View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Edinburgh Research Explorer

THE UNIVERSITY of EDINBURGH

Edinburgh Research Explorer

Tissue-specific contribution of macrophages to wound healing

Citation for published version:
Minutti, C, Knipper, J, Allen, J & Zaiss, D 2017, 'Tissue-specific contribution of macrophages to wound healing' Seminars in Cell and Developmental Biology. DOI: 10.1016/j.semcdb.2016.08.006

Digital Object Identifier (DOI):
10.1016/j.semcdb.2016.08.006

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Seminars in Cell and Developmental Biology

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 05. Apr. 2019

Elsevier Editorial System(tm) for Seminars in Cell and Developmental Biology
Manuscript Draft

Manuscript Number: YSCDB-D-16-00178R1

Title: Tissue-specific contribution of macrophages to wound healing

Article Type: SI: Innate immune pathways in wound heal

Keywords: Macrophage, Tissue Repair, Extra-cellular matrix, inflammation, lung, skin

Corresponding Author: Prof. Judith Allen,

Corresponding Author's Institution: University of Edinburgh

First Author: Carlos M Minutti

Order of Authors: Carlos M Minutti; Johanna A Knipper; Judith Allen; Dietmar M. W. Zaiss

Manuscript Region of Origin: UNITED KINGDOM

Abstract: Macrophages are present in all tissues, either as resident cells or monocyte-derived cells that infiltrate into tissues. The tissue site largely determines the phenotype of tissue-resident cells, which help to maintain tissue homeostasis and act as sentinels of injury. Both tissue resident and recruited macrophages make a substantial contribution to wound healing following injury. In this review, we evaluate how macrophages in two fundamentally distinct tissues, i.e. the lung and the skin, differentially contribute to the process of wound healing. We highlight the commonalities of macrophage functions during repair and contrast them with distinct, tissue-specific functions that macrophages fulfill during the different stages of wound healing.

Minor suggested changes/corrections/typos:

1. Introduction, paragraph 1: In the sentence referring to Figure 1, perhaps a minor modification can be made to highlight that Figure 1 specifically depicts the various phases of wound healing in the skin. For example, "……..and during "tissue remodelling" wound re-organization restructures the tissue [2], as is shown for the skin in Figure 1."

Change made as suggested. Highlighted in yellow

2. In the section "Architecture and function of the skin", paragraph 1: should the authors indicate that there are three layers of skin, rather than two (i.e. include hypodermis/subcutaneous layer)? This also applies to the figure legend for Fig 2.

Most literature still considers the skin to have only two layers, but we have now specifically added reference to the subcutaneous tissue lying beneath the dermis. Highlighted in yellow

3. In the section "Tissue resident macrophage populations during the immediate responses to wounding", paragraph 2: the sentence "As a consequence, release of intracellular ATP can promote macrophage proliferation and enhance wound repair [27]" seems a little out of place here. The cited study showed the therapeutic effects of administration of ATP-encapsulated in lipid vesicles. Whilst the study is relevant to this review and could be included and discussed, this would not seem to be relevant to the biology of ATP release from cells, as pertains to this paragraph. In fact, ATP, released as a DAMP, can trigger macrophage cell death via P2X7. Perhaps the authors could alter this paragraph slightly, by elaborating on biological roles of P2Y vs P2X receptors, and moving the sentence in question elsewhere (or providing a clearer flow of logic in relation to the rest of this paragraph or removing altogether).

We have reworked this section. Highlighted in yellow

4. In the section "The inflammatory response and influx of monocyte populations": although this section focuses on monocyte recruitment, there is also some discussion of neutrophil recruitment. The authors might like to consider describing the essential role of perivascular macrophages in the initial wave of neutrophil recruitment to the skin, since this has been very elegantly demonstrated in a bacterial infection model: Abtin et al, Nature Immunol, 2014, PMID: 24270515.

We have now mentioned this study. Highlighted in yellow

All the minor corrections requested have been made.

main text
Click here to view linked References

Tissue-specific contribution of macrophages to wound healing

Carlos M. Minutti[1]*, Johanna A. Knipper[1]*, Judith E. Allen[2#] & Dietmar M. W. Zaiss[1#]

[1]Centre for Immunity, Infection and Evolution, and the Institute for Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3FL, UK.

[2] School of Biological Sciences, Faculty of Biology, Medicine & Health & Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, Manchester M13 9PT United Kingdom

[*] *These authors contributed equally*

[#]Correspondence should be addressed to:

Dietmar M. Zaiss, *Institute for Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3FL United Kingdom, Tel.: +44 (0) 161 306 0480, e-mail: Dietmar.Zaiss@ed.ac.uk*

Judith E. Allen, *School of Biological Sciences, University of Manchester, Manchester M13 9PT United Kingdom, Tel.: +44 (0) 161 306 1347, e-mail: judi.allen@manchester.ac.uk*

Abstract:

Macrophages are present in all tissues, either as resident cells or monocyte-derived cells that infiltrate into tissues. The tissue site largely determines the phenotype of tissue-resident cells, which help to maintain tissue homeostasis and act as sentinels of injury. Both tissue resident and recruited macrophages make a substantial contribution to wound healing following injury. In this review, we evaluate how macrophages in two fundamentally distinct tissues, i.e. the lung and the skin, differentially contribute to the process of wound healing. We highlight the commonalities of macrophage functions during repair and contrast them with distinct, tissue-specific functions that macrophages fulfill during the different stages of wound healing.

Keywords:

Macrophage, Tissue Repair, Extra-cellular matrix, inflammation, lung, skin

Introduction

Wound healing is a highly dynamic and tightly coordinated process to achieve restoration of tissue integrity after infection or physical trauma. Fundamentally, this process can be divided into three overlapping but distinct phases. These phases have been defined as “coagulation and inflammation”, “tissue formation” and “tissue remodeling” [1]. During “coagulation and inflammation” the wound is provisionally closed by a blood clot and recruitment of inflammatory cells is initiated; during “tissue formation” pro-inflammatory signals decline and cell proliferation is initiated by local growth factors; and during “tissue remodeling” wound re-organization restructures the tissue [2] as shown for the skin in Figure 1. Cells from the monocyte/macrophage lineage are critical players following tissue damage, and depletion of macrophages results in impaired wound healing [3-5]. In particular the capacity of myeloid cells to regulate inflammation, to remove apoptotic cell debris and to promote cell proliferation have supported the notion that macrophages critically orchestrate the repair and healing response of damaged tissues [6]. Macrophages are present during all stages of the repair process and conditional depletion during the different phases of wound healing has revealed that macrophages fulfill distinct functional roles at the different stages, and emphasizes the great diversity and plasticity macrophages display during this process [7].

While many processes during wound repair are evolutionary conserved and follow similar mechanisms in different organisms and individual tissues, many other aspects of wound repair are regulated in a tissue specific context. Accordingly, tissue resident macrophage populations differ substantially from each other and may contribute to local wound healing in a tissue specific fashion. In this review, we compare the contribution of different macrophage populations to wound healing in two fundamentally distinct tissues, the skin and the lung. In this way, we separate common macrophage functions in wound healing from tissue-specific functions of macrophages, and review their distinct roles during the different phases of wound healing. Based on these different aspects, we propose a common model for the function of macrophages during wound healing.

Architecture and function of the skin

The skin forms a physical barrier between the organism and its environment, and thus a pivotal function of the skin is the protection from chemical and physical assaults, as well as from pathogen invasion and to prevent unregulated water loss [8]. Mammalian skin is composed of mainly two layers, the epidermis and the dermis, with a fat-rich subcutaneous tissue lying beneath the dermis (Figure 2a). The epidermal layer is rich in cells and comprises a physical, chemical and immunological barrier, and the dermis, which is rich in extracellular matrix that provides tensile strength and elasticity. The epidermis is a squamous epithelium composed of different layers, supported by self-renewing proliferating tissue-stem cells within the basal layer [9]. The dermis is tightly connected to the epidermis by the basement membrane and consists of different cell types, including fibroblasts and endothelial cells. Appendages such as hair roots, sebaceous glands and sweat glands are located in the dermal part.

Two types of macrophage populations reside in the skin: Langerhans cells (LCs) which are mainly located in the epidermal layer, and dermal macrophages, which are the dominant macrophage population in the dermis. LCs originate from yolk-sac derived progenitors and fetal liver monocytes, which are recruited to the epidermis before birth [10]. Under steady state conditions, LCs self-renew and only upon inflammation do blood monocytes replenish the epidermal LC population [11, 12].

LCs are defined by their expression of the lectin receptor langerin (CD207). In addition, LCs express CD11c and CD11b and are positive for F4/80 and MHCII. In contrast, dermal macrophages express F4/80 and CD11b but no CD11c or Langerin and only low levels of MHCII [13]. While the contribution of macrophages during wound healing is well established, the specific repair function of LCs is yet to be determined [14]. It is known that LCs re-populate the epidermis in the process of wound closure and increased numbers have been correlated with healing in diabetic foot ulcer patients [15] suggesting LCs are beneficial.

