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Review

Do mast cells affect villous architecture? Facts and conjectures

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Summary. In adult life, the architecture of the intestinal villus is maintained by a complex series of epithelial-stromal interactions that involve different types of fixed and mobile cells located in the intestinal mucosa. Mast cells (MC) are normal constituents of the small bowel mucosa where they reside in the villous and pericryptal lamina propria as well as within the columnar epithelial cell layer. Besides being involved in numerous immune and inflammatory reactions in the context of both innate and acquired host defence, MC are known to exert important non-immunological functions like wound repair, extracellular matrix remodelling, angiogenesis and neurotrophism as well as modulation of fibroblast, epithelial cell and smooth muscle cell activity. These pleiotropic functions put MC in a central, strategic position to organize tissue defence, restore tissue damage and maintain tissue homeostasis. This review summarizes the most recent advances concerning the functional anatomy of the crypt-villus unit and discusses the way intestinal MC might become part of the instructive circuits that ultimately lead to the maintenance of a proper villous shape.

Key words: Mast cell, Crypt-villus epithelial cell, Intestinal subepithelial myofibroblast, Microvasculature, Nerve fibres, Growth factors, Regulatory cytokines

Introduction

Mast cells (MC) are tissue secretory cells that originate from circulating bone marrow-derived precursors and express critical functions in inflammation and immunity (Metcalfe et al., 1997). MC are found in almost all of the major organs and tissues of the body, particularly in association with connective tissue structures such as blood vessels, lymphatic vessels and nerves, and in proximity to surfaces that interface the external environment, such as those of the respiratory and gastrointestinal system and the skin (Crivellato et al., 2004). This selective accumulation at tissue sites where foreign material attempts to invade the host suggests that MC are among the first cells to initiate defensive mechanisms. MC indeed synthesize and release a myriad of proinflammatory and immunoregulatory molecules and express a wide spectrum of surface receptors for cytokines and chemokines, which enable them to exert important functions in the context of both innate and acquired host defence (Mekori and Metcalfe, 2000; Benoist and Mathis, 2002). They are also implicated as effector cells in the pathogenesis of many allergic disorders. In addition, an increasing body of evidence has accumulated suggesting active participation of MC to distinct non-immunological functions, like wound repair, tissue remodelling and fibrosis as well as tissue angiogenesis (Church and Levi-Schaffer, 1997). MC store in their secretory granules and release in the surrounding micro-environment an impressively broad array of preformed mediators and signalling molecules that affect the functional profile of different resident tissue cells, like fibroblasts, smooth muscle cells, endothelial cells, epithelial cells and nerve fibres (Thabrew et al., 1996; Gruber et al., 1997; Nilsson et al., 1997; Ribatti et al., 2001). In addition, they synthesize and release both serine- and metallo-proteases, which cause extracellular matrix degradation and tissue remodelling (Levi-Schaffer and Pe'er, 2001). These functional properties put MC in a central, strategic position to organize tissue defence, restore tissue damage and maintain tissue homeostasis (Maurer et al., 2003).

Recently, a significant association has been recognized between villous architecture and the number of MC in the small bowel mucosa. Indeed, high values of total and tryptase-reactive MC in the lamina propria of human duodenum were found to be associated with normal villous profile while low values were associated with defective or atrophic villi (Crivellato et al., 2003). These findings suggest participation of MC in the

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complex network of cellular and molecular signals regulating villous structure. The present review will focus on how MC may fit in and impact on the process of villous patterning. The functional anatomy of the crypt-villus unit and the pericryptal fibroblastic sheath will first be considered. Then, the presentation will deal with the potential contribution of mucosal MC to homeostasis of villous architecture.

