The Journal of Immunology

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J Immunol 2017; 199:17-24; ;
doi: 10.4049/jimmunol.1700223
http://www.jimmunol.org/content/199/1/17

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Brief Reviews

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Macrophage-Mediated Inflammation in Normal and Diabetic Wound Healing

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The healing of cutaneous wounds is dependent on the progression through distinct, yet overlapping phases of wound healing, including hemostasis, inflammation, proliferation, and resolution/remodeling. The failure of these phases to occur in a timely, progressive fashion promotes pathologic wound healing. The macrophage (MΦ) has been demonstrated to play a critical role in the inflammatory phase of tissue repair, where its dynamic plasticity allows this cell to mediate both tissue-destructive and -reparative functions. The ability to understand and control both the initiation and the resolution of inflammation is critical for treating pathologic wound healing. There are now a host of studies demonstrating that metabolic and epigenetic regulation of gene transcription can influence MΦ plasticity in wounds. In this review, we highlight the molecular and epigenetic factors that influence MΦ polarization in both physiologic and pathologic wound healing, with particular attention to diabetic wounds. *The Journal of Immunology*, 2017, 199: 17–24.

Maintenance of skin integrity is crucial to survival, and tissue repair is an elaborate process that must be tightly regulated. Although many cell types are involved in tissue repair, the macrophage (MΦ) has been demonstrated to play a critical regulatory role in the healing process, particularly during the inflammatory phase of healing. MΦs represent a phenotypically heterogeneous set of innate immune cells, demonstrating a broad range of functions in both health and disease. Notably, the MΦ is active in both the initiation and the resolution of inflammatory processes. Early in wound repair, transcriptional changes in MΦs lead to production of inflammatory cytokines and clearance of pathogens and debris. During later stages of the inflammatory phase of wound healing, MΦs contribute to the resolution of inflammation and promote tissue repair. It is this paradox—that the MΦ exists both to promote tissue destruction and later repair—that makes the MΦ one of the most fascinating and important cells in the wound-healing process.

MΦ plasticity is crucial for wound repair. In this review, we will consider how MΦs in the wound regulate inflammation and tissue repair. Comparatively, we will examine a pathologic setting where loss of MΦ plasticity can lead to dysregulated inflammation and impaired wound healing.

MΦs and wound healing

Wound healing is exceedingly complex, and it represents the evolutionary layering of numerous genetic, epigenetic, and molecular processes to accomplish this goal. The process of human tissue repair is tightly regulated, and it proceeds in an orderly manner under normal conditions. This stepwise, regular order in which tissue healing takes place is divided into four stages, corresponding roughly to the four waves of predominant cell type appearing in the wound bed (1). These are the hemostasis, inflammatory, proliferative, and maturation phases.

In the hemostasis phase, platelets and circulating coagulant factors accumulate at the site of tissue injury. The interactions of these factors, along with activation of the endothelium, promote the initial invasion of immune cells, specifically polymorphonuclear leukocytes (PMNs). The arrival of these cells characterizes the beginning of the inflammatory phase (2). This rapid, initial influx of PMNs is followed by successive waves of infiltrating monocytes that differentiate within the wound into MΦs and dendritic cells. As injured tissue progresses through the inflammatory phase, the predominant MΦ phenotype in the wound bed changes as well. These changing populations result from successive waves of circulating monocyte invasion, as well as changes in the local tissue environment that direct the phenotypic expression in the tissue-resident MΦ (trMΦ) (3–5). Through this plasticity, MΦs both direct inflammation and tissue destruction and promote tissue repair and transition to the proliferative phase of healing. After resolution of the inflammatory phase, the proliferative phase ensues, in which fibroblasts and other cells reconstitute the tissue matrix, via deposition of collagens, primarily type III early and type I later as healing progresses (6, 7). Contractile myofibroblasts contribute to wound contraction (8). The final phase of healing is the maturation or remodeling phase, where the extracellular matrix expands and

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Received for publication February 13, 2017. Accepted for publication April 20, 2017.

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Abbreviations used in this article: ATM, adipocyte tissue MΦ; HDAC, histone deacetylase; H3K, histone H3 lysine; IRF, IFN-regulatory factor; MΦ, macrophage; M1, proinflammatory MoMΦ; M2, anti-inflammatory MoMΦ; MMP, matrix metalloproteinase; MoMΦ, monocyte-MΦ; PMN, polymorphonuclear leukocyte; SIRT, sirtuin; T2D, type 2 diabetes; trMΦ, tissue-resident MΦ.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1700223

tissue strength returns, although ultimately reaching only 70% of its initial strength (7).