Pulmonary environment and lung macrophages

The main function of the lung is to allow for oxygen uptake (Figure 2b). To achieve this function the human lung has an enormous exposed area - approximately the size of a tennis court [16]. This exposure carries the risk that the lung will initiate inflammation upon inhaled bacteria, viruses, oxidants, pollutants and allergens, compromising tissue integrity. However, a protective barrier of innate immune components functions to maintain a tolerant state towards innocuous antigens present in the inhaled air. This barrier is made up of mucus and the alveolar fluid, which is rich in pulmonary surfactants and opsonins including immunoglobulins, complement and collectins (Surfactant proteins A and D) [16]. Additionally, different types of macrophages (bronchial macrophages, interstitial macrophages and alveolar macrophages) reside in distinct spaces within the lungs [17]. Alveolar macrophages are embedded in the alveolar fluid, covering the alveolar surface (Figure 2b) and are defined in the mouse by their high expression of the sialic acid-binding immunoglobulin-type lectin F (Siglec F) and the alpha integrin CD11c. In addition, alveolar macrophages express typical macrophage markers such as CD11b, F4/80, MerTK and CD64. In contrast, interstitial macrophages, which are the dominant macrophage population in the lung interstitium, express F4/80, CD11b and MHCII, but express low levels of CD11c [17]. Under steady state conditions, alveolar macrophages comprise 90-95% of the cells in the alveolar space and similar to LCs are foetal liver monocyte-derived and self-renewing [18, 19]. Alveolar macrophages are required for surfactant catabolism and maintaining lung homeostasis [20]. In addition, alveolar macrophages remove antigenic particles and through a variety of mechanisms actively down-regulate inflammation and promote a state of immune

tolerance [16, 21]. In contrast to alveolar macrophages, interstitial macrophages are located within the lung tissue and are not in direct contact with the alveolar fluid and the exterior environment. Although the interstitial macrophages are a cell population that remains largely uncharacterized, it has been suggested that they can join alveolar macrophages in their role of dampening inflammation [22].

Tissue resident macrophage populations during the immediate responses to wounding

A number of evolutionary conserved reactions lead to a rapid activation of tissue resident macrophages and initiate an inflammatory response upon wounding (Figure 1). This first response to tissue injury involves a rapid release of calcium by damaged cells that travels as a wave via through adjacent cells to activate NADPH oxidase [23]. The NADPH oxidase mediates the production of hydrogen peroxide H_2O_2, known to be an immediate damage signal [24]. Calcium and hydrogen peroxide are thus the earliest known signals after tissue damage that mobilize epithelial cells and bring immune cells to the site of wounding [24, 25]. In monocytes/macrophages H_2O_2 has been shown to trigger the active release of high-mobility group box 1 protein (HMGB-1) [26]. HMGB-1 release is a potent mediator of systemic inflammation, when passively released by necrotic cells, and is reported to be one of the first amplifiers of the pro-inflammatory cascade upon wounding [26].

Other factors that lead to immediate activation of tissue resident macrophages are cell-endogenous molecules that are constitutively expressed and released upon tissue damage. These 'danger-associated molecular patterns' (DAMPs) can for instance be molecules such as HMGB-1 or ATP. ATP in blood normally has a half-life of less than 40 seconds. However, intracellular ATP released by wounding can be rapidly recognized by two different classes of purigenic receptors, P2Y (a G-protein coupled receptor) and P2X (a ligand-gated ion channel), which are both expressed on a number of tissue resident macrophages. Although P2YR signaling may contribute to repair through the uptake of apoptotic cells, both P2YR and particularly P2XR have strong pro-inflammatory effects, promoting recruitment of inflammatory cells to the site of injury[27]. In addition, a number of cytokines are pre-stored in tissue cells, which are then rapidly processed and released upon injury. These 'alarmins' including

IL-1α and IL-33, target tissue resident macrophages, such as alveolar macrophages, and induce their activation [28].

In parallel, damage of blood vessels causes blood leakage that then rapidly activates a coagulation cascade resulting in the formation of a clot (Figure 1). This clot serves as a first protective shield against invading pathogens and ongoing blood loss. It consists of platelets that are embedded in cross-linked fibrin fibers derived by thrombin cleavage of fibrinogen [1]. In the lung, coagulation occurs not only intra-vascularly in a milieu containing the complete plasma clotting system, but also extra-vascularly within the alveolar cavities [29]. Alveolar macrophages directly contribute to this pro-coagulant activity [30], potentially via the production of tissue factor and thereby help initiate the coagulation cascade [31]. Beyond the essential function of preventing blood loss, coagulation also contributes directly to the subsequent inflammatory and fibro-proliferative responses during wound healing via activation of resident macrophages [32]. For example, clot formation in itself releases substantial amounts of free heme, a DAMP that causes oxidative damage and acts on macrophages to promote a pro-inflammatory cascade [33].

All these different pathways induce an immediate pro-inflammatory environment around the wound, which within hours after injury leads to the rapid influx of additional leukocyte populations, such as neutrophils and monocytes [34]. Since this immediate response to injury is so highly conserved in all tissues, macrophages in both the skin and the lungs act as sentinels of tissue homeostasis and both have the capability to recognize immediate danger signals and to initiate the inflammatory phase of wound healing.

The inflammatory response and influx of monocyte populations

The induction of a pro-inflammatory environment within the ruptured tissue leads to the influx of neutrophils and monocytes (Figure 3a). In response to tissue-specific signals these monocytes then differentiate into macrophages and join tissue resident macrophage populations in driving the restoration of tissue integrity. The differentiation of monocyte populations during wound healing has been studied particularly well in skin injury models, using reporter mouse strains and *in vivo*

imaging. It has been shown that by the induction of chemokines, activated perivascular macrophages are major players in the process of neutrophil recruitment to inflamed skin [35]. Furthermore, it had been assumed that within the first hours after damage it was exclusively neutrophils that infiltrate the damage site, followed by monocytes as early as 24 to 48 hrs post injury [36]. However, recent imaging data by Rodero and colleagues revealed that an initial wave of monocytes enters the wound at the same time as neutrophils are accumulating and that monocytes infiltrate the wound bed through micro-hemorrhages caused by disrupted vessel integrity. These monocytes directly crawl trough vascular leakage and spread randomly into the wound bed [37].

In the skin, this first sequence of monocyte infiltration is followed by a second phase of monocyte infiltration starting 24hrs post wounding, whereby the type of monocytes that infiltrate the wound changes over time (Figure 3a). Two fundamentally different subsets of monocytes have been identified to infiltrate the wound bed. One monocyte population is characterized by the expression of the chemokine receptor CCR-2 (which are equivalent to CD14 expressing monocytes in humans), and the other is characterized by the expression of the chemokine receptor CX3CR-1 (which are equivalent to CD16 expressing monocytes in humans) [38-40]. Using CCR-2/GFP reporter mice it was shown that CCR-2 expressing monocytes are predominantly recruited to skin wounds in the first days following wounding. Accordingly, the absolute number of macrophages at the site of wounding is significantly reduced in CCR-2 deficient mice during the early phase of wounding [41]. As CCR-2 deficient mice had reduced blood vessel formation within the wound bed, this subset of monocytes has been assumed to be pro-angiogenic. Other processes of wound healing, such as wound closure rate and scar formation, were not altered in CCR-2 deficient mice [41]. The early influx of monocytes gives rise to macrophage that express iNOS, IL-1b and IL-6 [41] (Figure 3a). It is thus assumed that a key function of monocyte-derived cells, along with neutrophils during the inflammatory phase is to contribute to the defense against microorganisms, which might have entered the wound.

In contrast to the early influx of CCR-2 expressing monocytes, an influx of CX3CR-1 expressing monocytes is observed at later stages of skin wound repair (Figure 3a). Consistent with the different kinetics of infiltration, in CX3CR-1 deficient mice, unlike CCR-2 deficient mice, impaired healing was most pronounced at the late stage of repair in models of excisional or burn wounds [42, 43]. These findings strongly suggest that the two wound-infiltrating monocyte populations have distinct functions during wound repair, with CCR-2 expressing monocytes substantially contribute to neo-angiogenesis of the wound bed, while CX3CR-1 expressing monocytes contribute more to wound closure and the deposition of extracellular matrix component within the wound. However, in neither CCR-2 nor CX3CR-1 deficient mice were wound macrophages completely abrogated suggesting a level of redundancy between the populations with both contributing to the wider macrophage pool present in the wound bed [42].

Despite their distinct contributions to wound healing, the exact fate of the different infiltrating monocyte populations remains poorly understood. In addition, the tissue-specific mechanisms underlying the transition from an inflammatory monocyte into tissue-resident macrophages and the extent to which different infiltrating monocyte populations contribute to the macrophage pool remain to be fully understood. However, for both locations, in the skin as in the lung, it has become apparent that monocyte-derived macrophages in both tissues have specific functions during tissue repair, which separate them from tissue-resident macrophages.

Role of macrophages during the tissue formation phase

The tissue formation phase of wound healing is characterized by dynamic cellular proliferation and differentiation of cells along with the creation of new extracellular matrix (ECM) and deposition of collagen to support the new cells (Figure 1). The process is orchestrated by cell-cell and cell-ECM interactions. Additionally, an integral part of this process is that inflammation and new cell recruitment must be actively suppressed [44]. Data from excisional punch biopsy of the skin, show that macrophages are the dominant immune cells during the tissue formation phase [7]. When cells of the myeloid lineage are conditionally depleted at a very early inflammatory stage, mice exhibit reduced vascularization of the wound bed and

impaired re-epithelialization. However, depletion at the tissue formation stage, when several different monocyte population have already infiltrated the wound, has devastating effects on the progression of wound healing and leads to massive hemorrhage [7]. The critical role of macrophages to efficient wound healing is thus particularly apparent during the tissue-formation phase [4, 7] because they contribute to all aspects of this highly complex orchestra of cell growth, matrix deposition and controlled inflammation.

