Inflammation and Metabolism in Tissue Repair and Regeneration

Sabine A. Eming[1,2,3*], Thomas A. Wynn[4*], and Paul Martin[5,6,7*]

[1]Department of Dermatology, University of Cologne, 50937 Cologne, Germany;

[2]Center for Molecular Medicine Cologne, University of Cologne, Cologne 50931, Germany;

[3]Cologne Cluster of Excellence on Cellular Stress Responses in Aging-Associated Diseases, University of Cologne, Cologne 50931;

[4]Immunopathogenesis Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892;

[5]Schools of Biochemistry and Physiology & Pharmacology, University of Bristol, Bristol, U.K.

[6]School of Medicine, Cardiff University, Cardiff, U.K.

[7]Lee Kong Chian School of Medicine, Nanyang Technical University, Singapore.

[*]All authors contributed equally

***Corresponding Authors:**

Sabine A. Eming, Department of Dermatology, University of Cologne, Kerpenerstr. 62, 50937 Köln, Germany; sabine.eming@uni-koeln.de

Thomas A. Wynn, Immunopathogenesis Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA; TWYNN@niaid.nih.gov

Paul Martin, Schools of Biochemistry and Physiology & Pharmacology, University of Bristol, Bristol BS8 1TD, U.K.; pyxpm@bristol.ac.uk

Abstract

Tissue repair following injury is a complex metabolically demanding process. Depending on the tissue's regenerative capacity and quality of the inflammatory response, the outcome is generally imperfect with some degree of fibrosis, which is defined by aberrant accumulation of collagenous connective tissue. Inflammatory cells multitask at the wound site by facilitating wound debridement and by producing chemokines, metabolites, and growth factors. If this well-orchestrated response becomes dysregulated, it can become a chronic wound or progressively fibrotic, with both outcomes impairing tissue function that can ultimately lead to organ failure and death. Here, we review the current understanding of the role of inflammation and cell metabolism in tissue regenerative responses, highlight emerging concepts that may expand therapeutic perspectives and briefly discuss where important knowledge gaps remain. (Final abstract should not exceed 125 words.)

Introduction

Effective tissue repair is critical for the survival of all living organisms (*1*). After injury, necrotic debris, the clotting reaction, and any invading microbes collectively activate an inflammatory response that is propagated by the local release of chemotactic factors. Neutrophils, monocytes, and other innate immune cells are recruited to the wound site, to clear cell debris and infectious organisms, and subsequently help orchestrate the tissue repair response. The degree and duration of the response varies, and this influences the final outcome. There are major benefits from raising an inflammatory response but there are also negative consequences including the activation of a fibrotic response (define the fibrotic response for the non specialist), which is defined by the excessive and aberrant accumulation of collagenous connective tissues that can debilitate tissue function and in some cases lead to organ failure.

Inflammation following injury is normally modular in nature, with three distinct phases facilitating the restoration of normal tissue architecture. These stages include an early pro-inflammatory stage, where elements of the innate immune response initiate the repair response by mobilizing the recruitment of key inflammatory cells. In the second major phase, the pro-inflammatory response begins to subside, with key inflammatory cells like macrophages switching to a reparative phenotype. In the final stage, tissue homeostasis is restored when the inflammatory cells either exit the site of injury or are eliminated through apoptosis. (The subsequent paragraph is outlining some of the topics and discussion that will be presented throughout the article. At the start of this paragraph,

identify the piece as a review article that will explore some of the elements and concepts of inflammation in repair and regeneration so the reader is not looking for reference citations here.)

This review article will explore some of the newer concepts of inflammation in repair and regeneration and how they offer insights for potential therapeutic modulation to improve healing of tissues in the clinic. Because of recent advances in live imaging of translucent model organisms like zebrafish larvae the dynamic behavior of inflammatory cells can now be viewed as they are drawn to wounds and interact with local cell populations. These approaches along with recent mammalian tissue repair studies in multiple organs from skin to liver have revealed how leukocytic cells undergo behavioural switching from a pro-inflammatory to an anti-inflammatory state (define phenotypic switching; split this sentence into two?)and demonstrate how this impacts their dynamic interaction with host tissue cells to either drive fibrosis or mediate its resolution (*2*). Beyond elucidating spatial and temporal cues that govern immune cell function, recent studies have also identified previously unappreciated molecular determinants that orchestrate immune cell responses. In this context, evidence in the nascent field of immunometabolism has revealed how metabolic adaptation is not simply regulated by nutrient availability, but is also controlled by immune signals that govern immune cell function (*3*). Therefore, the rapid response of immune cells to tissue injury and the need to adjust their metabolic reprogramming during the repair response may in turn regulate immune cell function at the wound site. Thus, targeting metabolic pathways in immune cells may offer new opportunities to therapeutically modulate the inflammatory response and improve the overall outcome of wound healing responses.

New insights emerging from live imaging of the wound response

The immune response to tissue damage and infection was first observed by Metchnikoff in his studies of translucent waterborne creatures in the early 20th century (*4*). His classic rose thorn puncture of a starfish embryo was the first evidence for a "wandering" population of cells that are drawn to invading foreign bodies (**Fig 1A**). In recent years, fluorescent tagging of macrophages in *Drosophila* embryos, and neutrophils as well as macrophages in zebrafish larvae, which are both translucent but more genetically tractable than starfish, has enabled us to gain profound new live imaging insights into the biology of the wound inflammatory response (**Fig 1B—remove the lower diagram if not discussed in text**).

