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REVIEW

Stromal regulation of the intestinal barrier

Marvin Sylvestre[†], Selene E. Di Carlo[†] and Lucie Peduto[✉]

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The intestinal barrier is a complex structure that allows the absorption of nutrients while ensuring protection against intestinal pathogens and balanced immunity. The development and maintenance of a functional intestinal barrier is a multifactorial process that is only partially understood. Here we review novel findings on the emerging role of mesenchymal cells in this process using insights gained from lineage tracing approaches, Cre-based gene deletion, and single-cell transcriptomics. The current evidence points toward a key organizer role for distinct mesenchymal lineages in intestinal development and homeostasis, regulating both epithelial and immune components of the intestinal barrier. We further discuss recent findings on functional mesenchymal heterogeneity and implications for intestinal regeneration and inflammatory intestinal pathologies.

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INTRODUCTION

The intestine constitutes a surface barrier between the host and the environment, which contains an abundant microbiota, food antigens, and potential pathogens. As cells at barrier sites are constantly exposed to microbial and damaging elements that may compromise tissue function, a major challenge is to efficiently eliminate pathogens and promote repair while avoiding chronic inflammation. Dysregulation of the intestinal barrier leads to pathological responses to tissue damage and the microbiota, which is associated with several chronic diseases and systemic disorders, including inflammatory bowel diseases (IBDs), intestinal fibrosis, and cancer.

A functional intestinal barrier requires a tightly regulated crosstalk between a self-renewing epithelial layer and a subepithelial compartment containing innate and adaptive immune cells, blood vessels, and stromal mesenchymal cells (MCs). The intestinal epithelial layer continuously self-renews from intestinal epithelial stem cells (IESCs) located in the crypts¹. IESCs generate proliferating transit-amplifying (TA) cells that differentiate into distinct epithelial lineages, including absorptive enterocytes, enteroendocrine cells (EECs), antimicrobial peptide-secreting Paneth cells, and mucus-producing Goblet cells, altogether providing a first line of intestinal defense^{2,3}. Terminally differentiated epithelial cells undergo apoptosis at the villus tip and are shed into the intestinal lumen or sampled by phagocytes in the small intestine lamina propria (LP)^{2,4}. Intestinal homeostasis requires WNT/ β -catenin signaling and prostemness factors to maintain IESCs in the crypts and bone morphogenetic proteins (BMPs) along the crypt-villus axis to promote epithelial differentiation⁵. Paneth cells are closely adjacent to IESCs and produce the WNT ligand WNT3A, Notch ligands (DLL4, DLL1), and epidermal growth factor (EGF)⁶, sug-

gesting that they constitute the niche for IESCs. However, *in vivo* depletion of Paneth cells or ablation of *Wnt3*, or *Porcn*, and *Wls* (essential for WNT secretion) in epithelial cells did not impact intestinal homeostasis^{7–10}. In the colon, which lacks Paneth cells, deep crypt secretory cells activate Notch signaling in IESCs¹¹ but do not produce canonical WNT ligands, which are indispensable for IESCs. These findings support the existence of non-epithelial sources for WNT signals and other factors required for IESCs.

Beneath the intestinal epithelium lies the LP. The LP contains a dense network of MCs, which include subepithelial, perivascular, and interstitial mesenchymal populations, as well as fibroblastic reticular cells (FRCs) of the gut-associated lymphoid tissues (GALTs)^{12,13}. As their distinct localization suggests, MCs interact with various cell populations, including epithelial, immune, and vascular cells, and are essential to support their function. When dysregulated, MCs promote chronic inflammation, fibrosis, and cancer^{14–16}. MCs lack expression of hematopoietic (CD45), endothelial (CD31), and epithelial (EpCAM) markers; however, the resulting CD45[−]CD31[−]EpCAM[−] population, historically called fibroblast (α SMA[−]) or myofibroblast (α SMA^{low}), is neither homogeneous nor defining one single cell type¹⁴. Additional markers, such as platelet-derived growth factor receptor alpha (PDGFR α), Podoplanin (PDPN, GP38), or Thy1 (CD90), can be useful in visualizing the extensive and diverse mesenchymal network of the LP, as they are broadly expressed by several mesenchymal populations. Due to paucity of specific markers, intestinal MCs were mostly studied *in vitro* until recently, either from bulk primary cells isolated from organs and selected based on their adherence and growth capacity or using cell lines. Although these studies provided important information, notably on the crosstalk with immune cells¹⁷, they

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were not reflecting the heterogeneity and functional diversity of the mesenchymal compartment *in vivo*.

The recent expansion of single-cell transcriptomics (scRNA-seq) and development of new mouse models allowing for genetic lineage tracing and Cre-based gene/cell depletion of mesenchymal subsets is shedding new light on the functional diversity of intestinal MCs *in vivo* (Table 1). The resulting evidence suggests a complex mesenchymal microenvironment, with emerging key roles in postnatal maturation of the intestinal barrier, mucosal immunity, and tissue repair. We will further discuss these results in the context of intestinal inflammatory diseases, as increasing evidence suggests a role for MCs as information relays, responding to immune cell-derived signals, and directing immune function.

MESENCHYMAL-EPIHELIAL CROSSTALK IN INTESTINAL MORPHOGENESIS

A bidirectional mesenchymal-epithelial crosstalk is required for intestinal development. Intestinal morphogenesis starts in the embryo and follows a similar order of events in most vertebrates. Hedgehog (Hh) activity directs several intestinal patterning events, such as left/right patterning, radial patterning, smooth muscle differentiation, mesenchymal expansion, and villus morphogenesis¹⁸. The Hh signaling is paracrine, as Hh ligands are produced by epithelial cells, whereas their main receptor Ptch1 is exclusively non-epithelial. Starting at E13.5 in mice (weeks 8–10 in humans), the mesenchymal-epithelial crosstalk results in the formation of villi, which represents a milestone in intestinal development¹⁸. Villus formation massively increases the intestinal epithelial surface area, which will be required for sufficient nutrient absorption. Before villus morphogenesis, the pseudostratified epithelium is uniformly proliferative, but upon villi formation, epithelial proliferation becomes restricted to intervillous areas. Initiation of villus morphogenesis starts through the formation of subepithelial mesenchymal clusters, initiated by Hh ligands secreted by the intestinal epithelium. The villus mesenchymal clusters, visualized using PDPN or COL6A1, express PDGFR α , PTCH1, and the transcription factors GLI1 and GLI2, which leads to BMP2 and BMP4 expression, blocking epithelial proliferation above each mesenchymal cluster^{18–21}, while epithelial cells located between adjacent clusters remain proliferative. Several rounds of villus morphogenesis occur as new mesenchymal clusters form adjacent to the intervillous domain (Fig. 1). The Hh pathway is critical for the initiation of MC clustering, whereas both Hh ligands and PDGF-A secreted by the epithelium are essential for subsequent mesenchymal proliferation. Genetic deletion of the Hh target genes *Foxf1*, *Foxf2*, and *Foxl1* in MCs resulted in reduced villus development^{22,23}, and PDGF-A^{-/-} mice have major defects in villus morphogenesis and development of pericryptal MCs and Goblet cells¹⁹. Ligands of the Hh pathway, such as SHH (Sonic hedgehog) and IHH (Indian hedgehog), produced by epithelial cells, also mediated differentiation of α SMA^{mid} myofibroblasts and α SMA^{high} smooth muscle cells (SMCs) of the intestinal muscle layer¹⁸. In the human fetal intestine, α SMA⁺ SMCs are localized in proximity to proliferating crypts and are a source for RSPOs (R-spondins) and WNT ligands²⁴.