In both the lung and the skin macrophages are major sources of growth factors [41, 45-49]. Macrophage-derived growth factors not only induce differentiation and proliferation of cells but also induce ECM deposition [50]. Because ECM can bind growth factors, it regulates their activity. ECM functions as a reservoir for growth factors, concentrates their activity in the vicinity of cells and protects them from degradation. A key player in this orchestra is heparan sulfate, which as a component of the newly formed ECM underneath the wound, binds and enriches a number of different growth factors [50]. Heparan sulfate can potentiate the biological activity of VEGF, the basic fibroblast growth factor (FGF-2), the EGF-like growth factor Amphiregulin or the transforming growth factor-β (TGF-β). These growth factors target structural and progenitor cell populations such endothelial cells, mesothelial cells and potentially pericytes [51] to proliferate or survive as well as differentiate into one or more cell types contributing to wound repair [50, 52].

One prominent example demonstrating the pivotal role of macrophages during this phase of tissue repair is VEGF mediated neo-angiogenis of the wound bed. Wound angiogenesis, which is sprouting of capillaries from existing blood vessels into the wound bed, are promoted by growth factors such as VEGF-A. Angiogenesis is vital for healing and repair, because it supplies the newly formed tissue with nutrients and oxygen. The source of VEGF within the wound has been particularly well studied in a model of skin repair [41]. Using reporter mice, macrophages were identified as the major source of VEGF during an early healing stage – while, at later stages, most VEGF expressing cells were found within the neo-epithelium itself [41]. Accordingly, mice deficient for VEGF within the myeloid lineage ($VEGF^{fl/fl}$ x LysM-cre) showed impaired vascularization in early phases of wound healing. Since vascularization

finally recovered in these mice, these data suggest that myeloid-derived VEGF is important for the induction of wound angiogenesis, whereas at later stages epidermal cells might be able to take over as the critical source of VEGF during skin repair [41].

Resolution of inflammation during the tissue formation phase

Resolution of inflammation is a necessary step in the process that allows the formation and growth of new tissue, and macrophages actively contribute by the secretion of macrophage-derived anti-inflammatory cytokines. One of the first steps to initiate both resolution of inflammation and the initiation of tissue formation is the ingestion of apoptotic neutrophils by macrophages. Mice which are impaired in their ability to take up apoptotic neutrophils exhibit delayed healing of excisional skin wounds [53]. Phagocytosis of apoptotic cell debris triggers the release of growth factors such as vascular endothelial growth factor (VEGF) [54] or hepatocyte growth factor (HGF) [55], which are known to be crucial for tissue repair after injury. Additionally, it has long been known that ingestion of apoptotic neutrophils stimulates the release of anti-inflammatory mediators by macrophages [56], in particular TGF-beta, a cytokine that exhibits both anti-inflammatory activity and control of cell proliferation and differentiation [57]. IL-10 is also induced in macrophages on the uptake of apoptotic neutrophils and contributes to the resolution of lung injury [58]. In the skin, IL-10 is needed for an appropriately controlled repair response as IL-10-deficient mice show a sustained inflammatory response, which subsequently leads to increased collagen deposition in scar tissue [59]. Overall, IL-10 is increasingly recognised as central to quality tissue repair, at least in part through its ability to suppress inflammation [60].

Another strategy to dampen inflammation is the induction of interleukin-1 receptor antagonist (IL-1Ra) [61]. IL-1Ra is a member of the IL-1 family that binds to IL-1 receptors but does not induce any intracellular response. IL-1Ra therefore is able to inhibit the effects of IL-1α and IL-1β [62, 63], which are pro-inflammatory cytokines released at a very early phase of wounding upon tissue disruption [64]. In particular the sustained and sometimes excessive influx and activation of neutrophils into the wound is detrimental for the wound healing process [1]. Macrophage-derived IL-1ra dampens the local release of the chemokine MIP-2 and the local expression of the

epithelial adhesion molecule ICAM-1, in this way attenuating neutrophil recruitment. Since IL-1β is a critical survival signal for neutrophils, the local blockade of IL-1β by macrophage-derived IL-1ra within the wound may also induce apoptosis in wound-resident neutrophils [56, 61]. In addition has it been shown that by expressing the apoptosis-inducing ligand TRAIL, macrophages can directly induce apoptosis in neutrophils [65].

The intricate connection between repair and resolution of inflammation is demonstrated by a number of macrophage-derived cytokines that show a dual functionality. Best known is TGFβ, which directly contributes to the process of wound healing, while at the same time suppressing local inflammation. Another good example is the Epidermal Growth Factor (EGF)-like growth factor Amphiregulin [66]. Amphiregulin can induce the differentiation of tissue precursor cells within wounds [67]. At the same time, Amphiregulin can enhance local regulatory T cell function [68, 69] thereby suppressing local inflammation. When Amphiregulin is produced by activated alveolar macrophages, it can protect against LPS-induced acute lung injury [70] and mice with a regulatory T cell specific deficiency of the EGF-R (FoxP3cre x EGF-R$^{fl/fl}$), which cannot respond to Amphiregulin, show a marked delay in skin wound repair [71]. Further, intra-nasal administration of Amphiregulin protects mice from lethal lung infections by promoting tissue integrity and repair [72, 73]. Although Tregs themselves are important source of Amphiregulin during wound healing [74], the exact cellular sources need further study.

Macrophage-derived arginase is another excellent example of a molecule that demonstrates the close relationship of tissue repair activities to suppression of inflammation. Arginase converts arginine into ornithine, which can serve as a precursor for the synthesis or proline and hydroxyproline, which are central constituents of collagen. Additionally, ornithine feeds into the biosynthetic pathway for polyamines, which are required for cellular proliferation [75]. The activity of arginase also suppresses the inflammatory response because arginase competes with iNOS for their common substrate, arginine. Additionally by reducing the extra-cellular arginine concentration, arginase suppresses activated T cells, which are highly arginine-dependent [76].

IL-4 receptor signaling to macrophages during tissue repair

Arginase is considered a marker for macrophages activated via the IL-4receptor (M(IL-4)) [77] and thus M(IL-4) or 'alternatively activated' macrophages have long been considered to have tissue repair function [78]. However, arginase can be induced by many different factors including IL-10, and thus the specific importance of M(IL-4) to tissue repair has been a matter of some debate. Recent studies have ended this debate by providing direct evidence for the contribution of M(IL-4) to tissue repair along with detailed mechanistic insight. In mice lacking the IL-4Rα on macrophages, skin wound healing was delayed and vascular stability impaired [79]. Knipper et al. further showed that the IL-4Rα-inducible protein RELMα when produced by macrophages instructed fibroblasts to produce lysyl hydroxylase 2 (LH2) [79]. LH2 is an enzyme that directs collagen cross-links that are biochemically more stable and less likely to be degraded by enzymes than those found in uninjured skin. These findings demonstrate that although fibroblasts are the main matrix producing cells, macrophages critically regulate the synthesis and organization of de-novo collagen depositions in the wound.

Assessing the contribution of M(IL-4) in the lung during wound healing has been somewhat complicated by the fact that alveolar macrophages already express markers typically associated with M(IL-4), such as Ym1 and CD206, and rapidly secrete typical M(IL-4) growth factors, such as Amphiregulin [80-82]. While alveolar macrophages in the steady state exhibit STAT-6 activation and arginase expression (C. Minutti, unpublished data), they have little or no basal iNOS expression and STAT1 phosphorylation [83], typical markers of classical activation of macrophages derived from other tissues. Therefore alveolar macrophages are considered to be somewhat biased even at steady-state. Indeed, the anti-inflammatory properties of alveolar macrophages, as well as M(IL-4) macrophages may contribute to the ability of the lung to avoid over-reaction to innocuous substances. Consistent with this concept, depletion of CD206+ macrophages substantially exaggerated lung injury in endotoxemic mice [84].

More direct evidence for the contribution of M(IL-4) in promoting lung repair came from studies of the lung migrating nematode, *Nippostrongylus brasiliensis*, which leads to the expansion of large numbers of highly activated M(IL-4) [85]. Depletion of macrophages exacerbated the hemorrhaging caused by worm migration and led to more severe acute lung injury. Macrophage depletion also resulted in a significant reduction in insulin-like growth factor 1 (IGF-1) and arginase, both of which are induced by IL-4 and are important for repair in this lung injury model [85]. IGF-1 stimulates the proliferation and survival of fibroblasts and myofibroblasts to promote matrix production and wound closure [86]. In addition to IGF-1 and arginase many other repair proteins are regulated by IL-4 or IL-13 in macrophages [87]. Taken together, the studies on M(IL-4) suggest that in both tissues, the skin as well as the lung, their involvement is likely to be most prominent during the tissue formation stage as they appear to contribute to cellular proliferation, regulation of fibroblasts, collagen production and cross-linking while exerting broadly anti-inflammatory functions.