Recently, there has been a significant focus on the characterization of the various expressions of in vivo MΦ phenotypes. Consistent identification of monocyte-MΦ (MoMΦ) subtypes in vitro and in vivo has been controversial in the literature because of poor consensus on nomenclature and cell marker definitions (9). For example, NOS2 has traditionally been used to identify proinflammatory MoMΦs by immunohistochemistry, whereas Arg-1 has been used to identify anti-inflammatory MoMΦs (10). These definitions are problematic, however, because Arg-1 is also expressed in both proinflammatory MoMΦs and some trMΦs (11). Other markers have been suggested in defining these MoMΦ populations, for example, CCR2 for proinflammatory MoMΦs and CD163 or CD206 (mannose receptor) for anti-inflammatory MoMΦs, all with varying success (10).

MΦ phenotype identification, particularly in vivo, has remained elusive, because wound MΦs coexpress the same markers across subtypes and alter their surface marker expression profile in subtly different environments. Thus, in 2014, the International Congress of Immunology held a conference in Milan, Italy, to develop guidelines for MΦ characterization (9). Although the intricacies of these guidelines are beyond the scope of this review, the consensus recommended the use of multiple markers—cell surface, cytokine, and transcription factor—for the clear identification of specific MΦ subsets (9).

More recently, many laboratories have switched from looking at MΦ markers to looking more directly at monocyte markers. Murine and human blood monocytes have been shown to exist as distinct populations with specific effector functions. For instance, identification by flow cytometry demonstrates that murine CX3CR1^{Lo}, Ly6C^{Hi} (Ly6C^{Hi}) MoMΦs (and human correlate CD14^{+}CD16^{-}) track to inflamed tissues and produce inflammation for host defense, whereas CX3CR1^{Hi}, Ly6C^{Lo} (Ly6C^{Lo}) MoMΦs (and human correlate CD14^{lo} CD16^{+}) participate in tissue repair (12–15). Many in vivo studies have demonstrated the plasticity of these cells, suggesting Ly6C^{Hi} cells may differentiate into proinflammatory MoMΦs and Ly6C^{Lo} cells may differentiate into anti-inflammatory MoMΦs (16). Indeed, recent publications have corroborated these findings demonstrating that Ly6C^{Hi} and Ly6C^{Lo} MoMΦs are recruited to myocardium after myocardial infarction, liver parenchyma after hepatocellular injury, and skin after wounding to fulfill roles similar to both proinflammatory and anti-inflammatory MoMΦs, respectively (17, 18). Thus, the more readily detectable CD11b^{+} Ly6G^{-}Ly6C^{Hi} and CD11b^{+}Ly6G^{-}Ly6C^{Lo} MoMΦ populations represent more practical and promising cell surface definitions for future characterization of proinflammatory and anti-inflammatory MoMΦs, respectively. However, transcriptomic studies have suggested that even this definition is imperfect, because gene expression profiles do not necessarily correlate with Ly6C$^{Hi/Lo}$ status (19). Although the authors recognize the complexity of the experimental identification of MoMΦ subsets, for the purposes of this brief review, we will use the traditional, simplified nomenclature (i.e., proinflammatory MoMΦ = M1; anti-inflammatory MoMΦ = M2).

MΦ populations in wounds after injury. Although ostensibly both circulating MoMΦs and trMΦs represent the same cell type, it has been well demonstrated that trMΦs and MoMΦs are in fact quite different in gene transcription and in their function within the wound bed (20, 21). These are, in fact, independently maintained populations during wound inflammation and subsequent resolution. The origins of these two populations differ: trMΦs are, with the exception of those in the gastrointestinal tract, of embryonic origin, originating in the fetal liver and on embryonic day 9 in the yolk sac and seeding cutaneous and organ tissues early in mammalian development (22–25). In contrast, MoMΦs are hematopoietic cells of the myeloid lineage (22–25). trMΦs are thought to have tissue-specific roles in the healing process (26) and are, along with mast cells, dendritic cells, and stromal cells, activated in the immediate phase of injury by a number of cellular damage signals. These damage signals are released by direct cellular injury, pathogen entry, or toxic and oxidative stressors (27). For example, alarmins, a diverse group of molecules that play a variety of intracellular roles in the steady-state, are released upon injury into the extracellular milieu, where they activate local inflammatory cells through pattern recognition receptors (28, 29). This early activation of trMΦs is effected by receptors on their surface for so-called pathogen- and damage-associated molecular patterns (30, 31); among these receptors are TLRs, Nod-like receptors, and RIG-like helicases (26). Examples of damage-associated molecular patterns include markers of cell damage such as purinergic signals (ATP) and HMGB-1. Fetal cutaneous wounds heal without scars under normal circumstances, but the exogenous administration of alarmin HMGB-1 causes leukocyte influx and scar formation (28). Intracellular purines released from ruptured tissues signal MΦ activation through receptors, namely P2Y and P2X (32). All of these steps mark the beginning of the inflammatory phase.

