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## Inflammation and wound healing: The role of the macrophage

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### Abstract

The macrophage is a prominent inflammatory cell in wounds, but its role in healing remains incompletely understood. Macrophages have been described to have many functions in wounds, including host defense, 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 phenotypes. While the macrophage is beneficial to the repair of normally healing wounds, this pleotropic cell type may promote excessive inflammation and/or fibrosis in certain circumstances. Emerging evidence suggests that macrophage dysfunction is a component of the pathogenesis of non-healing and poorly healing wounds. Due to advances in the understanding of this multi-functional cell, the macrophage continues to be an attractive therapeutic target both to reduce fibrosis and scarring, and to improve healing of chronic wounds.

### Keywords

Macrophage; wound healing; inflammation; phagocytosis; neutrophils; fibrosis

### Introduction

The inflammatory response following tissue injury plays important roles both in 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 as well as 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

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##### Further reading

Barrientos, S., O. Stojadinovic, et al. (2008). "Growth factors and cytokines in wound healing." *Wound Repair Regen* 16(5): 585-601. This review examines the specific roles of these growth factors and cytokines during the wound healing process.

Brancato, S. K. and J. E. Albina (2011). "Wound macrophages as key regulators of repair origin, phenotype, and function." *Am J Pathol* 178(1): 19-25.

This article focuses 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. *Pharmacol Res* 58(2):112-6. This article explains how different immune cell lineages function in the various stages of repair.

also promotes inflammation, as hypoxia stimulates numerous cell types, including macrophages, to produce mediators important to 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 leukocytic infiltration into the wound develops that is similar to other acute inflammatory conditions (Figure 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 lymphocytes appear in the wound bed, and may influence the resolution and remodeling of the wound (Ref. 5, 6). As inflammation resolves and the number of leukocytes diminishes, the wound undergoes a lengthy period of remodeling 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 (Ref. 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 five years. The emerging picture demonstrates that wound macrophages are multi-functional and able to influence nearly all phases of repair. Modulation of macrophage function, then, is a logical and rapidly emerging target for wound therapeutics.

## The 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 (Ref. 9, 10, 11, 12). Early studies utilized guinea pigs depleted of macrophages by treatment with both anti-macrophage antiserum and glucocorticoids to study the role of this cell in the healing wound (Ref. 9). Because glucocorticoids have a myriad of 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 critical 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 prior to the placement of wounds (Ref. 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 VEGF and TGF-beta. In addition, depletion of macrophages resulted in reduced levels of myofibroblasts, which are a contractile cell important to 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 hemorrhage, 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

Similar 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 trans-migration through the endothelium into the extravascular space. Since 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 preferential infiltration of monocytes represents the response to a locally produced chemotactic gradient that specifically favors these cells. One important group of chemoattractants that are produced within the wound are the chemokines, 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. A large number of 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 (Ref. 16, 17, 18, 19, 20). The role of chemokines in the recruitment of leukocytes is complicated, as 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 pro-inflammatory mediators. Upon exposure to pro-inflammatory cytokines, interferons, LPS or other microbial products, or DAMPS such as heat-shock proteins, high mobility group box proteins, and molecular fragments of the extracellular matrix, macrophages acquire a pro-inflammatory, or “classically activated” phenotype (Ref. 22). Following activation, proinflammatory macrophages themselves produce a large number of mediators and cytokines including interleukin-1, interleukin-6, interleukin-12, TNF $\alpha$ , and inducible nitric oxide synthase (iNOS) (Ref. 23, 24). Because many of these mediators have been shown to be present in the early wound environment, macrophages seem a likely source (Ref. 23). Macrophages also produce chemoattractants, including chemokines, that recruit additional leukocytes (Ref. 25).

