Immunological roles of intestinal mesenchymal cells

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Summary

The intestine is continuously exposed to an enormous variety and quantity of antigens and innate immune stimuli derived from both pathogens and harmless materials, such as food and commensal bacteria. Accordingly, the intestinal immune system is uniquely adapted to ensure appropriate responses to the different kinds of challenge; maintaining tolerance to harmless antigens in the steady-state, whilst remaining poised to deal with potential pathogens. To accomplish this, leucocytes of the intestinal immune system have to adapt to a constantly changing environment and interact with many different non-leucocytic intestinal cell types, including epithelial and endothelial cells, neurons, and a heterogenous network of intestinal mesenchymal cells (iMC). These interactions are intricately involved in the generation of protective immunity, the elaboration of inflammatory responses, and the development of inflammatory conditions, such as inflammatory bowel diseases. Here we discuss recent insights into the immunological functions of iMC under homeostatic and inflammatory conditions, focusing particularly on iMC in the mucosa and submucosa, and highlighting how an appreciation of the immunology of iMC may help understand the pathogenesis and treatment of disease.

Keywords: homeostasis; immune response; inflammation; intestine; mesenchymal stromal cell.

Introduction

Mesenchymal cells (MCs) are non-epithelial, non-endothelial, non-haematopoietic cells that differentiate from mesenchymal stem cells and form the framework on which all mammalian tissues are built. However, this is no silent scaffolding. MCs are also able to act as sentinels, poised to provide physiological and immunological support in response to environmental cues. In the intestine,

MCs are found in all anatomical compartments, including the mucosa, submucosa and muscle layers of the intestinal wall (Fig. 1). MCs are also abundant in the associated secondary lymphoid tissues, where the priming of local immune responses takes place. These include the isolated lymphoid follicles (ILF) and Peyer's patches of the gut-associated lymphoid tissue (GALT), as well as the draining mesenteric lymph nodes (MLN). Although MCs in the GALT and MLN play crucial roles in the development,

Abbreviations: ACKR, atypical chemokine receptor; bFGF, basic fibroblast growth factor; CD, Crohn's disease; COX2, cyclo-oxygenase 2; CTGF, connective tissue growth factor; DAMP, damage-associated molecular pattern; DC, dendritic cell; DSS, dextran sodium sulphate; ECM, extracellular matrix; EGF, epidermal growth factor; Eos, eosinophils; EPO, eosinophil peroxidase; FDC, follicular dendritic cell; GALT, gut-associated lymphoid tissue; GM-CSF, granulocyte macrophage colony-stimulating factor; IBD, inflammatory bowel disease; ICAM, intercellular adhesion molecule; IGF-1, insulin-like growth factor-1; IGFBP3, IGF binding protein 3; ILC, innate lymphoid cell; iMC, intestinal mesenchymal cell; LPS, lipopolysaccharide; MAMP, microbe-associated molecular pattern; M-CSF, myeloid colony-stimulating factor; MLN, mesenteric lymph node; MMP, matrix metalloproteinase; NLR, NOD-like receptor; PDGF, platelet-derived growth factor; PDL, programmed death ligand; PGE_2, prostaglandin E_2; PMN, polymorphonuclear cells; PRR, pattern recognition receptor; RA, retinoic acid; RALDH, retinaldehyde dehydrogenase; ROS, reactive oxygen species; SCFA, short-chain fatty acid; scRNAseq, single-cell RNA sequencing; SMA, smooth muscle actin; TACI, transmembrane activator and CAML interactor; TIMP-1, tissue inhibitor of metalloproteinase-1; TLR, toll-like receptor; TNSF, TNF superfamily; UC, ulcerative colitis; VCAM, vascular cell adhesion protein

structure and immunological function of these organs, these properties are similar to those of MCs in other secondary lymphoid tissues that have been reviewed extensively elsewhere.[1]

Different anatomical locations contain phenotypically and functionally distinct MC populations, and substantial MC heterogeneity exists within tissues. This is particularly true in the intestine, where numerous populations of intestinal mesenchymal cells (iMC) occupy distinct niches and perform site-specific physiological functions. As well as secreting the extracellular matrix (ECM) components