Real-time fluorescent imaging of GFP-tagged neutrophils has revealed the dynamic influx and resolution of these cells in mammalian skin wounds (*5*). In mice, studies have also shown that not all wound inflammatory cells are tissue resident or come from the bloodstream. Indeed, a recent sterile liver injury study employing intravital microscopy identified the peritoneum as an important source of macrophages and the authors showed that this pool of macrophages was essential to repair (*6*). Other murine imaging studies have captured leukocytes as they engage with and then breach the venular wall (Fig 1C and(*7*)), or revealed novel in vivo responses following tissue damage including neutrophil "swarming" behaviors after skin wounding (*8*).

Model organism studies reveal some of the damage attractants and how inflammatory cells respond to these

At a fundamental cell biology level, we know that immune cells respond to attractant cues via Rho family small GTPase regulation of their actin cytoskeletons. If Rac is knocked down in *Drosophila* macrophages, they fail to make proper lamellae and efficiently migrate to the wound. If Rho is knocked down, then macrophage-directed migration is unperturbed, but the cells cannot contract and detach their uropod or tail end and thus remain tethered to the spot (*9*). Spatial activation of these small GTPase switches thus enables cells to migrate towards cues as, for example, highlighted in zebrafish larval experiments in which light activation of a genetically encoded Rac is used to artificially turn a neutrophil in vivo (*10*). (Why would you want it to turn experimentally?) (The lower part of Fig 1B could be moved to after 1C. That panel also seems to have too much text so more description here would be helpful—or eliminate unnecessary labels/text.)

The damage attractants identified in these models include small molecules like H_2O_2 (*11*) but these are clearly not the only attractants. Indeed, mathematical modeling of macrophage response behaviors in a *Drosophila* pupal wound reveals the nature of the key attractant(s) that trigger these cells to turn towards the wound; these signals travel back through wound fluids/tissues much too slowly to be a small molecule such as ATP or H_2O_2 (*12*). (The preceding sentence is far too long/complex. Please recast.) Immune cell attractants must therefore involve other signals, which will likely include growth factors and chemokines, as well as complement components, and metabolites of polyunsaturated fatty acids. Naive immune cells can not respond to a wound attractant;

they first need to be primed by other cues that include previous engulfment of apoptotic corpses (*13*). Studies in fly and fish are beginning to reveal the mechanisms by which inflammatory cells detect and integrate multiple priming and guidance cues in space and time, en route to the damage site. The translation of these findings will be important in mammalian wound scenarios where the distances travelled are greater and more complex than for the fly and fish model systems.

Resolving the inflammatory response

After healing is complete, the resolution of inflammation is not simply a passive process. Although there is clear evidence in mammalian wounds that many neutrophils undergo apoptosis at the site of inflammation, there is also evidence for the reverse migration of zebrafish neutrophils from sites of inflammation. Tracking studies have suggested that neutrophils, which have already been exposed to one damage site (other description than "experienced a wound site"?) can remain responsive to secondary insults (*14*). (What does hyper-responsive mean here?) Still other studies have suggested that neutrophils play an active role in the resolution of inflammation by depleting the chemoattractants that initially drew them to the wound site (*15*). These findings along with our increasing understanding of pro-resolving molecules produced by macrophages and other cells at sites of inflammation offer new therapeutic angles for modulating the trafficking of inflammatory cells (*16*).

Reliance of tissue repair and regeneration on the wound inflammatory response

The role of the inflammatory response, and specifically the function of macrophages, is not clear-cut. Knockdown of macrophages in rabbits with anti-macrophage serum led to severely impaired healing (*17*), whereas no major impact was observed when neutrophils were depleted. Still other studies have suggested that repair can happen independently of any inflammatory response. For example, wound re-epithelialization is so rapid in adult zebrafish that it is almost complete before the inflammatory response is initiated. Similar observations have also been reported with injured mammalian embryos that lack a mature inflammatory response (1). Indeed, neonatal mice that are genetically missing innate immune cell lineages repair injured tissues faster and typically without any evidence of a scar (*1*). However, the situation is dramatically different in the adult mouse where innate immune cell lineages play critical roles during the repair/regeneration process. In parallel studies of injured liver (which can fully regenerate) and skin (which can repair but leaves behind a scar), macrophages play distinct roles depending on what stage of repair they are depleted. In the liver, macrophage depletion during early repair reduces scar formation; however, if these cells are depleted after the extra cellular matrix (ECM) (define ECM) is laid down, then the normal resolution of fibrosis is impaired (*18*).In the skin, early macrophage depletion following injury prevents normal granulation tissue formation and re-epithelialization, whereas mid-stage depletion results in reduced normal vessel development leading to haemorraging; late-stage depletion of macrophages alters the pattern of scar formation (*19*). (Revise preceding sentence for clarity.)

