expert reviews
in molecular medicine

Inflammation and wound healing: the role of the macrophage

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The macrophage is a prominent inflammatory cell in wounds, but its role in healing remains incompletely understood. Macrophages have many functions in wounds, including host defence, the promotion and resolution of inflammation, the removal of apoptotic cells, and the support of cell proliferation and tissue restoration following injury. Recent studies suggest that macrophages exist in several different phenotypic states within the healing wound and that the influence of these cells on each stage of repair varies with the specific phenotype. Although the macrophage is beneficial to the repair of normally healing wounds, this pleotropic cell type may promote excessive inflammation or fibrosis under certain circumstances. Emerging evidence suggests that macrophage dysfunction is a component of the pathogenesis of nonhealing and poorly healing wounds. As a result of advances in the understanding of this multifunctional cell, the macrophage continues to be an attractive therapeutic target, both to reduce fibrosis and scarring, and to improve healing of chronic wounds.

The inflammatory response following tissue injury has important roles in both normal and pathological healing. Immediately after injury, the innate immune system is activated, setting in motion a local inflammatory response that includes the recruitment of inflammatory cells from the circulation. This rapid response begins with the degranulation of platelets that arrive at the site and the injury-induced degranulation of resident mast cells. Local immune cells, including resident macrophages, are activated by proinflammatory mediators released in response to injury, as well as damage-associated molecular pattern molecules (DAMPs) (Ref. 1). The hypoxic environment of the wound also promotes inflammation, because hypoxia stimulates numerous cell types, including macrophages, to produce mediators that are important for inflammation (Ref. 2). In response to these many signals, the levels of leukocyte chemoattractants increase substantially, further enhancing leukocyte recruitment.

As the recruitment of leukocytes from the circulation begins in earnest, a pattern of leucocytic infiltration into the wound develops that is similar to other acute inflammatory

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Accession information: doi:10.1017/S1462399411001943; Vol. 13; e23; July 2011

conditions (Fig. 1). Neutrophils, the most abundant white cell in the circulation, infiltrate the wound quickly and are the dominant leukocyte in the earliest stages (Ref. 3). Concomitantly with the influx of neutrophils, circulating monocytes enter the wound and differentiate into mature tissue macrophages (Ref. 3). Mast cell numbers in the wound also increase, with most of the infiltrating mast cells originating in the adjacent tissue (Ref. 4). In the late inflammatory phase of wound repair, T cells appear in the wound bed, and may influence the resolution and remodelling of the wound (Refs 5, 6). As inflammation resolves and the number of leukocytes diminishes, the wound undergoes a lengthy period of remodelling and resolution. Although inflammation is not prominent during this resolution phase, many studies suggest that the events of the inflammatory phase have profound effects on the final wound outcome (Refs 7, 8). Studies in many different anatomical systems suggest that scar formation and fibrosis may derive from inflammatory cell activity (Ref. 8).

Among immune cells in the wound, the role of the macrophage has been the subject of intensive investigation, yielding more than 600 published articles on the topic within the past 5 years. The emerging picture demonstrates that wound macrophages are multifunctional and able to influence nearly all phases of repair. Modulation of macrophage function, then, is a logical and rapidly emerging target for wound therapeutics.

Role of macrophages in wound healing

Landmark studies in the early 1970s and 1980s demonstrated that macrophages are critical to wound healing, and the ability of macrophages to produce factors that stimulate angiogenesis and fibroplasia has been firmly established (Refs 9, 10, 11, 12). Early studies used guinea pigs depleted of macrophages by treatment with both antimacrophage antiserum and glucocorticoids to study the role of this cell in the healing wound (Ref. 9). Because glucocorticoids have several additional effects that might influence repair, these early observations were limited in interpretation. Recent advances in the use of genetically modified mice have overcome this limitation. These techniques allow a highly selective and specific depletion of macrophages in wounds and have confirmed a crucial role for macrophages in wound healing. Two separate groups have used murine strains bearing macrophage-restricted expression of the human receptor for diphtheria toxin to effect a toxin-mediated selective depletion of macrophages before the placement of wounds (Refs 13, 14). The wounds of mice depleted of macrophages in this manner exhibited delayed wound closure, decreased granulation tissue formation and angiogenesis, decreased collagen synthesis, and decreased levels of growth factors, including vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGF-β). In addition, depletion of macrophages resulted in reduced levels of myofibroblasts, which are contractile cell types that are important for wound closure.

More recently, another group, again using a diphtheria toxin system, undertook a temporal selective depletion of macrophages at sequential times during the healing process (Ref. 15). The depletion of macrophages during the early inflammatory phase resulted in impaired wound closure and granulation tissue formation. Depletion during the early proliferative stage caused severe haemorrhage and curbed later wound closure and tissue maturation. Macrophage depletion that was limited to the tissue maturation phase had no significant effect on healing. These studies are some of the first to delineate the different roles of macrophages during the phases of the healing process and support the concept that this cell exhibits different functional phenotypes as repair progresses.

