Immune Cell Circuits in Mucosal Wound Healing: Clinical Implications

Sebastian Zundler^{a, b} Verena Tauschek^a Markus F. Neurath^{a, b}

^aDepartment of Medicine 1, University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Kussmaul Campus for Medical Research & Translational Research Center, Erlangen, Germany; ^bDeutsches Zentrum Immuntherapie, University Hospital Erlangen, Erlangen, Germany

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Abstract

Background: An intact mucosal barrier is essential for homeostasis in the gastrointestinal tract. Various pathological conditions such as infection or immune-mediated inflammation as well as therapeutic interventions like bowel surgery can result in injury of the intestinal mucosa. To counteract potential negative sequelae and to restore integrity of the tissue, a tightly regulated machinery of mechanisms exists, which crucially depends on the presence and absence of various immune cell subsets in different phases of intestinal wound healing. Cell trafficking is an increasingly acknowledged process that steers the localization of cells in tissues and the circulation. Thus, such cell circuits also crucially impact on the recruitment of immune cells in wound healing. **Summary:** We performed a selective literature research. In our review, we will shortly delineate some basic principles of intestinal immune cell trafficking before discussing the contribution of different immune cells to wound healing. Finally, we will discuss potential clinical implications of immune cell trafficking and wound healing interactions in inflammatory bowel disease (IBD) and bowel surgery. **Key Messages:** Intestinal wound healing has immense importance in pathological conditions like IBD, anastomotic healing, and others. Immune cell trafficking is indispensable for the correct temporal and spatial interaction of the cells involved. Further research is required to understand the final consequences of interfering with immune cell trafficking for intestinal wound healing.

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Introduction

The intestine is a highly complex organ fulfilling several seemingly conflicting functions at the same time. It provides a barrier against potentially harmful substances or species like toxins or bacteria present in the lumen, while allowing nutrients, minerals, and vitamins to cross this border.

These functions are secured by synergy and interaction of a variety of cells such as epithelial cells, immune cells, and fibroblasts. They are collaborating in the specialized and sophisticated tissue architecture of the intestinal mucosa, in which a single layer of epithelial cells provides a physical barrier that acts both as a first line of defense, but is also selectively permeable. Beneath, the meshwork of the lamina propria harbors different immune cells, including macrophages and T lymphocytes as well as vessels allowing the transport of absorbed nutrients into the circulation, but also the trafficking of immune cells from and to the gut.

A prerequisite for these aspects of homeostasis in the gut is the integrity of the epithelial barrier and the mucosal tissue architecture. If breaches occur due to physiological processes, pathological conditions, or medical intervention, a complex machinery of events is actuated in an effort to quickly seal such defects or wounds. These processes also crucially rely on the presence and timely interaction of different immune cell subsets, which need to traffic to the site in a coordinated manner. Hence, mucosal wound healing and intestinal immune cell trafficking are closely interconnected.

The following paragraphs will summarize the current evidence on both processes on their own before dissecting their meshing and describing their importance in selected clinical situations.

Fig. 1. Overview of immune cell trafficking in intestinal mucosal wound healing. In case of intestinal mucosal injury, immune cells like granulocytes, classical monocytes (CLMs), non-classical monocytes (NCMs), T helper cells (T_H s), or regulatory T cell populations (rTs) may be recruited to the mucosa, where these cells or descendants like inflammatory (M1-like; I) or wound healing (M2-like; W) macrophages functionally contribute to specific phases of tissue repair. Please refer to the main text for details.

Intestinal Immune Cell Trafficking: An Overview

Immune cell trafficking is an indispensable prerequisite for almost all functions of our immune system. The term describes all processes that control the localization of immune cells in our body. Thus, it subsumes aspects like the alerting and extravasation of innate immune cells to sites of acute insults, the patrolling of naïve and memory lymphocytes through different lymphoid or tissue compartments, as well as the retention of immune cells in peripheral tissues [1].

