Intestinal stromal cells in mucosal immunity and homeostasis

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A growing body of evidence suggests that non-hematopoietic stromal cells of the intestine have multiple roles in immune responses and inflammation at this mucosal site. Despite this, many still consider gut stromal cells as passive structural entities, with past research focused heavily on their roles in fibrosis, tumor progression, and wound healing, rather than their contributions to immune function. In this review, we discuss our current knowledge of stromal cells in intestinal immunity, highlighting the many immunological axes in which stromal cells have a functional role. We also consider emerging data that broaden the potential scope of their contribution to immunity in the gut and argue that these so-called “non-immune” cells are reclassified in light of their diverse contributions to intestinal innate immunity and the maintenance of mucosal homeostasis.

INTRODUCTION

Immune responses at the intestinal mucosa are tightly controlled to remain tolerant of the vast commensal microbiota, while simultaneously retaining the capacity to respond appropriately to harmful insult. Multiple cellular and molecular processes have evolved that contribute to this critical balance, although there is still much to be revealed regarding the precise mechanisms by which immunity and homeostasis in the gut is achieved.

The vast majority of our knowledge regarding intestinal immune homeostasis and its breakdown in inflammatory disease has concerned the role of specialized hematopoietic “conventional” immune cell populations—both in isolation and in concert with the epithelial monolayer that comprises the functional barrier between the host and the microbiota.¹ This focused research has revealed several mechanisms by which aberrant hematopoietic immune cell functional pathways contribute to human disease, including defective nucleotide-binding oligomerization domain-containing protein 1 (NOD1) and NOD2—induced autophagy and bacterial handling by myeloid cells;^{2,3} functional polymorphisms in the interleukin (IL)-23 receptor (IL-23R);⁴ loss-of-function mutations in the IL-10R;⁵ and variants in the inflammasome activator NOD-like receptor family, pyrin domain-containing 3 (NLRP3),⁶ alongside many others. Although they have a crucial role in

immunity and tolerance in the gut, hematopoietic immune and epithelial cells do not function in isolation but rather as part of a complex cellular network that contains several other distinct non-hematopoietic cell populations.

Residing in the sub-epithelial compartment and providing much of the structural framework of the intestine are multiple populations of non-hematopoietic mesenchymal cells, distinct from the epithelium, that can be grouped as stromal cells—a nomenclature similar to that assigned to the cellular networks at the heart of secondary lymphoid organs.⁷ Intestinal stromal cells dynamically interact with both epithelial and hematopoietic immune cells at this mucosal site and, although not considered “professional” immune cells, there is mounting evidence that they can perform many of the functions often solely attributed to their hematopoietic immune cell counterparts.⁸ In this review, we move beyond the previously well-documented role of stromal cells in intestinal fibrosis, tumor progression, and wound healing, focusing instead on the immunological functions that support their re-designation as *de facto* intestinal immune cells.

Intestinal stromal cells: ontogeny, location, and support of the intestinal epithelium

The mesenchymal compartment of the intestinal lamina propria contains multiple stromal cell populations with distinct

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localization, phenotype, and function. These include fibroblasts, myofibroblasts, pericytes, endothelial cells, and smooth muscle cells,⁹ in addition to less well-characterized populations that may represent distinct stromal subsets and suggests hitherto unknown phenotypic and functional heterogeneity within this compartment. The vast majority of published studies relating to immunological functions of the intestinal mesenchymal cell compartment have focused on cells of the fibroblast family, in particular, the sub-epithelial myofibroblast. Although these cells likely have a major role in this context, taking a similar intellectual approach to that used in lymphoid tissue stromal cell biology,⁷ other intestinal mesenchymal cell subsets—including smooth muscle cells, vascular endothelial cells, and lymphatic endothelial cells—can also be considered as stromal cell populations. As our current understanding of the phenotypic features and anatomical location of intestinal stromal cell subsets have been extensively covered recently,^{8,9} in this review we consider intestinal stromal cells in a very broad sense and focus on the functional contributions of multiple subsets to innate immunity and homeostasis in the gut.