Recruitment of monocytes and macrophages at sites of injury

Similarly to leukocyte migration at almost any site of inflammation, monocyte recruitment into wounds involves the sequential steps of endothelial cell activation, cell-to-cell interaction, and transmigration through the endothelium into the extravascular space. Because monocytes represent only about 3% of circulating leukocytes, the rate of monocyte influx into wounds is far from stoichiometric. Initially, monocytes may be recruited by factors produced quickly after injury, such as split products from the coagulation cascade, factors released from platelet degranulation and activated complement components. But most monocyte infiltration occurs later, and the

Accession information: doi:10.1017/S1462399411001943; Vol. 13; e23; July 2011

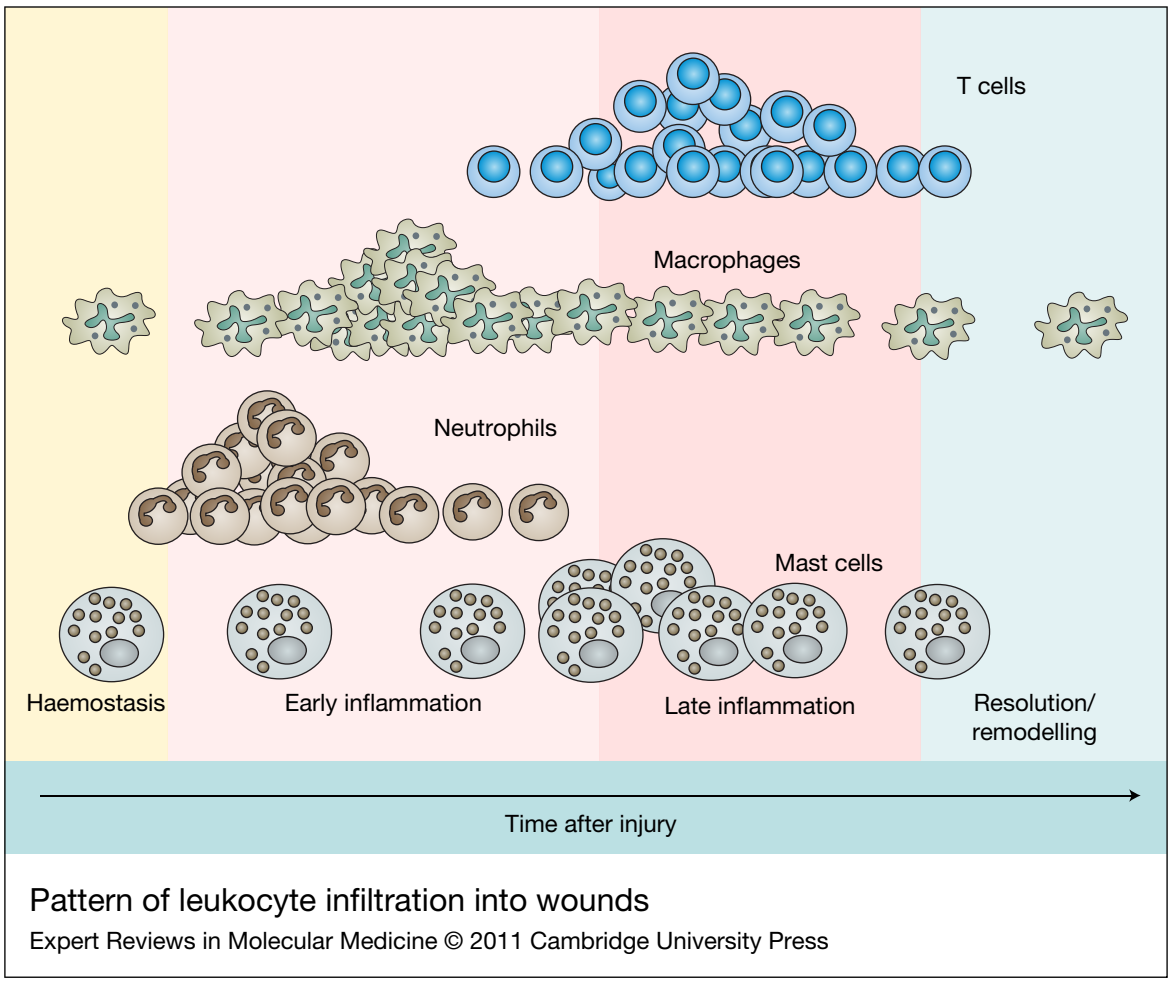

Figure 1. Pattern of leukocyte infiltration into wounds. Inflammatory cells are present during each of the phases of wound repair, represented here as haemostasis (yellow panel), early inflammation (light pink panel), late inflammation (dark pink panel) and resolution/remodelling (blue panel). The relative density of the four most prominent types of leukocytes in wounds (mast cells, neutrophils, macrophages and T cells) is depicted. Whereas neutrophils and lymphocytes disappear, low numbers of resident mast cells and macrophages are present during the lengthy remodelling phase.

preferential infiltration of monocytes represents the response to a locally produced chemotactic gradient that specifically favours these cells. One important group of chemoattractants produced within the wound are the chemokines, which are a group of related small proteins that display highly conserved cysteine amino acid residues. Chemokines can be produced by many cell types, and individual chemokines may preferentially recruit particular populations. Several studies have examined the expression and function of chemokines in healing wounds, and the general patterns of chemokine expression correlate with the movement of leukocytes, including monocytes, into wounds (Refs 16, 17, 18, 19, 20). The role of chemokines in the recruitment of leukocytes is complicated, because more than 40 chemokines have been identified. The complexity of this process has been well described by others (Ref. 21).

Specific macrophage functions in wounds

Promotion of inflammation

Resting macrophages produce only low levels of proinflammatory mediators. On exposure to proinflammatory cytokines, interferons, lipopolysaccharides or other microbial products, or DAMPs (such as heat-shock proteins, high-mobility group box proteins and molecular fragments of the extracellular matrix),

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macrophages acquire a proinflammatory or 'classically activated' phenotype (Ref. 22). Following activation, proinflammatory macrophages themselves produce a large number of mediators and cytokines, including interleukin (IL)-1, IL-6, IL-12, tumour necrosis factor-α (TNF-α) and inducible nitric oxide synthase (Refs 23, 24). Because many of these mediators have been shown to be present in the early wound environment, macrophages seem to be a probable source (Ref. 23). Macrophages also produce chemoattractants, including chemokines, that recruit additional leukocytes (Ref. 25).