that maintain intestinal architecture, myofibroblasts, subepithelial and crypt-associated fibroblasts regulate epithelial cell function. Pericytes and smooth muscle cells sustain blood and lymphatic vessels, while a novel group of submucosal fibroblasts may play a role in lymphangiogenesis. Smooth muscle cells and the interstitial cells of Cajal drive peristalsis and gut motility (Fig. 1; Table 1). We have long known that iMC populations play fundamental roles in the maintenance of intestinal architecture and function, but until recently their contribution to immune responses has largely been overlooked.
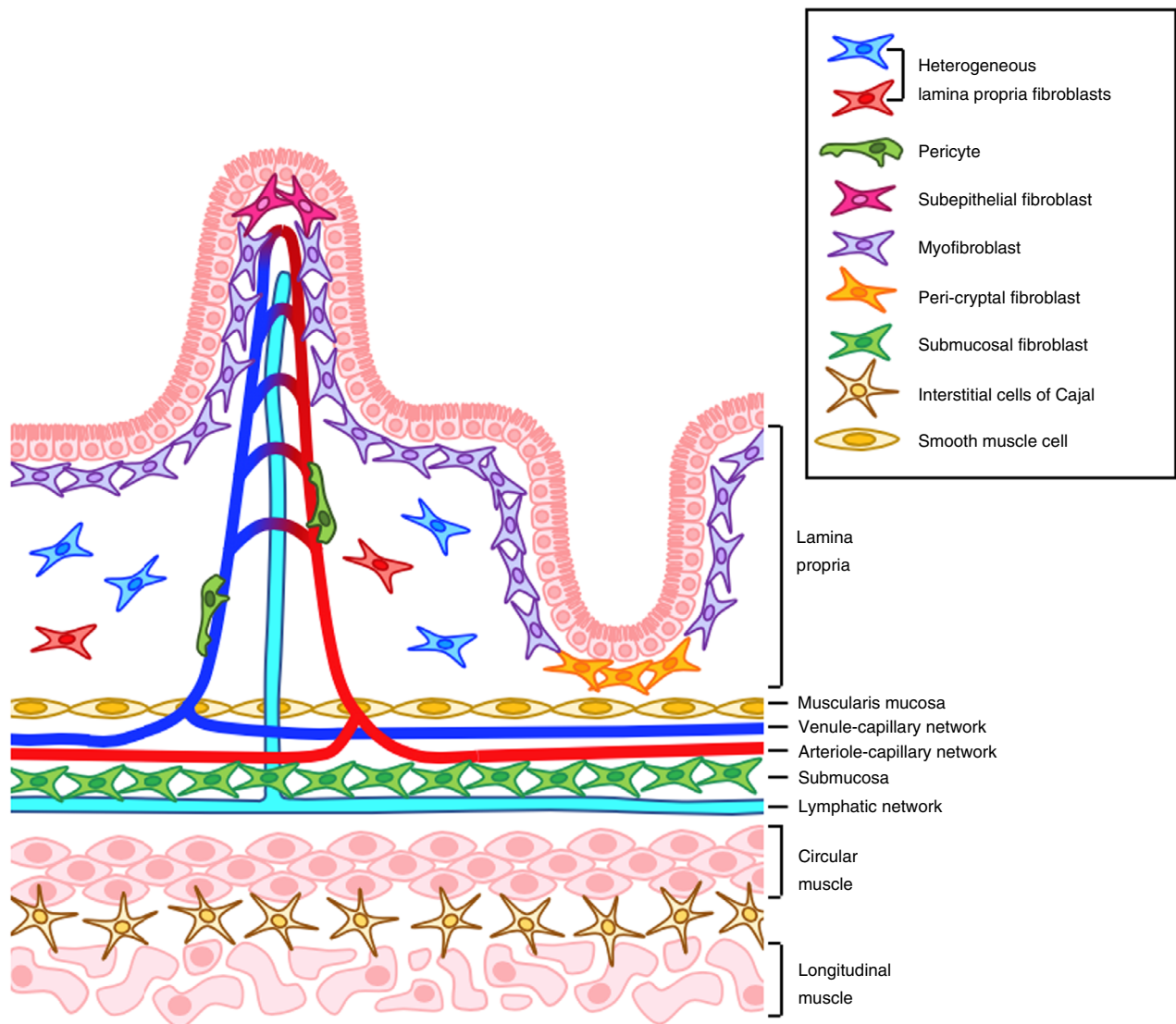

Figure 1. Mesenchymal stromal cells in the intestine. Several populations of fibroblasts exist in the lamina propria, where they secrete the extracellular matrix (ECM) components that are essential for maintaining the structural integrity of the gut. The true extent of lamina propria intestinal mesenchymal cell (iMC) heterogeneity remains to be fully established. Adjacent to the mucosal barrier, myofibroblasts, subepithelial and crypt-associated fibroblasts regulate the turnover, differentiation and movement of epithelial cells,[2,22] while pericytes and smooth muscle cells maintain the growth and function of vascular and lymphatic endothelial cells. Submucosal fibroblasts reside in close proximity to, and physically interact with, collecting lymphatic vessels.[6] These fibroblasts specifically express genes encoding endothelial regulators, so may play a role in lymphangiogenesis. Smooth muscle cells and the interstitial cells of Cajal drive peristalsis and gut motility.

Table 1. Physiological functions of iMC in mucosa and submucosa

iMC subset	Markers used for classification	Function
Myofibroblasts	α-SMA^{+}, Vimentin^{+}, PDGFRα^{+}, CD34^{−}, Myosin-11^{+}, CD90^{+}	Contractile function supports enterocyte movement along crypt-villus axis[109]
		Production of the basement membrane[109]
		Epithelial support
Lamina propria fibroblasts	α-SMA^{−}, Vimentin^{+}, PDGFRα^{+}, CD34^{+}, ICAM^{+/−}, CD90^{+/−}, CD55^{+/−}	ECM production and remodelling
		Heterogeneous population of cells[2,3]
		May include precursors[2]
Subepithelial iMC	α-SMA^{−}, PDGFRα^{+}, CD34^{+}, F3^{+}, SOX6^{hi}	Production of hedgehog molecules[110]
		Epithelial homeostasis?[2,110]
Peri-cryptal iMC	α-SMA^{−}, PDGFRα^{+}, CD34^{+}, F3^{+}, SOX6^{+}, Wnt-2b^{+}, Wnt-5a^{+}	Maintain epithelial regeneration via trophic effects on epithelial step cell niche[2,7,22]
Submucosal fibroblasts	PDGFRα^{+}, CD34^{+}, ACKR4^{+}, CD55^{+}, CD90^{+} (col), CD90^{−} (SI)	Modulation of lymphangiogenesis[6]
		Maintenance of chemokine gradients[6]
Pericytes	α-SMA^{+/−}, PDGFRα^{−}, CD146^{hi}, ESAM^{hi}, CD34^{−}, CD36^{+}, α7 integrin^{+}	Vascular contraction and support
Smooth muscle cells	α-SMA^{+}, PDGFRα^{−}, Desmin^{+}, Vimentin^{lo}	Smooth muscle contraction
		Mechanical support

ACKR, atypical chemokine receptor; ECM, extracellular matrix; ICAM, intercellular adhesion molecule; iMC, intestinal mesenchymal cells; PDGF, platelet-derived growth factor; SMA, smooth muscle actin.

Furthermore, we have lacked knowledge of the specific markers required to fully explore iMC heterogeneity and the functions of individual subsets. However, this is now changing, and single-cell RNA sequencing (scRNA seq) technology is beginning to enhance our understanding of these cells.[2,3] Here we will focus on the immunological roles of iMC, specifically fibroblasts, myofibroblasts, pericytes and smooth muscle cells. Moreover, we will address how phenotypically and spatially distinct iMC populations might perform unique immune regulatory functions in the intestinal mucosa and submucosa, before considering how these properties may contribute to inflammatory responses in the gut, including inflammatory bowel diseases (IBD). Finally, we will discuss how iMC may be modulated by the biggest environmental factor in their locale, the microbiome.