Contribution of macrophages to tissue re-organisation

The final stage of wound healing is the “tissue-remodeling” phase, during which a process of wound re-organization restructures the tissue back to its original form (Figure 1). The re-organization of wounded tissue is a multi-step process and during this phase, which can last for weeks or even months, tissue resident endothelial cells, macrophages and myo-fibroblasts undergo apoptosis, newly formed blood vessels regress and remodeling and re-organization of the newly formed collagen layer underneath the wound occurs (Figure 1). During this phase of wound healing, macrophages contribute to wound healing by ingesting cell debris and contributing to the degradation of excess ECM that had built up in and around the wound [88]. Nevertheless, depletion of skin macrophage in the tissue-remodeling phase does not have a substantial impact on the outcome of wound healing, suggesting that macrophages are part of a redundant system of tissue re-organisation and at this stage of wound repair their functions could be taken over by other wound resident cell populations [7].

Macrophages have been suggested to have a major role in the breakdown of matrix fragments by phagocytic uptake and intracellular degradation [89]. However, matrix fragments are also degraded enzymatically by secreted metalloproteinases, cysteine proteinases (cathepsin B and L) or serine proteases (eg plasmin). In particular, matrix metalloproteinases (MMPs) represent a group of enzymes involved in the degradation of most of the components of the collagen layer underneath wounds [90]. MMPs can be produced by macrophages (Figure 3a), but can also be produced by other cells at the wound site such as neutrophils, keratinocytes or fibroblasts. A specific function of macrophage-derived MMPs was recently identified for MMP-10 [91]. MMP-10 knock out mice produce a stiffer and more collagenous scar tissue compared to wild type mice indicating that MMP-10 is critical for collagen breakdown in skin wound healing. Macrophage depletion and adoptive transfer experiments demonstrated that macrophage-derived MMP-10 was critical for the collagenolytic activity in part by regulating the production of other MMPs by macrophages, particularly MMP13 [91].

Alveolar macrophages have been shown to produce MMP1, MMP2, MMP7 and MMP12 [92-94] (Figure 3a). In addition, it has been shown that macrophage-derived 12-lipoxygenase metabolites induce MMPs by lung fibroblasts during lung inflammation [95]. Furthermore, it has been observed that alveolar macrophages contain evident collagen by-products in animal models of emphysema, suggesting that alveolar macrophages can contribute to collagen catabolism [96]. Overall, the specific contribution of macrophages to this final repair stage requires further investigation, in particular whether macrophages are absolutely required, and if so, what cytokines or factors drive their reparative phenotype.

Conclusion and Outlook

The comparison of the role of macrophages in the process of wound healing in two distinct tissues has highlighted key similarities that likely reflect evolutionarily conserved pathways in tissue repair. In both the lung and the skin, the process of wound healing transitions through similar phases with macrophages performing similar functions (Figure 1). Tissue-resident macrophages in either tissue are an essential sentinel, which recognizes tissue damage and substantially contributes to the initiation of a local pro-inflammatory environment. The pro-inflammatory

environment then allows the influx of monocyte populations into the wound of both tissues, where they then differentiate in a tissue-specific manner and perform essential tissue repair functions that tissue-resident macrophage populations could not perform on their own. The reliance of both tissues on macrophage-derived growth factors for cell proliferation and differentiation within the wound, is also a shared characteristic, although lung resident macrophages are from the start more polarized towards a wound healing and inflammation-resolving phenotype. In both tissues, macrophages then actively contribute to the resolution of local inflammation and to the re-organization of the wounded tissue.

There is a fundamental limitation to the comparison of macrophage function between different tissues, and that is the very different nature of the experiments that are performed. Skin wound healing is often based on well-defined incisional or excisional wounds. In contrast, wounding in experimental lung models is often introduced either by a general physical disruption of lung structure, for instance, via forced ventilation or exposure to a strong cell irritant, such as bleomycin, or by the transition of the nematode *Nippostrongylus brasiliensis* through the lung tissue. Another current limitation is the fact that key experiments, such as tracking of different monocyte populations into the healing wound, have exclusively been performed in one model system but not in the other. Nevertheless, a number of differences in macrophage function in the two tissues have become apparent. For example, while wounds in both tissues go through very similar phases of wound repair, the dynamics of these different phases are different (Figure 3b). Puncture induced wounding in the skin can require weeks to complete the process of wound healing. In contrast, lungs punctured by migrating worm larvae, fully recover within days [85, 97, 98]. While the different phases of wound repair within the skin are clearly distinguishable and follow a clear chronological order, these phases in the lungs overlap considerably. There is a strong indication that the "tissue formation" phase with its macrophage mediated secretion of growth factor is already initiated in the lung before the "coagulation phase" has been completed [99] (Figure 3b). This rapid initiation of wound repair may be a consequence of the fact that alveolar macrophages are already skewed towards wound repair. The unique environment of the lung as a highly exposed organ significantly contributes to the phenotype of alveolar macrophages by providing local factors that

increase their activation threshold and thereby imprints the characteristic tolerogenic response in the lung. This characteristic of the lung may also explain another difference between the two tissues, which is the distinct regenerative capacity of these different tissues to re-organize wounded tissue back into its original structure. Wound repair in skin tissue is focused on restoration of skin integrity, however, many aspects of original skin structure with their highly differentiated compartmentalization is not re-created. In contrast, lung tissue, even after severe damaging or the surgical removal of parts of lung lobes, has a remarkable regenerative capacity that can lead to restoration of alveolar architecture [51, 100].

Our review of the literature also revealed a number of unanswered questions. One of the most pressing of these questions is the exact role that different monocyte populations and monocytes-derived macrophage populations play during wound healing and how they function in comparison to tissue resident macrophages (Figure 3a). CCR-2 and CX3CR-1 expressing monocytes in skin models have distinct functions but also immigrate to the wound at different stages of wound repair and therefore encounter fundamentally distinct local inflammatory conditions (Figure 3a). While CCR-2 expressing monocytes immigrate during a pro-inflammatory environment CX3CR-1 expressing monocytes enter at a stage when the pro-inflammatory environment has started to resolve and when wound-resident macrophage populations become polarized towards a more alternatively activated phenotype. CCR-2 expressing monocytes recruited to allergic skin can also acquire an alternative polarized phenotype, highlighting monocyte plasticity and context-dependence of monocyte differentiation and macrophage polarization [101]. In the lung, with its more polarizing environment recruited monocytes rapidly differentiate into fully functional alveolar macrophages [102, 103]. However, regardless of the dynamics of differentiation, for both locations, in the skin as in the lung, it became apparent that monocyte-derived macrophages in both tissues have specific functions during tissue repair, which separate them from tissue-resident macrophages.

In conclusion, we have aimed to describe the contribution of macrophages to processes involved in appropriate tissue repair under normal physiological conditions. However, it is critical to note that failure at any of the steps discussed above can result

in a pathological outcome (Figure 3b). Thus inflammation must be initiated for efficient healing [104], but if it is not resolved the consequence can be non-healing ulcers, or a constant battle between inflammation and matrix deposition leading to severe fibrosis [82, 105]. Similarly, collagen deposition must stop or otherwise scar tissue and loss of tissue function will ensue, and finally the breakdown and rebuilding of matrix that characterizes the final stages must be appropriated controlled or the continual remodeling of tissue will compromise it's functional integrity. Because of their central role in the orchestration of efficient and high quality tissue repair, macrophages are also central to the processes that fail. Thus by fully understanding the contribution of tissue-specific as well as common functions of macrophages that ensure proper repair, macrophages will be increasingly attractive targets for therapy against diseases that result from poor quality repair.

Acknowledgements: The figures were designed by Carmen González: http://www.unapiranha.com/. Research in the Zaiss laboratory is supported by the European Union (CIG: ''EGFR for Immunity'') and the Medical Research Council (MR/M011755/1). D.M.W.Z. is a member of The Wellcome Trust supported Center for Immunity, Infection and Evolution (CIIE). Research in the Allen lab is supported by the MRC (MR/K01207X/1), Wellcome Trust (106898/A/15/Z) and MRF-Asthma UK (MRFAUK-2015-302). JEA is a member the Wellcome Trust Center for Cell-Matrix Research.

[1] P. Martin, Wound healing--aiming for perfect skin regeneration, Science 276(5309) (1997) 75-81.
[2] B.K. Sun, Z. Siprashvili, P.A. Khavari, Advances in skin grafting and treatment of cutaneous wounds, Science 346(6212) (2014) 941-5.
[3] D.M. Simpson, R. Ross, The neutrophilic leukocyte in wound repair a study with antineutrophil serum, The Journal of clinical investigation 51(8) (1972) 2009-23.
[4] S.J. Leibovich, R. Ross, The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum, The American journal of pathology 78(1) (1975) 71-100.
[5] R. Mirza, L.A. DiPietro, T.J. Koh, Selective and specific macrophage ablation is detrimental to wound healing in mice, The American journal of pathology 175(6) (2009) 2454-62.