Functional anatomy of the crypt-villus unit

The mucosa of the small intestine is made up of a single-layered columnar epithelium of endodermal origin and an underlying mesenchymal lamina propria. The epithelial lining invaginates to form gland structures, called intestinal crypts of Lieberkühn, and protrudes to generate finger-shaped or leaf-like projections named villi. Epithelial cells arise from a resident stem cell compartment located in the lower part of the intestinal crypts (Bjerkens and Cheng, 1981; Potten et al., 1982; Millis and Gordon, 2001). Stem cells give rise to four primary epithelial cell types: enterocytes, goblet cells, enteroendocrine cells and Paneth cells (Cheng and Leblond, 1974; Bjerkens and Cheng, 1999). With the exception of Paneth cells, most members of the other three lineages complete their terminal differentiation during a highly ordered upward migration from each crypt to adjacent villi (Brittan and Wright, 2002). Migrating cells form distinct columns with sharply defined borders. It has recently been recognized that the multipotent stem cell gives rise to a daughter that produces the enterocytic lineage, and to descendants that generate secretory lineages (goblet, Paneth, and enteroendocrine cells) (Bjerkens and Cheng, 1999). In the human small intestine, the journey from the crypt to the villus tip is completed in 3-6 days and terminates in cell apoptosis and/or exfoliation. Thus, regular cellular renewal maintains an equilibrium between cell birth and cell death, the gut epithelium being one of the tissues with the most rapid proliferative rates in the body (MacDonald et al., 1964). Villous architecture is maintained by epithelial-stromal interactions, which are still poorly understood (Fritsch et al., 1997; Del Buono et al., 1992). Different local factors have been implicated in the regulation of crypt epithelial cell proliferation and differentiation: the resident and inflammatory cells in the lamina propria, the molecular composition of the extracellular matrix, and the pericryptal microvasculature and neuron plexus (Simon-Assmann et al., 1995; Bjerkens and Cheng, 2001; Paris et al., 2001).

The intestinal subepithelial myofibroblast

Intestinal crypts of Lieberkühn are surrounded by a prominent fibroblastic sheath (Fig. 1A). This forms a layer of specialized smooth muscle cell-like fibroblasts known as pericryptal fibroblasts or intestinal subepithelial myofibroblasts (ISEMF) (Powell et al., 1999b). These cells can be identified by certain characteristic features of the cytoskeleton, particularly by the expression of α-smooth muscle actin and vimentin (Sappino et al., 1990) (Fig. 1 B). ISEMF form a three-dimensional network immediately subjacent to the subepithelial basal lamina (Joyce et al., 1987). These mesenchymal cells make fundamental contributions to crypt-villus morphogenesis during embryonic life because they release specific growth and differentiating factors, such as platelet-derived growth factor (PDGF)-A (Goyal et al., 1998; Karlsson et al., 2000). In adult life, ISEMF are crucial elements in remodelling villous structure due to their production of cytokines and growth factors - like hepatocyte growth factor (HGF), tumour growth factor (TGF)-ß, granulocyte/monocyte colony-stimulating factor (GM-CSF), epidermal growth factor (EGF) and keratinocyte growth factor (KGF) - capable of stimulating crypt and villus epithelial cell proliferation and differentiation (Halttunen et al., 1996; Powell et al., 1999a; Brittan and Wright, 2002; Sennikov et al., 2002). During their replication and differentiation, ISEMF move upward from the base of the crypt to the tip of the villus (Marsh and Trier, 1974). This puts them in a strategic position to orchestrate a correct epithelial cell proliferation and migration rate. ISEMF may also indirectly affect epithelial cell growth and differentiation by secreting extracellular matrix molecules and various components of the basement membrane, such as type I and IV collagen, fibronectin, tenascin and proteoglycans (Schitt-Gräff et al., 1994; Finch et al., 1995; Powell et al., 1999a). In addition, ISEMF play a major role in inflammatory responses and wound repair processes occurring in the intestinal mucosa (Powell et al., 1999b).

MC in the small bowel mucosa

MC are important cell components of human intestinal mucosa and submucosa. Under normal conditions, they account for 2-3% of cells in the lamina propria and approximately 1% in the submucosa (Bischoff, 2000). In the normal adult duodenum and jejunum, there are up to 300 MC/mm^2 of mucosa (Strobel et al., 1981; Crivellato et al., 2003). Mucosal MC reside both in the pericryptal zone and villous axis (Fig. 2). Pericryptal MC are more numerous and densely packed than MC in the villous stroma. They often lie adjacent to blood capillaries, lymphatics or nerves, or close to crypt epithelium. Interestingly, subepithelial pericryptal MC present an elongated fibroblast-like profile that seems to embrace the crypt wall (Fig. 1C,E). A variable proportion of intraepithelial MC is also observable (Fig. 1D,F). Microscopical examination indicates that the number of MC within the columnar epithelial cell layer of the crypts is higher than the number of MC in the epithelium overlying the villi.