Reparative and anti-inflammatory function of wound macrophages

In vitro studies suggest that macrophages are capable of transitioning from a proinflammatory to an 'alternatively activated', or reparative, phenotype (Refs 26, 27). The alternative phenotype is characterised in part by expression of anti-inflammatory mediators such as IL-1 receptor antagonist, decoy IL-1 receptor type II and IL-10, and by the production of growth factors such as TGF-β, VEGF and insulin-like growth factor-1. The transition of macrophages to an alternative phenotype has been assumed to be requisite for the switch from inflammation to proliferation in the healing wound (Ref. 28).

The canonical factors for inducing the alternative phenotype are IL-4 and IL-13. Curiously, however, recent studies suggest that IL-4 and IL-13 are not requisite for the modulation of macrophage phenotypes in wounds in vivo (Ref. 29) Anti-inflammatory cytokines, glucocorticoids and modulators of glucose and lipid metabolism induce a broad spectrum of 'alternative' macrophage phenotypes, including those that exhibit non- or anti-inflammatory and pro-tissue repair functions. Recent studies suggest another pathway in which M1 macrophages are induced to develop into a novel M2-like phenotype in a manner that is independent of IL-4 and IL-13 (Ref. 30). In these in vitro studies, initial activation occurred by the engagement of toll-like receptors: an action that induces expression of the adenosine 2A receptor (A2AR). Subsequent interaction of A2AR with adenosine completes the activation, leading to an M2-like cell. This M2-like cell, dubbed the M2d macrophage, has been suggested to be important to wound-healing outcomes. As mentioned above, other factors, including IL-10, glucocorticoids, prostaglandins, metabolites and the process of efferocytosis (discussed in detail below), might also induce M2-like phenotypes. In the context of the healing wound, the role of each potential stimulus to the phenotypic switch and resolution of inflammation is not completely understood.

Until recently, the existence of discrete macrophage phenotypes within wounds was largely assumed rather than proven. However, recent studies of macrophages derived from skin wounds, as well as sponges implanted subcutaneously in mice, demonstrate that macrophages do exhibit multiple phenotypes that change over time (Ref. 29). These studies suggest that M1-like cells, which are characterized by the production of TNF-α, IL-1 and IL-6, are common in the early phases of repair, whereas M2-like cells, with less proinflammatory cytokines and elevated markers of alternative activation, including CD206 and arginase 1, are common in the later stages of repair. However, these in vivo phenotypes appear far from simple, and do not completely mimic the previously described in vitro macrophage classifications. For example, populations of macrophages were found to exhibit both markers of alternatively activated macrophages, such as mannose receptors and cytokines (TNF-α, IL-6) associated with a classically activated phenotype. At any single time point, then, the wound bed may contain several discrete macrophage phenotypes or hybrid macrophage phenotypes.

Several studies now suggest that alterations in macrophage phenotypes have a critical role in the pathogenesis of chronic wounds. For example, in a murine wound model, iron overloading of macrophages has been shown to create dominant proinflammatory M1-like macrophage populations, with resultant impaired wound healing (Ref. 31). This study has significant clinical implications because iron overload has been previously associated with human chronic venous ulcers and iron-overloaded macrophages have been identified in chronic venous ulcers (Refs 31, 32). Thus, at least for venous ulcers, iron might create a persistant proinflammatory macrophage phenotype that is critical to the failure to heal. In addition, our preliminary studies indicate that the transition from a proinflammatory to a prohealing phenotype is

Accession information: doi:10.1017/S1462399411001943; Vol. 13; e23; July 2011

impaired in excisional wound macrophages from diabetic db/db mice. However, the factors involved in maintaining the persistent proinflammatory wound macrophage phenotype in diabetic mice remain to be elucidated.

Removal of neutrophils and reduction of apoptotic load in the wound

One important function of wound macrophages is the capacity to facilitate the nonphlogistic removal of neutrophils. Neutrophils are abundant in early wounds and are essential for effective decontamination. Yet a large body of evidence suggests that neutrophils negatively influence repair, probably because this cell type is capable of destroying normal tissue (Ref. 33). Neutrophil proteases, such as elastase and cathepsin G, can degrade most components of the extracellular matrix as well as proteins as diverse as clotting factors, complement, immunoglobulins and cytokines (Refs 34, 35, 36). Because the extracellular matrix serves as a supporting scaffold for infiltrating cells, modification of the extracellular matrix by neutrophils could have important consequences for repair. Neutrophils also produce a large number of free oxygen radicals, and thus are capable of inducing considerable oxidative stress on the wound. Mediators such as superoxide and hydrogen peroxide can cause additional tissue damage, delaying the repair process and modifying healing outcomes (Ref. 37). The large load of neutrophils is removed primarily by apoptosis. The removal of apoptotic cells by phagocytosis, a process that is termed efferocytosis, prevents secondary necrosis of these cells and is thought to be essential for complete repair (Ref. 38).

Macrophages are unique in wounds, because they represent the single most effective means of neutrophil removal. Macrophages assist in the removal of neutrophils from sites of injuries in several ways. They respond to neutrophils and their products, and can induce apoptosis in neutrophils (Ref. 39). Perhaps more importantly, macrophages recognise and actively ingest apoptotic neutrophils, thus helping to resolve wound inflammation (Refs 38, 40, 41, 42). Several studies suggest that the phagocytosis of neutrophils influences macrophage phenotype, causing a switch from proinflammatory to a growth-promoting, reparative phenotype (Ref. 43).