### The reparative and anti-inflammatory function of wound macrophages

In vitro studies suggest that macrophages are capable of transitioning from a pro-inflammatory to an “alternatively activated”, or reparative, phenotype (Ref. 26, 27). The alternative phenotype is characterized in part by expression of anti-inflammatory mediators, such as IL-1R antagonist, decoy IL-1 receptor type II and IL-10, and by the production of growth factors such as TGF- $\beta$ 1, vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF)-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/IL-13. Curiously, though, recent studies suggest that IL-4/IL-13 is not requisite for the modulation of wound macrophage phenotypes 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 via an IL-4/IL-13-independent manner (Ref. 30). In these in vitro studies, initial activation occurred via the engagement of toll-like-receptors (TLRs), an action that induces adenosine 2A receptor expression. Subsequent interaction of A2AR by adenosine completed 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) may 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, characterized by the production of TNF-alpha, IL-1 and IL-6, are common in the early phases of repair, while M2-like cells, with less pro-inflammatory cytokines and elevated markers of alternative activation, including CD206 and arginase 1 (Arg1), were common in later stages of repair. However, the described 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 as well as cytokines (TNF-alpha, IL-6) associated with a classically activated phenotype. At any single time point, then, the wound bed may contain multiple discrete macrophage phenotypes or hybrid macrophage phenotypes.

Several studies now suggest that alterations in macrophage phenotypes play 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 an dominant proinflammatory M1-like macrophage populations with resultant impaired wound healing(Ref. 31). This study has significant clinical implications, as iron overload has been previously associated with human chronic venous ulcers, and iron overloaded macrophages have been identified in chronic venous ulcers(Ref. 31, 32). Thus, at least for venous ulcers, iron may create a persistent proinflammatory macrophage phenotype that is critical to the failure to heal. In addition, our initial studies indicate that the transition from a proinflammatory to a pro-healing phenotype is impaired in excisional wound macrophages from diabetic db/db mice, which is associated with impaired healing (Mirza and Koh, unpublished data). However, the factors involved in maintaining the persistent proinflammatory wound macrophage phenotype in diabetic mice remain to be elucidated.

### **Removal of neutrophils/apoptotic load in the wound**

One critically important function of wound macrophages is the capacity to facilitate the non-phlogistic 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 (Ref. 34, 35, 36). Since 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 the 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, as they represent the single most effective means of neutrophil removal. Macrophages assist in the removal of neutrophils from sites of injuries in several ways. Macrophages respond to neutrophils and their products, and can induce apoptosis in neutrophils (Ref. 39). Perhaps more importantly, macrophages recognize and actively ingest apoptotic neutrophils, thus helping to resolve wound inflammation (Ref. 38, 40, 41, 42). Several studies suggest that the phagocytosis of neutrophils influences macrophage phenotype, causing a switch from pro-inflammatory to a growth promoting, reparative phenotype(Ref. 43).

Recent studies suggest that a failure in removal of inflammatory cells, such as neutrophils, plays a role in the pathogenesis of non-healing wounds (Ref. 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. Importantly, this deficit was associated with higher levels of apoptotic cells and pro-inflammatory 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 aging (Ref. 45) Successful efferocytosis by macrophages, then, 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 remodeling. A multitude of factors that are known to be present in healing wounds have been shown to be produced by macrophages (Ref. 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 via the production of VEGF. VEGF is a potent pro-angiogenic factor that has been shown to contribute 50% or more of the proangiogenic activity in wounds(Ref. 48). Macrophages certainly have pro-angiogenic capabilities, and are well documented to produce abundant amounts of VEGF (Ref. 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 in wounds (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 due to the development of genetic mutants with selective deficiencies. A recent study employing



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 may 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 via an autocrine effect that promotes scar formation (Ref. 53).