While the functional importance of all these events is reviewed in greater detail elsewhere [2], it is important to note that, in principle, immune cell trafficking can be broken down into some basic mechanisms that are combined in different ways to enable the complexity of trafficking as a whole. These basic mechanisms include homing, recirculation, and retention.

Homing is the targeted and active extravasation of cells from high endothelial venules of the peripheral circulation to a tissue [3]. Cells passively trafficking with the blood stream may initiate contact with the endothelium of such vessels through interaction of selectins (e.g., L-selectin) with selectin ligands or integrins such as $\alpha 4 \beta 7$ in low-affinity confirmation with cell adhesion molecules like mucosal addressin cell adhesion molecule-1 (MAdCAM-1) to commence so-called rolling. By slowing down the immune cells, this facilitates the binding of tissue chemokines to chemokine receptors on these cells, resulting in cell activation and conformational changes in surface integrins as a prerequisite for firm adhesion to cell adhesion molecules and cell arrest at the vessel wall. Homing is subsequently completed by trans- or paracellular migration to the tissue [4].

Once arrived there, immune cells may also recirculate by leaving the tissue again. Two options exist for recircu-

lation: either cells access the local lymph vessels in a C-C chemokine receptor type 7 (CCR7)-dependent process and travel via draining lymph nodes to efferent lymphatics finally entering the venous system, or they are attracted directly back to the blood through sphingosine-1 phosphate, which is present in high concentrations in the peripheral blood and has chemotactic effects via sphingosine-1 phosphate receptor 1 [5].

Alternatively, cells may be retained in the tissue. In the case of T cells, this is effectuated by induction of the expression of CD69, which counteracts sphingosine-1 phosphate receptor 1 [6, 7]. An additional mechanism can be the upregulation of CD103 binding to epithelial E-cadherin resulting in mechanical retention [8].

Principles of Mucosal Wound Healing

The complexity of mucosal wound healing varies with size and depth of the mucosal defect. Very small breaches in the epithelial layer are a physiological event due to apoptosis associated with its continuous turnover and “only” require spreading of neighboring epithelial cells, but do not involve further cell types [9]. However, larger wounds also affecting the lamina propria need to be healed in a longer and complex process with the contribution of several further cell types. In such cases, three main phases of wound healing can be distinguished: inflammation, proliferation and remodeling [10]. While those phases actually overlap, they are characterized by specific contributions from different cell types. The contribution of the most important cells will be discussed in the next paragraphs (Fig. 1).

Epithelial Cells

Epithelial wounds that only involve one or very few cells can be very quickly resealed by division of neighboring cells. In addition, coordinated movement of adjacent epithelial cells in a so-called purse-string procedure may help to close such gaps. Here, the cells next to the wound form an actomyosin ring within a few minutes, which subsequently contracts and pulls cells from the neighborhood together [11]. In the case of larger defects, additional mechanisms are necessary and epithelial cells at the wound edges dedifferentiate and migrate to the wound area, where they proliferate, re-differentiate, and finally form a homogenous epithelial layer again after establishing tight junctions [12]. This process is regulated by a variety of luminal substances, including trefoil factors, growth factors, and bacterial products, as well as several cell-intrinsic pathways and mechanisms [13, 14]. At least in theory, this does not lead to activation of the mucosal immune system as long as the basement membrane is intact [9].

Since epithelial cells are not involved in cell trafficking circuits between different compartments, a broader discussion of the mechanisms of epithelial wound healing is beyond the scope of this review. However, it is important to note that epithelial cells are directly and indirectly involved in the regulation of immune cell function and cell trafficking. One of the most important aspects in the context of intestinal mucosal healing in this regard is the constant interaction of the epithelium with intestinal microbiota. Although the composition and function of the intestinal microbiome is still far from being understood, its crucial role in homeostasis as well as pathological conditions is beyond doubt. Thus, it is naturally also involved in mucosal wound healing in the gut [15]. Here, it can have both restorative and destructive functions. While commensal microbiota are able to support intestinal wound healing through a variety of mechanisms, such as the production of short-chain fatty acids to promote the generation of regulatory T cells [16], the induction of IL-22 secretion [17], or the induction of epithelial restitution [18], dysbiosis may lead to inflammation. Therefore, specific epithelial cells that secrete antimicrobial peptides such as α -defensins [19], β -defensins [20], and/or cathelicidins [21] shape the intestinal microbiome and consequentially regulate the immune responses and associated immune cell circuits in mucosal wound repair. Importantly, this may sometimes be an even direct impact, since it has been shown that many chemokines that control leukocyte trafficking and which are partly also produced by epithelial cells have intrinsic antimicrobial activity themselves [22].