Regenerative inflammation: an evolutionarily conserved mechanism

In lower vertebrates that regenerate whole appendages, such as fins and limbs, and even regions of damaged CNS, most studies suggest that inflammation is necessary. For example, if macrophages are depleted during the period of salamander limb blastema formation, regeneration fails and the amputated limb simply heals over (*20*). Tail fin regeneration is similarly blocked in adult zebrafish if macrophages are genetically ablated during the period of blastema proliferation. Once blastema formation is complete, however, macrophages are no longer critical (*21*).

Whereas neuroinflammation in the damaged mammalian brain is generally considered to be a negative influence (what is meant by viewed negatively?) because it triggers glial scarring that hinders axon rewiring, the situation in zebrafish is completely different. Indeed, the adult zebrafish brain and heart can completely regenerate following injury but in both cases the repair is dependent on inflammation (*22, 23*). Macrophages also influence the repair of vascular structures in the brain following injury. For example, live imaging of zebrafish brains showed that macrophages facilitate the ligation of injured vessels by positioning themselves between the two severed ends (*24*). The capacity to regenerate damaged cardiac tissue is also seen in mice but only until five days post birth. In this neonatal period, macrophage influx is again essential for repair (*25*). The difference between this neonatal period where regeneration is scar-free (use term other than perfect) and afterwards when hearts heal with a scar is the source and phenotype of

the inflammatory macrophage. In the neonate, they are an expanded population of embryonic-derived macrophages, whereas, in the damaged adult heart they are supplemented by pro-inflammatory monocyte-derived macrophages (*26*). Interestingly, inhibition of the monocyte-derived source of macrophages enhances adult cardiac repair (*26*), as does increasing the numbers of IL-4/IL-13-activated macrophages during post-infarct repair (*27*), thereby supporting the view that IL-4/IL-13-mediated polarization of macrophages is the more pro-regenerative phenotypic state. Nevertheless, emerging data has shown that macrophage activation is complex and influenced by ontogeny, local environmental factors and epigenetic changes that enable profound transcriptional reprogramming that influence their functional plasticity (*28*).

Dysregulated tissue repair and inflammation promote pathological fibrosis

Although the wound inflammatory response drives many aspects of tissue repair and regeneration, it can become dysregulated or chronic, and this may lead to the development of pathological fibrosis or scarring that can disrupt normal tissue architecture and function (*29*). As discussed above it is becoming increasingly apparent that monocytes and macrophages may play distinct roles in tissue repair with recruited monocytes often contributing to collateral tissue injury (*30*), while the resident tissue macrophage population appears to exert more beneficial properties by exhibiting pro-resolving, anti-inflammatory, and pro-regenerative activities (*31*). Nevertheless, there is substantial overlap between both populations, as some studies have shown that inflammatory monocytes can quickly adopt to a homeostatic mode. In some injured

tissues – the most extreme example being toxic liver injury - the recruited monocytes may supplement or permanently replace the resident macrophage population (*32*). Because the transformation of pro-inflammatory monocytes and macrophages to the pro-resolving phenotype is believed to be critical for normal wound repair and reduction of fibrosis, recent studies have focused on elucidating the mechanisms that license these distinct activation states (*33*).

Core immunological pathways of repair and fibrosis in mammals

Although numerous mechanisms can contribute to the development of fibrosis, two key immunological drivers of tissue fibrogenesis are TGF-β and the type-2 cytokines IL-4 and IL-13 (*34*). TGF-β1 has long been identified as a critical mediator of tissue repair and fibrosis following type 1- and type 17- driven inflammatory responses, which are characterized by the production of the pro-inflammatory cytokines IFN-γ and IL-17A, respectively. Moreover, several studies have concluded that the IL-17A/IL-1β/TGF-β1 axis is important to the development of fibrosis (*35*, *36*). In addition, TGF-β1 promotes tissue repair and fibrosis via two distinct mechanisms. It suppresses the production of pro-inflammatory mediators that can worsen tissue injury while simultaneously activating myofibroblasts that facilitate wound closure but also deposit aberrant levels and pattern of collagen at the site of repair (*37*). IL-13 exhibits similar and overlapping activity in that it functions as both a potent anti-inflammatory cytokine and driver of myofibroblast activation. In contrast to TGF-β1 however, IL-13 serves as the dominant mediator of tissue repair and fibrosis during a sustained type 2-polarized inflammatory response,

characterized by IL-4, IL-5, and IL-13. Consequently, dysregulated type-1/17 and type-2 inflammatory responses can each lead to the development of pathological fibrosis; however, distinct mechanisms dominate in each case (*38*) (**Fig. 2**). The mechanisms that initiate each response also appear to be distinct with IL-1 and IL-17A activating the TGF-β1 pathway and IL-25, IL-33, and TSLP serving as key initiators of type 2-dependent fibrosis (*36, 39*). Indeed, expansion of IL-13-producing type 2 innate lymphoid cells and $CD4^+$ Th2 cells by IL-25, IL-33, and TSLP has been shown to promote fibrosis in multiple tissues including the skin, lung, and liver (*40-43*). $CD11b^+$ macrophages also contribute to the development of IL-13-driven fibrosis by serving as key sources of inflammatory chemokines (*44*). Nevertheless, although macrophages are important for the initiation of type 2 fibrosis, studies dissecting the specific contribution of IL-4/IL-13 polarized macrophages (M2) concluded that the M2 subset primarily suppresses the development of fibrosis in the lung and liver (*45*). A more recent study of skin injury determined that M2 cells also influence collagen fibril assembly in fibroblasts by inducing lysyl hydroxylase-2 that directs collagen cross-linking and the stabilization of collagen (*46*). In contrast to liver, the dermal component of the skin has limited regenerative capacity in mammals, and healing leaves a scar. Hence, the studies in different organs corroborate the principal tissue protective role of type 2 immunity, but, the different outcome/quality of the healing-promoting activity will likely be tissue specific.