Immunological functions of iMC in steady-state

Intestinal mesenchymal cells are not simply bystanders in intestinal immune responses, and even in the steady-state they are involved in active, two-way communication with neighbouring leucocytes (Fig. 2). Here we will discuss the evidence that iMC can orchestrate leucocyte migration into, within and out of the intestine by producing and/or displaying chemokines, or by scavenging them from the extracellular environment. We will then describe how iMC help to maintain a tolerogenic environment in the steady-state intestine.

Regulation of leucocyte migration

Fibroblasts, myofibroblasts and pericytes have all been implicated in driving leucocyte migration into and within the gut in response to insult or injury, and they are thought to play a similar role in the steady-state. At the most basic level, ECM produced by iMC acts as a scaffold that leucocytes adhere to and crawl along after extravasating from the blood.[4] These fibres are rich in glycosaminoglycans that can bind extracellular chemokines and present them to patrolling leucocytes to trigger motility.[5] iMC can also directly contribute to leucocyte migration by producing chemokines, and they express numerous chemokine genes in the steady-state intestine of both humans and mice.[2,6,7] These include the genes encoding CXCL13, the B-cell and lymphoid tissue inducer cell chemoattractant;[6,7] the myeloid cell chemoattractants CCL2, CCL8, CCL11 and CCL13;[2,3,7] and the pleiotropic primordial chemokines CXCL12 and CXCL14.[2,6,7] Lamina propria iMC in the steady-state human colon have also been reported to express genes encoding the neutrophil chemoattractants CXCL1 and CXCL2,[2] despite the fact that neutrophils are rarely present in the healthy gut. Although few studies have examined chemokine production by iMC at the protein level, the steady-state intestine is highly enriched with eosinophils (Eos),[8] and is one of the few tissues where resident macrophages are continuously replenished from circulating monocytes.[9] Thus, it seems likely that the production and presentation of chemoattractants by iMC helps regulate the turnover of these populations in situ.

In addition to orchestrating leucocyte migration, some of the chemokines associated with resting iMC are reported to have bactericidal properties. The most well characterized is CXCL14, which is structurally similar to antimicrobial β-defensins due to its positive charge at neutral pH and presence of anti-parallel β-sheets, a C-terminal α-helix and several cationic residues.[10,11] Fibroblast-specific CXCL14 production has been suggested to provide protection against pathogens at other epithelial sites, such as the skin and lungs,[12,13] and known bacterial
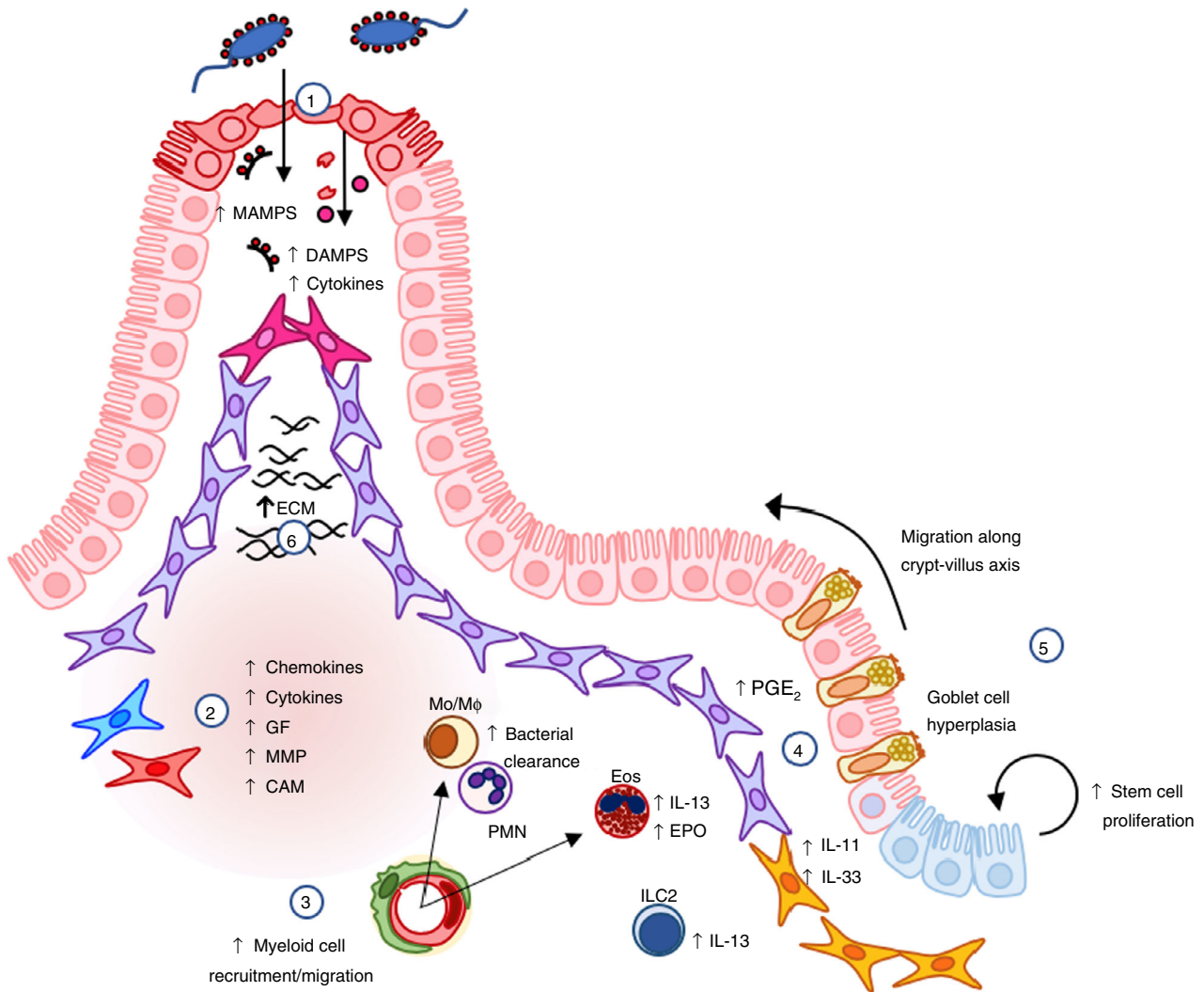
Figure 2. Role of intestinal mesenchymal cells (iMC) in intestinal inflammation and repair. 1. Following mechanical injury, infection or inflammation, iMC become activated by pro-inflammatory cytokines and pattern recognition receptor (PRR) agonists, such as microbe-associated molecular proteins (MAMPs; from the resident microbiota) and damage-associated molecular patterns (DAMPs; from the damaged epithelium). 2. This results in increased production of cytokines, such as interleukin (IL)-6, IL-11 and IL-33; prostaglandin E_2 (PGE_2); myeloid cell chemoattractants, such as CXCL1, CXCL2, IL-8/CXCL8, CCL2 and CCL11; growth factors (GF) myeloid colony-stimulating factor (M-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF); cellular adhesion molecules (CAM); and matrix metalloproteinases (MMPs).[41–44,46–49] 3. This enhances recruitment and migration of monocytes/macrophages (Mo/MØ), polymorphonuclear cells (PMN) and eosinophils (Eos), which facilitate bacterial clearance and bolster innate immune responses in the gut. 4. IL-11, IL-33 and PGE_2 may contribute to local inflammatory responses, but can also promote barrier repair through their pro-survival and proliferative effects on the epithelial stem cell niche.[47,68,108] 5. IL-33 also increases IL-13 production by innate lymphoid cells (ILC)2s and eosinophils, driving goblet cell hyperplasia and enterocyte migration up the crypt-villus axis.[70,71] IL-33-mediated induction of IL-13 and eosinophil peroxidase (EPO) can amplify cytokine production by iMC. 6. Cytokines in the inflamed mucosa increase the production of extracellular matrix (ECM) components, contributing to normal wound healing.

