrier defence, *Nature* (2024). DOI: 10.1038/s41586-024-07288-1

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