Fibrosis and tissue regeneration are distinctly regulated by type 2 immunity

IL-4 and IL-13 activated macrophages are important producers of a variety of growth factors including TGF-β1, insulin-like growth factor-1, VEGF-α, PDGF, and Relm (*31*). Therefore, they have long been implicated in tissue repair, regeneration, and fibrosis (*47*, *48*). In addition, other non-immune cells, including fibroblasts, epithelial cells, hepatocytes, and various stem and tissue progenitor cells have emerged as critical targets of IL-4 and IL-13 signaling following injury. For example, a recent study exploring the mechanisms of liver regeneration following injury showed that IL-4 receptor signaling in hepatocytes is required for proliferation following injury, with eosinophils delivering the IL-4 (*49*). Another study exploring the mechanisms of hepatic fibrosis following infection identified an important role for IL-4Rα expression in fibroblasts (*50*), with IL-13 rather than IL-4 identified as the critical regenerative mediator, and instead of promoting hepatocyte proliferation, IL-13 targeted bile duct epithelial cells (cholangiocytes) and hepatic progenitor cells directly, leading to increased numbers of bile ducts (*50*). A similar pro-regenerative role for IL-4 has also been reported in a model of skeletal muscle injury where, muscle damage triggered the recruitment of IL-4-producing eosinophils, which activated the regenerative actions of muscle resident fibro/adipocyte progenitor cells that support myogenesis (*51*).

Linking metabolism to immune cell repair functions

Why some immune pathways exhibit tissue protective activity and others play pathogenic roles currently remains unclear. Recent discoveries in the field of immunometabolism have generated considerable excitement because they suggest immune cell metabolic

signatures may impact cell plasticity and function and thus, might provide new mechanistic insights into how the immune system regulates repair (*52*).

As outlined above, macrophages and other cells of the innate and adaptive immune response contribute to tissue repair. However, the various mechanisms that modulate macrophage functional complexity during the different stages of healing are not fully understood. Local microenvironmental conditions found at sites of injury may be inhospitable to effective repair due to a combination of infection, hypoxia, and accumulating metabolites (Fig. 3). Although macrophages adapt rapidly to these demanding conditions to combat infection and facilitate tissue repair, their exact bioenergetic needs in these scenarios are still poorly understood. However, these bioenergetic needs are characterized by the activation of multiple metabolic pathways including glycolysis, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway as well as fatty acid synthesis and oxidation (Fig. 3) (*53, 54*). Nevertheless, the impact of these pathways on tissue repair, regeneration, and fibrosis remain largely unknown.

Glycolysis mediates anti-microbial and inflammatory activity of type 1 immune responses

The transcription factor HIF-1a that is activated by hypoxia was identified as a key regulator that coordinates the transcriptional programs of glycolytic metabolism and pro-inflammatory cytokine production in type 1-activated macrophages (*55*). Recent work has unraveled the regulatory network linking HIF-1a, energetic metabolism, and

inflammation. Interestingly, succinate that accumulates as a consequence of a broken TCA cycle in inflammatory conditions, stabilizes HIF-1a, which sustains the pro-inflammatory phenotype (*56*). In addition, mitochondrial succinate oxidation by succinate dehydrogenase (SDH) maintains LPS-induced inflammation (*57*) . Along these lines, itaconate, an abundant metabolite of LPS-activated macrophages, dampens inflammation by inhibiting SDH (*58*). Collectively, these studies highlight succinate metabolism as a critical mechanism controlling the macrophage inflammatory state. Indeed, inactivation of HIF-1a in myeloid cells dramatically reduced expression of the glucose transporter Glut1 and initiation of the glycolytic cascade. Accordingly, production of lactate and ATP, in response to LPS was reduced in HIF-1a-deficient macrophages, resulting in compromised antimicrobial activity (*59*). These findings demonstrated that type-1-activated macrophages exploit glycolysis to fuel their short-lived antimicrobial and pro-inflammatory functions. In addition, IL-1β and VEGF synthesis, both factors integrally linked to the initiation of the wound healing response was also reduced in HIF-1a-deficient macrophages (*55*). Together, these studies revealed how a single transcription factor serves as a key regulatory node and critically controls anti-microbial activity, energy metabolism, inflammation and key tissue repair mechanisms such as angiogenesis. (Please check over the figure to ensure that all labels/features are called out. To simplify the figure, eliminate ones that are not discussed.)