targets of CXCL14 include the intestinal pathobionts *Escherichia coli* and *Candida albicans*.[10,12] The chemokine concentrations required for antimicrobial action are high. Nevertheless, the constitutive expression of the gene encoding CXCL14 by subepithelial fibroblasts[2,6] may provide rapid bactericidal support in the event of bacterial translocation.

In addition to producing chemokines, some iMC express atypical chemokine receptors (ACKR), which are known to scavenge extracellular chemokines and target them for intracellular degradation. ACKR4 binds and internalizes the CCR7 ligands CCL19 and CCL21,[14,15] which are crucial for the mobilization of mature dendritic cells (DCs) from the intestine to the draining MLN.[16] ACKR4 also scavenges CCL25,[15] the only known CCR9 ligand, which is produced constitutively by the small intestinal epithelium, and is a key player in the recruitment of T and B lymphocytes and plasmacytoid DCs to

the tissue.[17–19] In recent studies, we found that in the colon and small intestine of mice, ACKR4 is exclusively expressed selectively by a population of CD34^{+} fibroblasts in the submucosa.[6] We have also observed that a large proportion of iMC express *Ackr3,* which encodes an ACKR specific for CXCL11 and 12 (Carolyn Thomson, unpublished data). The functional significance of ACKR expression by iMC subsets remains unclear, and there were no obvious immunological defects in the intestine of steady-state *Ackr4*-deficient mice.[6] However, it is possible that by scavenging chemokines, iMCs modulate chemokine gradients *in vivo* to finely tune leucocyte recruitment to, positioning within, or egress from the intestine, as has been described for ACKR4 in the skin and skin-draining lymph nodes where it is expressed by keratinocytes and lymphatic endothelial cells.[20,21] Alternatively, the immunological effects of ACKR4 in the intestine may only become more apparent following an infectious or inflammatory insult, which are known to be associated with increased CCR7- and CCR9-dependent leucocyte migration and *de novo* transcription of *Ccl19* by CD34^{+} and CD34^{−} iMC subsets.[22]

Regulation of leucocyte activation and differentiation

It has been reported that iMC are capable of expressing both MHC class I and II molecules, but there are conflicting reports about whether iMC express MHCII molecules at rest.[23,24] As well as finding that some subepithelial myofibroblasts can express HLA-DR *in situ* in the resting colon, Saada and colleagues showed that primary cultures of these cells could induce MHCII-dependent proliferation of allogeneic CD4^{+} T-cells *in vitro*. This required myofibroblast expression of the co-stimulatory molecules CD80 and CD86, which were induced by MHCII-mediated interaction with allogeneic T-cells.[24] As interferon (IFN)γ is a major driver of MHCII expression by human intestinal myofibroblasts,[25] it is possible that MHCII expression by iMC *in vivo* is dependent on the local levels of IFNγ. In turn, this may be determined by the exact composition of local microbiota, thus accounting for discrepancies in reports of MHCII expression by iMC *in situ*. Alternatively, MHCII expression may be lost following isolation and culture. Indeed, the earlier experiments that failed to find steady-state expression of MHCII on iMC[23] used cells that had been cultured for several weeks *in vitro*, whereas Saada and colleagues assessed MHCII expression *in situ* and on freshly isolated cells.[24]

It is not clear what impact cognate interactions between MHCII-expressing myofibroblasts and CD4^{+} T-cells would have in the steady-state mucosa, where the rarity of naïve T-cells means that presentation of antigen by iMC is unlikely to be important in initiating immune responses. However, as non-professional antigen-presenting cells are normally found to induce T-cell tolerance, it is possible that iMC contribute to the tolerogenic environment of the steady-state intestine. Via their expression of the programmed death ligands PDL1 and PDL2, iMC can also regulate the function of the fully differentiated PD-1^{+} effector/memory T-cells that dominate the lamina propria.[26–28] CD90^{+} colonic iMC use this mechanism to drive Treg polarization, suppress T effector cell proliferation and dampen IFNγ production *in vitro*.[26,27,29] A similar antiproliferative phenotype has been described for tissue-resident fibroblasts in other organs, including skin and lung[30] and, interestingly, severe T-cell-mediated autoimmune enteritis is a significant side-effect of PDL1 antibody blockade.[31]