Neutrophil Granulocytes

In case of acute insults to the organism, whether tissue damage or infection, an urgent mechanism of host defense is the recruitment of neutrophils to the scene. Neu-

trophils are endowed with a number of powerful effector mechanisms, which include the release of neutrophil extracellular traps [23], degranulation of vesicles containing factors such as proteases, phagocytosis, or the production of reactive oxygen species [24]. In the case of intestinal mucosal wound healing and tissue repair in general, these mechanisms are helpful to fight the threat of pathogen translocation and are a key feature of the first inflammatory phase of healing. Very early signals called danger-associated molecular patterns (DAMPs), such as H_2O_2 [25] or ATP [26] released from necrotic cells, or chemokines, such as those from the CXCL8 family, which are produced in the surrounding tissue [27], lead to early recruitment of some “scouting” neutrophils. If those encounter DAMPs in the lamina propria, they are stimulated to release further factors that result in an amplification of neutrophil recruitment called “neutrophil swarming.” Leukotriene B4 is considered to be one of the key drivers of this step [28, 29]. In the case of actual infection, this signal may be further intensified by the presence of pathogen-associated molecular patterns, while it is limited in sterile wounds to allow timely resolution of inflammation in favor of proliferation and remodeling. One mechanism to discriminate between injury and infection is based on CD24, which specifically recognizes DAMPs and negatively regulates the NF- κ B pathway by interacting with Siglec-G [30, 31]. Interestingly, resolution of inflammation is not necessarily associated with apoptosis of the neutrophils present in the wound area, since there is evidence that they may also leave the tissue again in a process called reverse transendothelial migration [32].

However, the influx of neutrophils must not be simply viewed as a means to prevent infection. It is also an essential prelude to later restorative phases of wound healing, since several pieces of evidence indicate that neutrophil depletion or recruitment blockade impair tissue repair [33, 34]. Moreover, neutrophils are not only effector cells, but also enter into crosstalk with their environment, e.g., by producing reactive oxygen species they deplete oxygen in their neighborhood leading to stabilization of hypoxia-inducible factor-1 α , which induces the production of trefoil factors by epithelial cells [35, 36] promoting intestinal epithelial restitution [12]. Importantly, neutrophils are also a source of IL-22, which has essential functions in the restoration of epithelial integrity and will be discussed in detail later [37]. In addition, several further mechanisms by which neutrophils regulate the recruitment or function of other cells exist [38].

Macrophages

While it seems that the importance of neutrophils is predominantly restricted to the early inflammatory phase of wound healing, macrophages play essential roles in all phases of wound repair. This is reflected in the variety of

macrophage phenotypes that have been observed. While macrophages have long been grouped into pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages with proliferative and homeostatic functions, recent work has shown that these phenotypes are only the fringes of a broad spectrum of macrophage subtypes with a specific transcriptional signature [39]. To understand wound healing in a clinical context, however, it still seems reasonable to discriminate between rather pro-inflammatory M1-like macrophages and M2-like wound healing macrophages with anti-inflammatory properties.