Human intestinal MC, like MC in the other peripheral tissues, develop from bone marrow-derived MC committed precursors (Metcalfe et al., 1997). These precursors circulate as mononuclear agranular cells,

traverse the microvascular endothelium and differentiate into mature intestinal MC upon the action of growth and differentiating cytokines secreted by fibroblasts, ISEMF and endothelial cells in the lamina propria (Mierke et al., 2000).

According to the content of serine-proteases, three MC phenotypes are distinguishable. The tryptase- and chymase-containing MC subset (MC_{TC}) forms the largest proportion (60-65%) of MC in the human intestinal mucosa, followed by MC containing tryptase but no chymase (MC_T) (~30%) and MC containing chymase but no tryptase (MC_C) (~10%) (Bischoff et al., 1996). Thus, MC capable of releasing the enzyme tryptase account for 90-95% of total mucosal MC whereas chymase-secreting MC form a portion of 70-75%. These figures are of the utmost importance in the light of the potential role of MC in the process of villus remodelling because both tryptase and chymase exert relevant effects on fixed stromal cells, extracellular matrix proteins and microvasculature.

MC participate in a wide spectrum of both physiological functions and pathological conditions affecting the small bowel (Fenoglio-Preiser et al., 1999). The most important include immune responses and proinflammatory reactions, regulation of mucous secretion and intestinal mucosal barrier, blood flow and