Another way that iMC may regulate local T-cell responses is through their production of retinoic acid (RA) via retinaldehyde dehydrogenase (RALDH)-mediated metabolism of dietary vitamin A. iMC-derived RA can drive expression of the P2-purinoceptor component P2X7 by CD4^{+} memory/effector T-cells in the lamina propria, rendering them more sensitive to nicotinamide adenine dinucleotide-induced apoptosis.[32] This may enhance the ability of iMC to promote local tolerance. Furthermore, iMC-mediated production of RA in combination with granulocyte macrophage colony-stimulating factor (GM-CSF) induces the expression of RALDH2 by intestinal DCs,[33] and plays a crucial role in the development and transcriptional programming of the CD103^{+}CD11b^{+} DCs that are unique to the intestinal mucosa.[33,34] However, iMC are not the only source of these immunoregulatory mediators in the mucosa, as epithelial cells express RALDH1 and contribute to DC imprinting,[35] while innate lymphoid cell (ILC)3 can produce GM-CSF in response to microbial colonization.[36] RA from colonic iMC can also induce upregulation of P2X7 in bone marrow-derived mast cells, together with several genes associated with mucosal mast cells. Thus, contact between local iMC and mast cell precursors may be involved in mucosal mast cell development in the intestine.[37]

Recent evidence suggests that small intestinal iMC may be unique in their ability to drive the differentiation of B-cells into IgA-secreting plasma cells. This was mediated by B-cell-activating factor (BAFF) as well as other unidentified soluble mediators.[38] It is possible that RALDH activity in iMC plays a role in this, as has been shown for RALDH[+] mucosal DCs in the draining MLN.[39]

Together these findings indicate that iMC have a number of properties that allow them to contribute to the maintenance of homeostasis in the steady-state intestine. However, the importance of iMC in steady-state leucocyte trafficking and immune homeostasis *in vivo* is unclear, and the precise roles of individual subsets of iMC remain to be determined.

Immunological functions of iMC in intestinal infection and inflammation

Following mechanical injury or exposure to pathogens and their products, leucocytes and tissue-resident stromal cells in the intestine undergo a series of changes designed to facilitate pathogen clearance, protect the epithelial barrier and promote wound repair (Fig. 2). Important components of these responses include amplification of local inflammatory responses, reorganization of the ECM and modulation of the villus-crypt unit structure in response to insult or injury. These innate and adaptive responses are tightly regulated and, in most circumstances, self-limiting. Failure to control intestinal inflammation and fully repair the epithelial barrier can result in the development of IBD, such as Crohn's disease (CD) and ulcerative colitis (UC). Prolonged colonic inflammation also increases susceptibility to colorectal cancer but, as the role that iMC play in tumours has been reviewed extensively elsewhere,[40] we will not consider it here. Instead we will focus on the role of iMC-derived cytokines, chemokines and growth factors in intestinal injury, infection, inflammation and repair.

Pro-inflammatory effects of iMC

As in the steady-state, the lack of specific markers has meant that there is little definitive information on how individual subsets of iMC contribute to intestinal inflammation. Nevertheless, iMC as a whole can express a number of cytokine and chemokine receptors, as well as pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs), indicating that they have the potential to react to changes in their environment.

Because of their proximity to the lumen, many studies have concentrated on the ability of subepithelial myofibroblasts to respond to microbe- and danger-associated molecular patterns (MAMPs and DAMPs).[41,42] Indeed, transcriptional profiling has revealed that cultured primary αSMA[+] iMC from the human colon express mRNA encoding TLR1−9, as well as NOD1 and NOD2.[41−45] Although the exact range of PRR expressed by other subsets of iMC remains unclear, cultured human and murine myofibroblasts produce the inflammatory cytokine IL-6, upregulate the neutrophil chemoattractants IL-8/CXCL8 or CXCL1, and upregulate cyclo-oxygenase 2 (COX2) to produce prostaglandin E_2 (PGE$_2$) in response to lipopolysaccharide (LPS).[41,42,46] LPS also stimulates murine iMC to upregulate *Cxcl2*, *Cxcl5*, *Ccl2* and *Ccl7* transcripts *in vitro*.[43] Less is known about if and how iMC respond to PRR ligands other than LPS, although murine iMC can produce IL-6 in response to synthetic lipoproteins, including Pam3CSK4, heat-killed *Listeria monocytogenes* and FSL-1, indicating that they can respond to

ligation of TLRs 1, 2 and 6.[43] Furthermore, colonic iMCs from mice infected with *Citrobacter rodentium* produce CCL2 in response to NOD2 ligands.[44]

Interestingly, iMC and leucocytes may respond differently to PRR ligation. For instance, TLR2 expression by non-haematopoietic cells is required for clearance of *C. rodentium* and, rather than amplifying local inflammatory responses, this helps maintain mucosal integrity, reduces bacterial dissemination and limits intestinal inflammation.[47] In contrast, activation of TLR2 on leucocytes amplifies the inflammatory response in this model, leading to increased morbidity and mortality.[47] Although it was not shown directly that iMC were the tissue-resident cells responding to TLR2 ligands during *C. rodentium* infection, IL-11 was upregulated by α-SMA[+] iMC in the muscularis mucosa in a TLR2-dependent manner, and protection against *C. rodentium* could be restored by treating TLR2-deficient mice with recombinant IL-11.[47] Thus, although iMC and other intestinal stromal cells are known to express TLR2, their downstream response appears to be distinct from that of leucocytes.

The induction of pro-inflammatory cytokines and chemokine production by iMC is not limited to ligation of PRR, and they can also respond to other extracellular stimuli such as cytokines. Interestingly, current evidence seems to indicate that iMC may be hard-wired to produce a similar repertoire of inflammatory mediators regardless of the exogenous stimulus. Thus stimulation of human colonic iMC with IL-1α, IL-1β, TNFα or IL-17 *in vitro* triggers the production of the same mediators that are produced following TLR ligation;[42,43,46] namely IL-6, chemokines CXCL1, IL-8/CXCL8 and CCL2, and growth factors myeloid colony-stimulating factor (M-CSF) and GM-CSF.[46,48,49] A similar phenomenon has been observed in mice, where iMC stimulated with IL-36γ, or isolated following acute dextran sodium sulphate (DSS)-mediated colitis *in vivo*, produced IL-6, CXCL1, CXCL2, CCL2 and GM-CSF.[22,50] Thus, iMC may contribute to host defence and inflammation via generic, pre-programmed production of cytokines and the release of chemokines that recruit innate immune cells such as monocytes, neutrophils and Eos. Migration of leucocytes through the tissue may be aided by the upregulation of adhesion molecules intercellular adhesion molecule (ICAM) and vascular cell adhesion protein (VCAM) by iMC, as has been observed at a transcript level during DSS-mediated colitis.[22] Furthermore, the growth factors and chemokines produced by iMC may trigger the mobilization of innate leucocytes from the bone marrow, as indicated by the GM-CSF-driven expansion of Eos in experimental IBD.[51]