During late morphogenesis, the mesenchymal-epithelial crosstalk shapes intestinal regionalization. PDGFR α ^{high} MCs promoted a WNT signaling gradient, which impacted SHH epithelial expression, anterior-posterior regionalization, and villus formation²⁵. Additional signals from subepithelial MCs, including BMPs

and FGFs (Fibroblast growth factor), further drive intestinal epithelial fates and differentiation^{26–28}. In mice, differentiation into enterocytes, Goblet cells, and EECs starts in the fetus, whereas crypts containing IESCs and rapidly proliferating TA cells develop after birth (around 10–12 days postnatal), concomitant to Paneth cells²⁹. PDGFR α ⁺ MCs, partially co-expressing Foxl1, PDPN, CD34, COL6A1, and GREM1, expand around the crypts in the first postnatal weeks to promote WNT signaling^{20,21} (Fig. 1). In the colon, depletion of the Col6a1Cre⁺ mesenchymal lineage in adult mice decreased EEC differentiation²¹. Paneth cells were decreased when RSPO activity was reduced by adenoviral expression of decoy receptors or ablation of *Rspo3* in MCs³⁰. In contrast, the differentiation of Paneth cells and Goblet cells was increased in mice overexpressing interleukin (IL)-33, highly expressed by MCs and epithelial cells, and mice lacking *Il33* were more susceptible to infection with *Salmonella Typhimurium*³¹. In fetal intestine, PDGFR α ^{high} cells expressed Neuregulin (NRG1), which increased the differentiation of secretory lineages in developing human intestine enteroid cultures²⁴. As Paneth cells and Goblet cells produce notably antimicrobial peptides and mucus³², respectively, which are essential for intestinal homeostasis, dysregulation of intestinal morphogenesis might compromise the intestinal barrier.

STROMAL REGULATION OF INTESTINAL HOMEOSTASIS

Maintenance of IESCs within crypts requires a specific environment, as the fate of Lgr5⁺ IESCs is to differentiate unless both RSPO and WNT ligands are present to promote self-renewal⁵. Several stromal populations around the crypts express BMP antagonists, WNTs, and RSPO1 and RSPO2, which potentiate canonical WNT signaling by binding to Lgr4–6 receptors on IESCs⁵. The identification of novel surface markers, as well as advances in genetic lineage tracing, Cre-based gene/cell depletion, intestinal organoids co-cultures with MCs, and scRNAseq, allowed to better define the niche for IESCs. Recent findings identified pericryptal MCs and trophocytes, identified by expression of PDGFR α ^{low}, PDPN, CD34, and CD81, as major producers of canonical WNTs, the BMP antagonist GREM1, and RSPO1, RSPO2, RSPO3, which promote WNT signaling in IESCs⁵. They are localized around the crypts, as well as between the external muscle and the crypt bottom. PDGFR α ⁺ cells partially (co-)express FOXL1 and GLI1, and genetic ablation of factors required for WNT secretion in PDGFR α ⁺, FoxL1⁺, or GLI1⁺ MCs, as well as genetic depletion of FOXL1⁺ cells, induced loss of intestinal crypts and IESCs^{30,33–37}. In the villi, PDGFR α ^{high} PDPN⁺CD34⁻ subepithelial MCs, which share some similarities with telocytes previously described in several organs^{38,39}, promoted epithelial differentiation by secreting BMPs under the control of Hh ligands, produced by epithelial cells^{20,40,41} (Fig. 2). BMPs oppose WNT signaling and suppress IESCs signature genes independently of WNT signaling⁴².

ScRNAseq of the LP identified additional stromal populations in addition to PDGFR α ^{low} CD81⁺ CD34⁺ pericryptal cells/trophocytes and PDGFR α ^{high} Foxl1⁺Acta2^{low} subepithelial MCs/telocytes. These include abundant PDGFR α ^{low}CD81⁻ interstitial MCs, Cspg4 (NG2)⁺Pdgfrb⁺ pericytes, Acta2^{high}Myh11⁺Hhip⁺ SMCs in the villus and below the crypts, CD31⁺CD34⁺Lyve1⁻ vascular endothelium, CD31⁺CD34⁻Lyve1⁺ lymphatic endothelium, S100b⁺ glial cells, and Acta2^{high} Myh11⁺ Hhip⁻ external smooth muscle layer^{34,43,44}. Similar populations were found in the human colonic mesenchyme^{43,45}, although CD34 is not expressed in PDGFR α ^{low} cells, and CD81 is expressed by most

Table 1. Mice models and genetic modifications to assess the function of stromal subsets in intestinal homeostasis and disease.

