Chapter 7
Immunosuppression by Intestinal Stromal Cells

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Abstract This chapter summarizes evidence that intestinal myofibroblasts, also called intestinal stromal cells, are derived in the adult from tissue mesenchymal stem cells under homeostasis and may be replenished by bone marrow mesenchymal stromal (stem) cells that are recruited after severe intestinal injury. A comparison of mechanism of immunosuppression or tolerance by adult intestinal stromal cells (myofibroblasts) is almost identical with those reported for mesenchymal stem cells of bone marrow origin. The list of suppression mechanisms includes PD-L1 and PD-L2/PD-1 immune checkpoint pathways, soluble mediator secretion, toll-like receptor-mediated tolerance, and augmentation of Treg cells. Further, both mesenchymal stem cells and intestinal stromal cells express an almost identical repertoire of CD molecules. Lastly, others have reported that isolate intestinal stromal cells are capable of differentiating into bone and less well into chondrocyte, but not into adipocytes, a finding that we have confirmed. These findings suggest that intestinal stromal cells (myofibroblasts) are partially differentiated adult, tissue-resident stem cells which are capable of exerting immune tolerance in the intestine. Their role in repair of inflammatory bowel disease and immune suppression in colorectal cancer needs further investigation.

Keywords Mesenchymal stem cells · Tissue-resident adult mesenchymal stem cells · Myofibroblasts · Immune tolerance · PD-L1 · PD-L2 · Toll-like receptors · Inflammatory bowel disease · Colorectal cancer

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This research was supported by NIH – NCATS U54 TR001342-01 and NIDDK R01 DK103150.

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B. M.J Owens, M. A. Lakins (eds.), *Stromal Immunology*, Advances in Experimental Medicine and Biology 1060, https://doi.org/10.1007/978-3-319-78127-3_7

7.1 Introduction

There are a multitude of pleotropic functions of intestinal stromal cells (myofibroblasts, fibroblasts, and pericytes) that have been discovered and investigated over the past 20 years. Previous reviews document knowledge about these cells up until the current era [1–7]. Our chapter defines the role of mucosal stromal cells in gut tolerogenic responses, including immunosuppression by B7 suppressor molecules (PD-L1 and PD-L2) which are present on these MHC class II-expressing antigen-presenting cells, immunosuppression by soluble mediators secreted by stromal cells, role in altering Th17 cell and Treg formation, and toll-like receptor-mediated modulation of immunosuppression. The PD-L1/PD-1 signaling pathways have recently become famous with the discovery of immune checkpoint therapy for cancer, revolutionizing the field of oncologic immune therapy and bringing effective therapies to previously untreatable cancers [8–10]. Our interest in these molecules developed when we discovered that mucosal $CD90^+$ stromal cells in gastric, small intestinal, and colonic mucosa were novel, innate immune cells expressing MHC class II [11–13]. Seeking an antigen-presenting function for these cells, we found that the positive B7 co-stimulatory molecules CD80 and CD86 were not normally expressed, although CD86 could be demonstrated after engagement of T cells [13]. Nevertheless, the negative co-stimulatory molecules PD-L1 and PD-L2 were robustly constitutively expressed [14], suggesting that $CD90^+$ stromal cells were far more important in tolerance than in activation of immunity.

In attempting to understand why stromal cells of all types – intestinal, chondrocytes, synovial, lung, and skin fibroblasts – might have such potent immunosuppressive functions [15], an attractive hypothesis was found in the emerging concepts of the origin of intestinal stromal cells and the idea that they might be derived from adult or tissue-resident, adult mesenchymal stem cells (tMSC) or from the recruitment of bone marrow-derived mesenchymal stromal cells (BMMSC). This was especially true since the mechanisms of MSC-mediated immunosuppression and the ability of MSC, like intestinal stromal cells, to switch from inflammation to suppression have been more recently become better understood [16–18]. Therefore, before describing the information we have learned about the immune suppressive role of intestinal stromal cells, we will briefly review the current understanding of the origin of intestinal stromal cells and of the immune functions of MSC.

7.2 Origin of Intestinal Stromal Cells

It was once thought that parenchymal mesodermal cells in the embryo originated from the neural crest [19]. However, more recent lineage tracing experiments have defined the mesothelium as the embryological origin of tissue parenchymal (myo) fibroblasts, perivascular pericytes, and vascular smooth muscle cells [20, 21]. In the adult, the discussion has centered on whether the origin of subepithelial stromal cells (myofibroblasts), during homeostasis or after tissue damage, is from a

tissue-resident, adult mesenchymal stem cells (tMSC) or from engraftment of circulating bone marrow mesenchymal stem cell (BMMSC) [22]. Stappenbeck's laboratory presented evidence for a tMSC, identified by its avid expression of COX-2 (and thus prostaglandin secretion), located in the upper aspects of the lamina propria but seemingly homing to a pericryptal location adjacent to epithelial stem cells in response to toll-like receptor (TLR) signaling [23]. Prostaglandin secretion from these relocating cells was critical for repair of dextran sodium sulfate (DSS)-induced colitis and for repair of experimental colonic perforating wounds [23]. While these cells had the repertoire of stems cells, their origin remained unclear [22]. Strong evidence that these cells may be tMSC has recently been published by Worthely et al. [24] in impressive lineage-tracking experiments using Gremlin 1 (Grem 1) as a marker of subepithelial mesenchymal cells. Tamoxifen-induced expression in a Grem1-creER^{T} mouse identified single subepithelial cells in the small intestine isthmus, the region that serves as the transition from villi to crypts. These cells divided exceedingly slowly, incorporating BrdU over the course of a month and taking 3 months for these labeled cells to populate the entire pericryptal mesenchymal sheath and a year to completely populate the entire villus with smooth muscle α-actin-positive myofibroblasts and smooth muscle α-actin-negative, but not NG2-positive, stromal pericyte-like cells. These marked cells persisted for 2 years. Worthely named these cells intestinal reticular stem cells (iRSC) denoting the reticular network that they formed. This network was entirely distinct from the closely approximated s100b-/NES-positive glial network. Thus, this publication gives strong evidence for a slow cycling, tissue stem cell providing homeostasis for small intestinal epithelial and lamina propria small vessel function and for tissue structure. Although results of studies in colonic and gastric tissues were not reported, Worthely has stated that similar observations were made for colonic and gastric mucosa (personal communication).