Despite exhibiting features of non-hematopoietic mesenchymal cells, such as abundant collagen production, expression of vimentin and α -smooth muscle actin (α SMA) filaments, and a lack of surface CD45 expression,⁹ there is currently debate as to the precise origins of intestinal stromal cells. It appears that such cells can arise from both mesenchymal and hematopoietic stem cells,^{10,11} at least in murine models, although the relative contributions of each source to the differentiated stromal pool in the intestine are not yet defined. During chronic inflammation, tissue mesenchymal cell populations can also arise from circulating myeloid-lineage cells known as fibrocytes,¹² with several alternative sources of tissue stromal cells reported in both steady-state and disease contexts, including the inflamed colon.^{13,14} Furthermore, recent evidence suggests that in experimental animals, intestinal epithelial cells can act as a direct progenitor of fibroblasts during ongoing tissue fibrosis *in vivo*, converting to stromal cells via a process of epithelial-to-mesenchymal transition that is reciprocally regulated by transforming growth factor β 1 (TGF β 1) and bone morphogenetic protein-7.¹⁵ However, as such processes are currently still poorly defined for the intestine, detailed *in vivo* lineage tracing and fate mapping approaches are required in order to determine the precise ontogeny of intestinal stromal cell populations in both homeostatic and inflammatory situations.

Adding to the confusion over intestinal stromal cell origin, there is currently much interest in the therapeutic potential and immune functionality of mesenchymal stem cells (MSCs), often referred to in the literature as “mesenchymal stromal cells”.^{16,17} MSCs are multipotent progenitor cells that are distinct from fully differentiated, intestinal-resident non-hematopoietic cells. Nevertheless, a better understanding of MSC function is likely to be beneficial in the field of intestinal stromal cell biology, particularly with regard to the delineation of stromal cell differentiation *in vivo* and the modulation of resident mesenchymal cell abundance/function in the context of stem cell transplantation for inflammatory bowel disease (IBD).¹⁰

The sub-mucosal localization of intestinal stromal cells means that they are critically placed to contribute to a range of homeostatic processes in the gut. Stromal cells form a dense network directly adjacent to and underlying the epithelium of the small and large intestine (Figure 1a,b, Pinchuk *et al.*⁸ and Powell *et al.*⁹). As this localization suggests, they interact through the basement membrane with various epithelial cell populations and have a critical role in supporting their proliferation and function.¹⁸ This relies on multiple complex interactions, mediated mostly by soluble mediators, and includes communication between stromal cells and epithelial stem cells,¹⁹ with these essential interactions often disrupted as a result of IBD.²⁰ Recent data has clarified some of the mechanisms involved in this homeostatic axis, revealing a major role for stromal cell-derived *Wnt* in epithelial stem cell homeostasis.²¹ Interestingly, stromal cell support of intestinal epithelial differentiation and function is not merely a stationary, passive process. In an elegant study using a murine model of colitis, Brown *et al.*²² revealed that stromal cells actively redistribute to the peri-cryptal area during inflammation, produce prostaglandin- E_2 , and initiate epithelial renewal after damage to the barrier. However, these cells appear to have features of MSCs, rather than a differentiated stromal cell subset.²³

In addition, stromal–epithelial interactions appear to be bi-directional: Indian hedgehog produced by intestinal epithelium is critical for the regulation of stromal cell development and proliferation in the mouse, and these stromal cells, in turn, regulate the faithful differentiation and regeneration of the intestinal epithelium.²⁴ This process must be tightly regulated, as constitutive activation of hedgehog signaling in the colon leads to an accumulation of myofibroblasts, loss of epithelial precursors, and crypt abnormalities in mice,²⁵ highlighting the critical importance of dynamic stromal–epithelial interactions for intestinal barrier function and repair. Although it is currently unclear whether stromal cells have a similar role in regulating epithelial function or regeneration after challenge with a mucosal pathogen, the potential innate immune roles of stromal cells are discussed in more detail later in this review.

The beneficial contributions of intestinal stromal cells to the physiological processes of epithelial development and regenerative responses to barrier damage are clear; however, when inappropriately regulated or activated, stromal cells can also contribute to many of the pathological mechanisms operating in the context of intestinal fibrosis and dysregulated mucosal healing in the gut. These areas of intestinal stromal cell biology have been the focus of intensive research over the years and are reviewed in detail elsewhere.^{11,26,27} However, in direct relevance to a more “classical” immunological role, stromal cell activation and phenotypic modulation also appears to occur more generally during chronic inflammatory immune-mediated disease.

Activation of stromal cells during inflammatory pathology

Stromal cell activation is implicated in a number of chronic inflammatory disorders of multiple tissues, including the lung,

Figure 1 Intestinal stromal cells actively support the intestinal epithelium and are phenotypically modulated during inflammation. **(a)** Intestinal stromal cells form a dense network immediately underlying the epithelium of the small and large intestine. **(b)** Multiple bi-directional interactions between stromal cells and epithelial cells, mediated by a variety of soluble factors, can maintain homeostasis of the barrier. **(c)** Further, during inflammation stromal cells can actively redistribute to damaged areas and aide in mucosal repair. **(d)** In the TNF Δ ARE model of Crohn's disease, tumor necrosis factor α (TNF α) produced by the intestinal epithelium directly activates CD90⁺ α SMA⁺CD31⁻ ileal stromal cells that upregulate inflammatory mediator expression and thus significantly contribute to pathology. CXCL7, C-X-C motif chemokine ligand 7; ICAM-1, intercellular adhesion molecule-1; IL-24, interleukin-24; MMP, matrix metalloproteinase; α SMA, α -smooth muscle actin; TNFR1, tumor necrosis factor receptor 1.