The problem of fully understanding 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 transparency, provide the additional advantage of allowing real-time live imaging of leukocyte infiltration into sites of inflammation and injury. Prior 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 labeled with differential fluorescent labels into a tail fin injury site (Ref. 55). In this study, the two cell types exhibited 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 multiple 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 persons in the US suffer from problems with inadequate wound healing, and non-healing ulcers remain a serious problem that greatly impacts 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, most with limited success. When using single factors, difficulties with the optimum delivery systems, timing and concentrations 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. Since macrophages are a source of growth factors, augmented macrophage activity may stimulate cellular proliferation and angiogenesis in non-healing wounds. Increasing the number of macrophages in the wound might also influence the protease imbalance that occurs in some non-healing wounds, as macrophages can produce protease inhibitors. Finally, the addition of more macrophages might provide increased an 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 (Ref. 57, 58) improves healing outcomes. Glucans are polymers of beta-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-epithelialization, and wound strength. Similarly, the application of chemoattractants that recruit monocytes to wounds, such as MCP-1, 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 favor of such a strategy comes from Danon et al. (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 (Ref. 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 aging and diabetes. In particular, agents that could recruit and drive macrophages toward 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, non-healing wounds are an immense problem, and current treatments are frequently ineffective. Moreover, evidence indicates that macrophage dysfunction plays a role in the impaired healing seen in diabetic patients and in the elderly, both groups who are at great risk for the development of non-healing ulcers (Ref. 38, 45).

## Research in progress and outstanding research questions

### In the absence of other inflammation, are macrophages essential for healing?

While 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 (Ref. 7, 63). Certainly 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 all other aspects of the inflammatory response are suppressed. Several recent studies utilizing 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*<sup>-/-</sup> mice (previously known as *PU.1*<sup>-/-</sup>), which lack both macrophages and functioning neutrophils, little inflammation occurs at the wound site, and repair appears to be scar-free, similar to the fetus (Ref. 64). In addition, mice with a deletion of the *Smad3* gene, a molecule critical to the intracellular signaling of TGF- $\beta$ 1, exhibited accelerated cutaneous wound healing and significantly reduced influx of inflammatory cells (Ref. 65). Similarly, 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 (Ref. 66, 67). Together, these findings suggest that in the face of normal inflammation, which includes edema, mast cell degranulation, and neutrophil ingress, macrophages play an important balancing role. In 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, but, 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 signals (Ref. 68). In contrast, there are few studies of the actual macrophage phenotypes that exist in the *in vivo* wound and the phenotypes so far described appear highly complex (Ref. 29). Moreover, multiple 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 believed to be derived from circulating monocytes. Circulating monocytes in mice are known to exist in two main subsets, an “inflammatory” subset expresses Ly6C at high levels and a “non-inflammatory” subset expresses Ly6C at low levels (Ref. 69, 70). Recently, similar populations of proinflammatory CD14<sup>lo</sup> and non-inflammatory CD14<sup>hi</sup> monocytes have been described in humans (Ref. 71). Our unpublished data on macrophages isolated from excisional wounds in normally healing mice (Mirza and Koh, submitted) shows that wound macrophages exhibit high level Ly6C expression early following wounding, which is associated with a pro-inflammatory phenotype. As healing progresses, wound macrophages exhibit a transition to low level Ly6C expression, which is associated with a pro-healing 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 pro-inflammatory or pro-healing phenotypes, remains to be determined.

Another remaining question is the role of macrophage in mediating the resolution phase of healing. During this final phase, capillary regression and collagen remodeling are dominant features. Macrophages can produce factors that are anti-angiogenic (such as thrombospondin-1 and IP-10)(Ref. 72, 73), and others, including CXCR3 ligands, that direct the termination of the repair response in multiple ways (Ref. 20, 74). Other cells within the wound may also produce these concluding signals, but since macrophages can theoretically be a major source of these types of factors, these cells may play 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 healing wound can be suggested from the current literature (Figure 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 may be immense. Macrophages are likely important contributors to pathophysiology in non-healing wounds, delayed healing, and fibrosis and scar formation, and so the macrophage remains an attractive target for therapeutic strategies.