Mouse model	Genetic modification	Deletion (days)	Model	Impact	Reference
Ccl19-cre	<i>Myd88^{fllox}</i> <i>Il15^{fllox}</i>	from conception	Infection (MHV/C. Rodentium)	Antiviral ILC1 and NK cell responses in PPs/mLNs Intestinal inflammation	61
Ccl19-cre Col6a1-cre	<i>Ltbr^{fllox}</i> <i>Tnfr1^{fllox}</i> <i>Tnfr1^{cneo/-}</i> <i>Tnfsf11^{fllox}</i>	from conception	Steady-state Infection (M-CoV)	PPs development/stromal organization Antiviral immune response	77
Col6a1-cre Twist2-cre	<i>Tnfsf11^{fllox}</i>	from conception	Steady-state	Intestinal immune homeostasis	59,60
Col6a1-cre	<i>Tnfr^{ΔARE}</i> <i>Tnfr1^{flxneo}</i>	from conception	Steady-state	Intestinal inflammation Joint inflammation	105
Col6a1-cre	<i>Rosa26^{flstopiDTR}</i>	Adult (3d)	Steady-state Inflammation (DSS)	Reduced enteroendocrine cells/homeostatic proliferation No effect on intestinal regeneration	21
Col1a2-cre^{ERT2}	<i>Map3k2^{fllox}</i>	Adult (10d)	Inflammation (DSS)	Intestinal inflammation	99
Foxl1-cre Foxl1-hDTR	<i>Rosa26^{flstopiDTR}</i>	Adult (3d)	Steady-state	Reduced intestinal stem/progenitor cells	33
Foxl1-cre^{ERT2}	<i>Porcn^{fllox}</i>	Adult (1-3d)	Steady-state	Reduced intestinal stem/progenitor cells	35
Gli1-cre^{ERT2}	<i>Wls^{fllox}</i>	Adult (15-21d)	Steady-state	Reduced intestinal stem/progenitor cells	34
Grem1-cre^{ERT2}	<i>Rosa26^{flstopiDTR}</i>	Adult (2d)	Steady-state	Intestinal damage /lethal	41
Lepr-cre	<i>Rosa26^{flstopiDTR}</i> <i>Igf1^{fllox}</i>	Adult (5-10d) From conception	Steady-state Irradiation	Reduced intestinal stem/progenitor cells Impaired intestinal regeneration	49
Lgr5-cre^{ERT2}	<i>Rosa26^{flstopDTA}</i>	Adult (7d)	Steady-state	Dysregulated villus tip VEGFA signaling	52
Lgr5-GFP-DTR		Adult (1d)	Steady-state	Dysregulated villus tip enterocytes gene expression	51
Ltbr^{TA} x tetO-cre (LC1)	<i>Pdgfra^{fllox}</i>	from birth until 28d	Postnatal P28	Increased intestinal stem/progenitor cells Reduced Goblet cells Dysregulated inflammation and repair responses	85
Pdgfra-cre	<i>Porcn^{fllox}</i> <i>Rspo3^{fllox}</i>	from conception	Postnatal P5 Steady-state Inflammation (DSS)	Reduced intestinal stem/progenitor cells Reduced Paneth cells Impaired intestinal regeneration	30
Pdgfra-cre Grem1-cre^{ERT2} Col1a-cre^{ERT2}	<i>Il1r1^{fllox}</i>	from conception Adult	Infection (C. Rodentium) Inflammation (DSS)	Intestinal inflammation/regeneration	101
Twist2-cre Myh11-cre	<i>MiR-143/145^{fllox}</i>	from conception	Inflammation (DSS)	Impaired intestinal regeneration	92

DTR = diphtheria toxin receptor; DSS = Dextran sulfate sodium; Indo = Indomethacin; M-CoV = Murine coronavirus; MHV = Mouse hepatitis virus; ILC1 = Innate lymphoid cells 1; NK = Natural killer; mLNs = mesenteric lymph nodes; PPs = Peyer's patches .

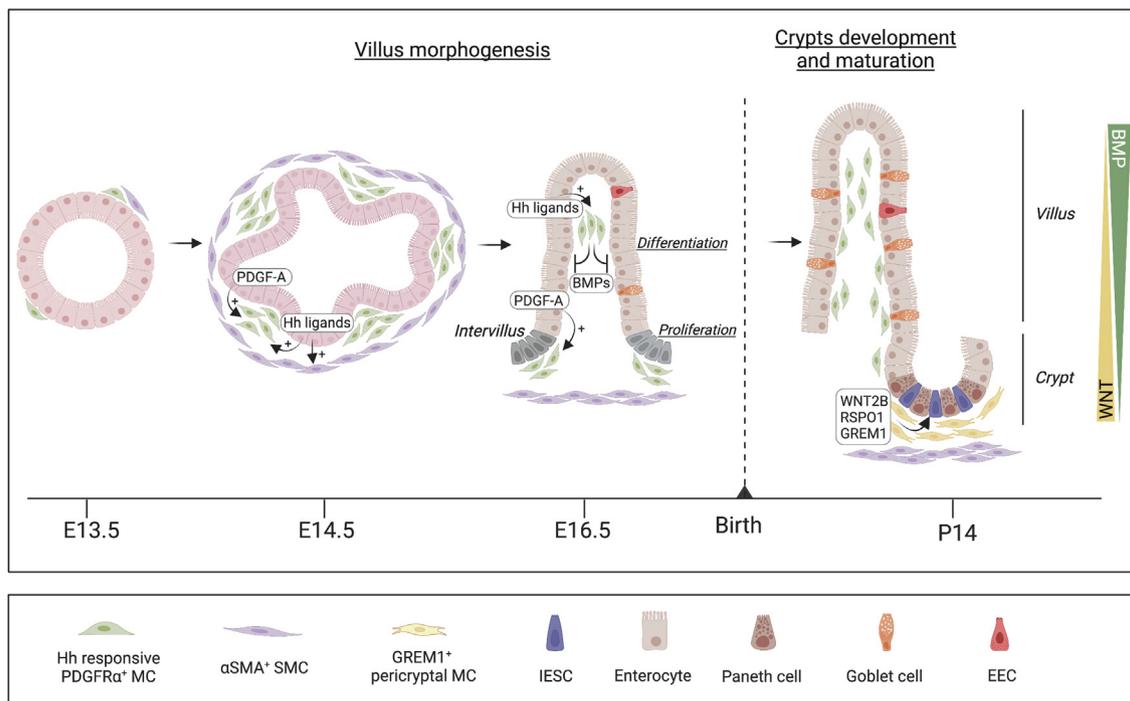


Fig. 1 Epithelial-mesenchymal crosstalk in mouse intestinal morphogenesis. Intestinal development starts in the embryo and requires a tightly regulated crosstalk between a developing epithelial layer and underlying Hh-responsive MCs and SMCs, which orchestrate epithelial cell proliferation, migration, and differentiation. In mice, differentiation into enterocytes, Goblet cells, and EECs starts in the fetus, whereas mature crypts containing IESCs develop after birth, concomitant to development of Paneth cells and a specialized stromal niche locally promoting WNT signaling. BMPs = bone morphogenetic proteins; EECs = enteroendocrine cells; Hh = Hedgehog; IESCs = intestinal epithelial stem cells; MCs = mesenchymal cells; PDGF-A = platelet-derived growth factor A; SMCs = smooth muscle cells.