liver, and pancreas.^{28–30} Perhaps best studied from a stromal cell angle, the diverse functional contributions of tissue-specific stromal cells to the immunopathology associated with rheumatoid arthritis (RA) are now well accepted. This includes tissue destruction, production of and response to inflammatory cytokines, activation of innate signaling pathways, and the initiation of angiogenesis and thus regulates the transition from acute-to-chronic inflammation (reviewed in Neumann et al.³¹ and Buckley et al.³²). Furthermore, stromal–stromal and stromal–leukocyte interactions have a major role in the pathophysiology of the inflamed joint.^{31,33,34} A cardinal feature of the modulated stromal cell phenotype during RA is that these alterations appear to be stable and persistent. This has also been observed in other contexts, such as in systemic sclerosis where there is a persistent activation of dermal fibroblasts.³⁵ Intriguingly, there is now mounting evidence that these stable alterations in stromal cell phenotype and function during persistent inflammatory disease may be as a result of imprinted epigenetic changes, with pro-inflammatory stromal epigenetic modifications reported in murine pulmonary inflammation,³⁶ human kidney fibrosis,³⁷ RA,³⁸ and keloid scarring.³⁹ Ospelt *et al.*⁴⁰ have, in fact, been led to propose that epigenetic changes in stromal cells during chronic inflammation lie at the heart of their capacity to have imprinted “inflammatory memories”. Although not yet addressed in as much detail as RA, evidence from mouse models of colitis and cultured human cells *ex vivo* suggest that stromal cells are also involved in the chronic pathology associated with intestinal inflammation (**Figure 1c**).

The activation of intestinal stromal cells by tumor necrosis factor- α (TNF α) is known to be sufficient for initiating intestinal pathology in the TNF Δ ARE mouse model of Crohn's disease (CD), via signaling through TNFR1 into resident tissue mesenchymal cells.⁴¹ This activation comprises rapid intercellular adhesion molecule 1 (ICAM-1) and matrix metalloproteinase (MMP) upregulation by ileal CD90⁺ α SMA⁺CD31⁻ stromal cells, before inflammatory cell infiltration, in a process driven by TNF α secretion by the intestinal epithelium.⁴² These inflammatory signals, driven by TNF α signaling, are equally pathogenic when targeted at either hematopoietic immune cells or tissue mesenchymal cells and therefore strongly support a major role for stromal cells in this model of IBD.⁴³ In the SCID T-cell transfer model of intestinal inflammation, persistent pro-inflammatory activation of colonic fibroblasts is also observed and may be enhanced by interferon- γ signaling and CD40 ligation.⁴⁴ However, other than generalized inflammatory signaling responses to TNF α activation and the enhanced expression of adhesion molecules, there is currently little mechanistic data as to how activated stromal cells contribute to immune-mediated pathology in models of chronic intestinal inflammation.

To date, evidence for the persistent activation of human intestinal stromal cells during chronic inflammation is more limited than in other human diseases. Although it is not necessarily indicative of a pro-inflammatory response, persistent changes in TGF β isoform expression by colonic stromal cells isolated from healthy or inflamed intestine have been

reported and appear to be stable across multiple culture passages *in vitro*.⁴⁵ Furthermore, stromal cells cultured from CD patients express higher levels of membrane-bound TNF α than those from uninflamed controls, and Infliximab has been reported to modulate their pro-inflammatory phenotype *in vitro*.⁴⁶ In addition, changes in gene expression are observed between uninflamed, stricture, and non-stricture fibroblasts from CD patients,⁴⁷ MMP production by stromal cells is stably enhanced during CD,⁴⁸ and persistent changes in S100A4 mRNA and protein expression by stricture fibroblasts has also been reported,⁴⁹ hinting at a potential for human intestinal stromal cells to exhibit stable phenotypic changes that are “imprinted” during chronic inflammation.