Recent studies suggest that failure to remove inflammatory cells, such as neutrophils, has a role in the pathogenesis of nonhealing wounds (Refs 38, 44). A deficit in the capability of macrophages to effectively remove neutrophils has recently been reported to be a critical component of the impaired healing seen in diabetes (Ref. 38). Macrophages derived from sponges implanted in diabetic mice showed a significant impairment in the phagocytosis of apoptotic cells. This deficit was associated with higher levels of apoptotic cells and proinflammatory mediators in wounds, a feature that was further validated in wound tissues of diabetic patients. A deficit in macrophage phagocytic capability has also been associated with the delayed repair that occurs with ageing (Ref. 45). Successful efferocytosis by macrophages therefore may be requisite for appropriate wound healing, both for removal of apoptotic neutrophils and for the generation of a macrophage phenotype that supports the proliferative aspects of repair.

Promotion of angiogenesis, fibroblast proliferation and ECM synthesis

Macrophages influence wound healing through the generation of growth factors that promote cell proliferation and protein synthesis (Ref. 46), as well as by the production of proteases and their inhibitors that influence ECM content and remodelling. Several factors that are known to be present in healing wounds have been shown to be produced by macrophages (Refs 23, 24, 47). In general, this information has been considered presumptive evidence for a macrophage-based influence on the healing process. However, direct evidence for the role of the macrophage as the critical source of these factors is difficult to obtain. Within the healing wound, macrophages are rarely the sole source of any of these described factors, and many other cell types within the wound, including other immune cells, keratinocytes, fibroblasts, endothelial cells and adipocytes, also produce the same factors.

One excellent example of this conundrum is the capability of wound macrophages to influence angiogenesis by the production of VEGF. VEGF is a potent proangiogenic factor that has been shown to contribute 50% or more of the proangiogenic activity in wounds (Ref. 48). Macrophages certainly have proangiogenic

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capabilities, and are well documented to produce abundant amounts of VEGF (Refs 11, 48, 49). However, in epithelial wounds, keratinocytes also produce plentiful amounts of VEGF, making it difficult to determine the relative contribution of macrophages versus keratinocytes in dictating the angiogenic phenotype (Ref. 50). A definitive answer to the question of the importance of macrophage VEGF during wound healing would require the selective temporal depletion of VEGF from the wound macrophage. Such experiments are increasing in feasibility because of the development of genetic mutants with selective deficiencies. A recent study using mice with a deletion of VEGF solely from cells of myeloid origin demonstrated that this deficiency yields delayed excisional wound healing, with little impact on incisional healing (Ref. 51).

Another caveat to the interpretation of the role of macrophage-derived factors is that many are known to have both direct and indirect effects on repair outcomes. For example, PDGF from wound macrophages might assist in the recruitment of progenitor cells and additional inflammatory cells (Ref. 52). More recently, PDGF has also been shown to cause fibroblasts to produce osteopontin, a factor that critically influences wound healing through an autocrine effect that promotes scar formation (Ref. 53).

A full understanding of the complex role of macrophage-derived factors is likely to benefit from emerging technologies. Future investigations to untangle the web of macrophage-derived factors might include global descriptions of macrophage mediator production patterns in healing wounds, along with analysis of direct and indirect effects of macrophage products in vivo and in vitro. Such large data sets, once generated, might benefit from advanced biostatistical analysis in order to develop models to explain these complex interactions.

One approach to the study of wound repair that continues to provide relevant information is the use of genetically tractable organisms, such as zebrafish and *Drosophila*. Zebrafish, owing to their near transparence, provide the additional advantage of allowing real-time live imaging of leukocyte infiltration into sites of inflammation and injury. Previous studies have examined macrophage infiltration into zebrafish wounds and have documented some of the cytoskeletal requirements for this migration (Ref. 54). A more recent study has demonstrated the simultaneous tracking of neutrophils and macrophages labelled with differential fluorescent labels into a tail fin injury site (Ref. 55). In this study, the two cell types showed marked differences in migration speed and kinetics of recruitment to the injury site. Notably, the study revealed a preferred pathway for macrophages along the abluminal surface of endothelial cells. The powerful approaches available in the zebrafish, including tracking of several inflammatory cell types, specific ablation of cell types, and the ability to perform mutagenesis and transgenesis, suggest that many difficult questions about macrophage function in wounds can be approached in this model system.

Clinical implications

Improving macrophage function to improve healing outcomes

An estimated 6 million people in the US have problems related to inadequate wound healing, and nonhealing ulcers remain a serious problem that greatly affects human health (Ref. 56). The improvement of wound healing therefore continues to be the target of many therapeutic strategies. Many attempts to augment the healing process have used single growth factors or cytokines, mostly with limited success. When using single factors, difficulties with optimum delivery systems, timing and concentration are daunting tasks. In addition, the chronic wound environment can be highly proteolytic, limiting the half-life of topically applied molecular factors.

One alternative to the use of molecular therapeutics for wounds is in situ activation, recruitment or addition of exogenous macrophages. Because macrophages are a source of growth factors, augmented macrophage activity may stimulate cellular proliferation and angiogenesis in nonhealing wounds. Increasing the number of macrophages in the wound might also influence the protease imbalance that occurs in some nonhealing wounds, because macrophages can produce protease inhibitors. Finally, the addition of more macrophages might provide an increased efferocytosis capacity.

In support of the therapeutic potential for increased macrophage activity in the healing wound, several early studies document that the topical treatment of wounds with the macrophage-activating agent glucan (Refs 57, 58)

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improves healing outcomes. Glucans are polymers of β-1,3-linked glucose that interact with polysaccharide receptors on the macrophage, causing cell activation. Glucan treatment of wounds has been shown to increase the number of macrophages, and promote fibroplasia, re-epithelialisation and wound strength. Similarly, the application of chemoattractants such as MCP-1 that recruit monocytes to wounds has been shown to promote healing (Ref. 59).