iMC and trained immunity

Altered iMC responses during inflammation may have long-term consequences for mucosal immunity, due to

the phenomenon of 'trained immunity' in which a primary innate stimulus alters subsequent responses by leucocytes and stromal cells.[52] Although often transient, trained immunity can also be a long-lasting consequence of epigenetic modification, and it has been shown that pro-inflammatory cytokines can induce histone acetylation in synovial fibroblasts, resulting in a heightened inflammatory response to secondary stimulation in vitro.[53,54] These long-lived changes to fibroblast function may contribute to the perpetuation of synovial pathology, and it would be interesting to determine whether similar processes occur during chronic intestinal inflammation. Indeed, epigenetic modifications have been observed in mucosal fibroblasts isolated from patients with fibrostenotic CD, with resulting changes in pathogenic gene expression.[55,56] Furthermore, early-life lymphotoxin-β receptor signalling has a life-long impact on the ability of MCs to promote IgA class-switching in the MLN.[57] Understanding whether trained immunity in iMC contributes to ongoing inflammation in the gut may be crucial for the development of novel therapeutics to tackle IBD.

Role of iMC in inflammatory bowel diseases

Mesenchymal cells are increasingly implicated in the pathogenesis and treatment of chronic inflammatory diseases with, for example, synovial fibroblasts thought to be central to the initiation and maintenance of pathology in rheumatoid arthritis.[58,59] Analogous processes of relapsing and remitting immunopathology are found in the IBDs CD and UC, in which inappropriate immune responses directed against harmless commensal microbes. This causes damage to the mucosal epithelium, impaired wound healing, and the formation of mucosal ulcers and strictures. Elegant studies using scRNAseq have shown that these features are accompanied by dramatic changes in colonic iMC, including the acquisition of a transcriptional profile consistent with a role in inflammation and in enhancing leucocyte recruitment to the mucosa.[2,3] As suggested from the in vitro studies described above,[40,42,46,48,49] this is likely mediated by inflammatory cytokines present in the mucosa and the increased abundance of endotoxin resulting from increased barrier permeability. Pericytes and putative peri-cryptal $WNT2B^{hi}$ fibroblasts from the colon of children with CD and UC showed increased expression of CCL2 and CCL8, while lamina propria and $WNT2B^{hi}$ fibroblasts expressed elevated levels of CXCL1 and CCL11.[3] In addition, a novel population of pro-inflammatory fibroblasts was found in UC, which was characterized by high levels of expression of the neutrophil chemoattractant CXCL5, as well as MMP3, IL1B, IL24 and IL6.[3] Again, it is interesting to note that the chemokine profile associated with iMC is highly selective for myeloid cells, underlining the

possibility that iMC may be pre-primed to mobilize this particular class of innate immune cells in response to an inflammatory insult. It has also been proposed that the pro-inflammatory iMC, which increase in numbers in IBD, may contribute to barrier damage by inhibiting the stem cell niche-associated iMC that promote epithelial renewal and differentiation.[2,3]

As well as altering the mucosal milieu in IBD, iMC may also drive the generation of tertiary lymphoid follicles, as indicated by increased numbers of FRC-like stromal cells in adults with UC. These cells expressed elevated levels of CCL19, as well as genes encoding CCL21, TNF superfamily (TNFSF) member 14 (LIGHT) and follicular DC secreted protein (FDC-SP).[2] A similar enrichment of these genes, as well as TNFSF13b (TACI), was observed in FRC-like fibroblasts isolated from children with IBD.[3] All these molecules are involved in the development, organization and immune function of tertiary lymphoid tissue, including naïve lymphocyte recruitment, modulation of germinal centre responses, and IgA class-switching.[60–62]

Thus, transcriptional analyses suggest that IBD is associated with expansion of pro-inflammatory and immunomodulatory stromal cells in both the LP and organized lymphoid structures. An important goal of future studies will be to identify exactly where these populations are located and the nature of the immune cells they interact with.

IMC-derived immunomodulators in intestinal repair and fibrosis

Mucosal inflammation is pivotal for host defence and pathogen clearance, but it comes at a potential cost, because the production of cytokines, matrix metalloproteinases (MMPs), reactive oxygen species (ROS) and nitrogen intermediates can lead to pathological ECM remodelling, epithelial cell death and ulceration. As a result, would healing and tissue repair play critical roles in ensuring healthy termination of acute inflammatory responses, and MCs are centrally involved in these processes. iMC are well known to promote wound healing and barrier repair via the production of ECM components, and via their trophic effects on epithelial cells and the stem cell niche (Fig. 2). These processes need to be tightly controlled, because excessive ECM production and iMC activation can lead to the development of fibrosis, scarring and stricture formation (Fig. 3). The important contributions iMC play in normal and pathological wound healing are largely physiological, and have been reviewed extensively elsewhere.[63] We will focus on the pathological roles that iMC-derived immunomodulators can play in intestinal inflammation, repair and fibrosis.