In uninjured skin, there are few recruited MoMΦs within the tissue, and trMΦs are the predominant MΦ population. As the early inflammatory phase progresses, the population of recruited MoMΦs rapidly exceeds the population of trMΦs (33, 34). This seems to have a 2-fold cause: an influx of newly recruited MoMΦs and an acute reduction of the trMΦ population after injury. Experimentally, recovery of trMΦs from injured tissue immediately postinjury is low. During the acute inflammatory phase, there is a distinct drop-off in the number of trMΦs, with restoration of those numbers roughly 2 d after the acute insult. This phenomenon has been termed the disappearance reaction, and it has been hypothesized to result from increased tissue adherence, emigration via lymphatics, and apoptosis (35).

Initially, studies seemed to indicate that the eventual repopulation of trMΦs derived from the influx and proliferation of circulating monocytes. However, in a 2011 study by Davies et al. (36), the authors elegantly demonstrated, in a mouse model of peritonitis, that trMΦs do in fact survive an initial insult and repopulate their numbers through local proliferation. Few trMΦs (defined as F4/80^{hi}Tim4^{+}) were recovered from the inflamed tissue on postinjury day 1, but returned to preinjury levels by postinjury day 3 secondary to a proliferative burst of self-renewal. Importantly, cell DNA content analysis demonstrated that these trMΦs were in proliferative phases of the cell cycle (36).

Regardless of origin, it is clear that MoMΦs are vital to wound repair. This point is validated by studies that interfere with MoMΦ function at various points during wound healing

and demonstrate profound effects on tissue repair. A reduction in myeloid-lineage cell recruitment via knockout of CX3CR1 resulted in delayed wound healing in cutaneous burns and excisional skin wounds (37, 38). Similarly, Lucas et al. (39) used an inducible Cre mouse to conditionally deplete MoMΦs at different points during the wound repair process. Depletion of MoMΦs during the inflammatory phase caused a significant reduction in the rate of wound closure and reduced the quantity of granulation tissue and keratinocyte proliferation, a marker of the transition from the inflammatory to the reparative phase of wound healing, at the wound margin during midphase of tissue repair. On the contrary, when MoMΦs are depleted during the maturation phase, wound healing is not impaired (40).

MΦ plasticity in wound healing. The inflammatory phase of wound healing is divided into early and late stages based upon the function of MoMΦs present in the tissue (41). Hours after the acute insult, there is an influx of PMNs into the wound. Although it was once believed that the PMN was the primary circulating cell involved in the immediate response to injury, it has recently been demonstrated that PMNs are joined by a very early wave of monocytes traveling via gaps in the endothelium (42). After this initial wave, large numbers of monocytes are recruited from the circulation into the wound bed (Fig. 1). These monocytes travel along chemokine gradients, localizing to the wound-adjacent circulation via the action of adhesion molecules and selectins (43–45), ultimately exiting the circulation to enter the wound bed. These recruited MoMΦs undergo significant phenotypic changes in response to cytokines and other mediators present in the local tissue environment (46, 47).

Classical MoMΦs are recruited to the site of inflammation within the first 24–48 h (48). The classical (Ly6C^{Hi} cells in mice; CD14^{+}CD16^{-} cells in humans) and nonclassical (Ly6C^{Lo} cells in mice; CD14^{+}CD16^{+} cells in humans) MoMΦ populations were originally defined as such in reference to their ability, or lack thereof, to respond to MCP-1 (MCP-1 or CCL2) and the time course of their activation during acute inflammation (48, 49). Classical MoMΦs exit the bone marrow in a CCR2-dependent fashion. Its ligands CCL2 and, more significantly, CCL7 maintain homeostatic levels of monocytes in circulation (50, 51). Moreover, the high expression of CCR2 on these cells mediates their recruitment in response to the CCL2/CCL7 gradient in the wound tissue (48, 51). Epelman et al. (52) used an angiotensin II infusion model to induce stress in cardiac myocytes and revealed that CCR2^{+} MoMΦs are the dominant MoMΦs driving the early inflammatory response in cardiac tissues postinjury.