Oxidative phosphorylation supports tissue protective type 2 immune responses

A recent study showed that type 2 cytokines serve as key activators of oxidative phosphorylation (OxPhos) in macrophages (*3*). OxPhos produces high-energy phosphates in mitochondria that are needed for energetically demanding cell replication, cell growth, and synthesis of macromolecules (Fig. 3). Microarray analysis and metabolic studies of type 1- and type 2-activated macrophages revealed that genes important for fatty acid oxidation (FAO) are preferentially expressed in IL-4- and IL-13-activated macrophages. Consistently, IL-4-stimulated macrophages showed a higher rate of FAO, mitochondrial mass and mitochondrial respiration. Inhibition of OxPhos dramatically attenuated the activation of the type 2-activated phenotype and resulted in increased expression of inflammatory markers. The type 2-activated phenotype could be rescued by transgenic expression of PPARγ coactivator 1β (PGC-1β), an important mediator of FAO and mitochondrial oxidative metabolism. Collectively, this study demonstrated that the metabolic shift from glycolysis towards OxPhos is paralleled by the transition from an inflammatory towards a pro-resolution M2-like phenotype, thus revealing new therapeutic options to modulate inflammation. An important unanswered question is whether under certain conditions oxidative metabolism might promote unsuccessful healing.

The in vitro studies above are now supported by *in vivo* studies showing that pharmaceutical inhibition of lysosomal lipolysis in murine macrophages results in a blockade of IL-4Rα-mediated macrophage activation (*60*). This study investigated the source of FA and discovered a previously unappreciated role for lysosomal acid lipase (LAL) in cell-intrinsic lipolysis in macrophages. LAL supported FAO, thus revealing an

integral role for this enzyme in the activation of the type 2 phenotype in macrophages. This study also showed that proper mitochondrial function is required for type-2-mediated release of Relm-a from macrophages, a factor involved in type 2-mediated skin fibrosis (*46*). Finally, a recent study also identified the Akt-mTORC1 axis as a key node calibrating the metabolic demands of type 2 cytokine activated macrophages (*61*).

Emerging concepts in pro-fibrotic mediator blockade

As type 2 immunity and TGF-β1 pathway activation both serve as core drivers of tissue repair and fibrogenesis, numerous therapeutic strategies that modulate specific components of each pathway are currently under development (*29, 62*). However, these pathways are also critically involved in normal tissue regeneration following injury (*29, 50*). IL-13 and TGF-β1 are also well known anti-inflammatory cytokines; therefore, therapeutics targeting these pathways may unleash rebound inflammatory responses that can impair healing, exacerbate tissue injury, and activate autoimmune disease (*63, 64*). Any successful anti-fibrotic strategy must mitigate these potential negative outcomes.

Whereas the clinical use of therapeutics that interfere with TGF-β signaling have been approved for treatment of a few fibrotic diseases, agents targeting specifically type 2 immunity have not yet been licensed. Instead, they are currently being investigated in the clinic for other repair-related indications, including atopic dermatitis (AD), idiopathic pulmonary fibrosis, and asthma. Phase 3 trials have demonstrated the efficacy and tolerance of an IL-4Rα-blocking antibody in AD (*65*), and these beneficial reports in AD

therapy may facilitate their investigation as anti-fibrotic therapies. Along these lines, a bi-specific antibody that neutralizes IL-4 and IL-13 has just been approved in a clinical trial for scleroderma (https://clinicaltrials.gov), a connective tissue disease for which no disease modifying treatment is currently approved.

(Please double check that all panels in Fig 3 are described or eliminate for simplicity. It might actually be nicer for the final layout to split Fig 3 into smaller portions to make a new Fig 4.)

Conclusions and Future Directions

It is still unclear how immune cells signal to stromal cells and what the cell and molecular changes are that allow responding cells to proliferate and cause scarring. There is growing evidence that monocyte/macrophage polarization dynamics are critical to instruct immune and non-immune tissue resident cells to both initiate and terminate healing responses. We next need to better understand the hierarchy of molecular determinants that orchestrate these divergent phenotypes that lead to different outcomes in the repair response.

Much has been learned in immune cell polarization and tissue regeneration from defined responses in experimental prototypic settings (e.g. helminth infection (*62*)), yet future studies need to clarify whether these mechanisms can be generalized. Current evidence suggests that the repair response triggered by core immune pathways is context dependent and is a function of a combination of multiple determinants including the

nature of the damage (e.g. pathogen induced, sterile injury or mixed injury pattern), the amplitude and duration of different polarization states as well as the intrinsic regenerative capacity of different organs and tissues.

In translational terms, the interaction between immune cells and stromal cells is an obvious therapeutic target for dampening the scar response. Tisue repair and fibrosis may also be influenced by directly modulating the inflammatory response and by manipulating known endogenous pro-fibrotic mediators such as TGF-β1, osteopontin, hedgehog signaling, and type 2 cytokines among others. In addition, augmenting the actions of pro-resolving molecules like IL-10 and resolvins at the wound site might be another way to encourage resolution after the essential functions of neutrophils and macrophages at the repair site have been accomplished (*11*). In vitro and in vivo screens are beginning to reveal small molecules/drugs that may trigger early resolution (*66*) and enable better controlled regeneration of the wound inflammatory response. Additionally, it may be possible to enhance tissue protective mechanisms that are upregulated at the wound site, including, for example, the enzymes downstream of nrf2 signalling that sequester ROS and shield repairing tissue from some of the negative consequences of sustained inflammation (*67*). All such therapeutic strategies must be engineered in a way that do not negatively impact pro-regenerative pathways.