PDGFR α ⁺ MCs. Recently, intestinal lymphatics have been shown to contribute to crypt homeostasis and repair by secreting pro-stemness factors RSPO3, WNT2, and Reelin^{46–48}. Therefore, the mesenchymal/vascular compartment seems to have a key role in coordinating the maintenance and repair of the IESCs niche (Fig. 2). As no single marker is restricted to a single mesenchymal or vascular cell type, Cre-driven gene or cell depletion should be interpreted accordingly. Nevertheless, most studies agree on the peri/subcryptal localization of GREM1⁺ MCs, which can be isolated by flow cytometry using coexpression of PDGFR α and CD81, or PDPN and CD34, within the CD45[−]CD31[−] population²⁰. A summary of the major mesenchymal and vascular intestinal populations and their markers is provided in Fig. 3.

Additional markers have been useful in characterizing the LP mesenchyme. In the crypt region, a subset of PDGFR α ⁺ FOXL1[−] GLI1[−] MCs expressing leptin receptor (LEPR⁺)^{49,50} has been shown to expand after irradiation. LEPR⁺ cells were essential for intestinal homeostasis and regeneration in a mechanism dependent on IGF1⁴⁹. Interestingly, the number of LEPR⁺ cells was modulated by diet, suggesting that alteration of niche MCs by diet (fasting, HFD) represents an additional mechanism to regulate IESC and intestinal homeostasis. In the villus tip, recent studies identified LGR5 positive PDGFR α ^{high} mesenchymal subsets. Subepithelial LGR5⁺ cells promoted enterocyte differentiation, presumably by affecting the production of BMP ligands, the non-canonical WNT ligand *Wnt5a* and the matrix⁵¹. Perivascular LGR5⁺ ADAMTS18⁺ cells maintained a functional endothelial cell polarization and fenestration in the villus tip by restricting fibronectin accumulation and regulating ECM-bound VEGFA⁵².

MESENCHYMAL CELLS OF GUT-ASSOCIATED LYMPHOID TISSUES

Effector intestinal immune cells disseminate throughout the LP and epithelial layer, whereas mostly naive cells cluster in the GALTs, such as Peyer's patches (PPs) in the small intestine and isolated lymphoid follicles (ILFs) in the small intestinal and colonic mucosa. GALTs are local sites for the generation of intestinal effector lymphocytes, such as cytokine-producing T cells and immunoglobulin (Ig) A-secreting B cells⁵³. In addition to immune cells, GALTs contain a fine reticulum of heterogeneous mesenchymal populations, mostly PDPN⁺ PDGFR α ^{med/low}, which share similarities with FRCs of secondary lymphoid organs (SLO)⁵⁴. In SLOs, FRCs are major producers of chemokines, matrix proteins, survival factors and adhesion molecules essential for DC-T cell interaction, antigen encounter, lymphocyte positioning, survival, and differentiation. FRCs of SLO and GALTs are essential players in immune homeostasis and induction/regulation of adaptive immunity^{55–57}.

In the small intestine, ILFs mesenchymal subsets include LEPR⁺ PDGFR α ⁺ telocytes at the top of follicle-associated epithelium, distinct from villus tip telocytes expressing *Lgr5*⁵⁸. Deletion of *Rankl* in TWIST2⁺ cells or COL6A1⁺ cells, which include several stromal and vascular populations, including MCs in the subepithelial dome (SED) of GALTs, impaired M cell-dependent antigen sampling and B cell-dendritic cell interaction in the SED, decreasing IgA production and microbial diversity^{59,60}. MCs of the CCL19⁺ lineage, which also labels FRCs of the PP, regulate the murine coronavirus (M-CoV) infection via IL15-mediated control of group 1 innate lymphoid cells (ILCs)⁶¹. Disruption of mechanosensing in CCL19⁺ MCs by the deletion of *Piezo1* disorganized the conduit associated perivascular FRCs and high