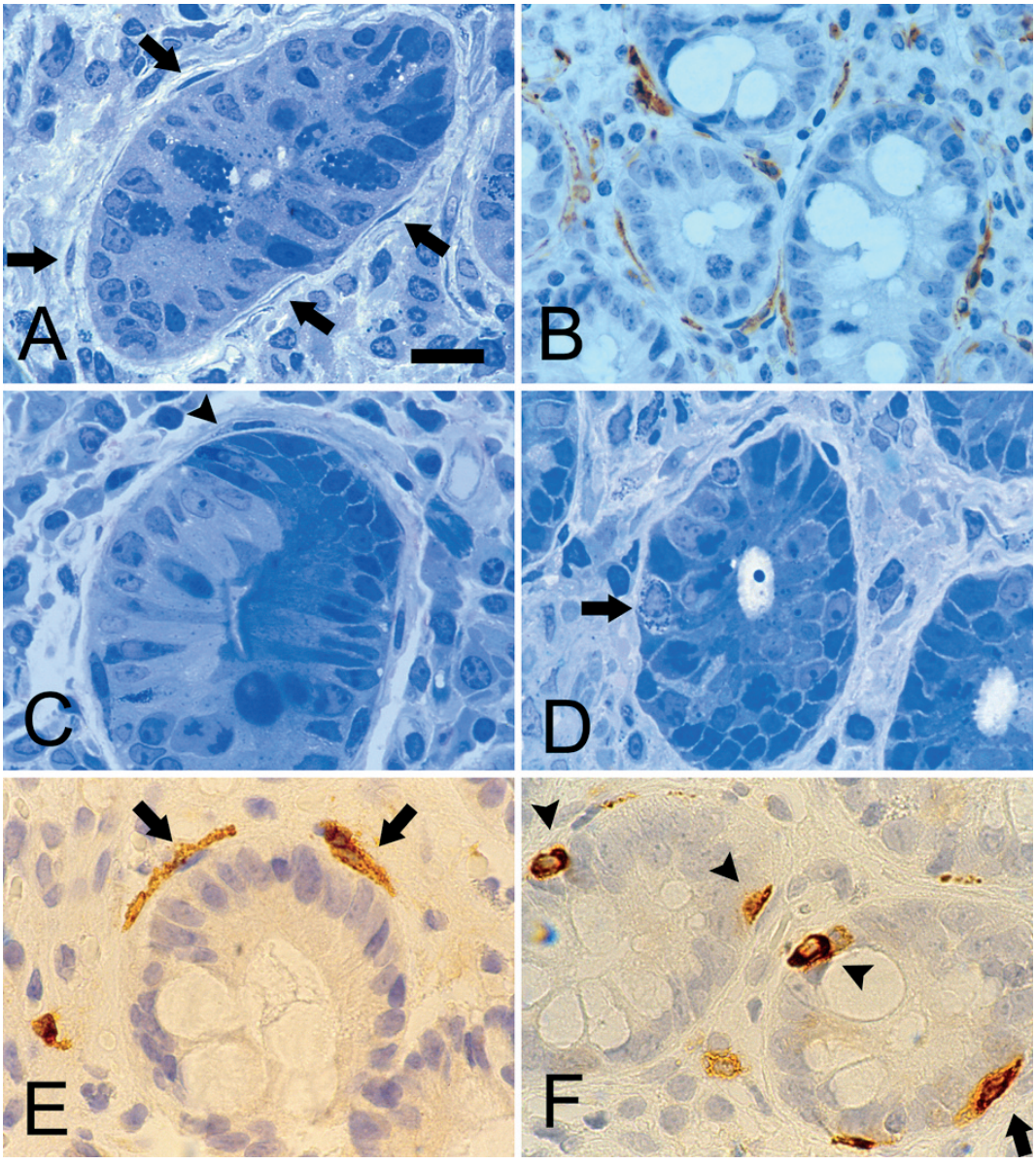

Fig. 1. Microscopic anatomy of the crypt epithelium and pericryptal lamina propria in the human small bowel mucosa. Each crypt is surrounded by a sheath of intestinal subepithelial myofibroblasts (arrows in **A)**, which express α-smooth muscle actin immunoreactivity **(B).** Mast cells with a fibroblast-like profile reside in the lamina propria close to crypts (arrowhead in **C,** arrows in **E** and **F)**. Other mast cells localize within the crypt epithelium (arrow in **D** and arrowheads in **F).** Mast cells in relation to crypts contain the proteolytic enzymes tryptase **(E)** and chymase **(F)**. Bar: 30 μm.

vascular permeability, and modulation of transmitter secretion by neuronal cells. MC play a fundamental role in food allergy, immediate hypersensitivity reactions, host responses to parasites and neoplasms, immunologically non-specific inflammatory and fibrotic conditions. In the mucosa of the small bowel, MC initiate acute inflammation and propagate chronic inflammatory reactions, modulating their cytokine response and adapting their mediator profile to distinct microenvironmental stimuli (Lorentz et al., 2000; Lorentz and Bischoff, 2001). Human intestinal MC are a rich source of proinflammatory cytokines as tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-8, as well as Th2 cytokines, such as IL-3, IL-5, IL-10 and IL-13 (Bischoff, 2000; Lorentz et al., 2000; Lorentz and Bischoff, 2001).

Cross-talk between MC and myofibroblasts

Several lines of evidence suggest that the dialogue between myofibroblasts and MC involves a bidirectional route of inductive signals. Myofibroblasts, indeed, have been shown to arise in a wide variety of settings - like wound healing and fibrosis - concurrently with a local increase in the number of tissue MC (Choi and Claman, 1987; Rothe and Kerdel, 1991; Hebda et al., 1993).

ISEMF and myofibroblasts from different anatomical sites produce chemokines, cytokines, growth and differentiating factors, inflammatory mediators as well as adhesion molecules that stimulate MC maturation and up-regulate MC function (Serini and Gabbiani, 1999; Powell et al., 1999a). ISEMF synthesize and release stem cell factor (SCF), the key growth factor for intestinal MC development and differentiation (Klimpel et al., 1996). This cytokine also plays a major role in driving "homing" and secretory activity of intestinal MC (Bischoff and Dahinden, 1992; Lorentz et al., 2002). ISEMF produce and release nerve growth factor (NGF), which is important for MC marker expression during *in vitro* culture of human umbilical cord blood cells (Welker et al., 2000). ISEMF secrete mediators with potent chemotactic activity on human MC, such as TGF-ß (Gruber et al., 1994), monocyte chemoattractant protein (MCP)-1 that also provokes MC activation (Casola et al., 1997), and RANTES (Casola et al., 1997; Romagnani et al., 1999). When activated, ISEMF express adhesion molecules like vascular adhesion molecule (VCAM)-1, which is responsible for MC docking on myofibroblasts by interacting with ß1 integrin VLA-4 on MC surface (Pang et al., 1994; Strong et al., 1998).