While it is possible that under conditions of significant intestinal damage these tMSC might be called to the damaged area by chemotaxis to repopulate the myofibroblast/fibroblasts and pericytes network, an alternative mechanism for rapid repair is also possible: homing of BMMSC. As demonstrated first by Britten and colleagues [25], and reviewed in detail by Mifflin et al. [5], using the Y chromosome from male bone marrow infused into female recipients under conditions of significant tissue wounding, BMMSC may reconstitute 40–60% of subepithelial myofibroblasts and pericytes within 10 weeks of transplantation. A similar phenomenon has been shown for the cancer microenvironment where 20% of cancer-associated fibroblasts in colorectal cancers are derived from BMMSC [26, 27]. Thus, one might reasonably postulate that tMSC are responsible for the homeostasis of the intestinal epithelium and lamina propria architecture, but that BMMSC replenishment serves as the mechanism for more rapid repair after acute damaging disease or trauma (Fig. 7.1).

More compelling proof that intestinal stromal cells are derived from MSC comes from the study of Signore et al. [28] who used CD146, a known MSC marker, to visualize lamina propria cells by confocal microscopy and to isolate them. The CD146-positive cells have the location and appearance of colonic CD90^{+} myofibroblasts/fibroblasts. Importantly, isolated colonic CD146 cells had the same marker phenotype as BMMSC but had a decreased intensity of the CD13, CD29, and

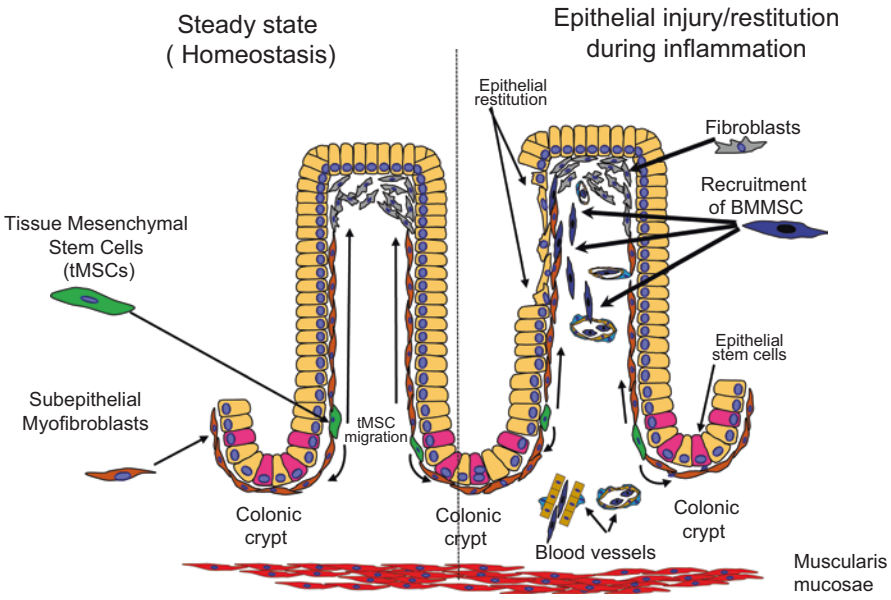

Fig. 7.1 Mesenchymal stem cell replacement of subepithelial myofibroblasts (stromal cells) during homeostasis (left) and following injury or damage from disease (right). Stromal cell replacement during homeostasis occurs by division of slow cycling tissue mesenchymal cells (tMSC) in a process that takes months to populate the top and bottom of the colonic crypts. After damage or disease, stromal cell replacement appears to be largely from recruitment of bone marrow-derived mesenchymal stem cells (BMMSC) which takes days or weeks. tMSC may also take part in stromal cells' replacement after damage

CD49c expression (Table 7.1). When these isolated CD146 colonic cells were place in differentiation media, they became osteocytes and differentiated less efficiently into chondrocytes, but not at all to adipocytes. Thus, we believe that, at least in normal intestinal mucosa, $CD90^{+}$ myofibroblasts/fibroblasts are restricted progenitor cells of mesenchymal stem cell origin. Functional differences between conventional MSC and intestinal stromal cells will no doubt be clarified over the coming years.

7.3 Immunosuppression by MSC

B7 Molecule-Mediated Suppression A fundamental property of MSC is their ability to alter the profile of dendritic cells, naive and effector T cells, and natural killer cells to induce an anti-inflammatory or tolerant phenotype [16]. While they express MHC class I constitutively, class II molecule expression must be induced. B7 co-stimulatory molecules CD80 (B7-1) and CD86 (B7-2) are robustly expressed by professional antigen-presenting cells (APCs) such as dendritic cells. These B7 ligands engage the T cell receptors CD28 (resting t cells) and CTLA-4 (activated T cells), although with a dramatically higher affinity (100- to 1000-fold higher) for

Table 7.1 Phenotypic comparison of BMMSC, gastrointestinal mucosal tMSC, and myofibroblasts