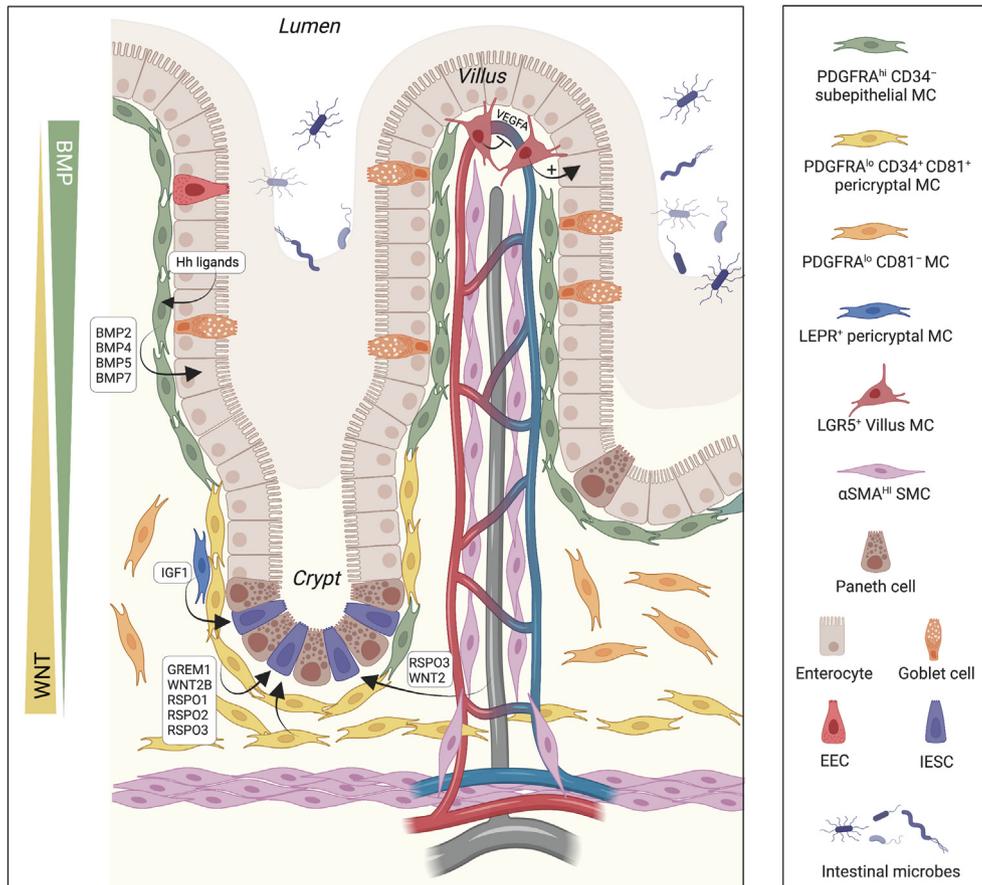


Fig. 2 Stromal regulation of intestinal homeostasis. Several stromal populations around the crypts contribute to IESC maintenance by locally promoting WNT signaling, including pericryptal and subcryptal PDGFR α^{low} CD34 $^+$ MCs (yellow), which are the major producers of RSPOs, WNT2B, and the BMP antagonist GREM1, as well as Lyve1 $^+$ lacteals (gray). In the villi, PDGFR α^{high} CD34 $^-$ subepithelial MCs (green) oppose WNT signaling and promote epithelial differentiation by secreting BMPs under control of Hh ligands, produced by epithelial cells. The expression of LGR5 and LEPR identifies specific subpopulations of subepithelial or pericryptal MCs. BMPs = Bone morphogenetic proteins; EECs = Enteroendocrine cells; Hh = Hedgehog; IESCs = Intestinal epithelial stem cells; LEPR = Leptin receptor; MCs = Mesenchymal cells; PDGFR = Platelet-derived growth factor receptor; RSPOs = R-spondins; SMCs = Smooth muscle cells.

endothelial venules, impairing lymphocyte entry into PPs and initiation of mucosal antibody responses⁶². Lymphotoxin-driven activation of CCL19 $^+$ FRCs also played a role in the maturation of cryptopatches (CPs) into ILFs, affecting ILC homeostasis and function⁶³. In the colon, CD34 $^-$ PDPN $^+$ MCs expressing the oxysterol 7 α ,25-OHC-synthesizing enzymes *Ch25h* and *Cyp7b1* were localized to ILFs, whereas CD34 $^+$ PDPN $^+$ MCs, which are abundant outside of ILFs, expressed higher levels of the oxysterol degrading enzyme *Hsd3b7*. The crosstalk between these two cell types might contribute to the localized 7 α ,25-OHC gradient required for migration of GPR183 $^+$ ILC3 and inflammatory tissue remodeling during colitis⁶⁴.

MESENCHYMAL/VASCULAR REGULATION OF THE POSTNATAL INTESTINAL BARRIER

In the perinatal period, the maturation of intestinal epithelial, immune, and vascular components is required to ensure digestive and barrier functions and balanced immunity (Fig. 4). Several of these processes are regulated by the intestinal microbiota that colonize the intestine after birth^{65–68}. The human gut mucosa exhibits a fully differentiated intestinal structure at birth in contrast to mice. Although there are differences

in gut maturity at birth, both humans and mice need to transition from a protected environment devoid of microbiota to a mature intestine, colonized with commensal and potentially pathogenic microorganisms. In the neonatal intestine, these structural differences, including lack of regenerative crypts/mature Paneth cells and decreased epithelial cell turnover have implications for infection. Indeed, rapid surface renewal in the adult intestine, as well as Paneth cell-derived antimicrobial peptides, represent a first line of protection from enteric pathogens^{69,70} (Fig. 4A).

This is particularly important as the murine neonatal intestine has an immature immune system, mostly devoided of adaptive immune cells at birth except for a fetal wave of $\gamma\delta$ T lymphocytes, a major source for IL17^{71,72}. These lymphocytes reside in the LP, as, unlike human neonates, mice do not contain epithelium-associated lymphocytes until weaning^{73,74}. Intestinal lymphoid tissue structures develop in the first few weeks after birth in contrast to mesenteric lymph nodes and Peyer's patches anlagen, which develop prenatally^{75,76}. During the first postnatal weeks, T and B lymphocytes recruited at the site of PP anlagen induce expansion and differentiation of PP-associated FRC-like cells. The mesenchymal network of the PP develops from fetal

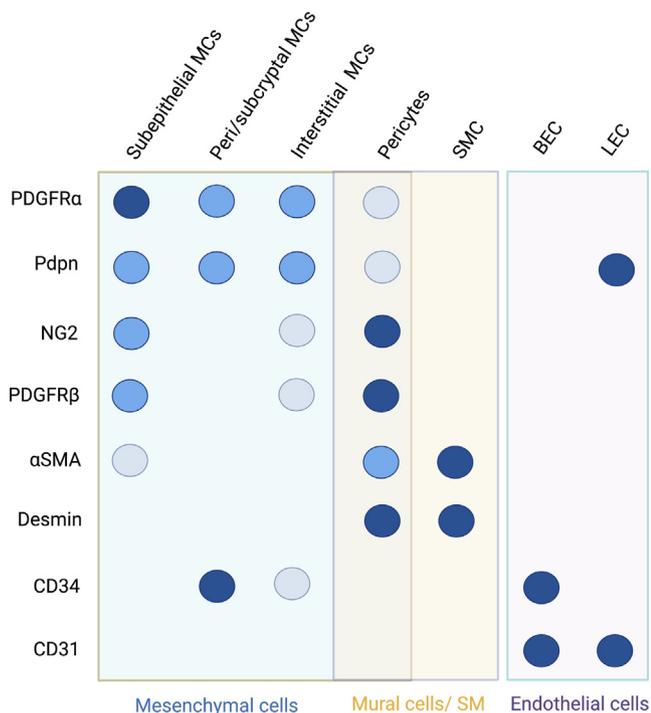


Fig. 3 Major mesenchymal and vascular cell types of the intestinal lamina propria. Subepithelial MCs, also known as telocytes or subepithelial myofibroblasts, express high levels of PDGFRα and are the major producers of BMPs. PDGFRα^{low/mid} cells are the most abundant and heterogeneous population, including pericryptal and subcryptal MCs (also known as trophocytes), which ensure a pro-stemness environment, interstitial MCs, and GALT-associated MCs. Mural cells refer to populations of the blood vessel wall, such as pericytes. It is noteworthy that, although the combination of several markers and localization allows for discrimination between these populations, no single marker identifies a unique cell type. Dark and light circles denote high and low expression. BECs = Blood endothelial cells; LECs = Lymphatic endothelial cells; MCs = Mesenchymal cells; PDGFR = Platelet-derived growth factor receptor; SMCs = Smooth muscle cells; SM = Smooth muscle.

perivascular CCL19⁺ and subepithelial COL6A1⁺ mesenchymal lineages, which colonize distinct zones of the PP in an LTBR and tumor necrosis factor (TNF)R1-dependent manner. Synergistic and partially compensatory mechanisms between both lineages ensure the IgA-dependent regulation of commensal microbiota and adaptive immune responses against intestinal viral infection⁷⁷.