Although not seen in CD, elevated expression of keratinocyte growth factor mRNA is observed in myofibroblasts cultured from ulcerative colitis (UC) patients,⁵⁰ further supporting the hypothesis that persistent alterations in the stromal compartment occur during chronic inflammation. This also suggests that diverse inflammatory stromal signatures are imprinted as a result of these distinct forms of intestinal pathology, although a more rigorous assessment of stromal cells isolated from multiple intestinal pathologies will be required to investigate this fully. Despite highly divergent anatomy, localization, and immune cell composition, persistent stromal cell activation has been recently suggested as a major factor in regulating the transition to chronic gut inflammation in patients with joint pathology linked to spondyloarthritis,⁵¹ and TNF α -dependent stromal activation contributes to the combined intestinal and synovial pathology in TNF Δ ARE experimental animals.⁴¹

The potential for imprinted pro-inflammatory alterations in stromal cell phenotype and function across a range of inflammatory disorders, highlights the timely need to apply modern high-throughput sequencing and immunological profiling techniques to cells isolated during human disease. Furthermore, determining the similarities and differences between the altered stromal cell phenotype of divergent inflamed tissues, such as the joint and gut, will allow for an assessment of the likelihood that shared pathological responses may operate at the level of the tissue mesenchyme, and such “inflammatory stromal” pathways are likely to provide multiple novel pathological axes to target during chronic inflammation.

Intestinal stromal cells and cytokine responses in health and disease

Although much of the previous mechanistic work concerning cytokine responses by intestinal stromal cells has focused on TNF α , these cells produce and respond to a far more diverse array of cytokines. Several distinct groups of these proteins are associated with various stromal homeostatic and immunological functions.

Cytokines have long been known to modulate the functional outcome of intestinal stromal cell–epithelial interactions,⁵² and this aspect of their behavior is seemingly dependent on several mechanisms (Figure 1b). Stromal cell production of IL-24 activates mucin expression by intestinal epithelial cells,⁵³ myofibroblast activation and MMP production stimulates

epithelial cells to produce C-X-C motif chemokine ligand 7 leading to neutrophil recruitment,⁵⁴ and colonic epithelial cells are able to activate the mesenchymal compartment via soluble Galectin-3 production.⁵⁵ This therefore suggests that a tripartite of epithelial, stromal, and hematopoietic immune cell-derived cytokines synergise for effective cellular function at the mucosal barrier.

Intestinal stromal cells are also sensitive to multiple conventional immune cell-derived cytokines with relevance to IBD (Figure 2). Intestinal stromal cells strongly respond to the pro-inflammatory cytokines IL-1 α and IL-1 β , with a variety of functional outcomes.^{56,57} In addition, the T helper type 17 (Th17)-associated cytokine IL-17A induces the activation and production of IL-6, IL-8, and C-C motif chemokine ligand 2 (CCL2) by intestinal stromal cells, through a mechanism involving nuclear factor (NF)- κ B.⁵⁸ Stromal responses to IL-17A and IL-17F appear to be strongest, as stimulation with other IL-17 family members (IL-17B/C/D/E) elicits a more limited capacity to enhance the IL-1 β -induced expression of inflammatory mediators.⁵⁹ In addition to being mediated through NF- κ B, the mechanism of IL-17A and IL-17F signaling has been reported to be as a result of ERK (extracellular signal-regulated kinase) and p38MAPK (p38 mitogen-activated protein kinase) activity, indicating that divergent signaling modules can be elicited in stromal cells by the same cytokines.

There is limited evidence that intestinal stromal cells can respond to the IL-10 cytokine family member IL-22, with one report describing a pro-inflammatory role for this cytokine in activating myofibroblasts.⁶⁰ If confirmed, this suggests that the impact of IL-22 signaling on the mesenchymal compartment is distinct from that of the epithelium, where IL-22 is generally considered to be involved in protective responses.⁶¹ Interestingly, combined stimulation with IL-17A and IL-4 leads to the synergistic induction of IL-6 release by stromal cells,⁶² suggesting a capacity to respond to cytokines associated with multiple classes of CD4⁺ helper T cell. Supporting this is evidence that intestinal stromal cells respond to the classical Th1 T-cell cytokine interferon- γ by increasing their expression of CD40 and MHCII (major histocompatibility complex class II), whereas downregulating α SMA expression, contributing to their functional activation *in vitro*.⁶³ It has been reported that IL-21 signalling can synergise with TNF α to drive MMP production by intestinal stromal cells⁶⁴ and attenuated STAT3 (signal transducer and activator of transcription factor 3) expression within colonic stromal cells is associated with reduced tumor burden in IL-21^{-/-} mice,⁶⁵ indicating a key role for this hematopoietic immune cell-derived cytokine in activating stromal cells that can contribute to cancer progression.