In contrast, the activation of nonclassical MoMΦs appears to be more variable, and their migratory behavior is less defined. Indeed, in the setting of cutaneous inflammation, many studies have suggested that classical MoMΦs will acquire phenotypic features of nonclassical MoMΦs after day 3 in the wound (53). In a recent study by Yona et al. (14), flow cytometric analysis after adoptive transfer with GFP-labeled

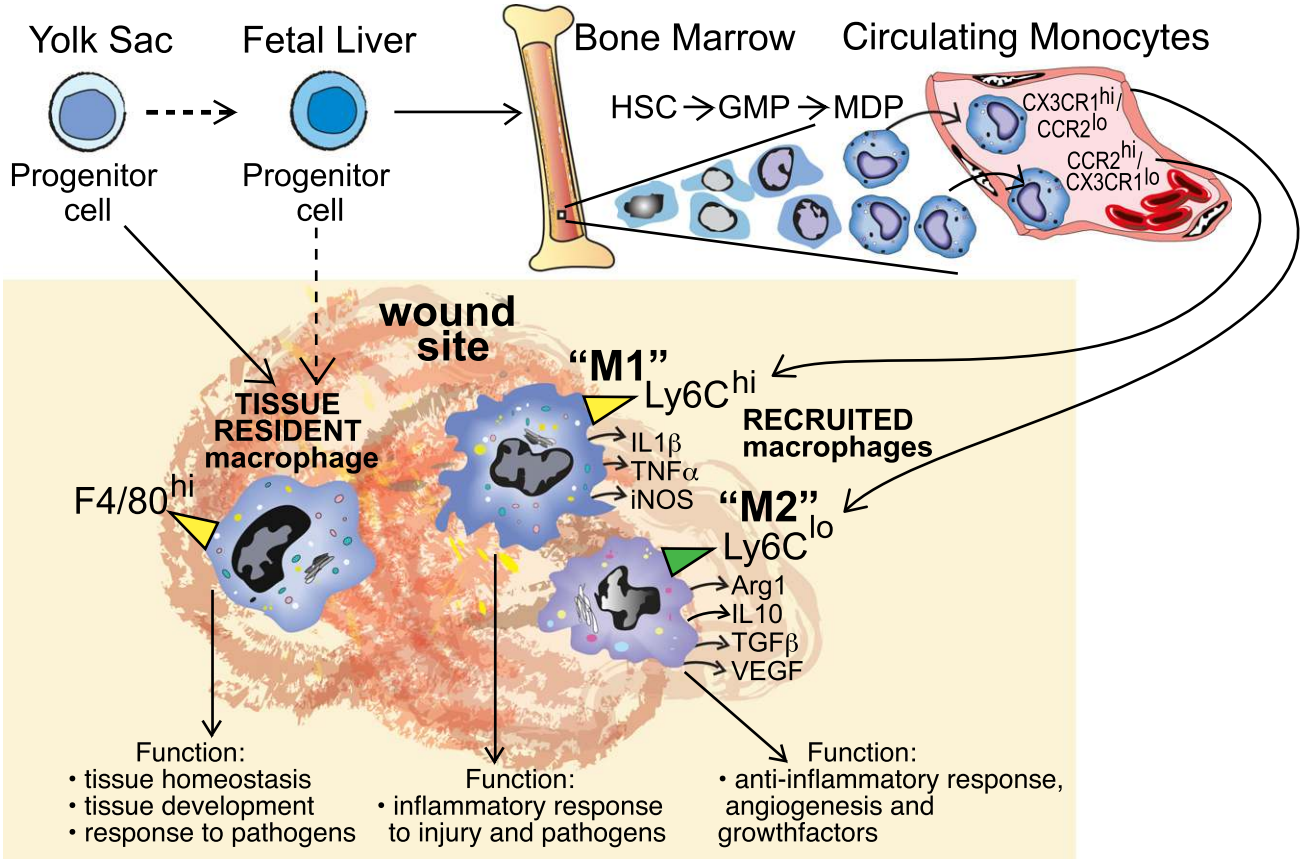

FIGURE 1. MΦ response in cutaneous wounds. trMΦs are derived from two sources: the yolk sac in the embryo and the fetal liver. Hematopoiesis occurs in these locations, giving rise to progenitors that seed tissues with F4/80^{hi} MΦs. The two populations of recruited monocytes originate from the bone marrow and are then released into the blood. After tissue injury, these two populations of monocytes are recruited to the site of injury within the peripheral tissues and differentiate into MoMΦs. The Ly6C^{hi} (M1) MoMΦs release proinflammatory mediators, whereas the Ly6C^{lo} (M2) MoMΦs secrete anti-inflammatory mediators. GMP, granulocyte-MΦ progenitor; HSC, hematopoietic stem cell; MDP, MΦ–dendritic cell progenitor.

