Dissecting the cellular states and fates of shed mouse intestinal cells

Analysis of cells shed from the mouse gut, using bulk and single-cell transcriptomics, as well as single-molecule FISH and intravital imaging, revealed that shed cells are diverse, remain viable for a few hours and upregulate anti-microbial gene expression programs.

This is a summary of:
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The question

Every day, the human gut sheds around 35 grams of cells, making up a substantial portion of the faeces[1]. It was commonly believed that the majority of these shed cells die soon after detaching from the tissue. However, our previous research, which employed spatially resolved transcriptomics, revealed that hundreds of genes are upregulated in epithelial cells only a few hours before shedding[2,3]. This finding prompted us to question why the body would expend so much energy on altering the states of cells that are destined to die shortly afterward. Furthermore, we wanted to know whether intestinal cells continue to function within the intestinal lumen after shedding, and if they do, whether they could potentially still perform a function, such as influencing the composition of the gut microbiota.

The observation

To determine the origin of shed cells, we collected tissue samples and faecal wash samples (containing the shed cells) from consecutive sections of the mouse gut (Fig. 1). We obtained the transcription profiles of host genes in both the tissue and the shed cells in faecal washes using bulk RNA sequencing (RNA-seq). To gain more insight into the cell types that are shed and their expression profiles, we performed single-cell RNA-seq (scRNA-seq) and single-molecule transcript imaging (single-molecule fluorescence in situ hybridization; smFISH) for the shed cells in the faeces. We also used intravital imaging to track the process of cell shedding in live mice. Moreover, we replicated these experiments in a mouse model of inflammatory bowel disease (dextran sodium sulfate (DSS)-induced colitis) and in mice treated with broad-spectrum antibiotics.

We found that shed cells remain viable and maintain their transcription activity for several hours after detachment. Based on transcription profiles, these shed cells comprise mainly epithelial cells, primarily originating from the tips of the villi. However, we also made the surprising discovery that diverse immune cells are consistently shed in physiological conditions. Interestingly, we observed that epithelial cells upregulate genes associated with antimicrobial activity on shedding, while the shedding of immune cells increases during episodes of inflammation in mice (induced by DSS treatment) and decreases upon antibiotic treatment. In addition, our research demonstrates that the alterations in gene expression detected in shed cells mirror the changes observed in mouse gut tissue in response to DSS treatment.

The implications

Our discoveries reveal a previously unrecognized phase in the life cycle of intestinal cells, which has major implications for understanding of the physiology and pathophysiology of the gut. The remarkable correspondence between the transcriptomic profiles of shed cells and the transcription changes observed in the intestinal tissue in states of inflammation suggests that faecal host transcriptomics could be utilized to diagnose various intestinal diseases in humans, as recently shown with bulk transcriptomics in patients with inflammatory bowel disease (IBD)[4,5].

Our study does not establish that shed cells are functional or that they actually modulate gut microbiota composition. To show that shed cells are functional, we plan to isolate these cells while they are still alive and demonstrate their functionality in vitro. Moreover, it remains to be seen whether faecal host RNA in humans also originates from live, viable shed cells.

Going forward, we hope to establish faecal host transcriptomics as a non-invasive modality to characterize human intestinal diseases, IBD, irritable bowel syndrome and colorectal cancer. Shed cell analysis will be particularly exciting for diseases such as coeliac disease, for which single-cell analysis of shed cells could identify specific domains along the villus axis that are more vulnerable to autoimmune damage.

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EXPERT OPINION

"Intriguingly, this study demonstrates that cells shed into the intestinal lumen include not only epithelial but also immune cells, that they stay alive after shedding, continue transcription and have a different transcriptional profile than tissue cells. These data are very interesting as they describe an unexplored cell population, providing important information, such as the fact that alive shed cells may perform specific functions once in the intestinal lumen." **Rocío López Posadas, University Hospital Erlangen, Erlangen, Germany.**

FIGURE

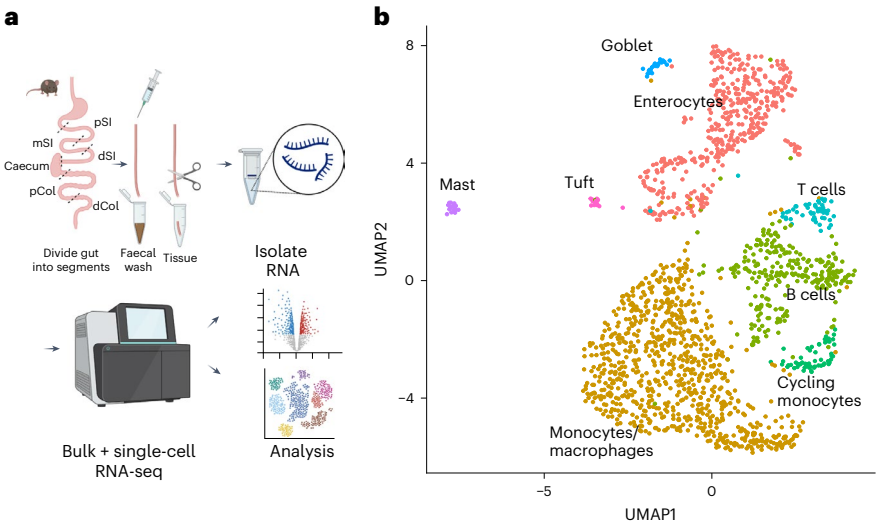

Fig. 1 | Host transcriptomics of shed intestinal cells. a, Schematic of the procedure for collection of faecal washes and tissue samples and subsequent transcriptomic analysis. pSI, proximal small intestine; mSI, mid small intestine; dSI, distal small intestine; pCol, proximal colon, dCol, distal colon. **b**, UMAP (uniform manifold approximation and projection) visualization and annotation of 1,717 single cells from faecal washes of the small intestine of four male mice, analysed by scRNA-seq. © 2023, Halpern, B. K. et al.

BEHIND THE PAPER

This study originated from Keren Bahar Halpern's hypothesis that gut cells might be functional after they are shed. The project involved addressing experimental challenges associated with isolating shed cells from faecal matter, particularly for scRNA-seq. A key challenge was establishing that viable cells in faecal washes were indeed cells shed in vivo and not cells mechanically dislodged during tissue flushing. To address this issue, we injected mice with a freely diffusing dye that labelled all tissue cells, which allowed us to demonstrate that the majority of shed cells did not contain the dye. This result provided evidence that the shed cells had already detached before the mice were sacrificed. Additionally, we conducted intravital imaging of the mouse gut in collaboration with Ziv Shulman and Adi Biram, which allowed us to observe the live shedding of immune cells from the gut. **S.I.**

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   This paper demonstrates that bulk transcriptomics of distal gut faecal washes can identify active inflammation in ileal Crohn's disease, in line with the stability of shed cells demonstrated in our study.

FROM THE EDITOR

"This study stood out to us because the authors not only found a way to analyse cell shedding in the intestine, but also because they discovered that these shed cells — contrary to previous belief — stay alive and transcriptionally active. The work opens up the possibility to study the function of shed intestinal epithelial and immune cells in gut homeostasis as well as in different disease states." **Editorial Team, *Nature Metabolism*.**