Marker	BMMSC	tMSC	Myofibroblasts	Description/function
CD4	nd	– [28]	nd	T cell coreceptor/interact with non-polymorphic regions of MHC class II and HIV protein gp120 [66]
CD10	+ [28]	+ [28]	nd	Metalloproteinase/development and cancer [67]
CD11b	nd	nd	– [11]	Integrin/adhesion, migration, phagocytosis, chemotaxis, cytotoxicity [68]
CD11c	–	nd	– [11]	Integrin/interacts with lipid A moiety of LPS [69]
CD13	++	+	±[a]	Aminopeptidase N/regulator of signals triggered by other receptors, apoptosis [70]
CD14	– [28]	– [28]	+[a]	Co-receptor for TLR4/implicated in LPS-induced skin fibroblast proliferation [71]
CD 24	– [28]	± [28]	+ [72]	Cell-cell and cell-matrix adhesion glycoprotein/ facilitates metastasis [73]
CD29	++ [28]	+ [28]	+[a]	Integrin β1/adhesion receptor for ECM components/epithelial differentiation, development and tissue organization [74]
CD31	– [28]	– [28]	–[a]	Platelet endothelial cell adhesion molecule/T cell homeostasis, effector function and trafficking [75]
CD34	– [28]	– [28]	–[a]	Hematopoietic progenitor cell Ag/cell adhesion regulation [76]
CD44	++ [28]	++ [28]	++[a]	Glycoprotein, a hyaluronic acid receptor/ regulates cell adhesion, proliferation, survival, migration, and differentiation [73]
CD45	+–[28]	– [28]	–[a]	Leukocyte common antigen, a transmembrane phosphatase/development and function of lymphocytes [77]
CD49a	++	++	nd	Integrin α1, heterodimerizes with the β1 subunit to form a cell-surface receptor for collagen and laminin/adhesion [78]
CD49c	++ [28]	++ [28]	+[a]	Integrin α3, heterodimerizes with the β1 subunit/ cell migration and adhesion, regulation of ECM components [79]
CD49d	++ [28]	++ [28]	++[a]	Integrin α4, heterodimerizes with the β1 subunit/ interact with VCAM-1; cell adhesion [80]
CD54	++ [28]	++ [28]	++ [81]	Glycoprotein, also known as intercellular adhesion molecule 1, ICAM-1/cell adhesion [80]
CD80	± [32]	nd	– [11]	B7 family co-stimulator/interacts with CD28, CTLA-4, and PD-L1/regulation of T and macrophage activity [30, 57]
CD86	± [32]	nd	± [11]	B7 family co-stimulator/interacts with CD28, CTLA-4/regulation of T and macrophage activity [30, 57]
CD90	++	++	++	Activation-associated cell adhesion molecule (Thy1)/cell adhesion [82]

(continued)

Table 7.1 (continued)

Marker	BMMSC	tMSC	Myofibroblasts	Description/function
CD105	++ [28]	++	±	Also known as an endoglin, accessory receptor for TGF-β/implicated in angiogenesis and neovascularization [83]
CD146	++ [28]	++ [28]	++[a]	Cell adhesion molecule (CAM)/implicated in development, cell migration, mesenchymal stem cells differentiation, angiogenesis, immune responses [84]
CD166	++ [28]	++ [28]	+[a]	Activated leukocyte adhesion molecule (ALCAM)/bind to CD6; adhesion and T cell activity regulation [85]
HLA-ABC	++ [28]	++ [28]	++[a]	MHC class I molecules/MHC class I restricted Ag presentation to $CD8^+$ T cells [86]
HLA-DR	− [28]	++ [28]	+ [14]	MHC class II molecule/MHC class II restricted Ag presentation to $CD4^+$ T cells [86]
PD-L1	+ [87]	+ [87]	+ [14]	B7 family co-inhibitor (B7-H1)/interacts with PD-1 and CD80; regulation of T and macrophage activity [30, 57]
PD-L2	+ [87]	+ [87]	+ [14]	B7 family co-inhibitor (B7-DC)/interacts with PD-1; regulation of T and macrophage activity [30, 57]
B7-H2	nd	nd	+[a]	B7 family co-stimulator (ICOSL)/interacts with ICOS; activate T cell proliferation and induction of T17 type responses [88]
a-SMA	− [6]	+ [28]	+ [11]	Alpha-smooth muscle actin-2/involved in cell motility, structure, and contractile apparatus [6]
vimentin	+	+	++ [89]	Type III intermediate filament protein/major cytoskeletal component of mesenchymal cells; cell adhesion and endothelial sprouting [90]

[a]Unpublished data
nd non-determined, *Ag* antigen, *ECM* extracellular matrix

CTLA-4 [29–31]. Ligation of CD28 by CD80 and/or CD86 enhances T cell proliferation, intensifies pro-inflammatory cytokine secretion, and upregulates anti-apoptotic genes. MSC have low or negative expression of the positive B7 co-stimulatory molecules CD80 and CD86 but are reported to express high level of B7 inhibitory molecules PD-L1 and PD-L2 [32, 33]. These inhibitory molecules are critically involved in suppression of activated T lymphocyte proliferation, thus contributing to the maintenance of peripheral tolerance [33, 34]. PD-L1 is also reported to be implicated in MSC-mediated suppression of Th17 cell differentiation [35]. Importantly, there is evidence that PD-L1 expression on MSC may be responsible for suppression of autoreactive T cells in experimental autoimmune type 1 diabetes [36] and in inducing immune tolerance to cardiac allografts when given in combination with rapamycin [37]. Recent reports have demonstrated that PD-L1 is involved in the regulation of inflammatory Th17 [38] and immunosuppressive $CD4^+$ $CD25^{high}$ $FoxP3^+$ regulatory T cell (Treg) responses [39]. MSC have been shown to contribute to the regulation of the Th17/Treg balance and may repress mature Th17 cells in a PD-L1-dependent manner [35]. Taken together, these properties allow MSC to

escape rapid immune rejection, and they establish the reason for the therapeutic value of MSC in the treatment of experimental and human immune-mediated diseases such as graft-versus-host disease, autoimmune encephalomyelitis, multiple sclerosis, type 1 diabetes mellitus, rheumatoid arthritis, systemic lupus erythematous, Crohn's disease, and cirrhosis, to name but a few [17, 40].

Soluble Mediator-Mediated Suppression The mechanisms that allow MSC-mediated immunosuppression were initially thought to occur only through secretion of soluble immune suppressors such as prostaglandin E2 (PGE2), indoleamine 2,3-dioxygenase (IDO, especially in human MSC), nitric oxide (NO, especially in murine MSC), and human leukocyte antigen (HLA)-G, as well as TGF-β, HGF, and hemoxygenase [17, 41–43]. Murine secretion of PGE_2 is upregulated by both interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α), while IDO upregulation requires IFN-γ [32]. Therefore, soluble factor secretions of immunosuppressant molecules together with PD-L1-/PD-L2-mediated signaling are among the critical immunosuppressive mechanisms exerted by MSC which contribute to immune tolerance.