[6] M. Lech, H.J. Anders, Macrophages and fibrosis: How resident and infiltrating mononuclear phagocytes orchestrate all phases of tissue injury and repair, Bba-Mol Basis Dis 1832(7) (2013) 989-997.
[7] T. Lucas, A. Waisman, R. Ranjan, J. Roes, T. Krieg, W. Muller, A. Roers, S.A. Eming, Differential roles of macrophages in diverse phases of skin repair, Journal of immunology 184(7) (2010) 3964-77.
[8] E. Proksch, J.M. Brandner, J.M. Jensen, The skin: an indispensable barrier, Experimental dermatology 17(12) (2008) 1063-72.
[9] E. Fuchs, S. Raghavan, Getting under the skin of epidermal morphogenesis, Nature reviews. Genetics 3(3) (2002) 199-209.
[10] G. Hoeffel, Y. Wang, M. Greter, P. See, P. Teo, B. Malleret, M. Leboeuf, D. Low, G. Oller, F. Almeida, S.H. Choy, M. Grisotto, L. Renia, S.J. Conway, E.R. Stanley, J.K. Chan, L.G. Ng, I.M. Samokhvalov, M. Merad, F. Ginhoux, Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages, The Journal of experimental medicine 209(6) (2012) 1167-81.
[11] F. Ginhoux, F. Tacke, V. Angeli, M. Bogunovic, M. Loubeau, X.M. Dai, E.R. Stanley, G.J. Randolph, M. Merad, Langerhans cells arise from monocytes in vivo, Nature immunology 7(3) (2006) 265-73.
[12] M. Merad, M.G. Manz, H. Karsunky, A. Wagers, W. Peters, I. Charo, I.L. Weissman, J.G. Cyster, E.G. Engleman, Langerhans cells renew in the skin throughout life under steady-state conditions, Nature immunology 3(12) (2002) 1135-41.
[13] M. Merad, F. Ginhoux, M. Collin, Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells, Nature reviews. Immunology 8(12) (2008) 935-47.
[14] I. Juhasz, G.F. Murphy, H.C. Yan, M. Herlyn, S.M. Albelda, Regulation of extracellular matrix proteins and integrin cell substratum adhesion receptors on epithelium during cutaneous human wound healing in vivo, The American journal of pathology 143(5) (1993) 1458-69.
[15] O. Stojadinovic, N. Yin, J. Lehmann, I. Pastar, R.S. Kirsner, M. Tomic-Canic, Increased number of Langerhans cells in the epidermis of diabetic foot ulcers correlates with healing outcome, Immunologic research 57(1-3) (2013) 222-8.
[16] J.R. Wright, Immunoregulatory functions of surfactant proteins, Nature reviews. Immunology 5(1) (2005) 58-68.
[17] M. Kopf, C. Schneider, S.P. Nobs, The development and function of lung-resident macrophages and dendritic cells, Nature immunology 16(1) (2015) 36-44.
[18] D. Hashimoto, A. Chow, C. Noizat, P. Teo, M.B. Beasley, M. Leboeuf, C.D. Becker, P. See, J. Price, D. Lucas, M. Greter, A. Mortha, S.W. Boyer, E.C. Forsberg, M. Tanaka, N. van Rooijen, A. Garcia-Sastre, E.R. Stanley, F. Ginhoux, P.S. Frenette, M. Merad, Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes, Immunity 38(4) (2013) 792-804.
[19] M. Guilliams, I. De Kleer, S. Henri, S. Post, L. Vanhoutte, S. De Prijck, K. Deswarte, B. Malissen, H. Hammad, B.N. Lambrecht, Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF, The Journal of experimental medicine 210(10) (2013) 1977-92.

[20] Y. Lavin, A. Mortha, A. Rahman, M. Merad, Regulation of macrophage development and function in peripheral tissues, Nature reviews. Immunology 15(12) (2015) 731-44.
[21] T. Hussell, T.J. Bell, Alveolar macrophages: plasticity in a tissue-specific context, Nature reviews. Immunology 14(2) (2014) 81-93.
[22] D. Bedoret, H. Wallemacq, T. Marichal, C. Desmet, F. Quesada Calvo, E. Henry, R. Closset, B. Dewals, C. Thielen, P. Gustin, L. de Leval, N. Van Rooijen, A. Le Moine, A. Vanderplasschen, D. Cataldo, P.V. Drion, M. Moser, P. Lekeux, F. Bureau, Lung interstitial macrophages alter dendritic cell functions to prevent airway allergy in mice, The Journal of clinical investigation 119(12) (2009) 3723-38.
[23] W. Razzell, I.R. Evans, P. Martin, W. Wood, Calcium flashes orchestrate the wound inflammatory response through DUOX activation and hydrogen peroxide release, Current biology : CB 23(5) (2013) 424-9.
[24] P. Niethammer, C. Grabher, A.T. Look, T.J. Mitchison, A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish, Nature 459(7249) (2009) 996-9.
[25] W. Wood, Wound healing: calcium flashes illuminate early events, Current biology : CB 22(1) (2012) R14-6.
[26] D. Tang, R. Kang, W. Xiao, L. Jiang, M. Liu, Y. Shi, K. Wang, H. Wang, X. Xiao, Nuclear heat shock protein 72 as a negative regulator of oxidative stress (hydrogen peroxide)-induced HMGB1 cytoplasmic translocation and release, Journal of immunology 178(11) (2007) 7376-84.
[27] M. Idzko, D. Ferrari, H.K. Eltzschig, Nucleotide signalling during inflammation, Nature. 509(7500) (2014) 310-17.
[28] H. Han, M.B. Headley, W. Xu, M.R. Comeau, B. Zhou, S.F. Ziegler, Thymic stromal lymphopoietin amplifies the differentiation of alternatively activated macrophages, Journal of immunology 190(3) (2013) 904-12.
[29] T. van der Poll, Tissue factor as an initiator of coagulation and inflammation in the lung, Critical care 12 Suppl 6 (2008) S3.
[30] G. Grunig, C. Hulliger, C. Winder, M. Hermann, T.W. Jungi, R. von Fellenberg, Spontaneous and lipopolysaccharide-induced expression of procoagulant activity by equine lung macrophages in comparison with blood monocytes and blood neutrophils, Veterinary immunology and immunopathology 29(3-4) (1991) 295-312.
[31] H.A. Chapman, Jr., C.L. Allen, O.L. Stone, D.S. Fair, Human alveolar macrophages synthesize factor VII in vitro. Possible role in interstitial lung disease, The Journal of clinical investigation 75(6) (1985) 2030-7.
[32] P.F. Mercer, R.C. Chambers, Coagulation and coagulation signalling in fibrosis, Biochimica et biophysica acta 1832(7) (2013) 1018-27.
[33] F.F. Dutra, M.T. Bozza, Heme on innate immunity and inflammation, Frontiers in pharmacology 5 (2014) 115.
[34] D. Kreisel, R.G. Nava, W. Li, B.H. Zinselmeyer, B. Wang, J. Lai, R. Pless, A.E. Gelman, A.S. Krupnick, M.J. Miller, In vivo two-photon imaging reveals monocyte-dependent neutrophil extravasation during pulmonary inflammation, Proceedings of the National Academy of Sciences of the United States of America 107(42) (2010) 18073-8.
[35] A. Abtin, R. Jain, A.J. Mitchell, B. Roediger, A.J. Brzoska, S. Tikoo, Q. Cheng, L.G. Ng, L.L. Cavanagh, U.H. von Andrian, M.J. Hickey, N. Firth, W. Weninger,

Perivascular macrophages mediate neutrophil recruitment during bacterial skin infection, Nature immunology 15(1) (2014) 45-53.
[36] M.H. Kim, W. Liu, D.L. Borjesson, F.R. Curry, L.S. Miller, A.L. Cheung, F.T. Liu, R.R. Isseroff, S.I. Simon, Dynamics of neutrophil infiltration during cutaneous wound healing and infection using fluorescence imaging, The Journal of investigative dermatology 128(7) (2008) 1812-20.
[37] M.P. Rodero, F. Licata, L. Poupel, P. Hamon, K. Khosrotehrani, C. Combadiere, A. Boissonnas, In vivo imaging reveals a pioneer wave of monocyte recruitment into mouse skin wounds, PloS one 9(10) (2014) e108212.
[38] F. Geissmann, S. Jung, D.R. Littman, Blood monocytes consist of two principal subsets with distinct migratory properties, Immunity 19(1) (2003) 71-82.
[39] F. Tacke, G.J. Randolph, Migratory fate and differentiation of blood monocyte subsets, Immunobiology 211(6-8) (2006) 609-18.
[40] C. Sunderkotter, T. Nikolic, M.J. Dillon, N. Van Rooijen, M. Stehling, D.A. Drevets, P.J. Leenen, Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response, Journal of immunology 172(7) (2004) 4410-7.
[41] S. Willenborg, T. Lucas, G. van Loo, J.A. Knipper, T. Krieg, I. Haase, B. Brachvogel, M. Hammerschmidt, A. Nagy, N. Ferrara, M. Pasparakis, S.A. Eming, CCR2 recruits an inflammatory macrophage subpopulation critical for angiogenesis in tissue repair, Blood 120(3) (2012) 613-25.
[42] Y. Ishida, J.L. Gao, P.M. Murphy, Chemokine receptor CX3CR1 mediates skin wound healing by promoting macrophage and fibroblast accumulation and function, Journal of immunology 180(1) (2008) 569-79.
[43] A.J. Clover, A.H. Kumar, N.M. Caplice, Deficiency of CX3CR1 delays burn wound healing and is associated with reduced myeloid cell recruitment and decreased sub-dermal angiogenesis, Burns : journal of the International Society for Burn Injuries 37(8) (2011) 1386-93.
[44] S.A. Eming, P. Martin, M. Tomic-Canic, Wound repair and regeneration: mechanisms, signaling, and translation, Science translational medicine 6(265) (2014) 265sr6.
[45] C. Henke, W. Marineili, J. Jessurun, J. Fox, D. Harms, M. Peterson, L. Chiang, P. Doran, Macrophage production of basic fibroblast growth factor in the fibroproliferative disorder of alveolar fibrosis after lung injury, The American journal of pathology 143(4) (1993) 1189-99.
[46] A.R. Medford, S.K. Douglas, S.I. Godinho, K.M. Uppington, L. Armstrong, K.M. Gillespie, B. van Zyl, T.D. Tetley, N.B. Ibrahim, A.B. Millar, Vascular Endothelial Growth Factor (VEGF) isoform expression and activity in human and murine lung injury, Respiratory research 10 (2009) 27.
[47] C. Song, H. Ma, C. Yao, X. Tao, H. Gan, Alveolar macrophage-derived vascular endothelial growth factor contributes to allergic airway inflammation in a mouse asthma model, Scandinavian journal of immunology 75(6) (2012) 599-605.
[48] T.A. Wynn, K.M. Vannella, Macrophages in Tissue Repair, Regeneration, and Fibrosis, Immunity 44(3) (2016) 450-62.
[49] A.J. Byrne, C.P. Jones, K. Gowers, S.M. Rankin, C.M. Lloyd, Lung macrophages contribute to house dust mite driven airway remodeling via HIF-1alpha, PloS one 8(7) (2013) e69246.