Some of the characteristic cytokines produced by iMC during inflammation, such as IL-11 and IL-33, can have

pro-repair and pro-inflammatory functions depending on the context. As noted above, IL-11 produced by subepithelial myofibroblasts and α-SMA⁺ iMC in the muscularis mucosa protects against the intestinal inflammation caused by *C. rodentium*.[47] This effect is mediated by STAT3 phosphorylation in neighbouring epithelial cells driving the expression of genes associated with survival and proliferation, thus promoting wound repair and preventing epithelial damage.[47,64] IL-11 also modulates cytokine production by mononuclear phagocytes, downregulating production of Th1-associate cytokines IL-12 and IFNγ.[65] This skews CD4⁺ T-cell polarization towards a Th2 phenotype, rather than a more inflammatory Th1 response. Recombinant IL-11 ameliorates inflammation and tissue destruction in several animal models of colitis,[47,64] and has subsequently been used with some success in clinical trials for CD.[66,67]

Interleukin-33 is expressed by numerous stomal cell populations in the steady-state intestine, including subepithelial and peri-cryptal iMC,[68,69] and it plays an important role in maintaining epithelial barrier integrity in the intestine.[68] IL-33 is upregulated by peri-cryptal iMC during *Salmonella typhimurium* infection in mice and, by binding to its ST2 receptor on epithelial progenitor cells, it helps protective immunity by driving expansion of secretory epithelial cells and promoting epithelial antimicrobial defence.[68] These direct effects of IL-33 on epithelial stem cells are complemented by its ability to induce the production of IL-13 by type 2 ILC2. This drives goblet cell hyperplasia and enhances epithelial cell migration from crypt to villus, both important aspects of barrier repair and protection against intestinal helminth infection.[70,71] IL-33 is also upregulated in the inflamed mucosa of patients with UC, predominantly by the α-SMA⁺ iMC that accumulate within lesions.[69,72] However, ST2 expression on epithelial cells is downregulated in IBD, possibly negating any protective effects of IL-33 under these circumstances.[73] It is important to note that IL-33 drives and amplifies type 2 inflammation and pathology,[74] and its expression correlates with disease severity in mixed Th1/Th2-mediated colitis associated with SAMP1/YitFc mice.[73] Furthermore, IL-33 increases
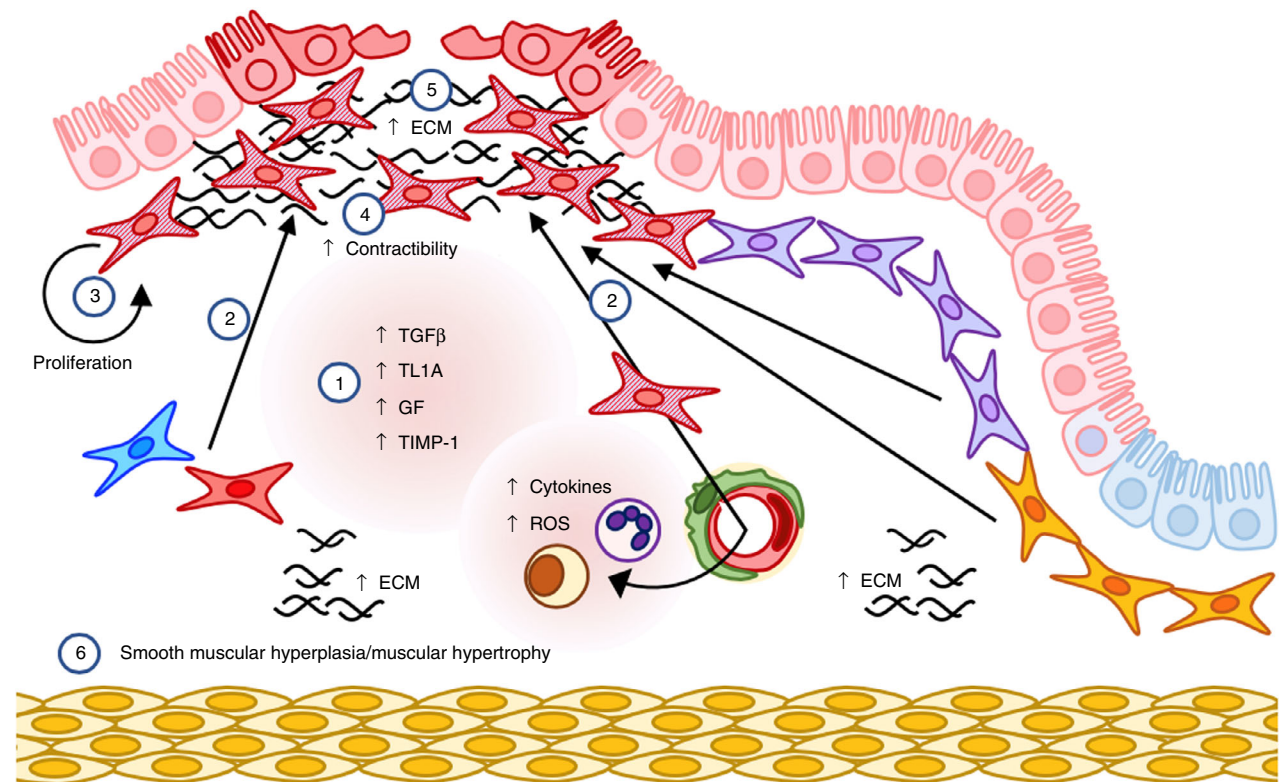

Figure 3. Fibrosis and pathological response to wounding. Dysregulated wound healing can lead to fibrosis and fibrostenosis, two major complications of inflammatory bowel disease (IBD). 1. Transforming growth factor (TGF)-β and TL1A, reactive oxygen species (ROS), basic fibroblast growth factor (bFGF), insulin-like growth factor-1 (IGF-I), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) are released by leucocytes and intestinal mesenchymal cells (iMC) during chronic inflammation. 2–4. These mediators drive the accumulation of numerous populations of iMC,[77,78,92] which then differentiate into contractile, pathologically activated myofibroblasts.[63] 5. Fibrosis-associated myofibroblasts lose their migratory potential and produce excess extracellular matrix (ECM) proteins resulting in pathological ECM deposition.[63,77–84] 6. Ultimately this leads to fibrosis, smooth muscle cell hyperplasia and hypertrophy of the muscularis.

the expression of IL-13 and Eos peroxidase by Eos, and this can lead to increased production of TNFα, IL-1β and IL-6 by IL-33-primed intestinal fibroblasts *in vitro*.[75] Therefore, whether iMC-derived IL-33 plays a harmful or beneficial role needs to be explored in individual forms of intestinal inflammation.