Multiple studies suggest that intestinal stromal cells are linked to mucosal Th2-type responses. After IL-1 β and TGF β stimulation, stromal cells are capable of abundant production of IL-11, a cytokine linked to the priming of Th2 responses.⁶³ Conversely, stromal cells are also able to respond to IL-31, a cytokine produced by activated Th2 cells, in similar ways to that seen with IL-17A.⁶⁶ The extent to which redundancy exists

Figure 2 Intestinal stromal cells dynamically interact with multiple hematopoietic immune cell populations and associated cytokines. Intestinal stromal cells interact through various means with immune cell populations, including T helper (Th) cells, regulatory T cells (Tregs), B cells and granulocytes. Intestinal stromal cells respond to multiple innate and adaptive immune cell-derived cytokines, as well as producing cytokines and chemokines that may influence myeloid cell differentiation, mature myeloid cell function, and the functional commitment of CD4⁺ T cells. Interactions between stromal cells and multiple hematopoietic immune cell populations (including innate lymphoid cell (ILC) subsets) is thought to lead to the initiation/stabilization of ectopic tertiary lymphoid structures in the gut, although the mechanisms are currently poorly defined. CCL, C-C motif chemokine ligand; DC, dendritic cell; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophages colony-stimulating factor; HLA, human leukocyte antigen; gp38, glycoprotein podoplanin; IFN, interferon; IL, interleukin; LT, lymphotoxin; PGE₂, prostaglandin-E₂; TGF, transforming growth factor; TLR, Toll-like receptor.

within these cytokine-induced activation pathways is not yet clear. Interestingly, levels of IL-33 are enhanced during UC and this Th2-associated alarmin appears to be preferentially expressed by intestinal myofibroblasts.^{67,68} It is not known whether this is a protective or pathological response, although the potential for IL-33 to exacerbate inflammatory pathology in the intestine will be discussed later in this review.

Intestinal stromal cell–hematopoietic immune cell interactions

As well as responding to and modulating intestinal inflammation in isolation, intestinal stromal cells also interact with multiple populations of conventional hematopoietic immune cells outlined below (Figure 2).

T CELLS

Intestinal stromal cells are known to interact with effector T cells *in vitro*, preventing their apoptosis via stromal production of IL-10⁶⁹ and supporting CD25⁺ regulatory T-cell expansion via expression of prostaglandin E₂.⁷⁰ Steady-state human colonic stromal cells also express human leukocyte antigen (HLA)-DR, can present model antigen, and activate a T-cell clone *in vitro*⁷¹—although nothing is known about the mechanisms employed by these cells to elicit antigen presentation after a more physiologically relevant challenge, for example, by an invasive bacterial species. It has been

proposed that intestinal stromal cells are capable of tolerance induction⁷² and can regulate CD4⁺ T-cell proliferation by inhibiting IL-2 production via stromal expression of PD-L1/2 (programmed death ligand 1/2),⁷³ with this function reminiscent of peripheral tissue antigen expression and tolerance induction by a subset of fibroblastic reticular cells (FRCs) in the peripheral lymph nodes.⁷⁴ It is not yet clear whether a subset of intestinal stromal cells can attenuate T-cell proliferation via provision of nitric oxide, as observed in peripheral lymph nodes.^{75–77}

In addition to regulating conventional T-cell activation and proliferation, human stromal cells contribute to intraepithelial lymphocyte proliferation and survival *in vitro* by an HLA-DR-independent, α4β1 integrin-dependent mechanism.⁷⁸ Currently, there is a lack of convincing evidence that stromal cells can directly induce T cells of a particular effector phenotype. However, it has been suggested that stromal cells can express IL-23p19 (at least at the mRNA level) in response to inflammatory stimuli⁷⁹ and as such could, in theory, support Th17 differentiation. This hypothesis has not yet been experimentally validated and whether this capacity for “myeloid” cytokine production is due to the differential origins (e.g., fibrocyte vs. MSC) of stromal populations is not known.

The many interactions between intestinal stromal cells and T cells have been reviewed in more detail recently;⁹ however, their functional interactions with other hematopoietic immune cells are less well studied.

B CELLS

Reports describing intestinal stromal cell–B cell interactions are more limited than those for T cells. A careful histological assessment of CD fistulae showed dense aggregates of CD20⁺ B cells in the granulation tissue of fistulae, often adjacent to a layer of myofibroblasts and CD68⁺ macrophages.⁸⁰ Whether such interactions are having a functional role in the tissue remodeling is not known. More recently, intestinal stromal cell–derived factors have been reported to co-operate with microbial stimuli in the generation of a unique (immunoglobulin A⁺) IgA⁺ “multifunctional” plasma cell population, capable of abundant TNF α and induced nitric oxide synthase production.⁸¹

Although the precise mechanisms are not known, LT β R signaling into intestinal stromal cells has been shown to be critical for IgA production,⁸² and intestinal stromal cells are able to induce B-cell IgM to IgA class switching *in vitro*,⁸³ supporting the argument that stromal activation and stromal cell–B cell interactions have a role in the production of mucosal IgA. However, as the limited evidence currently available attests, additional work will be required to determine whether stromal cell–B cell interactions have further roles in the complex immunobiology of the intestine.