Conceptually, the supplementation of wounds with exogenous macrophages could promote repair, particularly when macrophage function is deficient. Compelling evidence in favour of such a strategy comes from Danon and colleagues (Ref. 60), who demonstrated that the injection of macrophages into the wounds of aged mice could correct the age-related deficit in wound healing. Studies in human subjects have shown that monocyte-derived macrophages, obtained from peripheral blood, can improve the healing of pressure ulcers, as well as sternal chest wounds, in patients undergoing cardiac surgery (Refs 61, 62).

Taken together, these findings suggest that therapeutic strategies that increase macrophage accumulation could accelerate the wound-healing process, and might be particularly helpful in situations of impaired healing, such as ageing and diabetes. In particular, agents that could recruit and drive macrophages towards a reparative phenotype would promote tissue regeneration in the absence of destructive inflammation. The clinical implications of adding such a tool to the therapeutic arsenal could be great. As mentioned above, nonhealing wounds are an immense problem, and current treatments are frequently ineffective. Moreover, evidence indicates that macrophage dysfunction has a role in the impaired healing seen in diabetic patients and in the elderly, who are at great risk for the development of nonhealing ulcers (Refs 38, 45).

Research in progress and outstanding research questions

In the absence of other inflammatory cells, are macrophages essential for healing?

Although the studies described above suggest that macrophages are generally beneficial to repair, some controversy remains over the role of macrophages in the healing wound. Studies of healing in the early- to mid-gestation fetus show that early fetal wounds exhibit very little, if any, inflammatory response while healing in a scarless fashion (Refs 7, 63). Intrinsic differences in growth factor production, levels of stem cells and cellular proliferation capacity probably support fetal wound repair. However, the observed scarless repair in the absence of inflammation suggests that macrophages are not an essential feature of adequate repair, particularly if other aspects of the inflammatory response are suppressed. Several recent studies on mice genetically deficient in specific immune cells and molecules also support the concept that inflammatory cells are not needed for efficient tissue repair, as long as microbial contamination is controlled. In neonatal *Sfp1*$^{-/-}$ mice (previously known as *PU.1*$^{-/-}$), which lack both macrophages and functioning neutrophils, little inflammation occurs at the wound site, and repair appears to be scar free, similarly to that in the fetus (Ref. 64). In addition, mice with a deletion of the gene encoding Smad3, a molecule critical to the intracellular signalling of TGF-β1, exhibited accelerated cutaneous wound healing and significantly reduced influx of inflammatory cells (Ref. 65). Likewise, studies in a number of other systems have shown that soluble factors that reduce inflammation in wounds, such as the cytokine IP-10 and heparin-binding epidermal growth factor, are often beneficial to wound-healing outcomes in the adult organism (Refs 66, 67). Together, these findings suggest that in the face of normal inflammation, which includes oedema, mast cell degranulation and neutrophil ingress, macrophages have an important balancing role. By contrast, when the complete inflammatory response is severely suppressed and bacterial contamination is controlled, wounds appear to heal well. Thus, the role of macrophages must always be considered in the context of the specific wound environment in question. Reductionist approaches have provided a wealth of information about the function of wound macrophages; however, studies that focus on the integration of macrophage function with those of the many other cell types in the wound are required.

Macrophage phenotypes in the healing wound

As mentioned above, abundant in vitro studies suggest that macrophages can adopt discrete phenotypes in response to environmental

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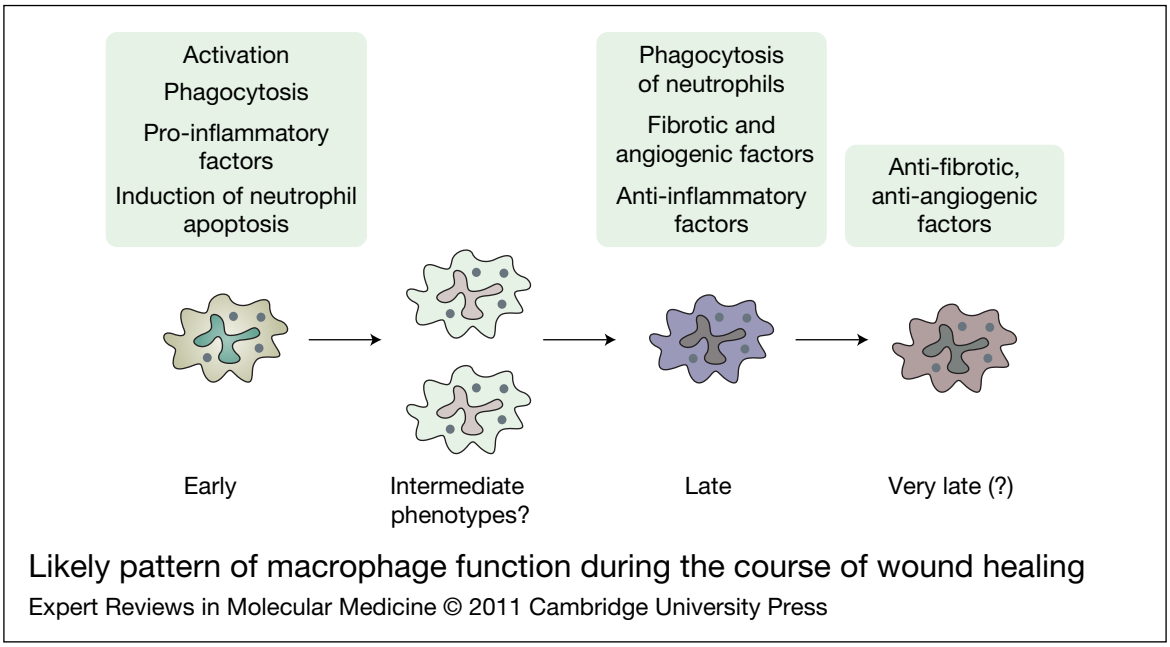
signals (Ref. 68). By contrast, there are few studies of the actual macrophage phenotypes that exist in the in vivo wound and the phenotypes described so far appear highly complex (Ref. 29). Moreover, several markers of macrophage phenotypes have been proposed, and there is a lack of agreement over which are the most critical or informative. Finally, investigations of wound macrophage phenotypes are limited by technical difficulties in obtaining truly representative sample populations of cells from specific locations in wounds.