Ly6C^{Hi} cells into wild type mice showed differentiation into Ly6C^{Lo} cells by day 3. The role of the nonclassical MoMΦs in tissue repair is context dependent, although they likely play an anti-inflammatory role (53). These express low levels of CCR2, but high levels of chemokine receptor CX3CR1 (54, 55). Although this population of MoMΦs has been shown to accumulate within the peritoneal cavity after injection of *Listeria* very early, and even before the first wave of neutrophils (56), in most other tissues, they appear at day 3 and beyond, after the initial wave of classical MoMΦs (17, 53, 57, 58).

At a basic level, blood monocyte subsets transition into proinflammatory and anti-inflammatory MoMΦs within the tissue. These phenotypes were originally described by Mills et al. (59) and provided the key, at least initially, to the paradox of the MΦ's role in both destruction and repair. It is important to note, however, that it has become clear through the work of the last two decades that such a precise dichotomy between phenotypes likely represents an oversimplification, although most recent studies continue to use this classification as a simple tool to describe the extremes of MoMΦ function (60). Although in vitro MoMΦs can be fully polarized toward an M1 phenotype (by LPS/IFN-γ) or M2 (by IL-4/IL-13/IL-10), in vivo they are exposed to a panoply of local signals and exist along a continuum of phenotypes (61).

In peripheral wounds, these differential phenotypic expressions exhibit a clear temporal cadence (62) (Fig. 2). The first wave of monocytes invading into the tissue transforms into inflammatory MoMΦs, secreting a number of cytotoxic and proinflammatory factors including IL-1β, TNF-α, and IL-6. They are involved in cell destruction by production of cytotoxic nitrogen intermediates from L-arginine via inducible NO synthase (NOS2) (63). Like PMNs, they are involved in the phagocytosis of microbes and of apoptotic and necrotic tissues.

This is followed by a second, later wave of anti-inflammatory MoMΦs, which plays a role in the promotion of tissue remodeling, fibrosis, and wound healing via production of TGF-β, IL-10, and other anti-inflammatory cytokines that are important for the transition from the inflammatory to the proliferative and remodeling phases of wound healing (64–66). Primarily, these MoMΦs produce growth factors and anti-inflammatory mediators, although they maintain their role in the clearance of apoptotic cells. In addition, they recruit endothelial stem cells and promote angiogenesis in the healing wound (67), allowing the development of granulation tissue and neovascularization.

A recent study on monocyte recruitment by Dal-Secco et al. (15) demonstrated this temporal phenotype shift in MoMΦ phenotype posthepatic injury. Using spinning-disk fluorescent confocal intravital microscopy to track the two subsets of monocytes from the peripheral blood into the tissues, the authors demonstrated that proinflammatory MoMΦs (CCR2^{Hi} CX3CR1^{Lo}) were recruited immediately to the wound and persisted for at least 48 h, after which they transformed to anti-inflammatory (CX3CR1^{Hi} CCR2^{Lo}) MoMΦs (15). These findings were replicated in other studies, both a mouse model of sterile wounding and skeletal muscle injury, demonstrating that the Ly6C^{Hi} MoMΦs that were initially recruited from circulation into the wound bed matured into Ly6C^{Lo} MoMΦs with reparative functions (58, 68). It appears that NR4A1, an orphan nuclear receptor, is crucial in the conversion of Ly6C^{Hi} to Ly6C^{Lo}, as demonstrated by Hilgendorf et al. (69). In this study, an NR4A1 knockout mouse after myocardial infarction had impaired cardiac function and limited expression of inflammatory mediators because of absence of conversion of Ly6C^{Hi} to Ly6C^{Lo} MoMΦs.

Mechanisms that influence the phenotypic shift of tissue MoMΦs. The mechanisms underlying MoMΦ phenotype plasticity in vivo are complex, but are under intense investigation. An early step in this transition of MoMΦs from the proinflammatory to the anti-inflammatory phenotype, and thus the initiation of tissue repair, is the ingestion of apoptotic PMNs and other immune cells by MoMΦs. Apoptotic neutrophils have been found to secrete a variety of signaling molecules, such as lysophosphatidylcholine, which attract MoMΦs via the chemotaxis receptor G2A, causing subsequent engulfment of these cells (70). As wound healing progresses, MoMΦs show increased expression of MerTK, a cell-surface tyrosine kinase involved in phagocytosis of apoptotic cells; these MerTK^{+}Ly6C^{lo} MoMΦs demonstrate the ability to produce VEGF and TGF-β (68). Indeed, it appears that phagocytosis of these apoptotic cells triggers the release of these growth factors and augments other tissue repair processes. In one study, Voll et al. (71) demonstrated that coculture of LPS-activated MoMΦs with apoptotic lymphocytes inhibited MoMΦ expression of proinflammatory mediators such as TNF-α and increased release of anti-inflammatory cytokines. These findings were replicated in a subsequent study in which cultured human peripheral blood monocytes did not upregulate expression of proinflammatory mediators IL-1β, IL-8, and TNF-α, but showed a 3-fold