A prerequisite for postnatal intestinal growth is the capacity to establish a functional vasculature. The microbiota has been shown to regulate the vasculature through different mechanisms, including signaling through a bacteria-sensing epithelial cell type⁷⁸, by promoting Tissue Factor (TF)–protease-activated receptor (PAR1) signaling⁷⁹, through activation of Toll-like receptors (TLRs) and NOD-like receptors in endothelial cells and MCs⁸⁰, as well as by inducing VEGF-C (Vascular endothelial growth factor C) in villus macrophages⁸¹. In zebrafish, which have similar intestinal organization, the microbiota promoted intestinal angiogenesis, mesenchymal metabolism, and induction of leucocytes recruiting chemokines in vascular SMCs⁸².

Concomitant to immune and vascular maturation, the perinatal intestinal epithelium undergoes major changes, including the

formation of crypts containing stem cells and the development of Paneth cells²⁹. PDGFRα⁺ MCs, partially co-expressing Foxl1, PDPN, CD34, COL6A1, and GREM1 expanded around the crypts in the first postnatal weeks^{20,21}. In agreement with a progenitor role of these cells, inducible lineage tracing of GREM1⁺ cells using the Grem1Cre^{ERT} mouse model showed that early GREM1⁺ MCs generate most subepithelial intestinal MCs within 1 year⁸³. PDGFRα^{-/-} mice are embryonic lethal, which prevented investigations of the role of PDGFRα in the postnatal maturation of the intestinal mesenchyme^{19,84}. This question was recently investigated using a novel mouse model allowing for inducible lineage tracing and gene depletion in Lymphotoxin β receptor (LTBR)-expressing cells⁸⁵. Lineage tracing of LTBR⁺ progenitors identified a distinct PDGFRα^{high} subepithelial mesenchymal lineage developing around 2 weeks after birth in the small intestinal villi in a process dependent on the microbiota. The PDGFRα^{high} LTBR lineage expressed higher levels of genes promoting immunoregulation and epithelial differentiation compared with PDGFRα⁺ MCs not generated from LTBR⁺ progenitors, which had a pro-stemness/pro-inflammatory gene signature. These distinct transcriptomic identities were maintained after injury, suggesting that the damage response of PDGFRα⁺ MCs is impacted by their origin or period of development⁸⁵ (Fig. 4A).

PDGFRα has a major impact on the postnatal mesenchymal maturation and fate. Indeed, conditional ablation of *Pdgfra* in the LTBR mesenchymal lineage prevented the acquisition of factors promoting epithelial differentiation and immunoregulation, including the Hh ligands receptor *Ptch1*, BMPs, basement membrane (BM) proteins, laminins, retinoic acid (RA) synthesis enzymes, *Il33*, as well as proper spatial arrangement within the crypt-villus⁸⁵. Such defect in mesenchymal maturation reduced postnatal growth and disrupted intestinal homeostasis, leading to increased IESCs and proliferative cells and decreased Goblet cells, mature enterocytes, and CD11b⁺CD103⁺ dendritic cells (DCs), which play a key role in neonatal immunity^{86,87}. Overall, lack of stroma maturation dysregulated repair responses and increased susceptibility to intestinal inflammation in young individuals⁸⁵ (Fig. 4B).

These data suggest that distinct PDGFRα⁺ lineages with pro-differentiation/immunoregulatory (PTCH1^{high}) or pro-stemness/inflammatory (PTCH1^{-/low}) functions are established in the first few weeks after birth and that their relative abundance determines the inflammatory status of the intestine. This is consistent with epithelial Hh ligands acting as key sensors for epithelial integrity and the observed downregulation of Hh signaling pathway in patients with IBDs^{88,89}. Therefore, postnatal maturation of intestinal mesenchymal/vascular elements seems to represent an essential step in the development of a functional intestinal barrier and is at least partially regulated by the microbiota (Fig. 4).

STROMAL REGULATION OF INTESTINAL REGENERATION AND INFLAMMATION

Tissue damage induces activation of a regenerative program to restore epithelial integrity. In the intestine, this response involves stem cells, as well as the secretory/absorptive lineages, which have the potential to revert to a stem cell state, and quiescent label-retaining cells⁹⁰. The intestinal regenerative response is tightly regulated by the microenvironment, including immune cells, enteric neuronal cells, the extracellular matrix, the diet, and the microbiome^{90,91}. In addition, the mesenchymal