A seminal work by Duffield et al. [40] in a model of liver injury demonstrated that different macrophage subsets with opposing functions are involved in tissue repair, since depletion of macrophages at different stages of restoration led to either decreased or increased injury. Although only partly supported by *in vivo* observations [41], it is generally perceived that M1-like macrophages contribute to the early inflammatory phase of wound healing by phagocytosis and the secretion of pro-inflammatory cytokines like IL-1 β , TNF- α , IL-12, or IL-23 [42, 43], while M2-like macrophages are essential for tissue proliferation and remodeling [44, 45]; e.g., such wound healing macrophages secrete vascular endothelial growth factor to promote neo-angiogenesis in the damaged tissue, thereby creating the prerequisites for the influx of cells and factors required for the generation of new tissue [46]. Moreover, they produce matrix metalloproteases, which help to reorganize the tissue [47], and anti-inflammatory cytokines like IL-10 and TGF- β limiting ongoing inflammation [43].

The importance of these aspects has also been shown for wound healing in the gut. Consistent with the above-described role of M2-like macrophages for repair and remodeling, differentiation of wound healing macrophages in STAT6-/- mice was reduced and wound healing following treatment with trinitrobenzene sulfonic acid was delayed, but could be restored by transfer of *ex vivo*-polarized M2-like macrophages [48]. Similarly, transfer of M2 macrophages to mice with dextran sodium sulfate-induced colitis accelerated the repair of ulcers [49].

It is important to note that, in contrast to other organs, macrophages in the intestine are not a self-renewing pool of resident cells derived from yolk sac precursors, but are continuously replenished from bone marrow-derived monocytes [50], which home to the gut and differentiate into macrophages or dendritic cells [51]. Thus, macrophage-controlled wound healing processes in the intestine critically rely on monocyte and macrophage trafficking, while plasticity of local macrophage subsets might not be as important as in other tissues.

Recent work has identified novel mechanisms by which intestinal macrophages support tissue repair; e.g., CD14+ macrophages have been shown to produce IL-

36 α , which was able to activate intestinal fibroblasts, to promote the proliferation of epithelial cells and the expression of antimicrobial peptides [52]. Moreover, IL-10 released from CD11c+ macrophages was demonstrated to be responsible for the secretion of WISP-1, which enhanced epithelial cell proliferation and wound closure [53]. These examples therefore further substantiate the view that intestinal wound repair is the result of a complex interplay of a variety of different players.

Lymphocytes

As adaptive immune cells that orchestrate immune responses, T lymphocytes play important roles in the regulation of wound healing. A very important mechanism is the secretion of IL-22 by T cell subsets like T_H17, T_H22, or Tr1 cells together with other cell types. IL-22 signals through the IL-22 receptor expressed on epithelial cells and induces pleiotropic protective functions [54]. Functionally, this results in a beneficial role in the context of chronic colitis [55] and a promotion of mucosal injury repair [37, 56, 57]. Regarding wound healing, this has been specifically linked to IL-22-induced activation of the transcription factor STAT3 in intestinal epithelial cells [56]. Recently, IL-22 was suggested to be involved in a cytokine network downstream of IL-36 γ inducing secretion of IL-23 in dendritic cells and finally resulting in production of IL-22 [17]. In addition, in contrast to Foxp3+ regulatory T cells, human regulatory Tr1 cells characterized by the secretion of IL-10, but not TGF- β , were shown to produce IL-22 and to promote tissue integrity in the gut [58].

Several other cytokines produced by T cells or acting on T cells have been implicated in wound healing; e.g., IL-15, which contributes to the shaping of tissue-resident memory T cells in the gut [6], was reported to induce the production of TGF- β in intraepithelial cells, leading to accelerated wound healing at the expense of increased susceptibility to infection [59].

However, as this example demonstrates, the restorative functions induced by T lymphocytes (and others) can be considered a double-edged sword. While protective mechanisms are necessary for tissue repair, they may also weaken host immunity or promote the development of cancer by inducing epithelial cell proliferation and inhibiting apoptosis. The latter link is nicely demonstrated by the role of IL-22 and its endogenous antagonist IL-22-binding protein, which impedes the pro-proliferative functions of IL-22 in steady state, but is downregulated in mucosal damage [57]. It is therefore critical in mucosal wound healing that such mechanisms happen in the right spatial and temporal context and are limited once the integrity is restored.

