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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Indian Hedgehog Suppresses a Stromal Cell–Driven Intestinal Immune Response

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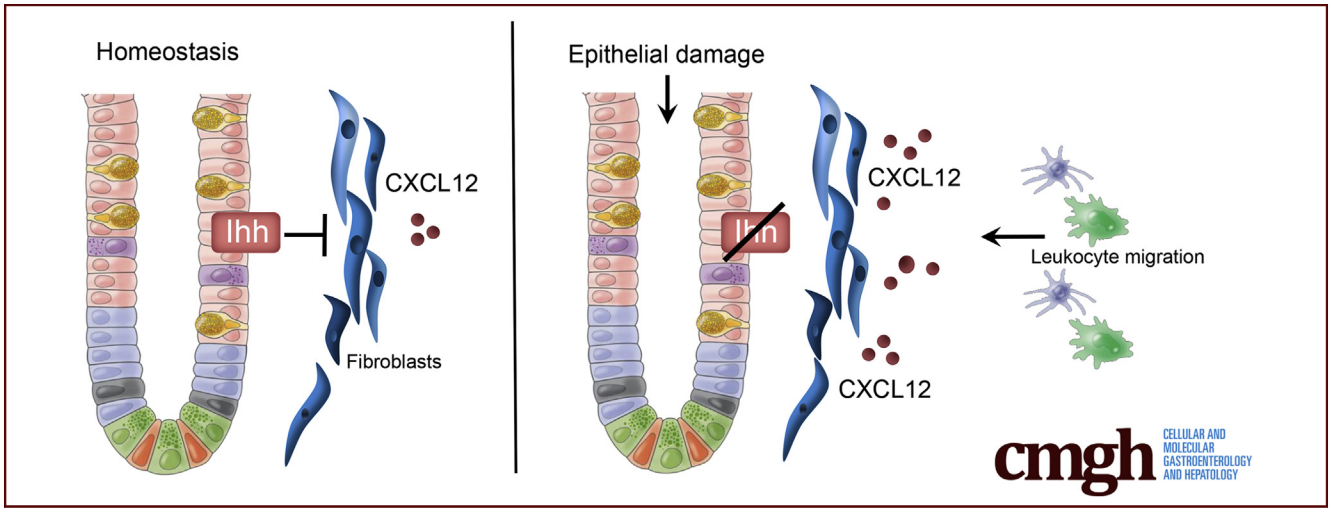

SUMMARY

We show that epithelium-derived Indian Hedgehog signals exclusively to fibroblasts in the intestine. Short-term loss of Indian Hedgehog leads to a rapid immune response with up-regulation of fibroblast-derived C-X-C motif chemokine ligand 12, and migration of immune cells into the lamina propria.

BACKGROUND & AIMS: Upon intestinal epithelial damage a complex wound healing response is initiated to restore epithelial integrity and defend against pathogenic invasion. Epithelium-derived Indian Hedgehog (Ihh) functions as a critical sensor in this process. Signaling occurs in a paracrine manner because the receptor for Ihh is expressed only in the mesenchyme, but the exact Hedgehog target cell has remained elusive. The aim of this study was to elucidate further the nature of this target cell in the context of intestinal inflammation.

METHODS: Hedgehog activity was modulated genetically in both cell type–specific and body-wide models and the resulting animals were analyzed for gene expression profiles and sensitivity for dextran sodium sulfate (DSS) colitis. To characterize the Hedgehog target cell, *Gli1-CreERT2-Rosa26-ZsGreen* animals were generated, which express ZsGreen in all Hedgehog-responsive cells. These cells were characterized using flow cytometry and immunofluorescence.

RESULTS: Loss of Indian Hedgehog from the intestinal epithelium resulted in a rapid increase in expression of inflammation-related genes, accompanied by increased influx of immune cells. Animals with epithelium-specific deletion of Ihh or lacking the Hedgehog receptor Smoothened from Hedgehog target cells were more sensitive to DSS colitis. In contrast, specific deletion of Smoothened in the myeloid compartment did not alter the response to DSS. This suggests that Hedgehog signaling does not repress intestinal immunity through an effect on myeloid cells. Indeed, we found that Hedgehog-responsive cells expressed gp38, smooth muscle actin, and desmin, indicating a fibroblastic nature. Ihh signaling inhibited expression of C-X-C motif chemokine ligand 12 (CXCL12) in fibroblasts in vitro and in vivo, thereby impairing the recruitment of immune cells.

CONCLUSIONS: We show that epithelium-derived Indian Hedgehog signals exclusively to fibroblasts in the intestine. Loss of Ihh leads to a rapid immune response with

up-regulation of fibroblast-derived CXCL12, and migration of immune cells into the lamina propria. *(Cell Mol Gastroenterol Hepatol 2017;■:■–■; http://dx.doi.org/10.1016/j.jcmgh.2017.08.004)*

Keywords: Intestine; Hedgehog; Stroma; Inflammation; CXCL12.

Immune cells in the intestinal lamina propria are in a state of basal tolerance. In response to epithelial damage, a switch to an activated status is required to limit the consequences of exposure to potentially dangerous luminal content. This control is maintained in part by the immune system itself and in part by the epithelial tissue. The intact epithelium provides factors that mediate immune suppression under steady-state conditions. Upon tissue damage, the loss of epithelial cells results in loss of these factors and relief of the active immunosuppression. Several epithelium-derived factors have been identified that can suppress the mucosal immune response, including thymic stromal lymphopoietin, transforming growth factor-β, semaphorin 7a, and interleukin 25 (IL25).[1–4] These factors mainly exert their effects through their influence on the development of dendritic cells and macrophages with tolerogenic properties such as production of IL10 or inhibition of IL17 and tumor necrosis factor-α.