Immune cell metabolism also represents a previously unexplored area for therapeutic intervention at sites of tissue repair. However, to date, little detailed information is known about how immune cell metabolism impacts repair mechanisms. In this respect, it is

intriguing to speculate that the distinct regenerative capacities of different tissues and organs may be explained in part by their unique metabolic demands. It will also be important to understand whether scarring or failed regeneration results from aberrant shifts in metabolic programming in immune or non-immune cells. Along these lines, a recent systems biology study found parallel metabolic and transcriptional pathways that control macrophage polarization (*68*), revealing potential pharmacological control points.

Finally, we still do not fully understand why scars are capable of resolving in some tissues such as liver but not in others like skin. Moreover, why organisms like zebrafish display excellent regenerative ability but mammals do not also currently remains unclear. Future studies should therefore address whether differences in macrophage origin (*28*), recruitment and activation may be an important contributing mechanism explaining this variation between different organs and species. Understanding precisely how these key inflammatory cells are influenced by the wound environment and themselves influence how stromal cells deposit matrix in the repairing tissue will undoubtedly guide us towards therapeutic strategies for managing and improving healing in the clinic.
(Please provide a brief final overview paragraph to conclude the piece.)

References

1. S. A. Eming, P. Martin, M. Tomic-Canic, Wound repair and regeneration: mechanisms, signaling, and translation. *Science translational medicine* **6**, 265sr266 (2014).
2. K. M. Vannella, T. A. Wynn, Mechanisms of Organ Injury and Repair by Macrophages. *Annu Rev Physiol*, (2016).
3. D. Vats *et al.*, Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab* **4**, 13-24 (2006).
4. E. Metchnikoff, Lectures on the comparative pathology of inflammation. . *Reprited by Dover Publications.*, (1968).
5. M. H. Kim *et al.*, Dynamics of neutrophil infiltration during cutaneous wound healing and infection using fluorescence imaging. *The Journal of investigative dermatology* **128**, 1812-1820 (2008).
6. J. Wang, P. Kubes, A Reservoir of Mature Cavity Macrophages that Can Rapidly Invade Visceral Organs to Affect Tissue Repair. *Cell* **165**, 668-678 (2016).
7. S. Nourshargh, R. Alon, Leukocyte migration into inflamed tissues. *Immunity* **41**, 694-707 (2014).
8. T. Lammermann *et al.*, Neutrophil swarms require LTB4 and integrins at sites of cell death in vivo. *Nature* **498**, 371-375 (2013).
9. W. Wood, P. Martin, Macrophage Functions in Tissue Patterning and Disease: New Insights from the Fly. *Dev Cell* **40**, 221-233 (2017).
10. S. K. Yoo *et al.*, Differential regulation of protrusion and polarity by PI3K during neutrophil motility in live zebrafish. *Developmental cell* **18**, 226-236 (2010).
11. 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**, 996-999 (2009).
12. H. Weavers *et al.*, Systems Analysis of the Dynamic Inflammatory Response to Tissue Damage Reveals Spatiotemporal Properties of the Wound Attractant Gradient. *Current biology : CB* **26**, 1975-1989 (2016).
13. H. Weavers, I. R. Evans, P. Martin, W. Wood, Corpse Engulfment Generates a Molecular Memory that Primes the Macrophage Inflammatory Response. *Cell* **165**, 1658-1671 (2016).
14. F. Ellett, P. M. Elks, A. L. Robertson, N. V. Ogryzko, S. A. Renshaw, Defining the phenotype of neutrophils following reverse migration in zebrafish. *Journal of leukocyte biology* **98**, 975-981 (2015).
15. L. Pase *et al.*, Neutrophil-delivered myeloperoxidase dampens the hydrogen peroxide burst after tissue wounding in zebrafish. *Current biology : CB* **22**, 1818-1824 (2012).
16. C. N. Serhan, N. Chiang, J. Dalli, The resolution code of acute inflammation: Novel pro-resolving lipid mediators in resolution. *Seminars in immunology* **27**, 200-215 (2015).