SECONDARY AND TERTIARY LYMPHOID ORGANS

The generation of several classes of secondary lymphoid structures in the gut relies on exquisitely controlled spatio-temporal interactions between innate lymphoid cell populations, such as lymphoid tissue inducer cells, and stromal organizer cell populations.⁸⁴ Unlike the development of secondary lymphoid organs in the periphery,⁷ the precise contributions of intestinal stromal cells to lymphoid structures in the gut are not yet known, nor is it fully clear whether the stromal subsets found in peripheral lymphoid organs are present within the intestine. Nevertheless, the isolation and culture of lymphatic endothelial cells (LECs) from human intestine has been reported,⁸⁵ with these cells bearing a phenotype analogous to the LEC stromal subset found within the peripheral lymph nodes.

Despite these processes being tightly regulated, the aberrant or ectopic accumulation of lymphoid structures in multiple organs occurs during inflammation,⁸⁶ including the intestine,⁸⁷ although the precise contribution of intestinal stromal cells to *de novo* ectopic lymphoid organogenesis is not known. However, during murine models of inflammation, activated ileal-resident stromal cells exhibit some features of lymphoid organ stromal cells, such as podoplanin (gp38) expression,⁸⁸ hinting at the possibility of a contribution to ectopic/tertiary lymphoid organogenesis in the gut. Although not fully characterized for CD, CCL21 mRNA and protein expression by stromal cells has been observed during UC,⁸⁹ and stromal cell activation and the presence of CCL21⁺ FRC-like cells in nascent T-cell zones associated with dendritic cell (DC) and lymphoid aggregates has been observed during other chronic inflammatory processes.^{90,91} Much is still to be clarified regarding the precise role of tissue stromal cells in the generation of tertiary lymphoid structures during chronic

intestinal inflammation, and there is a pressing need to investigate these mechanisms in humans. In addition to the interactions with adaptive immune T and B cells described, multiple functional interactions between innate immune cells and intestinal stromal cells have also been reported.

GRANULOCYTES

Intestinal stromal cells can support the survival of mast cells *in vitro*⁹² through a mechanism dependent on the production of IL-6.⁹³ Interestingly, the interaction between stromal cells and granulocytes appears to be bi-directional, as mast cell and eosinophil-derived factors can modulate intestinal stromal cell proliferation, phenotype, and function,^{93–95} at least *in vitro*. Although not yet determined for the gut, an IL-33-dependent interaction between dermal stromal cells and eosinophils enhances the pro-inflammatory activation of eosinophils *in vitro*,⁹⁶ suggesting that stromal cells can also directly modulate granulocyte function in some mucosal contexts. Indeed, mast cell activation as a result of enhanced stromal IL-33 production in the inflamed synovium is proposed to be a major pathological axis operating during experimental arthritis.⁹⁷ As UC is associated with enhanced IL-33 expression by intestinal stromal cells,^{67,68} this raises the intriguing possibility that stromal cell–granulocyte interactions may contribute to pathology associated with this form of IBD, by mechanisms similar to those recently proposed for Th2-driven intestinal responses more generally.⁹⁸ Further characterization of intestinal stromal cell and granulocyte interactions will be required to address this hypothesis.

MYELOID CELL DIFFERENTIATION AND FUNCTIONAL CONDITIONING

It appears that intestinal stromal cells are capable of producing factors required for the differentiation of myeloid populations, such as the NF- κ B-dependent induction of M-CSF (macrophage colony-stimulating factor) and GM-CSF (granulocyte macrophage colony-stimulating factor).⁹⁹ This can be regulated by cytokines implicated in IBD: indeed one of the earliest functions ascribed to human T-cell-derived IL-17A was the induction of cytokines from stromal cells that created a niche for myeloid cell differentiation.¹⁰⁰ Supporting the existence of this axis in the gut, IL-17 directly enhances TNF α -induced GM-CSF and G-CSF production by intestinal stromal cells *in vitro*,¹⁰¹ although whether stromal cells are capable of polarizing myeloid precursors or monocytes toward distinct effector phenotypes is still unclear.