TLR Signaling Modulates Suppressive Properties of MSC While the biological and functional properties of murine and human MSCs differ, MSC from both species express toll-like receptors (TLRs) and NOD-like receptors (NLRs) NOD1 and NOD2 [18, 44]. TLRs and NLRs are known to trigger an innate immune response against microbial stimuli [45]. It has been suggested that stimulation of TLR3 has opposing effects from that occurring after activation of TLR4-mediated signaling [18]. TLR3 ligation by its putative ligand, dsRNA, results in an anti-inflammatory MSC phenotype with secretion of high levels of soluble immune suppressants, including IDO, PGE2, and TGF-B, and enhances MSC capacity to induce suppressive regulatory T (Treg) cells and M2 (suppressive) macrophages. Conversely, activation of TLR4 by its putative receptor, LPS, results in reduction of soluble suppressor secretion and in an increase in lymphocyte-recruiting chemokine (PIP-1a, MIP-1b, RANTES, CXCL9, and CXCL10) production [46]. However, Chen et al. [42] could not reproduce a differential suppressive effect of TLR 3 or TLR 4. In their experiments, ligation of neither TLR3 nor TLR4 affected the self-renewal, apoptosis, or expression of stem cell markers on MSC, while stimulation of TLR3 enhanced MSC differentiation into adipocytes and osteocytes, but activation of TLR4 signaling inhibited MSC differentiation. Thus, further investigation of TLR signaling in both MSC and MSC progeny, stromal cells, is necessary.

7.4 Immunosuppression by Intestinal Stromal Cells

B7 Molecule-Mediated Suppression While recent study of MSC immune function brought attention to the immunosuppressive potential of these cells [47, 48], initial studies of intestinal stromal myofibroblasts/fibroblasts (MFs) focused on their role in antigen presentation. In 2006 our group reported that human colonic MFs were among the major cell phenotypes in the normal human colonic lamina

propria and were capable of presenting antigens in a MHC class II-dependent manner [13]. Expression of MHC class II was also observed on small intestinal and gastric MFs after stimulation with IFN-γ [12, 13]. In 2013, Owens et al. [49] demonstrated that, although somewhat limited when compared to dendritic cells, colonic MFs were able to uptake, phagocyte, and process *Salmonella typhimurium* antigens. Thus, MFs may possibly play a role of local APCs in the gastrointestinal mucosa.

MHC class II was shown to be involved in $CD4^+$ T cell proliferation induced by allogeneic and syngeneic MFs [11, 13, 50], but we observed that MFs isolated from healthy gut mucosa had a limited capacity to induce proliferation of naïve/resting $CD4^+$ T cells. Similar to the MSC, the limited capacity was thought to be due to constitutive absence of CD80 expression and low CD86 expression on MFs. These observations make it likely that CD86 mostly engages CTLA-4 on the activated effector T cells present in gut mucosa, and this engagement will contribute to the CTLA-4-mediated immunosuppression. Although further studies are required to understand the mechanisms and involvement of stromal cell CD86 expression in CTLA4-mediated suppression of activated T cells, a similar suppressor function has been proposed for immature dendritic cells which also have a low level of surface CD80 expression [51, 52].

Our finding of low levels of CD86 expression on normal human colonic MFs led us to hypothesize that these cells normally serve as "suppressors" of activated T cell responses in the healthy colon. MFs derived from normal colonic mucosa express strong basal level of PD-L1 and PD-L2 [14], and we found a similar robust expression of PD-L1 and PD-L2 in small intestinal and gastric MFs (unpublished data). As has been previously reported for MSC, PD-L1 and PD-L2 were found to be critically involved in MF-mediated suppression of the CD3-/CD28-activated $CD4^+$ T cell proliferation and IL-2 production [14].

Besides suppression of T cell proliferation, PD-L1 and PD-L2 are implicated in regulation of IFN-γ production by different immune cell subsets [53–56]. We demonstrated that PD-L1 is involved in the colonic MF-mediated suppression of the IFN-γ production by activated $CD4^+$T cells [57]. Recently we have observed that PD-L2 also contributes to MF-mediated suppression of both Th1 transcription factor T-bet expression and IFN-γ production by activated $CD4^+$T cells (unpublished data). Further studies are necessary to delineate the differences in the PD-L1- and PD-L2-mediated MF tolerogenic responses in the gut mucosa.

Suppression by Soluble Mediators While our laboratory has focused mostly on MF B7 molecule-mediated immunosuppression, similar to MSC, MFs in GI mucosa produce multiple soluble immunosuppressive cytokines, growth factors, and small metabolites (IL-10, IL-21, TGF-β, PGE_2, and IDO) [13, 50, 58, 59]. These molecules are known to contribute to the regulation of immune responses in the gut and are implicated in the regulation of Th1/Th17/Treg cell balance [5, 7]. Treg are especially important for maintaining gut mucosal tolerance [60]. We demonstrated that production of PGE_2 is critical to colonic MF-mediated induction of immunosuppressive Treg cells from naïve $CD4^+CD45RA^+$ T cells [50]. PD-L1 was minimally involved (contributing only ~10%) to colonic MF ability to induce Treg which

appeared to be also dependent on MF expression of MHC class II [50]. Further studies will allow better understanding of the role of the soluble immunosuppressive molecule produced by MFs in their ability to promote tolerogenic responses.

TLR-Like Receptor-Mediated Modulation of Tolerogenic Responses The GI tract is populated by a resident and transitory microbiome. The continuous presence of normal physiological microflora in the GI lumen and mucosal surface provides a significant source for TLR and NLR ligands [45]. Signaling through these innate immunity receptors is thought to play a major role in orchestrating mucosal tolerogenic responses [57, 61]. Because MFs are located just beneath the basement membrane of the epithelial layers, are exposed to luminal ligands when epithelial tight junctions are leaky, express TLR 1–9 and NOD 1/NOD 2, and actively participate in wound repairs in GI mucosa [62], it is likely that MF-mediated immunosuppression is modulated by microbiota at least during wound healing process. Indeed, we recently demonstrated that stimulation of TLR2, TLR4, and TLR5 enhances the immunosuppressive capacity of normal colonic MFs via an increase in PD-L1 expression [57].