Dysregulated wound healing can lead to fibrosis and fibrostenosis, which are major complications of IBD (Fig. 3). iMC such as fibroblasts, myofibroblasts and pericytes accumulate in chronically inflamed sites[63] and, in contrast to the relative lack of knowledge of their role in primary immune responses, there is much clearer evidence that iMC are centrally involved in IBD-associated fibrosis. In this context, iMC lose migratory capacity, differentiate into contractile myofibroblast-like cells, produce excess ECM proteins, and release inhibitors of the MMPs that control tissue remodelling. This disrupts the normal balance between ECM deposition and degradation, leading to fibrosis, smooth muscle cell hyperplasia, hypertrophy of the muscularis and eventually stricture formation.[76] Identifying which iMC are involved in these processes, and the mechanisms they use, could be an important advance in designing new therapies for treating IBD-associated fibrosis.

Transforming growth factor-β is a well-known product of MCs that can drive fibrosis by inducing the migration, differentiation and proliferation of myofibroblasts, as well as increasing collagen production by numerous iMC populations.[63,77–84] TGF-β also enhances the production of MMP inhibitors, such as tissue inhibitor of metalloproteinase-1 (TIMP-1),[80] and induces the expression of other profibrogenic molecules, such as fibronectin, connective tissue growth factor, platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), IL-11 and IGF binding protein 3 (IGFBP3).[78,85–88] Although TGF-β is expressed constitutively in the steady-state mucosa, overexpression of TGF-β isoform 1 (TGF-β_1) drives progressive fibrosis and stricture formation in mice.[81] TGF-β expression is greatly increased in the stricture-associated and inflamed mucosa of patients with CD and UC, respectively,[80,89,90] and this production has been attributed to myofibroblasts and lamina propria lymphocytes.[80,91]

Other profibrogenic factors elevated in the mucosa of IBD patients include insulin-like growth factor-1 (IGF-I), PDGF, epidermal growth factor (EGF), bFGF and fibronectin, as well as inflammatory cytokines such as tumour necrosis factor (TNF)α, IFNγ, IL-13, IL-17A and IL-33. Fibronectin, IGF-I, PDGF and EGF can all stimulate myofibroblast migration *in vitro*,[77,78,92] whereas TNFα and IFNγ reduce their migratory potential.[93] These dichotomous roles could account for the accumulation and subsequent retention of activated myofibroblasts within a fibrotic site *in vivo*. Importantly, these cytokines and growth factors can also drive fibroblast production of ECM proteins, predominantly collagen.[75,94–97] Although TNFα can increase the production of potentially protective MMPs,[98] it also induces the expression of TIMP-1 by myofibroblasts.[95] Moreover, TNFα-induced MMP production is inhibited in the presence of IL-13, which is overexpressed in fibrotic CD tissue, resulting in excessive collagen deposition.[98] Another novel player involved in inflammation-mediated fibrosis is TNFSF member TL1A. IBD development has been associated with variants of the TL1A coding gene *TNFSF15* that result in increased TL1A protein expression,[99,100] and TL1A can drive the expression of the profibrogenic factors TGF-β_1, connective tissue growth factor (CTGF) and IGF-I.[97] TL1A inhibition reverses established fibrosis in two different murine models of colitis,[97] whereas transgenic overexpression drives the development of fibrostenosis in experimental colitis.[101] Collectively, myofibroblasts and smooth muscle cells responding to local mediators in intestinal inflammation play important roles in fibrosis when inflammation does not resolve.

What role do microbes play in iMC responses?

The intestine is a prime example of a tissue whose cellular components are exposed to and imprinted by a highly complex microenvironment. Several local factors are known to be involved in these processes, including the microbiota, dietary products and metabolites, epithelial cell-derived mediators and neurological factors. Of these, only the microbiota has been explored to any extent for an effect on iMC. Importantly, this 'forgotten organ', constituting trillions of microbial cells from thousands of different species of bacteria, viruses, protozoa and archaea, has major effects on shaping immune function throughout life. The maternal microbiota can even shape fetal immune development *in utero*. Most studies of microbiota-driven imprinting have focused on cells of haematopoietic origin. Interestingly, however, human fetal myofibroblasts have a more inflammatory phenotype than those isolated from infants, perhaps indicating that acquisition of the microbiota at birth may dampen iMC activity.[25] The mechanisms and pathways underlying these effects are largely unknown. However, microbial signals are reportedly required for RALDH activity and RA production in iMC so this pathway might make a contribution.[33] Furthermore, microbially derived short-chain fatty acids (SCFA) dampen chemokine production by myofibroblasts, and attenuate their production of MMPs in response to cytokine stimulation *in vitro*.[102–104] Thus, regulation of iMC inflammatory processes by microbial mediators may help maintain a tolerogenic environment in the steady-state gut.

Microbial metabolites can also regulate other aspects of iMC function. For instance, SCFA and urolithins can modulate prostaglandin synthesis by human myofibroblasts in

culture, suggesting that the microbiome may contribute to how iMC influence barrier function and mucus production *in vivo*.[105,106] Evidence is also beginning to emerge that the microbiota may play a role in iMC-mediated pathologies. For example, the ability of transgenic overexpression of TL1A to induce intestinal fibrosis requires an intact microbiome, and fibroblasts isolated from germ-free mice showed impaired migration and collagen production in response to TL1A or bacterial products.[107] Thus, microbial priming of iMC may shape their responsiveness to certain stimuli, shifting them from a pro-inflammatory to a more tolerogenic, wound-healing phenotype.

Future perspectives

Studies in recent years have revealed some of the roles that specific populations of iMC play in intestinal immunity, both in steady-state and during infection and inflammation. However, our knowledge is far from complete and stems predominantly from observations *in vitro*. Recent studies using scRNAseq have characterized iMC heterogeneity in unprecedented detail, and will no doubt help allow the function of specific iMC populations to be investigated extensively *in vivo*. Furthermore, this recent work suggests that pro-inflammatory populations of iMC expand in IBD, and further studies are now required to establish how these discrete populations collaborate with the immune system during homeostasis and inflammation, and whether novel targeted therapeutics can be developed for intestinal diseases, such as IBD, that are characterized by persistent iMC activation and defective function. Moreover, it will be key to understand how the microbiota impacts iMC phenotype and function because, by educating the stromal cell compartment, microbe-—iMC interactions could have important implications for the development of IBD and other intestinal pathologies.del AppSup.bat

Disclosures

The authors have no competing interests to declare.

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