In this regard, regulatory T cells have crucial functions. They have been shown to limit neutrophil effector func-

tions by preventing their recruitment or inducing their apoptosis [60–62]. Moreover, they limit the production of pro-inflammatory cytokines from neutrophils and macrophages and promote the phenotype switch of macrophages to wound-healing macrophages during wound healing [63]. Finally, they also counteract the pro-inflammatory functions of effector T cells and support matrix remodeling in the late phases of wound healing [64].

In addition to T lymphocytes, innate lymphoid cells (ILCs) have also been implicated in wound healing. ILC2s are a potent source of amphiregulin, which acts as a growth factor to restore tissue integrity and is induced by IL-33 released from epithelia [65]. Another pathway applies to ILC3s, in which TNF-like ligand 1A can induce the secretion of IL-22 [66]. Specifically, IL-22 released from ILCs has been shown to promote the intestinal stem cell niche and thereby to promote epithelial renewal in the case of damage [67–69].

Clinical Relevance of the Interplay of Trafficking and Wound Healing

The fact that only a portion of the above-mentioned cells implicated in intestinal wound healing is resident within the tissue implies that trafficking is necessary to ensure the local and timely coordination of repair processes. Few studies have so far directly addressed how cell trafficking affects wound healing and vice versa. However, a number of observations provide important insights into the interconnection of both aspects. In the following paragraphs, we will discuss these observations with regard to two obvious clinical fields in which healing of the intestinal mucosa is crucial.

Inflammatory Bowel Diseases

Inflammatory bowel diseases (IBDs) are relapsing-remitting inflammatory diseases of the intestinal tract, with the main entities being ulcerative colitis and Crohn's disease. Although it is currently unclear to which extent this is a primary and/or secondary event, mucosal defects in the form of ulcers or erosions are an intrinsic feature of IBD [70]. Notably, the endoscopic finding of mucosal healing, i.e., the absence of mucosal damage, is currently considered the main treatment target in IBD [71]. Moreover, IBDs are the leading indication in which biological therapies directed against molecules involved in leukocyte trafficking, such as the anti- $\alpha 4\beta 7$ integrin antibody vedolizumab or the anti- $\beta 7$ integrin antibody etrolizumab, are used or developed [2]. Thus, the interplay of trafficking and healing is of enormous clinical relevance in IBD.

Recently, evidence on monocyte trafficking in this context has been presented. Work from the Nusrat group

revealed that the formyl peptide receptor 2 is implicated in a CCL6-CCR20-dependent recruitment pathway of monocytes in a mouse punch biopsy model, since lack of the receptor resulted in delayed wound healing [72]. Importantly, monocyte subsets with different homing properties can be distinguished. The recruitment of “classical” monocytes (CD14⁺⁺CD16[–] in humans, Ly6C^{hi}CX3CR1^{lo} in mice) is thought to depend on CCR2, while “non-classical” monocytes (CD14⁺CD16⁺ in humans, Ly6C^{lo}-CX3CR1^{hi} in mice) relies on CX3CR1 [51]. Recently, we have shown that human classical monocytes express high levels of $\alpha L\beta 2$ and $\alpha M\beta 2$ integrins, while non-classical monocytes express these integrins to a lesser extent and a portion of them expresses $\alpha 4\beta 1$ and $\alpha 4\beta 7$ integrins [73], rather considered as integrins controlling lymphocyte recruitment. When considering the effects of tissue homing of these cells on wound healing, the differentiation process into macrophages has also to be taken into account. While this is very difficult with regard to the plethora of macrophage subtypes and the different cues from the microenvironment monocytes may encounter, some evidence from skin, heart, and intestinal wound healing models suggests that non-classical monocytes are skewed towards an M2-like phenotype [46, 73, 74]. Consistently, in the setting of inflammation, Ly6C^{hi} monocytes give rise to pro-inflammatory macrophages [75]. Thus, although it would be a clear oversimplification to claim that classical monocytes give rise to inflammatory macrophages in the early phase after wounding and non-classical monocytes produce wound healing macrophages later on, a certain bias of these cells in the respective directions seems to exist. Interestingly, this coincides with earlier recruitment of classical monocytes to injured tissue [38, 43].