Many MC-derived mediators are, conversely, capable of affecting myofibroblast development and/or activation. MC are a source of PDGF and SCF, which are both responsible for myofibroblast differentiation from embryological stem cells in different anatomical sites (Bernex et al., 1996; Bostrom et al., 1996). Along with IL-4, NGF and tryptase, all MC key products, PDGF is also one of the most important factors for the transdifferentiation of adult lung, skin and renal fibroblasts into mature myofibroblasts (Yamakage et al., 1992; Schitt-Gräff et al., 1994; Doucet et al., 1998; Micera et al., 2001). MC synthesize and release TGF-ß, which is crucial in generating phenotypically and functionally activated myofibroblasts (Taipale et al., 1995; Fritsch et al., 1997). In addition, latent TGF-ß bound to matrix can be released by MC-derived chymase (Taipale et al., 1995). Interestingly, myofibroblasts express receptors for histamine, as documented by studies in the skin and intestine (Berschneider and Powell, 1992; Taipale et al., 1995). Proinflammatory cytokines, such as IL-1, IL-6 and IL-8, all produced and secreted by human intestinal MC, have also been implicated in the process of myofibroblast activation and proliferation (Gailit et al., 2001). In addition, although TNF-α by itself is an insufficient stimulus for inducing myofibroblast transdifferentiation, it can facilitate the mechanism through which heparin stimulates the expression of a myofibroblast phenotype (Desmoulière et al., 1992; Schitt-Gräff et al., 1994; Jobson et al., 1998). Heparin is a major constituent of MC granule matrix (Metcalfe et al., 1997).

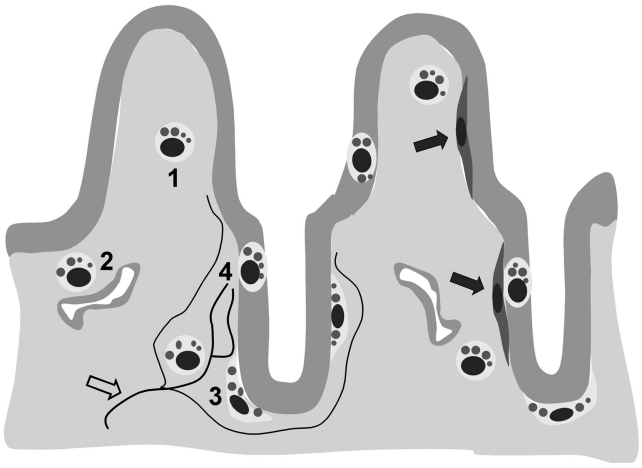

Fig. 2. Mapping of mast cell distribution in the small bowel mucosa. Mast cells may reside either in the villous axis (1), or in the perivascular and pericryptal lamina propria (2) (3), or within the crypt-villus epithelium (4). Pericryptal mast cells are situated close to the crypt wall. Solid arrows indicate intestinal subepithelial myofibroblasts. The empty arrow points to the pericryptal neural network.

MC and villous epithelium

As previously outlined, a small but significant proportion of intestinal MC localizes to crypt-villus epithelium (Fig. 1D,F). Under normal conditions, crypt epithelium harbours most of intraepithelial MC, although no definite quantitative data are available. Little is known about the functional significance of MC

residing between epithelial cells. They likely exert proinflammatory activities related to mucosal immunity. In the mouse small intestine, for instance, the number of MC located within the crypt-villus epithelial cell layer have been shown to rapidly increase during the acute phase of experimentally induced infection with *Trichinella Spiralis* and to subside shortly after the peak of inflammation (Friend et al., 1996). Under the same experimental conditions, intraepithelial MC undergo changes in their granule protease content. These findings suggest active participation of intraepithelial MC to inflammatory processes affecting the intestinal mucosa. The possibility should not be discarded, however, that this MC subset may also exert non-inflammatory functions. It is tempting to speculate that intraepithelial MC might partly regulate epithelial cell proliferation and differentiation, providing an additional contribution to villous remodelling. MC indeed are a source of mediators, such as PDGF, TGF-ß and GM-CSF, which are well recognized growth and differentiating factors for intestinal epithelial cells (Halttunen, 1996; Karlsson et al., 2000; Sennikov et al., 2002). In other anatomical sites, such as the skin and airway epithelium, MC-derived mediators modulate epithelial cell function in terms of proliferation and cytokine release (Cairns and Walls, 1997). Further studies are needed to clarify the potential contribution of intraepithelial MC to villus structural homeostasis. In addition, it is reasonable to conceive that not only intraepithelial MC but also MC residing immediately below the crypt basement membrane may affect intestinal epithelial cells through paracrine release of regulatory molecules (Fig. 1C,E).