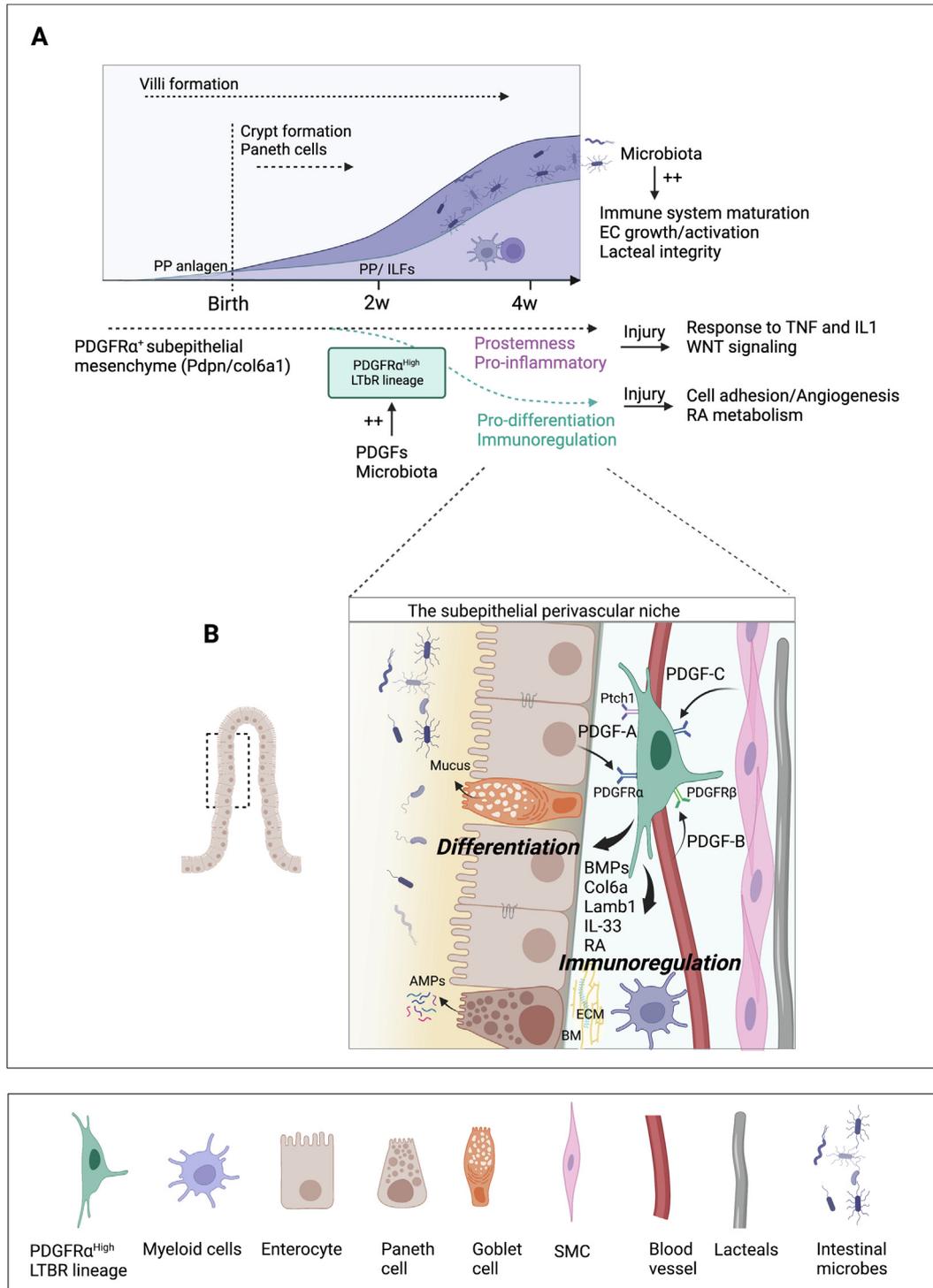


Fig. 4 Stromal/vascular regulation of the postnatal intestinal barrier. (A) Microbial colonization of the intestine after birth plays a major role in maturation of immune, vascular, and stromal components. At birth, the murine neonatal intestine is mostly devoid of adaptive immune cells. Intestinal lymphoid tissue structures develop in the first few weeks after birth, whereas mesenteric lymph nodes and Peyer's patches anlagen develop prenatally. In the first few weeks after birth, PDGFs play an essential role in the maturation of PDGFR α^{high} MCs by inducing pro-differentiation and immunoregulatory genes, including BMPs, RA, and laminins (B), as well as on spatial stromal organization within the crypt-villus. Such program is essential to promote intestinal homeostasis/barrier function and prevent dysregulated repair and inflammatory responses following injury. BM = Basement membrane; BMPs = Bone morphogenetic proteins; IL = Interleukin; PP = Peyer's Patch; MCs = Mesenchymal cells; EC = Endothelial cells; PDGFR = Platelet-derived growth factor receptor; LT β R = Lymphotoxin beta receptor; RA = Retinoic acid; SMCs = Smooth muscle cells; TNF = Tumor necrosis factor; AMPs = Antimicrobial peptides; ECM = Extracellular matrix.

compartment is recently emerging as a critical player in intestinal regeneration and inflammation.

Several factors essential for regeneration have been identified. Knockout of the microRNAs miR-143/145 using Twist2Cre mice, which mostly label SMCs of the muscularis propria and α SMA⁺ myofibroblasts, resulted in disorganized regenerative responses, loss of epithelial proliferation, and exacerbated dextran sodium sulfate (DSS)-induced colitis. MiR-143 functions as a cell-autonomous tumor suppressor in colon cancer and was found to derepress *Igfbp5*, which impaired IGF1 signaling after injury⁹². IGF1 is a potent growth and repair factor in intestinal regeneration following resection, radiation injury, and DSS-induced colitis. Using a diphtheria toxin-mediated cell depletion model, ablation of LEPR⁺ crypt MCs and their progeny in LeprCre^{Rosa26-IDTR} mice impaired regeneration following irradiation in a mechanism dependent on IGF1⁴⁹. LEPR⁺ cell-derived WNT2B also promoted regeneration during colitis⁵⁰.

An additional factor supporting colon regeneration after DSS-induced damage is prostaglandin E2 (PGE2), produced by intestinal MCs^{20,76,93}. PGE2 enhanced YAP (Yes1 associated transcriptional regulator) activity, which promoted colon regeneration by increasing the expression of the PGE2 synthesis enzyme COX-2 and the prostaglandin EP4 receptor⁹⁴ but also induced colon tumorigenesis through mechanisms dependent on YAP and Gs-axin- β catenin signaling pathways^{94–96}. Of note, *Sco2a1*-deficiency (which increases PGE2 concentrations) was associated with small intestinal inflammation/ulcerations in humans and increased sensitivity to colitis in mouse models⁹⁷, suggesting a double-edged mechanism. During the first days after injury in mice, the non-canonical WNT5A ligand produced by wound adjacent MCs was required for transient inhibition of epithelial proliferation, an essential step in crypts regeneration⁹⁸. Although the identity of the WNT5A-producing MCs remains unclear, the PDPN⁺CD34⁻ cells produced higher levels of *Wnt5a* than pericryptal PDPN⁺CD34⁺ cells in DSS-induced colitis²⁰.

The mesenchymal niche also maintains the stem cell compartment while regulating epithelial cell proliferation. Following DSS-induced inflammation, mesenchymal populations localized in the proximity of crypts, including PDPN⁺CD34⁺ MCs, trophocytes, GL1⁺ and PDPN⁺CD90⁺ MCs, overexpressed pro-stemness factors such as *Grem1*, *Rspo1*, and *Rspo3*^{20,34,41}. Several triggers activate such a protective mesenchymal response. Upregulation of *Rspo1* by PDPN⁺CD90⁺ MCs after DSS was dependent on *Map3k2* and ROS (Reactive oxygen species) signaling, and deletion of *Map3k2* in COL1A1-expressing cells using Col1a2Cre^{ERT2}*Map3k2*^{fl/fl} increased the severity of DSS-induced colitis⁹⁹. Additional factors promoting expression of pro-stemness factors include the pro-inflammatory cytokine IL1, consistent with the hypothesis that similar pathways are engaged in inflammation and regeneration^{85,90}. IL1R1 was highly expressed by pericryptal GREM1⁺PDGFR α ⁺ MCs¹⁰⁰, and IL1R1 activation induced upregulation of *Rspo3* during infection to protect stem cells.