Myeloid differentiation factor 88 (MyD88) serves as an adaptor for the majority of TLRs (except TLR3) and is required for the initiation of intracellular signaling in response to the binding of a microbial ligand to TLR [63]. Using primary human MF cultures and fibroblast-specific MyD88 conditional knockout mice, we demonstrated that both basal- and TLR-induced levels of PD-L1 on MFs in the colonic mucosa depend on MyD88 [57]. TLR4-mediated upregulation of MF PD-L1 resulted in enhanced suppression of $CD4^+$ effector T cell proliferation and IFN-γ production. Taking into consideration the key role of PD-L1 in the negative regulation of Th1 and IFN-γ production, the TLR-mediated increase in PD-L1 expression by MFs might serve the function of tuning the immune balance between inflammation and tolerance in the colonic mucosa and would serve to protect the colonic mucosa against overt inflammatory responses toward otherwise innocuous microflora.

7.5 Summary and Future Challenges

We have highlighted the current knowledge supporting an emerging concept that mucosal $CD90^+$ stromal cells are partially differentiated MSC, and like MSC they are key participants in gut mucosa tolerogenic responses. While more extensive work is needed to understand the functional differences between MFs and MSCs, it is clear that MFs derived from normal GI mucosa preserve several MSC immunosuppressive functions through expression of common immunosuppressive molecules: PD-L1, PD-L2, PGE_2, IDO, and TGF-β. However, MFs acquire some specific innate immunogenic effector functions that are, perhaps, relevant to the specific organs/tissue [64]. The similarity and difference in molecule expression by BMMSC, tMSC, and MFs are summarized in Table 7.1. For instance, although less efficient than professional APCs, the intestinal MFs express MHC class II and are

capable of the uptake, processing, and presenting of antigen to T cells [11, 49]. In contrast to the MSC, MFs express the positive co-stimulator CD86 (although this expression is limited) but strong constitutive expression of ICOSL (a.k.a. B7-H2) and B7-H3 (unpublished data) whose immune roles in MFs are unclear.

Multiple challenges must be overcome to better understand the role of these cells in the maintenance of health and in the development and progression of gastrointestinal inflammatory diseases. For example, although recently published evidence supports the mesenchymal origin of these cells, additional source of MFs has been described: epithelial to mesenchymal transition, endothelial to mesenchymal transition, and mucosal engraftment of circulating fibrocytes, presumably of hematopoietic origin. A better panel of MF-, tissue-, and lineage-specific markers is necessary to understand the stromal cell's role in chronic intestinal inflammatory diseases and cancers [5, 65]. Over the last decade, we have achieved some understanding on the role of the gastrointestinal MF in the regulation of the $CD4^+$ T cell responses. However, the role of these innate immune cells in the regulation of $CD8^+$ T cells, gamma/delta T cells, B cells, and professional APCs is unreported and will definitively be topics to clarify over the coming years.

Finally, recent published reports support the concept that mucosal stromal cells are innate immune cells contributing to the maintenance of the mucosal tolerance. A critical importance for stromal cells in inflammatory bowel disease and cancer has been suggested [5, 13, 50, 65]. Here, we have only discussed current knowledge of the immunological functions of stromal cells during homeostasis. However, we and others have observed that these cells appear to undergo hardwired phenotypic changes, switching from immunosuppressive to an inflammation-promoting phenotype at the chronic stage of the GI inflammatory diseases and cancers [13, 50]. Understanding these pathological processes will likely provide investigators with novel biomarkers and new therapeutic targets.

References

1. Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts. I. Paracrine cells important in health and disease. Am J Phys. 1999;277:C1–9.
2. Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts. II. Intestinal subepithelial myofibroblasts. Am J Phys. 1999;277:C183–201.
3. Furuya S, Furuya K. Subepithelial fibroblasts in intestinal villi: roles in intercellular communication. Int Rev Cytol. 2007;264:165–223.
4. De Wever O, Demetter P, Mareel M, Bracke M. Stromal myofibroblasts are drivers of invasive cancer growth. Int J Cancer. 2008;123:2229–38.
5. Powell DW, Pinchuk IV, Saada JI, Chen X, Mifflin RC. Mesenchymal cells of the intestinal lamina propria. Annu Rev Physiol. 2011;73:213–37.
6. Mifflin RC, Pinchuk IV, Saada JI, Powell DW. Intestinal myofibroblasts: targets for stem cell therapy. Am J Physiol Gastrointest Liver Physiol. 2011;300:G684–96.
7. Owens BM, Simmons A. Intestinal stromal cells in mucosal immunity and homeostasis. Mucosal Immunol. 2013;6:224–34.