MC and villous matrix

MC may regulate villous architecture by releasing multiple cytokines, proteolytic enzymes and growth factor mediators affecting the biochemical composition of the extracellular matrix. Tryptase and fibroblast growth factor (FGF)-2, which are synthesized and stored in the granules of human MC, promote differentiation of mesenchymal stem cells into mature fibroblasts, provoke fibroblast migration and proliferation, and induce collagen synthesis and deposition (Cairns and Walls, 1997; Gruber et al., 1997; Artuc et al., 2002). Overproduction of such mediators has been implicated in the pathogenesis of fibrotic diseases in many organs and may affect such bowel conditions like Crohn's disease (Gelbmann et al., 1999). On the other hand, MC secrete serine-proteases, in particular tryptase, which activate the pre-enzyme forms of some metallo-proteases (Gruber et al., 1989). These, in turn, are responsible for connective tissue degradation, extracellular matrix remodelling and cytokine mobilization. In addition, tryptase is also capable of cleaving fibronectin and type VI collagen as well as provoking the release of collagenase from fibroblasts (Kielty et al., 1993; Lees et al., 1994). Chymase may contribute to tissue remodelling by cleaving type I, IV and VI collagen, destructing vitronectin and fibronectin, and splitting epithelial-mesenchymal junctions (Kielty et al., 1993; Lees et al., 1994; Kofford et al., 1997). Chymase-dependent basement membrane degradation may have a major role in the process of villous elongation and epithelial cell migration from the base of the crypt to the tip of the villus.

MC and villous microvasculature

Vessels in the intestinal mucosa originate from submucosal arterioles. Some ramify into a capillary network that surrounds the crypts, others continue into the villus axis and arborize into a dense capillary bed that is closely apposed to the epithelial basement membrane. MC may affect villous structure by secreting survival and antiapoptotic factors for microvascular endothelial cells. Some experimental data point in this direction. It is well known indeed that radiotherapy causes abnormal villous architecture and villous atrophy. Radiation has been postulated to target epithelial stem cells within the crypts. However, it has recently been recognized that epithelial cell damage and exfoliation occurring after intestinal radiation are preceded by specific vascular alterations in the villous stroma (Paris et al., 2001). In the mouse model, indeed, microvascular endothelial apoptosis is the primary lesion leading to stem cell dysfunction. In addition, survival factors for endothelium, like vascular endothelial growth factor (VEGF) and FGF-2, prevents radiation-induced stem cell damage, villus denudation and atrophy, intestinal failure and animal death. This underlines the potential role of vascular-targeting MC mediators in maintaining villous homeostasis. Human intestinal MC are a major source of both VEGF and FGF-2 (Grutzkau et al., 1998; Qu et al., 1998). In addition, MC-derived tryptase and chymase are potent angiogenic and angiotrophic factors (Levi-Schaffer and Pe'er, 2001), which may be partly responsible for the maintenance of normal villous structure by preventing microvascular endothelium vulnerability to chemical and microbiological injury. Tryptase, in particular, stimulates proliferation of human vascular endothelial cells and promotes vascular endothelial cell tube formation in culture (Blair et al., 1997). Human intestinal MC produce and release other important angiogenic factors, such as histamine, heparin, TNF-α and IL-8 (Bischoff et al., 1999; Crivellato et al., 2004). In addition, the production of type VIII collagen by MC may influence villus micro-repair processes since this collagen is believed to facilitate the assembly of endothelial cell tubes and its synthesis precedes that of pro-collagen type I (Ruger et al., 1994). On the other hand, endothelial cells are potent regulators of MC functions, providing chemotactic, differentiating and survival factors for both MC precursors and mature MC (Mierke et al., 2000). Thus, MC-endothelial cell interactions in the intestinal lamina propria are likely of great relevance in the perspective of maintaining villous morphology.

MC and pericryptal nerves

The enteric nervous system is a complex network of interacting neurons and supporting glial cells that regulates intestinal motility, blood flow, secretion and immune functions. Enteric neurons are located in two distinct anatomical regions: the myenteric plexus and the submucosal plexus. Myenteric plexus neurons send most of their axonal projections to the muscle layers of the intestine. Submucosal plexus neurons send the majority of their projections to the subepithelial region, including the area surrounding crypts. Pericryptal nerves have recently been recognized as important players in the regulation of crypt epithelial cell progenitor proliferation rate (Bjerkens and Cheng, 2001). Glucagons-like peptide 2 (GLP-2) is known to prevent villus atrophy resulting from damaging agents, such as chemotherapy. Indeed, GLP-2 induces an epithelial hyperplasia reflected in increased crypt and villus size. Pericryptal nerves are the targets of GLP-2 (Bjerkens and Cheng, 2001). In fact, they express the GLP-2 receptor gene and respond to administration of GLP-2 by secreting an unspecified factor that induces proliferation of columnar, not mucous, crypt progenitors. Experimental evidence also suggests that MC are involved in maintaining trophism of enteric neurons. In the rat intestinal mucosa, for instance, reconstitution of nerve fibres after experimentally-induced inflammation and nerve fibre degeneration is accompanied by a significant increase in mucosal MC density (Stead et al., 1991). MC synthesize and release powerful neurotrophic and/or neurogenic cytokines, such as NGF and FGF-ß (Tsui-Pierchala et al., 2002), and microscopical studies have revealed close apposition of intestinal MC to nerve structures in the lamina propria (Stead et al., 1987, 1989). In the light of these data, it is tempting to speculate that mucosal MC may affect villous shape by cooperating in maintaining the trophic status of the pericryptal nerve plexus.