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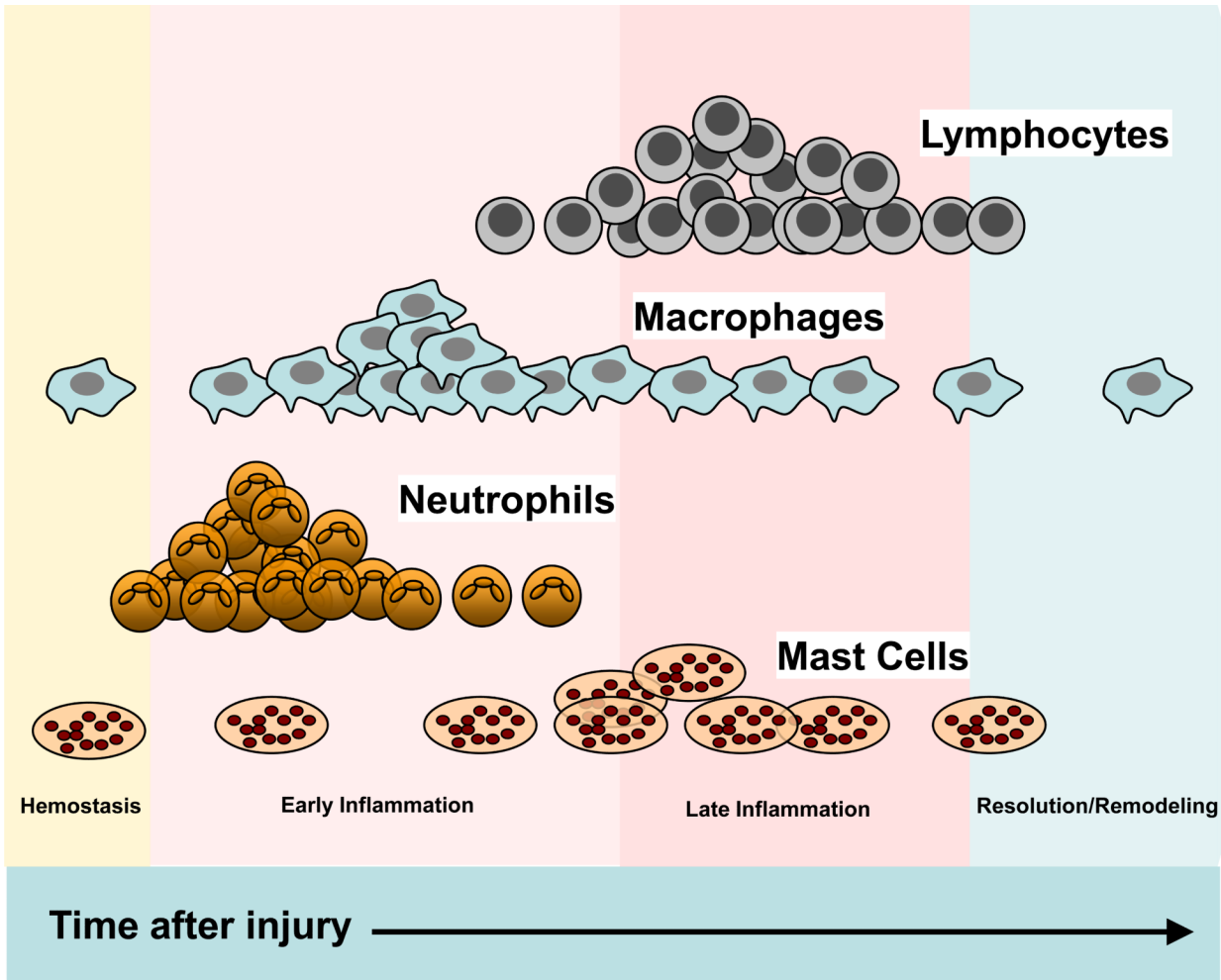
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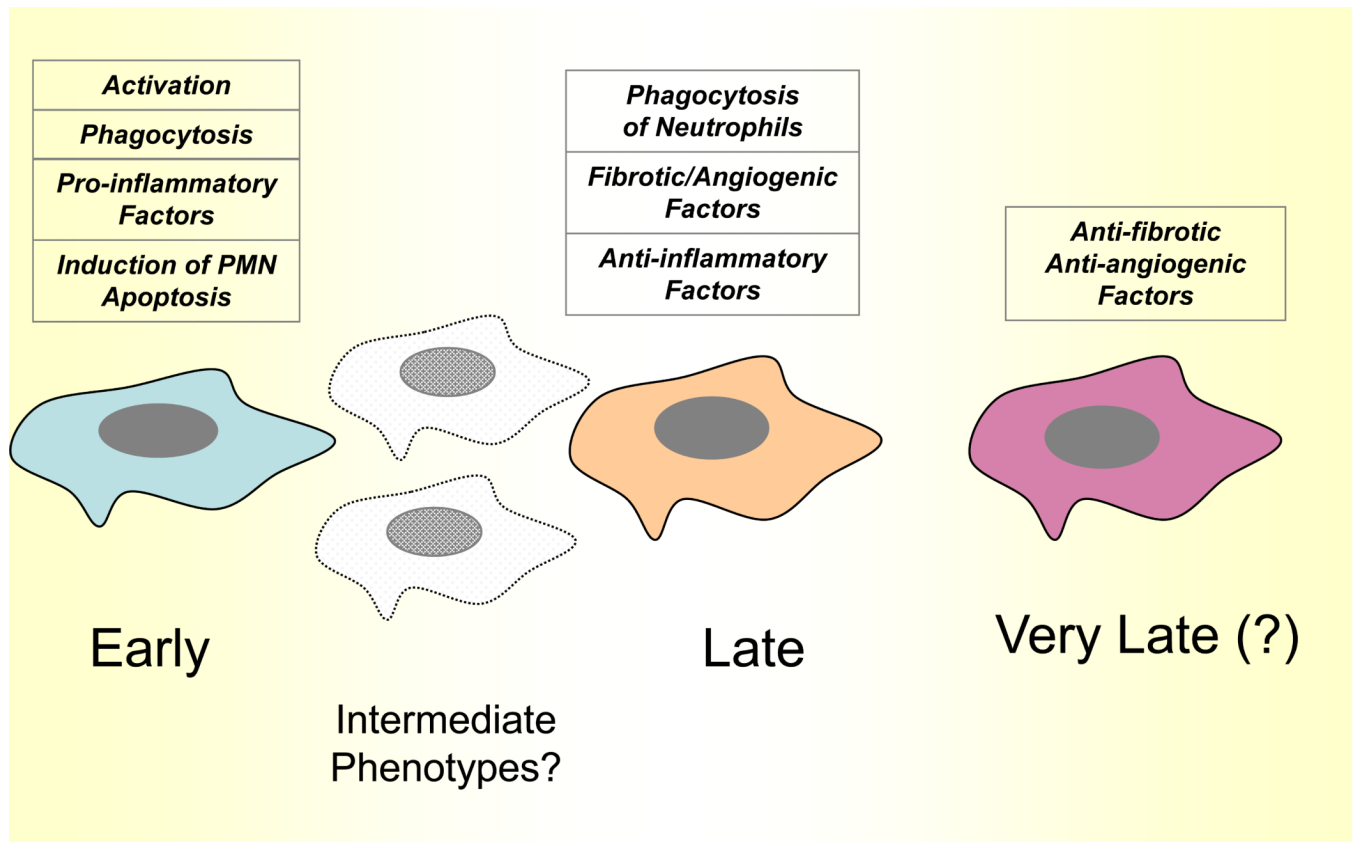
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**Figure 1. The pattern of leukocyte infiltration into wounds**

Inflammatory cells are present during each of the phases of wound repair, represented here as hemostasis (yellow panel), early inflammation (light pink panel), late inflammation (medium pink panel) and resolution/remodeling (blue panel). The relative density of the four most prominent types of leukocytes in wounds (mast cells, neutrophils, macrophages, and lymphocytes) is depicted. While neutrophils and lymphocytes disappear, low numbers of resident mast cells and macrophages are present during the lengthy remodeling phase.





**Figure 2. The 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 turning the wound towards a non-inflammatory, 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 remodeling by producing factors to promote capillary regression and collagen remodeling.