8. Brahmer JR, Hammers H, Lipson EJ. Nivolumab: targeting PD-1 to bolster antitumor immunity. Future Oncol. 2015;11:1307–26.
9. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366:2455–65.
10. Lipson EJ, Sharfman WH, Drake CG, Wollner I, Taube JM, Anders RA, Xu H, Yao S, Pons A, Chen L, Pardoll DM, Brahmer JR, Topalian SL. Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody. Clin Cancer Res. 2013;19:462–8.
11. Saada JI, Pinchuk IV, Barrera CA, Adegboyega PA, Suarez G, Mifflin RC, Di Mari JF, Reyes VE, Powell DW. Subepithelial myofibroblasts are novel nonprofessional APCs in the human colonic mucosa. J Immunol. 2006;177:5968–79.
12. Pinchuk IV, Beswick EJ, Saada JI, Suarez G, Winston J, Mifflin RC, Di Mari JF, Powell DW, Reyes VE. Monocyte chemoattractant protein-1 production by intestinal myofibroblasts in response to staphylococcal enterotoxin a: relevance to staphylococcal enterotoxigenic disease. J Immunol. 2007;178:8097–106.
13. Pinchuk IV, Morris KT, Nofchissey RA, Earley RB, Wu JY, Ma TY, Beswick EJ. Stromal cells induce Th17 during Helicobacter pylori infection and in the gastric tumor microenvironment. PLoS One. 2013;8:e53798.
14. Pinchuk IV, Saada JI, Beswick EJ, Boya G, Qiu SM, Mifflin RC, Raju GS, Reyes VE, Powell DW. PD-1 ligand expression by human colonic myofibroblasts/fibroblasts regulates CD4+ T-cell activity. Gastroenterology. 2008;135:1228–37.
15. Jones S, Horwood N, Cope A, Dazzi F. The antiproliferative effect of mesenchymal stem cells is a fundamental property shared by all stromal cells. J Immunol. 2007;179:2824–31.
16. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005;105:1815–22.
17. Singer NG, Caplan AI. Mesenchymal stem cells: mechanisms of inflammation. Annu Rev Pathol. 2011;6:457–78.
18. Bernardo ME, Fibbe WE. Mesenchymal stromal cells: sensors and switchers of inflammation. Cell Stem Cell. 2013;13:392–402.
19. Joseph NM, Mukouyama YS, Mosher JT, Jaegle M, Crone SA, Dormand EL, Lee KF, Meijer D, Anderson DJ, Morrison SJ. Neural crest stem cells undergo multilineage differentiation in developing peripheral nerves to generate endoneurial fibroblasts in addition to Schwann cells. Development. 2004;131:5599–612.
20. Wilm B, Ipenberg A, Hastie ND, Burch JB, Bader DM. The serosal mesothelium is a major source of smooth muscle cells of the gut vasculature. Development. 2005;132:5317–28.
21. Rinkevich Y, Mori T, Sahoo D, Xu PX, Bermingham JR Jr, Weissman IL. Identification and prospective isolation of a mesothelial precursor lineage giving rise to smooth muscle cells and fibroblasts for mammalian internal organs, and their vasculature. Nat Cell Biol. 2012;14:1251–60.
22. Powell DW, Saada JI. Mesenchymal stem cells and prostaglandins may be critical for intestinal wound repair. Gastroenterology. 2012;143:19–22.
23. Brown SL, Riehl TE, Walker MR, Geske MJ, Doherty JM, Stenson WF, Stappenbeck TS. Myd88-dependent positioning of Ptgs2-expressing stromal cells maintains colonic epithelial proliferation during injury. J Clin Invest. 2007;117:258–69.
24. Worthley DL, Churchill M, Compton JT, Tailor Y, Rao M, Si Y, Levin D, Schwartz MG, Uygur A, Hayakawa Y, Gross S, Renz BW, Setlik W, Martinez AN, Chen X, Nizami S, Lee HG, Kang HP, Caldwell JM, Asfaha S, Westphalen CB, Graham T, Jin G, Nagar K, Wang H, Kheirbek MA, Kolhe A, Carpenter J, Glaire M, Nair A, Renders S, Manieri N, Muthupalani S, Fox JG, Reichert M, Giraud AS, Schwabe RF, Pradere JP, Walton K, Prakash A, Gumucio D, Rustgi AK, Stappenbeck TS, Friedman RA, Gershon MD, Sims P, Grikscheit T, Lee FY, Karsenty G, Mukherjee S, Wang TC. Gremlin 1 identifies a skeletal stem cell with bone, cartilage, and reticular stromal potential. Cell. 2015;160:269–84.

25. Brittan M, Chance V, Elia G, Poulsom R, Alison MR, MacDonald TT, Wright NA. A regenerative role for bone marrow following experimental colitis: contribution to neovasculogenesis and myofibroblasts. Gastroenterology. 2005;128:1984–95.
26. Quante M, Tu SP, Tomita H, Gonda T, Wang SS, Takashi S, Baik GH, Shibata W, Diprete B, Betz KS, Friedman R, Varro A, Tycko B, Wang TC. Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. Cancer Cell. 2011;19:257–72.
27. Worthley DL, Si Y, Quante M, Churchill M, Mukherjee S, Wang TC. Bone marrow cells as precursors of the tumor stroma. Exp Cell Res. 2013;319:1650–6.
28. Signore M, Cerio AM, Boe A, Pagliuca A, Zaottini V, Schiavoni I, Fedele G, Petti S, Navarra S, Ausiello CM, Pelosi E, Fatica A, Sorrentino A, Valtieri M. Identity and ranking of colonic mesenchymal stromal cells. J Cell Physiol. 2012;227:3291–300.
29. Tirapu I, Huarte E, Guiducci C, Arina A, Zaratiegui M, Murillo O, Gonzalez A, Berasain C, Berraondo P, Fortes P, Prieto J, Colombo MP, Chen L, Melero I. Low surface expression of B7-1 (CD80) is an immunoescape mechanism of colon carcinoma. Cancer Res. 2006;66:2442–50.
30. Seliger B, Marincola FM, Ferrone S, Abken H. The complex role of B7 molecules in tumor immunology. Trends Mol Med. 2008;14:550–9.
31. Romo-Tena J, Gomez-Martin D, Alcocer-Varela J. CTLA-4 and autoimmunity: new insights into the dual regulator of tolerance. Autoimmun Rev. 2013;12:1171–6.
32. English K, Barry FP, Field-Corbett CP, Mahon BP. IFN-gamma and TNF-alpha differentially regulate immunomodulation by murine mesenchymal stem cells. Immunol Lett. 2007;110:91–100.
33. Jang IK, Yoon HH, Yang MS, Lee JE, Lee DH, Lee MW, Kim DS, Park JE. B7-H1 inhibits T cell proliferation through MHC class II in human mesenchymal stem cells. Transplant Proc. 2014;46:1638–41.
34. Chen L. Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. Nat Rev Immunol. 2004;4:336–47.
35. Luz-Crawford P, Noel D, Fernandez X, Khoury M, Figueroa F, Carrion F, Jorgensen C, Djouad F. Mesenchymal stem cells repress Th17 molecular program through the PD-1 pathway. PLoS One. 2012;7:e45272.
36. Fiorina P, Jurewicz M, Augello A, Vergani A, Dada S, La Rosa S, Selig M, Godwin J, Law K, Placidi C, Smith RN, Capella C, Rodig S, Adra CN, Atkinson M, Sayegh MH, Abdi R. Immunomodulatory function of bone marrow-derived mesenchymal stem cells in experimental autoimmune type 1 diabetes. J Immunol. 2009;183:993–1004.
37. Wang H, Qi F, Dai X, Tian W, Liu T, Han H, Zhang B, Li H, Zhang Z, Du C. Requirement of B7-H1 in mesenchymal stem cells for immune tolerance to cardiac allografts in combination therapy with rapamycin. Transpl Immunol. 2014;31:65–74.
38. Dulos J, Carven GJ, van Boxtel SJ, Evers S, Driessen-Engels LJ, Hobo W, Gorecka MA, de Haan AF, Mulders P, Punt CJ, Jacobs JF, Schalken JA, Oosterwijk E, van Eenennaam H, Boots AM. PD-1 blockade augments Th1 and Th17 and suppresses Th2 responses in peripheral blood from patients with prostate and advanced melanoma cancer. J Immunother. 2012;35:169–78.
39. Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, Sharpe AH. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med. 2009;206:3015–29.
40. Cao W, Cao K, Cao J, Wang Y, Shi Y. Mesenchymal stem cells and adaptive immune responses. Immunol Lett. 2015;pii: S0165-2478(15)00101-7. https://doi.org/10.1016/j.imlet.2015.06.003. [Epub ahead of print].
41. Ghannam S, Bouffi C, Djouad F, Jorgensen C, Noel D. Immunosuppression by mesenchymal stem cells: mechanisms and clinical applications. Stem Cell Res Ther. 2010;1–7.
42. Hoogduijn MJ, Popp F, Verbeek R, Masoodi M, Nicolaou A, Baan C, Dahlke MH. The immunomodulatory properties of mesenchymal stem cells and their use for immunotherapy. Int Immunopharmacol. 2010;10:1496–500.
43. Stagg J, Galipeau J. Mechanisms of immune modulation by mesenchymal stromal cells and clinical translation. Curr Mol Med. 2013;13:856–67.