As previously stated, intestinal stromal cells can produce both IL-10 and prostaglandin-E₂, similar to stromal cells from other organs, factors that in these distinct tissues can support the generation of regulatory DC populations.¹⁰² Conversely, stromal cell–derived IL-33—also abundantly expressed by intestinal stromal cells during UC—can modulate local pro-inflammatory myeloid cell function during RA.¹⁰³ These data highlight the possibility that stromal cells can functionally skew myeloid populations to both pro- and anti-inflammatory phenotypes, although the extent to which the stromal–myeloid

interactions apparent in other tissue sites are also operating in the gut will require extensive further investigation.

Unlike mucosal epithelial cells, evidence for the conditioning of mature myeloid cell function by intestinal stromal cells is limited. However, gastric and intestinal stromal factors, most likely derived from the extracellular matrix, can regulate human myeloid DC function, downregulating IL-12 production and blunting the induction of Th1 responses by T cells in co-culture.¹⁰⁴ Furthermore, stromal factors have also been implicated in the homeostatic regulation of TLR5 expression in mucosal DCs,¹⁰⁵ and stroma-conditioned media also regulates monocyte chemotaxis via IL-8 and TGF β .¹⁰⁶ Very little else is known for the gut, and many highly pertinent questions remain as to the extent of stromal cell–myeloid cell interactions and their functional impact under steady-state and inflammatory conditions.

Stromal cells as direct mediators of innate immune responses in the intestine

Growing evidence suggests that non-hematopoietic stromal cells of the intestine are capable of directly responding to pathogens in a cell-intrinsic fashion (Figure 3). Although direct *in vivo* evidence of the stromal contribution to microbial sensing is currently lacking, these cells appear to be equipped with many mechanisms to facilitate such a functional capacity.

Recent murine data support a functional role for stromal expression of innate immune receptors *in vivo*, as

NOD2-dependent CCL2 production by intestinal stromal cells is key for regulating inflammatory monocyte recruitment and thus pathogen clearance during *Citrobacter rodentium* infection, an experimental model of human Enterohemorrhagic/pathogenic *Escherichia coli* infection.¹⁰⁷ This function is associated with the direct sensing of live bacteria and is specifically linked to NOD2-dependent bacterial-driven CCL2 production, as TNF α production is unaffected when NOD2-deficient colonic stromal cells are infected with *C. rodentium* *in vitro*.

Data on the expression of other NLRs by murine intestinal stromal cells are limited; however, colonic myofibroblasts are the major intestinal cell type expressing the innate inflammation-activating receptor NLRP6 in mice, through which signaling regulates epithelial reconstitution after bacterial challenge *in vivo*.¹⁰⁸ Although yet to be experimentally validated, it is possible that sensing of bacteria by NLRs in the stromal compartment after a breach in the epithelial barrier leads to the direct production of—as yet unknown—mediators that facilitate epithelial repair. Interestingly, the re-localization of stromal cells during epithelial regeneration is dependent on MyD88 (myeloid differentiation primary response gene (88)),²² a fact that lends support to the notion of innate immune sensing having a part in driving stromal cells toward an epithelial regenerative/healing response after a breach in the barrier and exposure to luminal bacteria.

Human intestinal stromal cells are known to express mRNA encoding the cytosolic innate receptors NOD1 and NOD2, and

Figure 3 Intestinal stromal cells have a cell-intrinsic role in bacterial sensing at the intestinal barrier. Upon bacterial translocation across the epithelium, intestinal stromal cells are perfectly located to respond to the invading organisms. Murine stromal cells express NOD2 (nucleotide-binding oligomerization domain-containing protein 2), which when triggered by *Citrobacter rodentium* initiates the production of C-C motif chemokine ligand 2 (CCL2) by the intestinal stroma, recruitment of inflammatory monocytes, and the clearance of the organism. Human colonic stromal cells express functional Toll-like receptor 2 (TLR2) and TLR4 (in addition to many pathogen-recognition receptors at the message level), which facilitate the production of multiple cytokines and other mediators after challenge with live bacteria or isolated bacterial ligands. Given their strategic location and capacity to interact with epithelial cells, it is possible that stromal cells produce mucosal repair factors such as extracellular matrix (ECM) proteins, growth factors and other unknown factors after bacterial triggering, thus allowing the intestinal mesenchymal compartment to both recruit hematopoietic immune cells (via chemokines) as well as begin the process of epithelial regeneration. GM-CSF, granulocyte macrophages colony-stimulating factor; ICAM-1, intercellular adhesion molecule-1; MyD88, myeloid differentiation primary response gene (88); NLR, NOD-like receptor; PGE₂, prostaglandin-E₂; VCAM-1, vascular cell adhesion molecule-1.