The mesenchymal crosstalk with immune cells is tightly regulated and most likely adapted to the damage intensity. Indeed, IL1 stimulation of GREM1⁺ *Rspo3*-expressing cells was sufficient for epithelial repair in DSS-induced colitis but required IL22 production by ILCs, also expressing IL1R1, to promote epithelial proliferation and repair in intestinal infection driven by *Citrobacter rodentium*¹⁰⁰. In a model of *Listeria (Lm)* infection, the crosstalk

of several immune subsets and PDPN⁺ MCs, which produced IL11, was required for STAT3-dependent epithelial response and depletion of Goblet cells, overall leading to decreased *Lm* villus invasion but higher susceptibility to colitis¹⁰¹. In the colon, oxysterol production by PDPN⁺CD34⁻ MCs, abundant in ILFs and CPs, played a key role in the recruitment of ILC3s expressing GPR138. Increased oxysterol production during inflammation increased susceptibility to colitis through GPR183-mediated cell recruitment⁶⁴.

MESENCHYMAL CELLS IN INTESTINAL INFLAMMATORY DISEASES

In chronic conditions, continuous cycles of regeneration lead to dysregulated inflammatory and repair responses, as observed in IBDs, including Crohn's disease (CD) and ulcerative colitis (UC). Recent scRNAseq studies shed new light on the heterogeneity of the mesenchymal compartment in patients with IBD, suggesting a dysregulated mesenchymal crosstalk with leucocytes, epithelial cells, and the extracellular matrix (ECM).

Single-cell RNAseq analysis of the colonic mesenchymal compartment in healthy controls and patients with UC identified several mesenchymal clusters expressing non-fibrillar collagens, matricellular proteins, and BMP/WNT factors involved in the formation of basement membrane and epithelial stemness/differentiation. One cluster overexpressing PDPN, CCL19, and IL33 strongly expanded in patients with UC⁴³, suggesting a causal role in UC disease pathogenesis or a reaction to promote repair. A similar cluster in DSS-induced colitis overexpressed *Lox* and *Lox11*, which catalyze covalent crosslinking of collagen and elastin, generating hydrogen peroxide as a by-product⁴³. Blockade of *Lox* enzymes reduced the severity of DSS colitis most likely due to the impact on redox processes or matrix remodeling⁴³.

Genome-wide association studies (GWASs) revealed risk alleles for UC. A cell atlas from the colon mucosa of 18 patients with UC and 12 healthy individuals identified a subset of IL11⁺-IL13RA2⁺ inflammatory fibroblasts associated with resistance to anti-TNF treatment⁴⁵. One of the most enriched genes in inflammatory fibroblasts is the Oncostatin M receptor (OSMR), which is also a putative risk gene for IBD¹⁰². OSM is highly expressed in myeloid cells, which also expanded during inflammation, further providing a link between inflammatory fibroblasts and OSM-mediated resistance to anti-TNF treatment¹⁰³. Of note, TNF-induced response on MCs was sufficient to induce several chronic inflammatory pathologies, including in IBD¹⁰⁴. A similar myeloid-mesenchymal interaction was recently shown in patients who carry CD-risk alleles of *NOD2*¹⁰⁵. Loss of *NOD2* dysregulated homeostasis of activated fibroblasts and macrophages, notably through elevated STAT3 and gp130 receptor ligands such as OSM and IL6, potentially driving fibrosis¹⁰⁶. Therefore, the blockade of gp130 family members has been suggested to represent an alternative treatment for selected patients with CD by breaking up the circuit of fibroblast-immune cell interactions. In patients with ileal CD, scRNAseq identified pathogenic cellular modules associated with resistance to anti-TNF therapy, which included pro-inflammatory MCs and endothelial cells overexpressing IL6 and chemokines recruiting neutrophils and monocytes¹⁰⁷. Development of mesenchymal and endothelial cells enriched in inflammatory, matrix remodeling, and Type I interferon signatures was also identified in pediatric patients with IBD¹⁰⁸.

Increasing evidence points to a dysregulated crosstalk between activated MCs expressing cytokines such as IL6 and IL11, which respond to IL1 β and OSM (mostly expressed by myeloid cells), leading to dysregulated inflammation/regeneration cycles and epithelial barrier dysfunction, key characteristics of IBD. In addition, MCs are also major producers of structural and regulatory proteins of the ECM, including collagens, proteoglycans, BM proteins, and enzymes involved in ECM degradation and remodeling. As several elements of the ECM regulate immune cell recruitment, as well as blood vessels and epithelial cell integrity, local changes in ECM composition have most likely an impact on intestinal homeostasis and inflammation^{109,110}. Changes in the ECM might also have systemic implications, as studies suggested that the association between skin inflammation and IBD involves recognition by colonic MC of hyaluronan (HA) fragments released in skin inflammation¹¹¹. HA has also been suggested to play a role in reactive colon adipogenesis and host defense after injury¹¹².

CONCLUSION

As key regenerative cells of the intestine, IESCs receive signals from an “expanded” niche, which includes stromal cells, paneth cells, immune cells, and the microbiota¹¹³. The type of injury, intestinal stress, age, and differences in the microbiota might further affect their niche function. Concerning the recent scRNA-sequencing experiments, it will be interesting to investigate whether and to which extent the mesenchymal clusters identified in different studies are similar, map their localization within the tissue during disease progression and define lineage relationships.

Overall, it remains unclear how to specifically target fibrotic or inflammatory MCs in pathologies. In several organs, MCs close to blood vessels have been identified as major contributors to scar tissue and fibrosis^{14,114}. The development of more specific Cre-based mice models might help in identifying and targeting pathological mesenchymal subsets while protecting the normal mesenchyme (in the intestine as in other organs), which is required to maintain epithelial, vascular, and immune homeostasis and for tissue repair. A “normalization” of the mesenchymal compartment, rather than targeting specific molecules, represents a promising alternative approach but requires a better understanding of the biology of MCs, which is still only partially understood.

AUTHOR CONTRIBUTIONS

All authors contributed to defining the review content and figures. LP wrote the manuscript.

DECLARATION OF COMPETING INTERESTS

The authors have no competing interests to declare.

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