44. Kim HS, Shin TH, Yang SR, Seo MS, Kim DJ, Kang SK, Park JH, Kang KS. Implication of NOD1 and NOD2 for the differentiation of multipotent mesenchymal stem cells derived from human umbilical cord blood. PLoS One. 2010;5:e15369.
45. Wlodarska M, Kostic AD, Xavier RJ. An integrative view of microbiome-host interactions in inflammatory bowel diseases. Cell Host Microbe. 2015;17:577–91.
46. Chen X, Zhang ZY, Zhou H, Zhou GW. Characterization of mesenchymal stem cells under the stimulation of Toll-like receptor agonists. Develop Growth Differ. 2014;56:233–44.
47. Ishikura H, Dorf ME. Thymic stromal cells induce hapten-specific, genetically restricted effector suppressor cells in vivo. Immunobiology. 1990;182:11–21.
48. Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, Dazzi F. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood. 2003;101:3722–9.
49. Owens BM, Steevels TA, Dudek M, Walcott D, Sun MY, Mayer A, Allan P, Simmons A. CD90(+) stromal cells are non-professional innate immune effectors of the human colonic mucosa. Front Immunol. 2013;4:307.
50. Pinchuk IV, Beswick EJ, Saada JI, Boya G, Schmitt D, Raju GS, Brenmoehl J, Rogler G, Reyes VE, Powell DW. Human colonic myofibroblasts promote expansion of CD4(+) CD25(high) Foxp3(+) regulatory T cells. Gastroenterology. 2011;79:2737–45.
51. Lohr J, Knoechel B, Jiang S, Sharpe AH, Abbas AK. The inhibitory function of B7 costimulators in T cell responses to foreign and self-antigens. Nat Immunol. 2003;4:664–9.
52. Probst HC, McCoy K, Okazaki T, Honjo T, van den Broek M. Resting dendritic cells induce peripheral CD8+ T cell tolerance through PD-1 and CTLA-4. Nat Immunol. 2005;6:280–6.
53. Alvarez IB, Pasquinelli V, Jurado JO, Abbate E, Musella RM, de la Barrera SS, Garcia VE. Role played by the programmed death-1-programmed death ligand pathway during innate immunity against Mycobacterium tuberculosis. J Infect Dis. 2010;202:524–32.
54. Chang KC, Burnham CA, Compton SM, Rasche DP, Mazuski RJ, McDonough JS, Unsinger J, Korman AJ, Green JM, Hotchkiss RS. Blockade of the negative co-stimulatory molecules PD-1 and CTLA-4 improves survival in primary and secondary fungal sepsis. Crit Care. 2013;17:R85.
55. Liang SC, Greenwald RJ, Latchman YE, Rosas L, Satoskar A, Freeman GJ, Sharpe AH. PD-L1 and PD-L2 have distinct roles in regulating host immunity to cutaneous leishmaniasis. Eur J Immunol. 2006;36:58–64.
56. McAlees JW, Lajoie S, Dienger K, Sproles AA, Richgels PK, Yang Y, Khodoun M, Azuma M, Yagita H, Fulkerson PC, Wills-Karp M, Lewkowich IP. Differential control of CD4(+) T-cell subsets by the PD-1/PD-L1 axis in a mouse model of allergic asthma. Eur J Immunol. 2015;45:1019–29.
57. Beswick EJ, Johnson JR, Saada JI, Humen M, House J, Dann S, Qiu S, Brasier AR, Powell DW, Reyes VE, Pinchuk IV. TLR4 activation enhances the PD-L1-mediated tolerogenic capacity of colonic CD90+ stromal cells. J Immunol. 2014;193:2218–29.
58. Ferdinande L, Demetter P, Perez-Novo C, Waeytens A, Taildeman J, Rottiers I, Rottiers P, De Vos M, Cuvelier CA. Inflamed intestinal mucosa features a specific epithelial expression pattern of indoleamine 2,3-dioxygenase. Int J Immunopathol Pharmacol. 2008;21:289–95.
59. Ina K, Kusugami K, Kawano Y, Nishiwaki T, Wen ZH, Musso A, West GA, Ohta M, Goto H, Fiocchi C. Intestinal fibroblast-derived IL-10 increases survival of mucosal T cells by inhibiting growth factor deprivation- and fas-mediated apoptosis. J Immunol. 2005;175:2000–9.
60. Allez M, Mayer L. Regulatory T cells: peace keepers in the gut. Inflamm Bowel Dis. 2004;10:666–76.
61. Wang S, Charbonnier LM, Noval Rivas M, Georgiev P, Li N, Gerber G, Bry L, Chatila TA. MyD88 adaptor-dependent microbial sensing by regulatory T cells promotes mucosal tolerance and enforces commensalism. Immunity. 2015;43:289–303.
62. Otte JM, Rosenberg IM, Podolsky DK. Intestinal myofibroblasts in innate immune responses of the intestine. Gastroenterology. 2003;124:1866–78.
63. Deguine J, Barton GM. MyD88: a central player in innate immune signaling. F1000Prime Rep. 2014;6:97.