the Toll-like receptors (TLRs) 1–9, and have detectable protein levels of TLR2 and TLR4, allowing them to respond to lipoteichoic acid and lipopolysaccharide *in vitro*, as well as enabling them to produce IL-8 in response to bacterial challenge.¹⁰⁹ Indeed, lipopolysaccharide stimulation of intestinal stromal cells elicits a range of functional responses,^{110–112} including the expression of cytokines such as GM-CSF, IL-1 β , IL-6, and IL-10, and the upregulation of the adhesion molecules ICAM-1 and VCAM-1 (vascular cell adhesion molecule-1).¹¹³ It is not clear whether human stromal cells express functional NOD2, as they do in mice, nor whether this NLR drives similar patterns of stromal chemokine expression.¹⁰⁷ Interestingly, however, primary human stromal cells have been shown to produce CCL2 in response to staphylococcal toxins¹¹⁴—although whether innate receptors are responsible for this is not known and, in fact, this effect may be mediated by HLA-DR crosslinking. As it is also unclear whether signaling pathways downstream of PRR (pathogen-recognition receptor) triggering in stromal cells are shared with those of myeloid populations, much still needs to be clarified regarding the cell-intrinsic innate immune functions of these cells.

In addition to bacterial responses, intestinal stromal cell populations are also implicated in the regulation of anti-parasitic immune responses. Intestinal smooth muscle cell expression of the IL-4R α is critical for helminth expulsion in mouse models, and the mechanistic basis of this has been linked to both intestinal contractility and the coordination of Th2 responses.^{115,116} Whether other organisms directly influence intestinal stromal cell function, such as the obligate intracellular parasites *Toxoplasma gondii* and *Leishmania donovani*, will require further investigation, although intriguingly, the direct modulation of stromal cell populations in other organs has been suggested as a potential mechanism by which parasites can manipulate host immune responses.^{102,117}

As both epithelial and mesenchymal compartments of the gut are composed of radioresistant cells, it has not been possible to distinguish the relative contribution of epithelial vs. stromal sensing to host defence/inflammation where previous studies have used *in vivo* bone marrow chimeric approaches.¹¹⁸ This highlights the need to use tools which allow for the differential cell intrinsic innate immune functions of intestinal epithelial and stromal cells to be determined *in vivo*.¹¹⁹ Genetic targeting of intestinal mesenchymal cells has been successfully performed previously, using a variety of promoters to drive expression of Cre-recombinase by intestinal stromal cells. This has included Collagen VI,⁴¹ Collagen 1a2,¹²⁰ and FoxL1,¹²¹ although such experimental animals also show Cre activity in stromal cells of the joint,¹²² multiple organs,¹²⁰ and liver,¹²³ respectively. As such a “pure” intestinal mesenchymal cell-specific Cre-driver strain has remained elusive. Nevertheless, these existing tools, in addition to others such as podoplanin (gp38)-Cre mice,¹²⁴ will be useful to determine the role of stromal cell innate sensing to immunity in the intestine. Crossing such strains with mice bearing conditionally targeted alleles of pertinent innate immune receptors or key elements of their signalling pathways would allow for the ablation of these

proteins in the intestinal mesenchyme, an approach similar to the intestinal epithelial cell-specific deletion of MyD88 recently reported.¹²⁵

The future of stromal cells as non-hematopoietic intestinal immune cells

As this review has highlighted, there is still much to be revealed regarding the origin, heterogeneity and functions of intestinal stromal cell populations, with a pressing need to unravel their complex functionality *in vivo*. In particular, the application of advanced immunological techniques to the study of intestinal stromal cells holds great promise in further dissecting their roles in homeostasis and during inflammation. These approaches have recently been applied to the detailed analysis of the lymph node mesenchyme, identifying novel stromal subsets and revealing marked transcriptional changes in these populations as a result of inflammation,¹²⁶ as well as their anatomical location in the periphery.¹²⁷ Such exciting data highlights the requirement to assess in detail the potential immunological heterogeneity of stromal cell populations within the intestine.

Despite the need for extensive further work, we believe that the evidence compiled here strongly contests the historical notion that intestinal stromal subsets are simply “non-immune” cells. As such, we propose “non-hematopoietic immune cells” as a more appropriate nomenclature for intestinal stromal cells. These dynamic cells lie in a critical anatomical location, produce, and respond to a bewildering array of “immunological” cytokines, interact with multiple populations of conventional immune cells, and are functionally implicated in the initiation of inflammatory pathology. Furthermore, they express a wide array of innate immune receptors and can directly sense live pathogens, paving the way for them to have a major cell-intrinsic role governing host protection after bacterial translocation across the epithelium. A better understanding of the immunobiology of these fascinating cell populations is essential to unravel the complex pathophysiological mechanisms underlying intestinal inflammatory disease, and may, in time, lead to the identification of novel pathological axes amenable to therapeutic manipulation.

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DISCLOSURE

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