[50] G.S. Schultz, A. Wysocki, Interactions between extracellular matrix and growth factors in wound healing, Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 17(2) (2009) 153-62.
[51] D.N. Kotton, E.E. Morrisey, Lung regeneration: mechanisms, applications and emerging stem cell populations, Nature medicine 20(8) (2014) 822-32.
[52] D. Jiang, J. Liang, J. Fan, S. Yu, S. Chen, Y. Luo, G.D. Prestwich, M.M. Mascarenhas, H.G. Garg, D.A. Quinn, R.J. Homer, D.R. Goldstein, R. Bucala, P.J. Lee, R. Medzhitov, P.W. Noble, Regulation of lung injury and repair by Toll-like receptors and hyaluronan, Nature medicine 11(11) (2005) 1173-9.
[53] A. Sindrilaru, T. Peters, J. Schymeinsky, T. Oreshkova, H. Wang, A. Gompf, F. Mannella, M. Wlaschek, C. Sunderkotter, K.L. Rudolph, B. Walzog, X.R. Bustelo, K.D. Fischer, K. Scharffetter-Kochanek, Wound healing defect of Vav3-/- mice due to impaired {beta}2-integrin-dependent macrophage phagocytosis of apoptotic neutrophils, Blood 113(21) (2009) 5266-76.
[54] H.A. Golpon, V.A. Fadok, L. Taraseviciene-Stewart, R. Scerbavicius, C. Sauer, T. Welte, P.M. Henson, N.F. Voelkel, Life after corpse engulfment: phagocytosis of apoptotic cells leads to VEGF secretion and cell growth, FASEB journal : official publication of the Federation of American Societies for Experimental Biology 18(14) (2004) 1716-8.
[55] H. Amano, K. Morimoto, M. Senba, H. Wang, Y. Ishida, A. Kumatori, H. Yoshimine, K. Oishi, N. Mukaida, T. Nagatake, Essential contribution of monocyte chemoattractant protein-1/C-C chemokine ligand-2 to resolution and repair processes in acute bacterial pneumonia, Journal of immunology 172(1) (2004) 398-409.
[56] S. Herold, K. Mayer, J. Lohmeyer, Acute lung injury: how macrophages orchestrate resolution of inflammation and tissue repair, Frontiers in immunology 2 (2011) 65.
[57] M. Morikawa, R. Derynck, K. Miyazono, TGF-beta and the TGF-beta Family: Context-Dependent Roles in Cell and Tissue Physiology, Cold Spring Harbor perspectives in biology 8(5) (2016).
[58] Y. Zhao, Z. Xiong, E.J. Lechner, P.A. Klenotic, B.J. Hamburg, M. Hulver, A. Khare, T. Oriss, N. Mangalmurti, Y. Chan, Y. Zhang, M.A. Ross, D.B. Stolz, M.R. Rosengart, J. Pilewski, P. Ray, A. Ray, R.L. Silverstein, J.S. Lee, Thrombospondin-1 triggers macrophage IL-10 production and promotes resolution of experimental lung injury, Mucosal immunology 7(2) (2014) 440-8.
[59] S.A. Eming, S. Werner, P. Bugnon, C. Wickenhauser, L. Siewe, O. Utermohlen, J.M. Davidson, T. Krieg, A. Roers, Accelerated wound closure in mice deficient for interleukin-10, The American journal of pathology 170(1) (2007) 188-202.
[60] W. Ouyang, S. Rutz, N.K. Crellin, P.A. Valdez, S.G. Hymowitz, Regulation and functions of the IL-10 family of cytokines in inflammation and disease, Annual review of immunology 29 (2011) 71-109.
[61] S. Herold, T.S. Tabar, H. Janssen, K. Hoegner, M. Cabanski, P. Lewe-Schlosser, J. Albrecht, F. Driever, I. Vadasz, W. Seeger, M. Steinmueller, J. Lohmeyer, Exudate macrophages attenuate lung injury by the release of IL-1 receptor antagonist in gram-negative pneumonia, American journal of respiratory and critical care medicine 183(10) (2011) 1380-90.
[62] W.A. Verri, Jr., A.T. Guerrero, S.Y. Fukada, D.A. Valerio, T.M. Cunha, D. Xu, S.H. Ferreira, F.Y. Liew, F.Q. Cunha, IL-33 mediates antigen-induced cutaneous and

articular hypernociception in mice, Proceedings of the National Academy of Sciences of the United States of America 105(7) (2008) 2723-8.
[63] W.P. Arend, M. Malyak, C.J. Guthridge, C. Gabay, Interleukin-1 receptor antagonist: role in biology, Annual review of immunology 16 (1998) 27-55.
[64] L.A. Borthwick, The IL-1 cytokine family and its role in inflammation and fibrosis in the lung, Seminars in immunopathology (2016).
[65] E.E. McGrath, H.M. Marriott, A. Lawrie, S.E. Francis, I. Sabroe, S.A. Renshaw, D.H. Dockrell, M.K. Whyte, TNF-related apoptosis-inducing ligand (TRAIL) regulates inflammatory neutrophil apoptosis and enhances resolution of inflammation, Journal of leukocyte biology 90(5) (2011) 855-65.
[66] D.M. Zaiss, W.C. Gause, L.C. Osborne, D. Artis, Emerging functions of amphiregulin in orchestrating immunity, inflammation, and tissue repair, Immunity 42(2) (2015) 216-26.
[67] D. Burzyn, W. Kuswanto, D. Kolodin, J.L. Shadrach, M. Cerletti, Y. Jang, E. Sefik, T.G. Tan, A.J. Wagers, C. Benoist, D. Mathis, A special population of regulatory T cells potentiates muscle repair, Cell 155(6) (2013) 1282-95.
[68] C. Meulenbroeks, H. van Weelden, C. Schwartz, D. Voehringer, F.A. Redegeld, V.P. Rutten, T. Willemse, A.J. Sijts, D.M. Zaiss, Basophil-derived amphiregulin is essential for UVB irradiation-induced immune suppression, The Journal of investigative dermatology 135(1) (2015) 222-8.
[69] D.M. Zaiss, J. van Loosdregt, A. Gorlani, C.P. Bekker, A. Grone, M. Sibilia, P.M. van Bergen en Henegouwen, R.C. Roovers, P.J. Coffer, A.J. Sijts, Amphiregulin enhances regulatory T cell-suppressive function via the epidermal growth factor receptor, Immunity 38(2) (2013) 275-84.
[70] Y. Xu, C. Meng, G. Liu, D. Yang, L. Fu, M. Zhang, Z. Zhang, H. Xia, S. Yao, S. Zhang, Classically Activated Macrophages Protect against Lipopolysaccharide-induced Acute Lung Injury by Expressing Amphiregulin in Mice, Anesthesiology 124(5) (2016) 1086-99.
[71] A. Nosbaum, N. Prevel, H.A. Truong, P. Mehta, M. Ettinger, T.C. Scharschmidt, N.H. Ali, M.L. Pauli, A.K. Abbas, M.D. Rosenblum, Cutting Edge: Regulatory T Cells Facilitate Cutaneous Wound Healing, Journal of immunology 196(5) (2016) 2010-4.
[72] A.M. Jamieson, L. Pasman, S. Yu, P. Gamradt, R.J. Homer, T. Decker, R. Medzhitov, Role of tissue protection in lethal respiratory viral-bacterial coinfection, Science 340(6137) (2013) 1230-4.
[73] L.A. Monticelli, G.F. Sonnenberg, M.C. Abt, T. Alenghat, C.G. Ziegler, T.A. Doering, J.M. Angelosanto, B.J. Laidlaw, C.Y. Yang, T. Sathaliyawala, M. Kubota, D. Turner, J.M. Diamond, A.W. Goldrath, D.L. Farber, R.G. Collman, E.J. Wherry, D. Artis, Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus, Nature immunology 12(11) (2011) 1045-54.
[74] N. Arpaia, J.A. Green, B. Moltedo, A. Arvey, S. Hemmers, S. Yuan, P.M. Treuting, A.Y. Rudensky, A Distinct Function of Regulatory T Cells in Tissue Protection, Cell 162(5) (2015) 1078-89.
[75] S.M. Morris, Jr., Arginine metabolism: boundaries of our knowledge, The Journal of nutrition 137(6 Suppl 2) (2007) 1602S-1609S.
[76] B.S. Choi, I.C. Martinez-Falero, C. Corset, M. Munder, M. Modolell, I. Muller, P. Kropf, Differential impact of L-arginine deprivation on the activation and effector functions of T cells and macrophages, Journal of leukocyte biology 85(2) (2009) 268-77.