17. S. J. Leibovich, R. Ross, The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum. *Am J Pathol* **78**, 71-100 (1975).
18. J. S. Duffield *et al.*, Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. *The Journal of clinical investigation* **115**, 56-65 (2005).
19. T. Lucas *et al.*, Differential roles of macrophages in diverse phases of skin repair. *Journal of immunology* **184**, 3964-3977 (2010).
20. J. W. Godwin, A. R. Pinto, N. A. Rosenthal, Macrophages are required for adult salamander limb regeneration. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 9415-9420 (2013).
21. T. A. Petrie, N. S. Strand, C. T. Yang, J. S. Rabinowitz, R. T. Moon, Macrophages modulate adult zebrafish tail fin regeneration. *Development* **141**, 2581-2591 (2014).
22. A. S. de Preux Charles, T. Bise, F. Baier, J. Marro, A. Jazwinska, Distinct effects of inflammation on preconditioning and regeneration of the adult zebrafish heart. *Open Biol* **6**, (2016).
23. N. Kyritsis, C. Kizil, M. Brand, Neuroinflammation and central nervous system regeneration in vertebrates. *Trends Cell Biol* **24**, 128-135 (2014).
24. C. Liu *et al.*, Macrophages Mediate the Repair of Brain Vascular Rupture through Direct Physical Adhesion and Mechanical Traction. *Immunity* **44**, 1162-1176 (2016).
25. A. B. Aurora *et al.*, Macrophages are required for neonatal heart regeneration. *The Journal of clinical investigation* **124**, 1382-1392 (2014).
26. K. J. Lavine *et al.*, Distinct macrophage lineages contribute to disparate patterns of cardiac recovery and remodeling in the neonatal and adult heart. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 16029-16034 (2014).
27. M. Shiraishi *et al.*, Alternatively activated macrophages determine repair of the infarcted adult murine heart. *The Journal of clinical investigation* **126**, 2151-2166 (2016).
28. E. Gomez Perdiguero *et al.*, Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* **518**, 547-551 (2015).
29. T. A. Wynn, T. R. Ramalingam, Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med* **18**, 1028-1040 (2012).
30. M. T. Heneka *et al.*, NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nature* **493**, 674-678 (2013).
31. T. A. Wynn, K. M. Vannella, Macrophages in Tissue Repair, Regeneration, and Fibrosis. *Immunity* **44**, 450-462 (2016).
32. L. van de Laar *et al.*, Yolk Sac Macrophages, Fetal Liver, and Adult Monocytes Can Colonize an Empty Niche and Develop into Functional Tissue-Resident Macrophages. *Immunity* **44**, 755-768 (2016).
33. P. J. Murray, Macrophage Polarization. *Annu Rev Physiol*, (2016).
34. L. Barron, T. A. Wynn, Fibrosis is regulated by Th2 and Th17 responses and by dynamic interactions between fibroblasts and macrophages. *Am J Physiol Gastrointest Liver Physiol* **300**, G723-728 (2011).

35. T. Fabre, H. Kared, S. L. Friedman, N. H. Shoukry, IL-17A enhances the expression of profibrotic genes through upregulation of the TGF-beta receptor on hepatic stellate cells in a JNK-dependent manner. *J Immunol* **193**, 3925-3933 (2014).
36. M. S. Wilson *et al.*, Bleomycin and IL-1beta-mediated pulmonary fibrosis is IL-17A dependent. *J Exp Med* **207**, 535-552 (2010).
37. M. W. Ferguson, S. O'Kane, Scar-free healing: from embryonic mechanisms to adult therapeutic intervention. *Philos Trans R Soc Lond B Biol Sci* **359**, 839-850 (2004).
38. M. Kaviratne *et al.*, IL-13 activates a mechanism of tissue fibrosis that is completely TGF-beta independent. *J Immunol* **173**, 4020-4029 (2004).
39. H. Hammad, B. N. Lambrecht, Barrier Epithelial Cells and the Control of Type 2 Immunity. *Immunity* **43**, 29-40 (2015).
40. K. M. Vannella *et al.*, Combinatorial targeting of TSLP, IL-25, and IL-33 in type 2 cytokine-driven inflammation and fibrosis. *Sci Transl Med* **8**, 337ra365 (2016).
41. T. McHedlidze *et al.*, Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis. *Immunity* **39**, 357-371 (2013).
42. E. Hams *et al.*, IL-25 and type 2 innate lymphoid cells induce pulmonary fibrosis. *Proc Natl Acad Sci U S A* **111**, 367-372 (2014).
43. H. K. Jessup *et al.*, Intradermal administration of thymic stromal lymphopoietin induces a T cell- and eosinophil-dependent systemic Th2 inflammatory response. *J Immunol* **181**, 4311-4319 (2008).
44. L. A. Borthwick *et al.*, Macrophages are critical to the maintenance of IL-13-dependent lung inflammation and fibrosis. *Mucosal Immunol* **9**, 38-55 (2016).
45. K. M. Vannella *et al.*, Incomplete deletion of IL-4Ralpha by LysM(Cre) reveals distinct subsets of M2 macrophages controlling inflammation and fibrosis in chronic schistosomiasis. *PLoS Pathog* **10**, e1004372 (2014).
46. J. A. Knipper *et al.*, Interleukin-4 Receptor alpha Signaling in Myeloid Cells Controls Collagen Fibril Assembly in Skin Repair. *Immunity* **43**, 803-816 (2015).
47. P. J. Murray, T. A. Wynn, Protective and pathogenic functions of macrophage subsets. *Nat Rev Immunol* **11**, 723-737 (2011).
48. R. Mori, T. J. Shaw, P. Martin, Molecular mechanisms linking wound inflammation and fibrosis: knockdown of osteopontin leads to rapid repair and reduced scarring. *J Exp Med* **205**, 43-51 (2008).
49. Y. P. Goh *et al.*, Eosinophils secrete IL-4 to facilitate liver regeneration. *Proc Natl Acad Sci U S A* **110**, 9914-9919 (2013).
50. R. L. Gieseck, 3rd *et al.*, Interleukin-13 Activates Distinct Cellular Pathways Leading to Ductular Reaction, Steatosis, and Fibrosis. *Immunity* **45**, 145-158 (2016).
51. J. E. Heredia *et al.*, Type 2 innate signals stimulate fibro/adipogenic progenitors to facilitate muscle regeneration. *Cell* **153**, 376-388 (2013).
52. K. Ganeshan, A. Chawla, Metabolic regulation of immune responses. *Annu Rev Immunol* **32**, 609-634 (2014).