Relevant to the question of wound macrophage phenotypes is the question of the origin of wound macrophages. Most wound macrophages are thought to be derived from circulating monocytes. These cells in mice exist in two main subsets: an 'inflammatory' subset expresses Ly6C at high levels and a 'noninflammatory' subset expresses Ly6C at low levels (Refs 69, 70). Recently, similar populations of proinflammatory $CD14^{lo}$ and noninflammatory $CD14^{hi}$ monocytes have been described in humans (Ref. 71). Our preliminary research on macrophages isolated from excisional wounds in normally healing mice shows that wound macrophages exhibit high-level Ly6C expression early following wounding, which is associated with a proinflammatory phenotype. As healing progresses, wound macrophages exhibit a transition to low-level Ly6C expression, which is associated with a prohealing phenotype. Whether the observed phenotype transition is derived from an in situ response to differential environmental cues or from selective recruitment of monocyte populations that are already predisposed to proinflammatory or prohealing phenotypes remains to be determined.

Another question is the role of macrophages in mediating the resolution phase of healing. During this final phase, capillary regression and collagen remodelling are dominant features. Macrophages can produce factors that are antiangiogenic (such as thrombospondin-1 and IP-10) (Refs 72, 73) and others, including CXCR3 ligands, that direct the termination of the repair response in several ways (Refs 20, 74). Other cells within the wound may also produce these concluding signals, but



Likely pattern of macrophage function during the course of wound healing

Figure 2. Likely pattern of macrophage function during the course of wound healing. In the early wound, monocytes and resident macrophages become activated, undertake phagocytosis of microbes and perhaps early neutrophils, and produce proinflammatory mediators and chemoattractants. Macrophages also assist in the induction of apoptosis in neutrophils, thus steering the wound towards a noninflammatory, reparative state. In the later phases of wound repair, macrophages ingest apoptotic neutrophils, producing growth factors to support tissue restoration. In the very late stages, as the wound resolves, macrophages may guide tissue remodelling by producing factors to promote capillary regression and collagen remodelling.

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because macrophages can theoretically be a major source of these types of factors, these cells might have an active role in the termination of the wound-healing process. Little is known about how macrophages might shut down the healing response, and much remains to be learned about this phase.

A composite of macrophage functions throughout the time course of the healing wound is suggested from the current literature (Fig. 2). Whether wound macrophages fall into specific discrete phenotypes remains to be determined, yet the significance of gaining a true understanding of macrophage phenotypes within wounds could be huge. Macrophages are probably important contributors to pathophysiology in nonhealing wounds, delayed healing, and fibrosis and scar formation, thus the macrophage remains an attractive target for therapeutic strategies.

Acknowledgements and funding

Wound-healing research in L.A.D.'s laboratory is supported by National Institute of Health Grants RO1-GM50875 and P20-GM078426. Tissue repair research in T.J.K.'s laboratory is supported by the United States Army Medical Research and Materiel Command #W81XWH-05–1-0159. The authors thank the reviewers for their critical comments and suggestions.

References

1 Zhang, X. and Mosser, D.M. (2008) Macrophage activation by endogenous danger signals. Journal of Pathology 214, 161-178
2 Sen, C.K. (2009) Wound healing essentials: let there be oxygen. Wound Repair and Regeneration 17, 1-18
3 Ross, R. and Odland, G. (1968) Human wound repair. II. Inflammatory cells, epithelial-mesenchymal interrelations, and fibrogenesis. Journal of Cell Biology 39, 152-168
4 Artuc, M. et al. (1999) Mast cells and their mediators in cutaneous wound healing – active participants or innocent bystanders? Experimental Dermatology 8, 1-16
5 Barbul, A. et al. (1989) Wound healing in nude mice: a study on the regulatory role of lymphocytes in fibroplasia. Surgery 105, 764-769
6 Barbul, A. et al. (1989) The effect of in vivo T helper and T suppressor lymphocyte depletion on wound healing. Annals of Surgery 209, 479-483
7 Cowin, A.J. et al. (1998) Endogenous inflammatory response to dermal wound healing in the fetal and adult mouse. Developmental Dynamics 212, 385-393
8 Martin, P. and Leibovich, S.J. (2005) Inflammatory cells during wound repair: the good, the bad and the ugly. Trends in Cell Biology 15, 599-607
9 Leibovich, S.J. and Ross, R. (1975) The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum. American Journal of Pathology 78, 71-100
10 Polverini, P.J. et al. (1977) Activated macrophages induce vascular proliferation. Nature 269, 804-806
11 Hunt, T.K. et al. (1984) Studies on inflammation and wound healing: angiogenesis and collagen synthesis stimulated in vivo by resident and activated wound macrophages. Surgery 96, 48-54
12 Kovacs, E.J. and DiPietro, L.A. (1994) Fibrogenic cytokines and connective tissue production. FASEB Journal 8, 854-861
13 Goren, I. et al. (2009) A transgenic mouse model of inducible macrophage depletion: effects of diphtheria toxin-driven lysozyme M-specific cell lineage ablation on wound inflammatory, angiogenic, and contractive processes. American Journal of Pathology 175, 132-147
14 Mirza, R., DiPietro, L.A. and Koh, T.J. (2009) Selective and specific macrophage ablation is detrimental to wound healing in mice. American Journal of Pathology 175, 2454-2462
15 Lucas, T. et al. (2010) Differential roles of macrophages in diverse phases of skin repair. Journal of Immunology 184, 3964-3977
16 Fahey, T.J. 3rd et al. (1990) Cytokine production in a model of wound healing: the appearance of MIP-1, MIP-2, cachectin/TNF and IL-1. Cytokine 2, 92-99
17 DiPietro, L.A. et al. (1995) Modulation of JE/MCP-1 expression in dermal wound repair. American Journal of Pathology 146, 868-875
18 DiPietro, L.A. et al. (1998) MIP-1alpha as a critical macrophage chemoattractant in murine wound repair. Journal of Clinical Investigation 101, 1693-1698
19 Wetzler, C. et al. (2000) Large and sustained induction of chemokines during impaired wound healing in the genetically diabetic mouse: prolonged persistence of neutrophils and macrophages during the late phase of repair. Journal of Investigative Dermatology 115, 245-253
20 Engelhardt, E. et al. (1998) Chemokines IL-8, GROalpha, MCP-1, IP-10, and Mig are sequentially and differentially expressed during phase-specific infiltration of leukocyte subsets in human wound healing. American Journal of Pathology 153, 1849-1860