[77] P.J. Murray, J.E. Allen, S.K. Biswas, E.A. Fisher, D.W. Gilroy, S. Goerdt, S. Gordon, J.A. Hamilton, L.B. Ivashkiv, T. Lawrence, M. Locati, A. Mantovani, F.O. Martinez, J.L. Mege, D.M. Mosser, G. Natoli, J.P. Saeij, J.L. Schultze, K.A. Shirey, A. Sica, J. Suttles, I. Udalova, J.A. van Ginderachter, S.N. Vogel, T.A. Wynn, Macrophage activation and polarization: nomenclature and experimental guidelines, Immunity 41(1) (2014) 14-20.
[78] S. Goerdt, C.E. Orfanos, Other functions, other genes: alternative activation of antigen-presenting cells, Immunity 10(2) (1999) 137-42.
[79] J.A. Knipper, S. Willenborg, J. Brinckmann, W. Bloch, T. Maass, R. Wagener, T. Krieg, T. Sutherland, A. Munitz, M.E. Rothenberg, A. Niehoff, R. Richardson, M. Hammerschmidt, J.E. Allen, S.A. Eming, Interleukin-4 Receptor alpha Signaling in Myeloid Cells Controls Collagen Fibril Assembly in Skin Repair, Immunity 43(4) (2015) 803-16.
[80] K. Dai, L. Huang, J. Chen, L. Yang, Z. Gong, Amphiregulin promotes the immunosuppressive activity of intrahepatic CD4+ regulatory T cells to impair CD8+ T cell immunity against hepatitis B virus infection, Immunology (2014).
[81] I. Waern, J. Jia, G. Pejler, E. Zcharia, I. Vlodavsky, J.P. Li, S. Wernersson, Accumulation of Ym1 and formation of intracellular crystalline bodies in alveolar macrophages lacking heparanase, Molecular immunology 47(7-8) (2010) 1467-75.
[82] A.J. Byrne, T.M. Maher, C.M. Lloyd, Pulmonary Macrophages: A New Therapeutic Pathway in Fibrosing Lung Disease?, Trends in molecular medicine 22(4) (2016) 303-16.
[83] C.M. Minutti, B. Garcia-Fojeda, A. Saenz, M. de Las Casas-Engel, R. Guillamat-Prats, A. de Lorenzo, A. Serrano-Mollar, A.L. Corbi, C. Casals, Surfactant Protein A Prevents IFN-gamma/IFN-gamma Receptor Interaction and Attenuates Classical Activation of Human Alveolar Macrophages, Journal of immunology (2016).
[84] K. Kambara, W. Ohashi, K. Tomita, M. Takashina, S. Fujisaka, R. Hayashi, H. Mori, K. Tobe, Y. Hattori, In vivo depletion of CD206+ M2 macrophages exaggerates lung injury in endotoxemic mice, The American journal of pathology 185(1) (2015) 162-71.
[85] F. Chen, Z. Liu, W. Wu, C. Rozo, S. Bowdridge, A. Millman, N. Van Rooijen, J.F. Urban, Jr., T.A. Wynn, W.C. Gause, An essential role for TH2-type responses in limiting acute tissue damage during experimental helminth infection, Nature medicine 18(2) (2012) 260-6.
[86] W.N. Rom, P. Basset, G.A. Fells, T. Nukiwa, B.C. Trapnell, R.G. Crysal, Alveolar macrophages release an insulin-like growth factor I-type molecule, The Journal of clinical investigation 82(5) (1988) 1685-93.
[87] G.D. Thomas, D. Ruckerl, B.H. Maskrey, P.D. Whitfield, M.L. Blaxter, J.E. Allen, The biology of nematode- and IL4Ralpha-dependent murine macrophage polarization in vivo as defined by RNA-Seq and targeted lipidomics, Blood 120(25) (2012) e93-e104.
[88] G.C. Gurtner, S. Werner, Y. Barrandon, M.T. Longaker, Wound repair and regeneration, Nature 453(7193) (2008) 314-21.
[89] D.H. Madsen, D. Leonard, A. Masedunskas, A. Moyer, H.J. Jurgensen, D.E. Peters, P. Amornphimoltham, A. Selvaraj, S.S. Yamada, D.A. Brenner, S. Burgdorf, L.H. Engelholm, N. Behrendt, K. Holmbeck, R. Weigert, T.H. Bugge, M2-like macrophages are responsible for collagen degradation through a mannose receptor-mediated pathway, The Journal of cell biology 202(6) (2013) 951-66.

[90] A. Davey, D.F. McAuley, C.M. O'Kane, Matrix metalloproteinases in acute lung injury: mediators of injury and drivers of repair, The European respiratory journal 38(4) (2011) 959-70.
[91] M.G. Rohani, R.S. McMahan, M.V. Razumova, A.L. Hertz, M. Cieslewicz, S.H. Pun, M. Regnier, Y. Wang, T.P. Birkland, W.C. Parks, MMP-10 Regulates Collagenolytic Activity of Alternatively Activated Resident Macrophages, The Journal of investigative dermatology 135(10) (2015) 2377-84.
[92] R. Shaykhiev, A. Krause, J. Salit, Y. Strulovici-Barel, B.G. Harvey, T.P. O'Connor, R.G. Crystal, Smoking-dependent reprogramming of alveolar macrophage polarization: implication for pathogenesis of chronic obstructive pulmonary disease, Journal of immunology 183(4) (2009) 2867-83.
[93] I. Haq, G.E. Lowrey, N. Kalsheker, S.R. Johnson, Matrix metalloproteinase-12 (MMP-12) SNP affects MMP activity, lung macrophage infiltration and protects against emphysema in COPD, Thorax 66(11) (2011) 970-6.
[94] A.M. Wallace, A.J. Sandford, J.C. English, K.M. Burkett, H. Li, R.J. Finley, N.L. Muller, H.O. Coxson, P.D. Pare, R.T. Abboud, Matrix metalloproteinase expression by human alveolar macrophages in relation to emphysema, Copd 5(1) (2008) 13-23.
[95] T.J. Mariani, S. Sandefur, J.D. Roby, R.A. Pierce, Collagenase-3 induction in rat lung fibroblasts requires the combined effects of tumor necrosis factor-alpha and 12-lipoxygenase metabolites: a model of macrophage-induced, fibroblast-driven extracellular matrix remodeling during inflammatory lung injury, Molecular biology of the cell 9(6) (1998) 1411-24.
[96] M. Lucattelli, E. Cavarra, M.M. de Santi, T.D. Tetley, P.A. Martorana, G. Lungarella, Collagen phagocytosis by lung alveolar macrophages in animal models of emphysema, The European respiratory journal 22(5) (2003) 728-34.
[97] J.J. Reece, M.C. Siracusa, A.L. Scott, Innate immune responses to lung-stage helminth infection induce alternatively activated alveolar macrophages, Infection and immunity 74(9) (2006) 4970-81.
[98] T.E. Sutherland, R.M. Maizels, J.E. Allen, Chitinases and chitinase-like proteins: potential therapeutic targets for the treatment of T-helper type 2 allergies, Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology 39(7) (2009) 943-55.
[99] R.P. Marshall, G. Bellingan, S. Webb, A. Puddicombe, N. Goldsack, R.J. McAnulty, G.J. Laurent, Fibroproliferation occurs early in the acute respiratory distress syndrome and impacts on outcome, American journal of respiratory and critical care medicine 162(5) (2000) 1783-8.
[100] M. Herriges, E.E. Morrisey, Lung development: orchestrating the generation and regeneration of a complex organ, Development 141(3) (2014) 502-13.
[101] M. Egawa, K. Mukai, S. Yoshikawa, M. Iki, N. Mukaida, Y. Kawano, Y. Minegishi, H. Karasuyama, Inflammatory monocytes recruited to allergic skin acquire an anti-inflammatory M2 phenotype via basophil-derived interleukin-4, Immunity 38(3) (2013) 570-80.
[102] A.M. Guth, W.J. Janssen, C.M. Bosio, E.C. Crouch, P.M. Henson, S.W. Dow, Lung environment determines unique phenotype of alveolar macrophages, American journal of physiology. Lung cellular and molecular physiology 296(6) (2009) L936-46.

[103] L. van de Laar, W. Saelens, S. De Prijck, L. Martens, C.L. Scott, G. Van Isterdael, E. Hoffmann, R. Beyaert, Y. Saeys, B.N. Lambrecht, M. Guilliams, Yolk Sac Macrophages, Fetal Liver, and Adult Monocytes Can Colonize an Empty Niche and Develop into Functional Tissue-Resident Macrophages, Immunity 44(4) (2016) 755-68.
[104] M.F. Beers, E.E. Morrisey, The three R's of lung health and disease: repair, remodeling, and regeneration, The Journal of clinical investigation 121(6) (2011) 2065-73.
[105] N.X. Landen, D. Li, M. Stahle, Transition from inflammation to proliferation: a critical step during wound healing, Cellular and molecular life sciences : CMLS (2016).