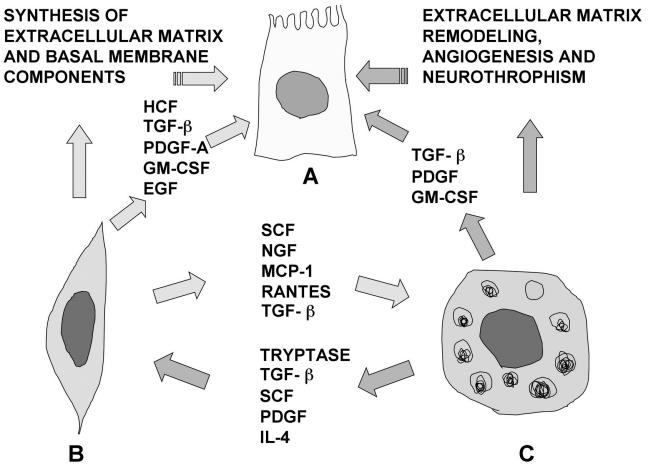

Fig. 3. Schematic drawing illustrating the potential cross-talk between myofibroblasts and mast cells in the intestinal lamina propria. Both cell types may affect crypt epithelial cells in a direct or indirect way. (1) Intestinal epithelial cell, (2) myofibroblast, (3) mast cell.

Concluding remarks and future perspectives

As outlined in this review, the maintenance of a proper villous architecture depends upon complex epithelial-stromal interactions. MC are likely to represent key elements in the regulation of such homeostatic processes. They are indeed a rich potential source of an impressively broad array of mediators stimulating ISEMF proliferation and differentiation, remodelling extracellular matrix proteins, affecting pericryptal vessel and nerve trophism, and modulating crypt and villus epithelial cell activity (Fig. 3). They are mobile cells that can be promptly recruited in the pericryptal tissue by endothelial cell- and ISEMF-derived chemokines. They localize to strategic positions either around crypts, or close to vessels and nerves in the lamina propria, or within the crypt and villus epithelium. The mucosa of the small intestine contains the largest peripheral pool of MC committed precursors (Guy-Grand et al., 1984). These cells can rapidly differentiate into mature MC upon the action of growth and differentiating factors released by pericryptal microvessels and myofibroblasts. Thus MC are likely candidates to play a crucial role in the context of signalling networks regulating villous structure.

Different interesting questions remain to be elucidated. It is known that myofibroblasts arise in a wide variety of settings concurrently with a local increase in the number of tissue MC. Therefore, what is the functional relationship between ISEMF and MC in the pericryptal and villous lamina propria? How do modifications in the ISEMF density correlate with changes in the number and phenotypes of mucosal MC? It can be speculated that ISEMF, thanks to their cytokine and chemokine repertoire, may attract both MC precursors and mature MC in the pericryptal zone stimulating MC development and up-regulation. MC, in turn, may induce ISEMF proliferation and activation with profound effects on villous architecture. Another intriguing issue concerns intraepithelial MC. What is the functional significance of MC residing within the crypt epithelium? Are they purely involved in proinflammatory activities or can they also operate in the context of epithelium remodelling? How do intraepithelial MC vary according to alterations in the villus profile and modification of the mitotic index in the crypt epithelial cells? As MC are heterogeneous cells with distinct tissue specialization and phenotypic profile, we also need to identify the exact cytokine repertoire actually expressed by intestinal MC upon different stimulation. Animal models, like genetically W/W^v MC-deficient mutant mice, will be useful in understanding the role of these cells during villous restitution after injury.

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