Accession information: doi:10.1017/S1462399411001943; Vol. 13; e23; July 2011

21 Bonecchi, R. et al. (2009) Chemokines and chemokine receptors: an overview. Frontiers in Bioscience 14, 540-551
22 Mosser, D.M. (2003) The many faces of macrophage activation. Journal of Leukocyte Biology 73, 209-212
23 Barrientos, S. et al. (2008) Growth factors and cytokines in wound healing. Wound Repair and Regeneration 16, 585-601
24 Diegelmann, R.F. and Evans, M.C. (2004) Wound healing: an overview of acute, fibrotic and delayed healing. Frontiers in Bioscience 9, 283-289
25 DiPietro, L.A. (1995) Wound healing: the role of the macrophage and other immune cells. Shock 4, 233-240
26 Brancato, S.K. and Albina, J.E. (2011) Wound macrophages as key regulators of repair origin, phenotype, and function. American Journal of Pathology 178, 19-25
27 Stein, M. et al. (1992) Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. Journal of Experimental Medicine 176, 287-292
28 Mosser, D.M. and Edwards, J.P. (2008) Exploring the full spectrum of macrophage activation. Nature Reviews. Immunology 8, 958-969
29 Daley, J.M. et al. (2009) The phenotype of murine wound macrophages. Journal of Leukocyte Biology 87, 59-67
30 Pinhal-Enfield, G. et al. (2003) An angiogenic switch in macrophages involving synergy between Toll-like receptors 2, 4, 7, and 9 and adenosine A(2A) receptors. American Journal of Pathology 163, 711-721
31 Sindrilaru, A. et al. (2011) An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. Journal of Clinical Investigation 121, 985-997
32 Zamboni, P. et al. (2006) The overlapping of local iron overload and HFE mutation in venous leg ulcer pathogenesis. Free Radical Biology and Medicine 40, 1869-1873
33 Dovi, J.V., He, L.K. and DiPietro, L.A. (2003) Accelerated wound closure in neutrophil-depleted mice. Journal of Leukocyte Biology 73, 448-455
34 Briggaman, R.A. et al. (1984) Degradation of the epidermal-dermal junction by proteolytic enzymes from human skin and human polymorphonuclear leukocytes. Journal of Experimental Medicine 160, 1027-1042
35 Dovi, J.V., Szpaderska, A.M. and DiPietro, L.A. (2004) Neutrophil function in the healing wound: adding insult to injury? Thrombosis and Haemostasis 92, 275-280
36 Li, W.Y. et al. (2003) Plasminogen activator/plasmin system: a major player in wound healing? Wound Repair and Regeneration 11, 239-247
37 Wilgus, T.A. et al. (2003) Reduction of scar formation in full-thickness wounds with topical celecoxib treatment. Wound Repair and Regeneration 11, 25-34
38 Khanna, S. et al. (2010) Macrophage dysfunction impairs resolution of inflammation in the wounds of diabetic mice. PLoS One 5, e9539
39 Meszaros, A.J., Reichner, J.S. and Albina, J.E. (2000) Macrophage-induced neutrophil apoptosis. Journal of Immunology 165, 435-441
40 Meszaros, A.J., Reichner, J.S. and Albina, J.E. (1999) Macrophage phagocytosis of wound neutrophils. Journal of Leukocyte Biology 65, 35-42
41 Daley, J.M. et al. (2005) Modulation of macrophage phenotype by soluble product(s) released from neutrophils. Journal of Immunology 174, 2265-2272
42 Savill, J.S. et al. (1989) Macrophage phagocytosis of aging neutrophils in inflammation. Programmed cell death in the neutrophil leads to its recognition by macrophages. Journal of Clinical Investigation 83, 865-875
43 Fadok, V.A. et al. (1998) Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. Journal of Clinical Investigation 101, 890-898
44 Acosta, J.B. et al. (2008) The pro-inflammatory environment in recalcitrant diabetic foot wounds. International Wound Journal 5, 530-539
45 Swift, M.E. et al. (2001) Age-related alterations in the inflammatory response to dermal injury. Journal of Investigative Dermatology 117, 1027-1035
46 Rappolee, D.A. et al. (1988) Wound macrophages express TGF-alpha and other growth factors in vivo: analysis by mRNA phenotyping. Science 241, 708-712
47 Burke, B. and Lewis, C.E. (eds) (2002) The Macrophage, Oxford University Press, Oxford UK
48 Nissen, N.N. et al. (1998) Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. American Journal of Pathology 152, 1445-1452
49 Knighton, D.R. et al. (1983) Oxygen tension regulates the expression of angiogenesis factor by macrophages. Science 221, 1283-1285