Figure 1: Sequent phases of physiological skin wound healing.

During the "Coagulation and Inflammation" phase, immediately after injury, tissue-resident macrophages contribute to the initiation of a local inflammatory response, which leads to the influx of high numbers of neutrophils into the wound. In parallel, a fibrin clot forms, around which a provisional protective matrix is formed. During the "Tissue Formation" phase, a highly vascularized granulation tissue develops within the wound, comprising of a high cell density of mainly macrophages, fibroblasts and endothelial cells. Keratinocytes migrate from the wound margins to close the epidermal gap. During the "Tissue Reorganisation" phase, macrophages contribute to the active remodelling of the extra-cellular matrix within the wound, leaving behind a scar tissue that is characterized by increased matrix deposition and reduced cellular density.

Figure 2: Architecture of skin vs. alveoli.

The skin is composed of two layers: the epidermis and the dermis. The epidermis provides a physical, chemical and immunological barrier from the outside environment. The epidermis is a squamous epithelium composed of different layers, supported by self-renewing proliferating tissue-stem cells. In contrast, the dermis is a structure rich in extracellular matrix that provides tensile strength and elasticity. The dermis is tightly connected to the epidermis by the basement membrane and consists of different cell types, including fibroblasts and endothelial cells. Appendages such as hair roots, sebaceous glands and sweat glands are located in the dermal part. Langerhans cells are located in the epidermal layer whereas dermal macrophages are the dominant macrophage population in the dermal layer.

The pulmonary alveoli are the terminal ends of the respiratory tree forming an anatomical structure that allows for oxygen uptake. The alveolar sacs are composed of a thin alveolar epithelium composed of two cell types: thin, squamous type I cells, which cover 95% of the alveolar surface and form the structure of the alveolar wall, and cuboidal type II cells that secrete pulmonary surfactant. Surrounding the alveolar sacs, the lung interstitium is a connective tissue-rich framework for the alveoli and contains the interstitial macrophages. Lining the airway lumen alveolar macrophages are embedded in the alveolar fluid rich in pulmonary surfactants providing a barrier for lung defence.

Figure 3:

In all three phases of wound repair (Coagulation and Inflammation, Tissue Formation and Tissue Reorganisation) resident macrophages and monocyte-derived macrophage populations play essential but distinct functions. a) Following injury, tissue-resident macrophage populations function as sentinels and become rapidly activated and contribute to a pro-inflammatory environment within the wound. During the "Coagulation and Inflammation" phase, this pro-inflammatory environment induces the influx of the first wave of CCR-2 expressing monocytes into the wound, which is then followed by a second wave of CX3CR-1 expressing monocytes. These recruited monocyte populations undergo marked phenotypic and functional changes in response to IL-4 as well as to tissue-specific factors. During this phase, the pro-inflammatory environment is resolved and macrophage derived growth factors contribute to tissue repair and regeneration. This resolving environment initiates the "Tissue Reorganisation" during which macrophages contribute to the degradation of cell debris and the re-organisation of excessive ECM material and thus the restoration of affected tissue. b) This process of wound repair is conserved in both the lung as the skin but the dynamics of the distinctive phases differ between the tissues, with the lung tissue recovering faster than the skin. Disruption of this process leads to pathology, which manifests itself as fibrosis or emphysema in the lung and as extended scar tissue in the skin.

Figure 1

Click here to download high resolution image

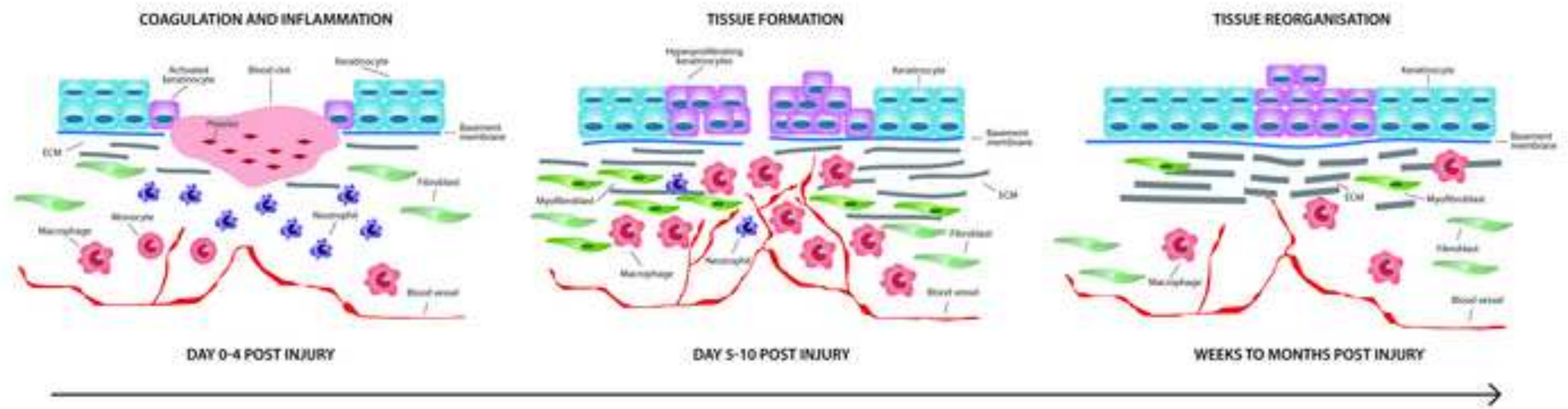
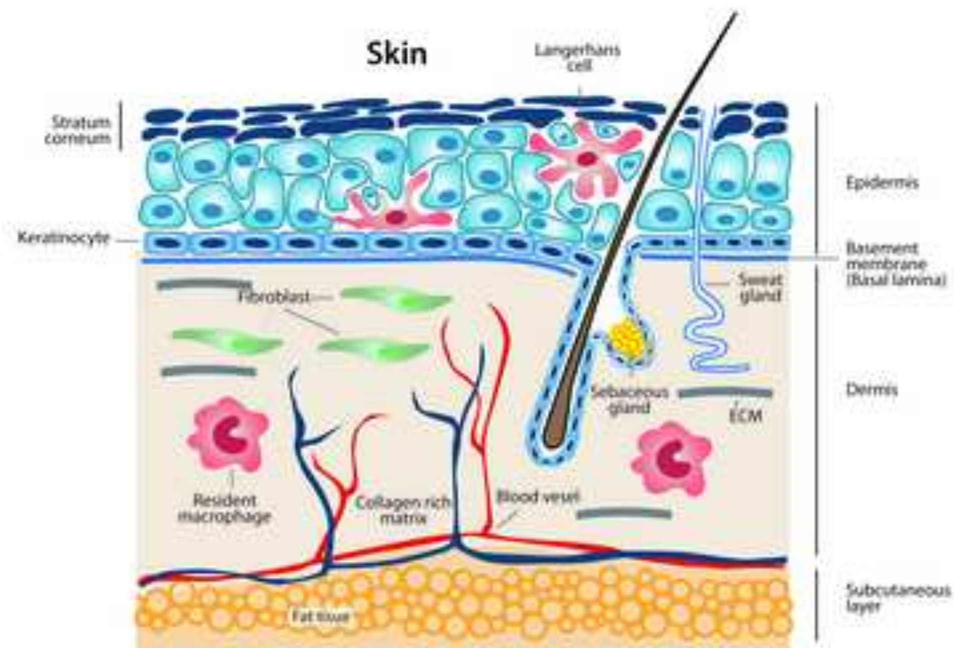


Figure 2
Click here to download high resolution image



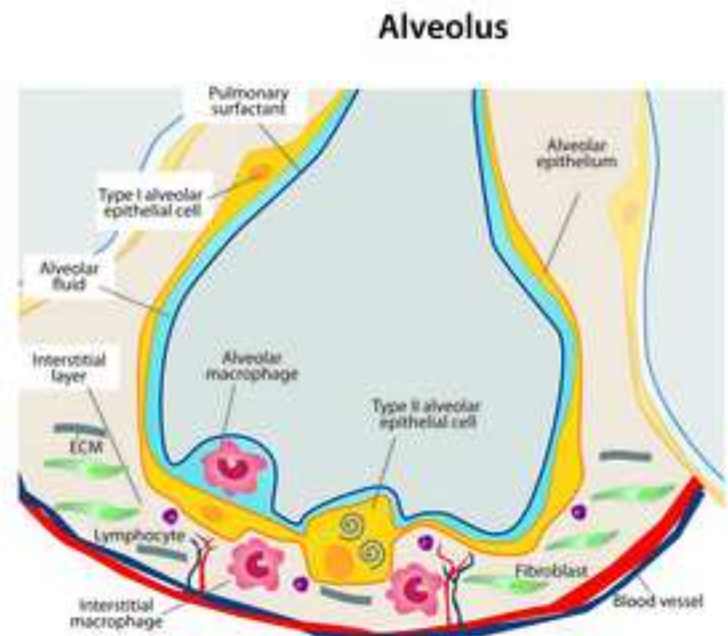


Figure 3
Click here to download high resolution image