53. L. A. O'Neill, E. J. Pearce, Immunometabolism governs dendritic cell and macrophage function. *J Exp Med* **213**, 15-23 (2016).
54. L. A. O'Neill, R. J. Kishton, J. Rathmell, A guide to immunometabolism for immunologists. *Nat Rev Immunol* **16**, 553-565 (2016).
55. T. Cramer *et al.*, HIF-1alpha is essential for myeloid cell-mediated inflammation. *Cell* **112**, 645-657 (2003).
56. G. M. Tannahill *et al.*, Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. *Nature* **496**, 238-242 (2013).
57. E. L. Mills *et al.*, Succinate Dehydrogenase Supports Metabolic Repurposing of Mitochondria to Drive Inflammatory Macrophages. *Cell* **167**, 457-470 e413 (2016).
58. V. Lampropoulou *et al.*, Itaconate Links Inhibition of Succinate Dehydrogenase with Macrophage Metabolic Remodeling and Regulation of Inflammation. *Cell Metab* **24**, 158-166 (2016).
59. S. C. Cheng *et al.*, mTOR- and HIF-1alpha-mediated aerobic glycolysis as metabolic basis for trained immunity. *Science* **345**, 1250684 (2014).
60. S. C. Huang *et al.*, Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. *Nat Immunol* **15**, 846-855 (2014).
61. A. J. Covarrubias *et al.*, Akt-mTORC1 signaling regulates Acly to integrate metabolic input to control of macrophage activation. *Elife* **5**, (2016).
62. T. A. Wynn, Type 2 cytokines: mechanisms and therapeutic strategies. *Nat Rev Immunol* **15**, 271-282 (2015).
63. D. F. Choy *et al.*, TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. *Sci Transl Med* **7**, 301ra129 (2015).
64. T. R. Ramalingam *et al.*, Enhanced protection from fibrosis and inflammation in the combined absence of IL-13 and IFN-gamma. *J Pathol* **239**, 344-354 (2016).
65. E. L. Simpson *et al.*, Two Phase 3 Trials of Dupilumab versus Placebo in Atopic Dermatitis. *N Engl J Med* **375**, 2335-2348 (2016).
66. A. L. Robertson *et al.*, A zebrafish compound screen reveals modulation of neutrophil reverse migration as an anti-inflammatory mechanism. *Sci Transl Med* **6**, 225ra229 (2014).
67. M. Telorack *et al.*, A Glutathione-Nrf2-Thioredoxin Cross-Talk Ensures Keratinocyte Survival and Efficient Wound Repair. *PLoS Genet* **12**, e1005800 (2016).
68. A. K. Jha *et al.*, Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* **42**, 419-430 (2015).

Figure legends:

Figure 1. Live imaging of the wound inflammatory response. A. Metchnikoff's discovery of damage triggered "inflammation" in starfish embryo (*4*). High magnification view (right) after wounding to illustrate recruitment of "wandering" cells. B. Timelapse series after laser wounding the *Drosophila* pupal wing epithelium to reveal macrophage (green with red nuclei) recruitment to the wound (courtesy of Helen Weavers, University of Bristol); schematic to illustrate those aspects of the wound inflammatory response that can be analyzed by dynamic imaging studies in *Drosophila* and zebrafish. C. Neutrophil diapedezing through the outer pericyte layer of a mouse vessel (courtesy of Tamara Girbl and Sussan Nourshargh, QMUL). Migratory tracks in B and C in blue.

Figure 2. Distinct mechanisms contribute to pathological tissue remodeling during highly polarized type 1 and type 2 responses. Sustained type 1 responses (IFN-γ and IL-17A) lead to substantial tissue damage. The injury in turn activates TGF-β, which suppresses the inflammatory response while activating ECM production by myofibroblasts that contribute to fibrosis. During a polarized type 2 response, IL-13 serves as an important driver of fibrosis, with the IL-13 decoy receptor (IL-13Rα2) and IL-10 exhibiting negative regulatory activity. Effective tissue regeneration is typically associated with less polarized immune responses.

Figure 3.: Integrated perspective of potential activation phenotypes and metabolic pathways in wound macrophages. Hif-1α activation and glycolysis are hallmarks of M1 activation. In contrast, mitochondrial biogenesis, FA degradation, an intact TCA cycle and oxidative phosphorylation lead to efficient production of large amounts of ATP that sustain the secretory and resolution phenotype of M2. How dynamics of macrophage polarization phenotypes (marker genes are depicted in triangles) and cellular metabolic pathways instruct the sequence of specific repair mechanisms at a tissue level remains open. Abbreviations used: electron (e^-); pentose phosphate pathway (PPP); pathogen recognition receptor (PRR); pathogen-associated molecular pattern (PAMP); damage-associated molecular pattern (DAMP); tricarboxylic acid (TCA); succinate dehydrogenase (SDH); triacylglycerol (TAG); free fatty acids (FFA), low-density lipoprotein (LDL); very-low-density lipoprotein (VLDL).