Therefore, although T cells are considered the main target of anti-adhesion therapies used in IBD [2], antibodies like vedolizumab, etrolizumab, or the anti-MAd-CAM-1 antibody ontamalimab might also affect intestinal recruitment of monocyte subsets. The fact that clinical studies have demonstrated superiority over placebo with regard to mucosal healing for anti-adhesion antibodies [76–78] points to the notion that this might be of rather minor importance in inflammation-associated mucosal injury. In this context, it might be more important that vedolizumab inhibits homing of T cells, particularly T_{H2} and T_{H17} cells, and etrolizumab, in addition, retention of CD103-expressing T_{H9} cells [8], which have all been described to promote inflammation [79].

As discussed below, the contribution of T cells and macrophages might be different in surgical settings. The mentioned clinical studies, however, definitely show that the alterations of cell trafficking which have been identified as mechanism for vedolizumab and etrolizumab [2, 8, 80] are able to reprogram immunological networks in

the gut in a way that supports mucosal restoration. Yet, the specific pathways by which the blockade of trafficking steps induces tissue repair are not yet understood and require further research.

Bowel Surgery

Iatrogenic damage to the bowel wall obviously also requires intestinal wound healing. While surgical anastomotic techniques provide mechanical stability as long as tissue continuity is missing, the immune system and trafficking of its cells are also of key relevance in this context. Importantly, patients with IBD often require resection with subsequent placement of an anastomosis. Thus, surgical intervention may also coincide with treatment with antitrafficking agents.

However, despite the obvious importance of cell circuits in bowel surgery of IBD and non-IBD patients, it is only possible to speculate on their functional role, since evidence is very limited in this regard.

One study investigated the role of CD18 (α L integrin)-dependent cell recruitment in the context of colonic anastomotic healing in rats. The authors showed that anti-CD18 treatment substantially reduced myeloperoxidase activity in the tissue, but did not affect the breaking strength of the anastomoses [81]. They concluded that neutrophils do not affect mucosal healing in the context of surgical defects. Yet, it should be considered that CD18 is also expressed on other immune cells like monocytes and T cells [3]. Thus, the overall outcome on anastomosis strength might be the net effect of altered gut homing of several different cell subsets with different impact on wound healing.

Interestingly, several studies have reported increased rates of surgical site infections after bowel surgery in IBD patients treated with vedolizumab [82–84]. This could be interpreted in the way that wound healing is impaired in these cases and predisposes to pathogen translocation over a weakened anastomosis. The findings summarized above on non-classical monocyte trafficking and macrophage differentiation [73] might provide a mechanistic explanation for these observations, since macrophages are also considered essential in the process of anastomotic healing [85]. However, it is important to underscore that other studies did not report increased postoperative complications in vedolizumab-treated patients [86], notably neither in anastomotic leakage. Thus, prospective clinical studies will be necessary to provide the final answer to this question.

As for vedolizumab, the effects of treatment with the anti-TNF- α antibody infliximab on perioperative complications have been discussed controversially. A recent meta-analysis concluded that there is no increased rate of complications in infliximab-treated patients [87]. Potentially increased complication rates in patients treated with

thiopurines have also been debated [88], while so far only very limited data are available for the anti-IL-12/23 antibody ustekinumab [89]. However, these drugs have mechanisms of action that are not associated to immune cell trafficking, but alter survival or signaling of immune cell signaling. Thus, potential effects of these agents can be regarded as downstream of immune cell circuits.

Conclusion

Collectively, intestinal wound healing is a clinically important process that is tightly regulated and depends on the coordinated temporal and spatial interaction of various cells, including immune cells. Thus, the orchestration of immune cell trafficking is an essential prerequisite for tissue repair. As a consequence, using antitrafficking agents to interfere with immune cell circuits is both a potential threat and a potential chance with regard to wound healing, since it might specifically impact on the balance of restorative and destructive immune cell subsets in the wound area. Further basic research and translational clinical studies are necessary to better understand these processes and to optimize clinical therapy.

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