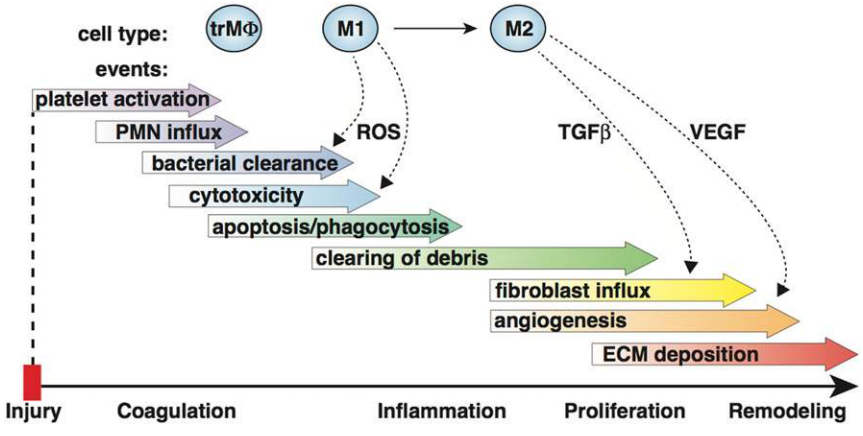

FIGURE 2. Stage-dependent function of MΦs in wound healing. ROS, reactive oxygen species.

induction of TGF-β expression in those MoMΦs that engulfed apoptotic PMNs compared with naive MoMΦs (72).

Pathologic inflammation in wound healing

In the pathologic state, the phenotypic switch from proinflammatory to anti-inflammatory MoMΦs can be delayed or fail to occur, resulting in pathologic phenotype skewing and preventing the transition from the inflammatory to the resolution phase of tissue repair. For example, TNF-α blocks phagocytosis-mediated conversion of inflammatory MoMΦs to reparative phenotypes (73). Residual iron in the wound bed accumulates in local inflammatory MoMΦs and supports TNF-α production (73). In a study by Sindrilaru et al. (74), patients with chronic venous ulcers had an increased accumulation of proinflammatory MoMΦs in the wound bed, and these MoMΦs demonstrated increased iron uptake. Most findings in this study were consistent with other studies in the literature that have found an iron-recycling phenotype in anti-inflammatory MoMΦs and an iron-sequestering phenotype in proinflammatory MoMΦs (75, 76).

MoMΦ phenotype skewing toward an inflammatory phenotype has been implicated in the pathogenesis of type 2 diabetes (T2D) and nonhealing diabetic wounds. Recent literature has supported the concept that diabetes and metabolic syndrome are systemic inflammatory diseases driven by adipose hypertrophy (77). In obesity, adipocyte hypertrophy occurs, which leads to lipotoxicity and excessive cytokine and chemokine production (78). Apoptosis of adipocytes in obesity causes release of inflammatory mediators that attract MoMΦs to the adipose tissue (79). This causes further immune cell infiltration, intensifying inflammatory mediator production, creating a feed-forward loop resulting in a systemic low-grade inflammatory state (80). It has been shown that obesity is associated with increased adipose MoMΦ infiltration, and that these MoMΦs express disproportionate amounts of inflammatory mediators within the adipose tissue (81). For example, 40–50% of the stromal cells of white adipose tissues in obese individuals are MoMΦs compared with only 10% in lean subjects (82).