Accession information: doi:10.1017/S1462399411001943; Vol. 13; e23; July 2011

50 Brown, L.F. et al. (1992) Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing. Journal of Experimental Medicine 176, 1375-1379
51 Stockmann, C. et al. (2011) A wound size-dependent effect of myeloid cell-derived vascular endothelial growth factor on wound healing. Journal of Investigative Dermatology 131, 797-801
52 Reuterdahl, C. et al. (1993) Tissue localization of beta receptors for platelet-derived growth factor and platelet-derived growth factor B chain during wound repair in humans. Journal of Clinical Investigation 91, 2065-2075
53 Mori, R., Shaw, T.J. and Martin, P. (2008) Molecular mechanisms linking wound inflammation and fibrosis: knockdown of osteopontin leads to rapid repair and reduced scarring. Journal of Experimental Medicine 205, 43-51
54 Redd, M.J. et al. (2006) Imaging macrophage chemotaxis in vivo: studies of microtubule function in zebrafish wound inflammation. Cell Motility and the Cytoskeleton 63, 415-422
55 Gray, C. et al. (2011) Simultaneous intravital imaging of macrophage and neutrophil behaviour during inflammation using a novel transgenic zebrafish. Thrombosis and Haemostasis 105, 811-819
56 Sen, C.K. et al. (2009) Human skin wounds: a major and snowballing threat to public health and the economy. Wound Repair and Regeneration 17, 763-771
57 Browder, W. et al. (1988) Effect of enhanced macrophage function on early wound healing. Surgery 104, 224-230
58 Leibovich, S.J. and Danon, D. (1980) Promotion of wound repair in mice by application of glucan. Journal of Reticuloendothelial Society 27, 1-11
59 DiPietro, L.A. et al. (2001) Modulation of macrophage recruitment into wounds by monocyte chemoattractant protein-1. Wound Repair and Regeneration 9, 28-33
60 Danon, D., Kowatch, M.A. and Roth, G.S. (1989) Promotion of wound repair in old mice by local injection of macrophages. Proceedings of the National Academy of Sciences of the United States of America 86, 2018-2020
61 Danon, D. et al. (1997) Treatment of human ulcers by application of macrophages prepared from a blood unit. Experimental Gerontology 32, 633-641
62 Orenstein, A. et al. (2005) Treatment of deep sternal wound infections post-open heart surgery by application of activated macrophage suspension. Wound Repair and Regeneration 13, 237-242
63 Hopkinson-Woolley, J. et al. (1994) Macrophage recruitment during limb development and wound healing in the embryonic and foetal mouse. Journal of Cell Science 107, 1159-1167
64 Martin, P. et al. (2003) Wound healing in the PU.1 null mouse – tissue repair is not dependent on inflammatory cells. Current Biology 13, 1122-1128
65 Ashcroft, G.S. et al. (1999) Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response [see comment]. Nature Cell Biology 1, 260-266
66 Luster, A.D. et al. (1998) Delayed wound healing and disorganized neovascularization in transgenic mice expressing the IP-10 chemokine. Proceedings of the Association of American Physicians 110, 183-196
67 Xia, G. et al. (2003) Upregulation of endogenous heparin-binding EGF-like growth factor (HB-EGF) expression after intestinal ischemia/reperfusion injury. Journal of Investigative Surgery 16, 57-63
68 Stout, R.D. et al. (2005) Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. Journal of Immunology 175, 342-349
69 Geissmann, F., Jung, S. and Littman, D.R. (2003) Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity 19, 71-82
70 Sunderkotter, C. et al. (2004) Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. Journal of Immunology 172, 4410-4417
71 Cros, J. et al. (2010) Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. Immunity 33, 375-386
72 DiPietro, L.A. et al. (1996) Thrombospondin 1 synthesis and function in wound repair. American Journal of Pathology 148, 1851-1860
73 Bodnar, R.J. et al. (2009) IP-10 induces dissociation of newly formed blood vessels. Journal of Cell Science 122, 2064-2077
74 Yates, C.C. et al. (2008) ELR-negative CXC chemokine CXCL11 (IP-9/I-TAC) facilitates dermal and epidermal maturation during wound repair. American Journal of Pathology 173, 643-652

Accession information: doi:10.1017/S1462399411001943; Vol. 13; e23; July 2011

Further reading, and resources and contacts

Barrientos, S. et al. (2008) Growth factors and cytokines in wound healing. Wound Repair and Regeneration 16, 585-601

This review examines the specific roles of these growth factors and cytokines during the wound-healing process.

Brancato, S.K. and Albina, J.E. (2011) Wound macrophages as key regulators of repair origin, phenotype, and function. American Journal of Pathology 178, 19-25

This article focuses on the phenotype of wound macrophages, and the functions attributed to the role of macrophages in healing wounds.

Wilgus, T.A. (2008) Immune cells in the healing skin wound: influential players at each stage of repair. Pharmcological Research 58, 112-116

This article explains how different immune cell lineages function in the various stages of repair.

Features associated with this article

Figures

Figure 1. Pattern of leukocyte infiltration into wounds.

Figure 2. Likely pattern of macrophage function during the course of wound healing.

Citation details for this article

Timothy J. Koh and Luisa Ann DiPietro (2011) Inflammation and wound healing: the role of the macrophage. Expert Rev. Mol. Med. Vol. 13, e23, July 2011, doi:10.1017/S1462399411001943

Accession information: doi:10.1017/S1462399411001943; Vol. 13; e23; July 2011