One of the key epithelium-derived factors required to maintain immune tolerance in the intestine is Indian Hedgehog (Ihh).[5] Ihh is secreted exclusively by intestinal epithelial cells[6,7] and signals in a paracrine manner to the inhibitory receptor Patched1 (Ptch1) on cells in the mesenchyme.[8] Binding of Ihh to Ptch1 alleviates the inhibitory effect on the second Hedgehog receptor Smoothened (Smo), resulting in translocation of the glioma-associated oncogene proteins (Gli) into the nucleus and subsequent transcription of the Hedgehog target genes such as *Gli1*, *Ptch1*, and the Hedgehog interacting protein (*Hhip*).[9]

Conditional loss of Ihh from the intestinal epithelium activates many aspects of an epithelial repair response such as crypt expansion and increased proliferation of progenitor cells.[10,11] Ultimately, unresolved loss of Ihh results in the development of severe enteritis with extensive fibrosis, mucosal damage, and the infiltration of macrophages and leukocytes. The Hedgehog pathway already was linked to mucosal inflammation when a single-nucleotide polymorphism in the gene that encodes transcription factor *Gli1* was found to predispose to inflammatory bowel disease (IBD).[12] This polymorphism results in a hypomorphic protein with diminished capacity for transcriptional activation. Furthermore, the expression of the Hedgehog targets *Gli1*, *Ptch1*, and *Hhip* is down-regulated in patients with IBD with active disease.[12,13] In addition, the association of reduced Hedgehog signaling and the risk of developing IBD was functionally tested in mice that were heterozygous mutant for *Gli1*. These mice developed more severe disease compared with controls in a murine model of colitis.[12] In vitro, the role of Ihh as an immune suppressor has been studied using cultured embryonic tissue.[11] Culturing intestinal lamina propria in the absence of epithelial cells, and therefore in the absence of Hedgehog, resulted in loss of Hedgehog signaling and significant activation of inflammatory genes such as IL1β, IL6, and Toll-like receptor 2, which was corrected by the addition of recombinant Hedgehog protein.

Despite the fact that Ihh seems to be a crucial sensor of epithelial integrity in the intestine, the identity of the Hedgehog-responsive cells and a precise anti-inflammatory pathway via which Ihh signals remain to be shown. Although it was suggested that lamina propria macrophages and dendritic cells can respond directly to Hedgehog signaling, functional evidence for such direct effects is lacking.[11,12] Other stromal cells such as fibroblasts, smooth muscle cells, blood vessels, and lymphatic vessels also may play a role. In fact, it has been shown that Hedgehog is a critical regulator of smooth muscle homeostasis[8,14] and is important for the maintenance of myofibroblasts in the intestine.[15]

Here, we identify fibroblast-like cells as the exclusive Hedgehog-responsive cells in the intestine. Short-term loss of Hedgehog signaling resulted in up-regulation of chemokine production by these cells, in particular C-X-C motif chemokine ligand 12 (CXCL12). Subsequently, various subsets of immune cells were recruited to the intestine that predisposed to the development of colitis.

Materials and Methods

Animals

Villin-CreERT2 mice[16] were crossed with *Ihh*$^{fl/fl}$ mice[17] and *Rosa26-ZsGreen* mice (007906; The Jackson Laboratory, Bar Harbor, ME) to generate *Villin-CreERT2-ZsGreen-Ihh*$^{fl/fl}$ animals. *Rosa26-CreERT2* mice[18] were crossed with *Ptch1*$^{fl/fl}$ animals[19] to generate the previously described *Rosa26-CreERT2-Ptch1*$^{fl/fl}$ animals.[7] *LysM-Cre* mice[20] (004781; The Jackson Laboratory) were crossed with *Rosa26-YFP* (006148; The Jackson Laboratory) and *Smo*$^{fl/fl}$ mice (004288; The Jackson Laboratory) to generate *LysM-Cre-YFP-Smo*$^{fl/fl}$ mice. *CD11cCre-GFP* mice (008068; The Jackson Laboratory) were crossed with *Smo*$^{fl/fl}$ mice to generate *CD11c-Cre-GFP-Smo*$^{fl/fl}$ mice. *Gli1-CreERT2* (007913; The Jackson Laboratory) and *Rosa26-ZsGreen* animals were crossed to generate *Gli1-CreERT2-Rosa26-ZsGreen* mice. *Gli1-CreERT2* and *Smo*$^{fl/fl}$ mice were crossed to generate *Gli1-CreERT2-Smo*$^{fl/fl}$ animals. Activation of CreERT2 and thus induction of the respective gene manipulations was performed by intraperitoneal administration of

***Authors share co-first authorship.**

Abbreviations used in this paper: **α-SMA, α smooth muscle actin; CXCL, C-X-C motif chemokine ligand; CXCR, C-X-C motif chemokine receptor; DMEM, Dulbecco's modified Eagle medium; DSS, dextran sodium sulfate; FCS, fetal calf serum; Gli, glioma-associated oncogene proteins; Hhip, Hedgehog interacting protein; IBD, inflammatory bowel disease; Ihh, Indian Hedgehog; *Ihh*$^{+/+}$, *Villin-CreERT2-ZsGreen-Ihh*$^{+/+}$; *Ihh*$^{-/-}$, *Villin-CreERT2-ZsGreen-Ihh*$^{fl/fl}$; IL, interleukin; MPO, myeloperoxidase; PBT, PBS/BSA/Triton; Ptch1, Patched1; RT-PCR, reverse-transcription polymerase chain reaction; Smo, Smoothened.**

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