These MoMΦs found in obese adipose tissue have been likened to M1 MΦs, whereas those found in lean adipose tissue tend to resemble M2 (83). This was further elucidated in an elegant study by Lumeng et al. (84), where they showed that murine adipocyte tissue MΦs (ATMs) undergo a phenotypic switch from an anti-inflammatory to a proinflammatory phenotype after chronic high-fat feeding. Inflammatory gene expression profiles from ATMs of obese mice demonstrated increased levels of proinflammatory mediators IL-6, TNF-α, NOS2, and CCL2, whereas ATMs in lean mice revealed expression of markers of M2 MΦs, such as IL-10, Arg-1, and Ym1 (84). Furthermore, ATMs from obese CCR2 knockout mice expressed M2 markers at similar levels as those in lean mice, suggesting that the inflammatory phenotype in obese mice is secondary to the CCR2-dependent influx of circulating monocytes. This study also found that IL-10, one of the anti-inflammatory cytokines that was overexpressed in lean ATMs, protected adipocytes from TNF-α–induced insulin resistance (84). This concept has been further demonstrated in work by Orr et al. (85) investigating iron handling of obese versus lean ATMs, which found that adipose tissue expansion alters iron metabolism gene expression, and that the impaired iron handling by the iron-rich ATM population led to adipocyte iron overload in obese mice. Thus, in diabetes and obesity, phenotype skewing of ATMs toward the inflammatory phenotype significantly augments systemic inflammation, supporting the development of insulin resistance and the metabolic syndrome.

Not only are ATMs altered in T2D and obesity, but wound MoMΦs have been found to demonstrate a similar predominance of the proinflammatory MoMΦ phenotype, contributing significantly to the impaired wound healing prevalent within this patient population. Increased numbers of PMNs and MoMΦs in nonhealing diabetic wounds have been demonstrated, and similarly an increased ratio of proinflammatory compared with anti-inflammatory MoMΦs in diabetic skin (86). This imbalance of proinflammatory and anti-inflammatory MoMΦs results in increased production of proinflammatory cytokines, preventing tissue repair (86).

Another family of mediators that plays a role in MoMΦ phenotype skewing is a class of deacetylase enzymes known as sirtuins (SIRTs). SIRT1 regulates MoMΦ inflammatory responses via deacetylation of the hematopoietic lineage–specific IFN-regulatory factor (IRF) 8 (87). Moreover, deletion of the gene encoding SIRT1 has been demonstrated to increase insulin resistance in high-fat diet–fed mice and stimulate proinflammatory polarization of ATMs (88). Suppression of IRF8 activity with a small interfering RNA in mice results in delayed wound healing, and although it does not affect overall MΦ numbers in the wound bed, IRF8 suppression leads to a decrease in expression of proinflammatory cytokines within the wound (89). Our laboratory has investigated the role of another SIRT, SIRT3, and found that SIRT3 affects MoMΦ polarization in wound tissues and influences inflammation in the wound bed (90).

In a recent work by Mirza et al. (91), they found that IL-1β expression is increased in diabetic wound MoMΦs from both mice and humans. They demonstrated that inhibiting the IL-1β pathway results in improved wound healing in mice via induction of a switch from the proinflammatory to a reparative MoMΦ phenotype (91). In human subjects, higher serum levels of TNF-α, MCP-1, matrix metalloproteinase (MMP)-9, and

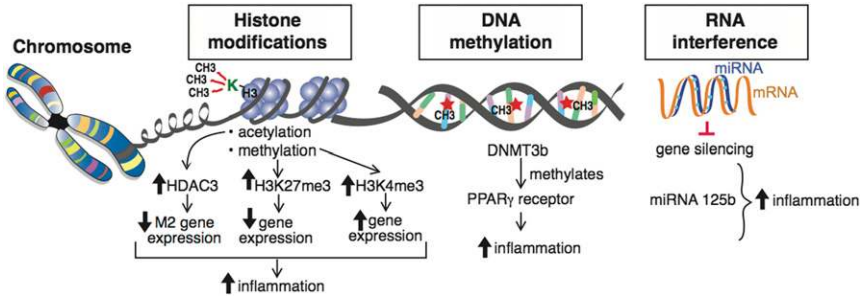

FIGURE 3. Epigenetic changes in wound healing. Histone modifications (acetylation and methylation), DNA methylation, and RNA interference are common epigenetic alterations that have been associated with changes in gene expression that influence inflammation during wound healing.

FGF-2 were found to have been associated with failure to heal in diabetic foot wounds (92). Furthermore, the abundance of proinflammatory cytokines increases MMP production and decreases inhibitors of MMPs; this imbalance further augments degradation of the extracellular matrix, impairs cell migration, and reduces fibroblast proliferation and collagen synthesis (93). One recent study compared the innate immune response in wound healing of both wild type and a genetic leptin-receptor–modified model of T2D (*db/db*) mice. They found that expression of M2-related genes, specifically Ym1 and Arg-1, was significantly reduced in the wounds of the *db/db* mice (94). Further PCR array analysis revealed altered cytokine expression in *db/db* wounds, with significantly increased IL-17 and IL-20, compared with wild type mice (94). In another genetic mouse model of T2D, *ob/ob* mice, poor wound healing has been linked to increases in autophagy in wound MoMΦs (95), which were polarized toward an inflammatory phenotype. Indeed, when 3-MA, an inhibitor of autophagy, was given, wound closure kinetics partially returned to that demonstrated by controls. The fact that this was only a partial response underscores the complexity of T2D wounds, and that multiple factors influence inflammation in these wounds.