64. Owens BM. Inflammation, innate immunity, and the intestinal stromal cell niche: opportunities and challenges. Front Immunol. 2015;6:319.
65. Rieder F, Fiocchi C. Intestinal fibrosis in inflammatory bowel disease: progress in basic and clinical science. Curr Opin Gastroenterol. 2008;24:462–8.
66. Dumas F, Preira P, Salome L. Membrane organization of virus and target cell plays a role in HIV entry. Biochimie. 2014;107(Pt A):22–7.
67. Maguer-Satta V, Besancon R, Bachelard-Cascales E. Concise review: neutral endopeptidase (CD10): a multifaceted environment actor in stem cells, physiological mechanisms, and cancer. Stem Cells. 2011;29:389–96.
68. Zhou H, Liao J, Aloor J, Nie H, Wilson BC, Fessler MB, Gao HM, Hong JS. CD11b/CD18 (Mac-1) is a novel surface receptor for extracellular double-stranded RNA to mediate cellular inflammatory responses. J Immunol. 2013;190:115–25.
69. Flaherty SF, Golenbock DT, Milham FH, Ingalls RR. CD11/CD18 leukocyte integrins: new signaling receptors for bacterial endotoxin. J Surg Res. 1997;73:85–9.
70. Mina-Osorio P. The moonlighting enzyme CD13: old and new functions to target. Trends Mol Med. 2008;14:361–71.
71. Yang H, Li J, Wang Y, Hu Q. Effects of CD14 and TLR4 on LPS-mediated normal human skin fibroblast proliferation. Int J Clin Exp Med. 2015;8:2267–72.
72. Lang M, Schlechtweg M, Kellermeier S, Brenmoehl J, Falk W, Scholmerich J, Herfarth H, Rogler G, Hausmann M. Gene expression profiles of mucosal fibroblasts from strictured and nonstrictured areas of patients with Crohn's disease. Inflamm Bowel Dis. 2009;15:212–23.
73. Jaggupilli A, Elkord E. Significance of CD44 and CD24 as cancer stem cell markers: an enduring ambiguity. Clin Dev Immunol. 2012;2012:708036.
74. Yeh YC, Lin HH, Tang MJ. A tale of two collagen receptors, integrin beta1 and discoidin domain receptor 1, in epithelial cell differentiation. Am J Physiol Cell Physiol. 2012;303:C1207–17.
75. Marelli-Berg FM, Clement M, Mauro C, Caligiuri G. An immunologist's guide to CD31 function in T-cells. J Cell Sci. 2013;126:2343–52.
76. Nielsen JS, McNagny KM. Novel functions of the CD34 family. J Cell Sci. 2008;121:3683–92.
77. Thiel N, Zischke J, Elbasani E, Kay-Fedorov P, Messerle M. Viral interference with functions of the cellular receptor tyrosine phosphatase CD45. Viruses. 2015;7:1540–57.
78. Gardner H. Integrin alpha1beta1. Adv Exp Med Biol. 2014;819:21–39.
79. Subbaram S, Dipersio CM. Integrin alpha3beta1 as a breast cancer target. Expert Opin Ther Targets. 2011;15:1197–210.
80. Mitroulis I, Alexaki VI, Kourtzelis I, Ziogas A, Hajishengallis G, Chavakis T. Leukocyte integrins: role in leukocyte recruitment and as therapeutic targets in inflammatory disease. Pharmacol Ther. 2015;147:123–35.
81. Musso A, Condon TP, West GA, De La Motte C, Strong SA, Levine AD, Bennett CF, Fiocchi C. Regulation of ICAM-1-mediated fibroblast-T cell reciprocal interaction: implications for modulation of gut inflammation. Gastroenterology. 1999;117:546–56.
82. Saalbach A, Wetzel A, Haustein UF, Sticherling M, Simon JC, Anderegg U. Interaction of human Thy-1 (CD 90) with the integrin alphavbeta3 (CD51/CD61): an important mechanism mediating melanoma cell adhesion to activated endothelium. Oncogene. 2005;24:4710–20.
83. Nassiri F, Cusimano MD, Scheithauer BW, Rotondo F, Fazio A, Yousef GM, Syro LV, Kovacs K, Lloyd RV. Endoglin (CD105): a review of its role in angiogenesis and tumor diagnosis, progression and therapy. Anticancer Res. 2011;31:2283–90.
84. Wang Z, Yan X. CD146, a multi-functional molecule beyond adhesion. Cancer Lett. 2013;330:150–62.
85. Chappell PE, Garner LI, Yan J, Metcalfe C, Hatherley D, Johnson S, Robinson CV, Lea SM, Brown MH. Structures of CD6 and its ligand CD166 give insight into their interaction. Structure. 2015;23:1426–36.
86. Nakayama M. Antigen presentation by MHC-dressed cells. Front Immunol. 2014;5:672.

87. Augello A, Tasso R, Negrini SM, Amateis A, Indiveri F, Cancedda R, Pennesi G. Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. Eur J Immunol. 2005;35:1482–90.
88. Lina TT, Pinchuk IV, House J, Yamaoka Y, Graham DY, Beswick EJ, Reyes VE. CagA-dependent downregulation of B7-H2 expression on gastric mucosa and inhibition of Th17 responses during Helicobacter pylori infection. J Immunol. 2013;191:3838–46.
89. Adegboyega PA, Mifflin RC, DiMari JF, Saada JI, Powell DW. Immunohistochemical study of myofibroblasts in normal colonic mucosa, hyperplastic polyps, and adenomatous colorectal polyps. Arch Pathol Lab Med. 2002;126:829–36.
90. Dave JM, Bayless KJ. Vimentin as an integral regulator of cell adhesion and endothelial sprouting. Microcirculation. 2014;21:333–44.