In addition to the cellular and molecular mechanisms underlying MΦ plasticity, the maturing field of epigenetics has become a new focus in the investigation of MΦ-mediated inflammation (Fig. 3). A number of epigenetic alterations have been associated with MΦ polarization (96, 97). Histone deacetylases (HDACs) are a group of epigenetic regulators associated with influencing MoMΦ phenotype via alterations in chromatin conformation. For example, HDAC3 has been found to promote proinflammatory and inhibit anti-inflammatory MoMΦ polarization (98–100). In Mullican et al. (99), mice lacking HDAC3 in MoMΦs displayed a gene expression profile recapitulating in vitro M2 activation. Furthermore, a chromatin immunoprecipitation assay revealed localization of HDAC3 to a subset of sites bound by PU.1, a major determinant of the MoMΦ epigenome (101, 102).

Epigenetic enzymes also play a role in MoMΦ phenotype skewing in T2D and metabolic syndrome. Hyperlipidemia has been shown to induce DNA methylation and repress anti-inflammatory cytokines; similarly, a genome-wide analysis for promoter methylation in MΦs revealed hypomethylation of M1 genes and hypermethylation of M2 reparative genes (103). DNA methyltransferase 3b has been found to promote M1 activation in obese mice by DNA methylation of peroxisome proliferator-activated receptor γ1 promoter. Furthermore, knockdown of DNA methyltransferase 3b enhances M2 activation and suppresses the M1 response (104).

Given the role of epigenetic factors in the systemic inflammatory milieu of T2D and metabolic syndrome, attention has recently turned to epigenetic alterations present within diabetic wounds. Alterations in histone methylation have been linked to impairments in diabetic wound healing. As mentioned previously, pathologic expression of IL-1β contributes to impaired wound healing (91). In a study by Kimball et al. (105), MΦs isolated from wounds of a diet-induced obese mouse model of T2D expressed increased IL-1β after stimulation with LPS, and chromatin immunoprecipitation analysis revealed increased histone H3 lysine (H3K) 4 (H3K4; activation mark) and decreased H3K27 (repressive mark) methylation, suggesting an epigenetic mechanism underlying IL-1β expression. Another epigenetic event that strongly influences healing is decreased global methylation through a reduction of (H3K27) trimethylation (106). Gallagher et al. (107) discovered that, in diet-induced obese mice, the *IL-12* promoter exhibited increased demethylation of the repressive H3K27me3 mark in bone marrow progenitor cells, circulating blood monocytes and tissue MΦs. This allowed increased expression of proinflammatory IL-12. Further, the histone demethylase enzyme JMJD3 was decreased at the *IL-12* gene, suggesting that these MΦs were epigenetically preprogrammed toward a proinflammatory phenotype, ultimately negatively impacting wound healing (107).

MicroRNAs regulate gene expression and interact with epigenetic enzymes through a complicated feedback network. It has recently been demonstrated that hyperglycemia leads to changes in the microRNA signature in wound healing, and they have been found to play a role in the dysregulated inflammation of diabetic wounds (108–110). For example, miR-125b epigenetically enhances MΦ activation by increasing the responsiveness to IFN-γ and concurrently inhibits the reparative phenotype by targeting the M2 transcription factor IRF4 (111). Thus, although this field of study is in its relative infancy, there is great potential for continued exploration of the epigenetic mechanisms in inflammatory disease and diabetic wound healing.

Conclusions

MΦ populations in wound tissue are complex, and the specific molecular and epigenetic mechanisms that program and sustain MΦ phenotypes has revealed novel insights into human disease. MΦ plasticity is essential in the initiation, repair, and remodeling of wounds, and the phenotype shifts within the tissues play a key role in the transition between the inflammatory and proliferative phases. Further, epigenetic reprograming of MΦs is intimately linked to their plasticity and function.

In pathologic states, such as T2D, failure to transition from the inflammatory to the proliferative phase can result in injured tissue entering into a state of chronic, unresolving inflammation. Hence, therapies that allow for regulated and programmed inflammation in wounds are a promising approach for the treatment of diabetic and other pathologic wounds. Ultimately understanding the intricacies of the pathways that program MΦ phenotypes in tissue can help guide therapeutic strategies in the future.

Acknowledgments

We thank Robin Kunkel for assistance with the graphical illustrations.

Disclosures

The authors have no financial conflicts of interest.

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