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Chasing the recipe for a pro-regenerative immune system

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Abstract

Identification of the key ingredients and essential processes required to achieve perfect tissue regeneration in humans has so far remained elusive. Injury in vertebrates induces an obligatory wound response that will precede or overlap any regeneration specific program or scarring outcome. This process shapes the cellular and molecular landscape of the tissue, influencing the success of endogenous repair pathways or for potential clinical intervention. The involvement of immune cells is also required for aspects of development extending beyond the initial inflammatory phase of wounding. It has now become clear from amphibian, fish and mammalian models of tissue injury that the type of immune response and the profile of immune cells attending the site of injury can act as the gatekeepers that determine wound repair quality. The heterogeneity among innate and adaptive immune cell populations, along with the developmental origin of these cells, form key ingredients affecting the potential for downstream repair and the suppression of fibrosis. Cell-to-cell interactions between immune cells, such as macrophages and T cells, with stem cells and mesenchymal cells are critically important for shaping this process and these exchanges, are in turn influenced by the type of injury, tissue location and developmental stage of the organism. Developmentally, mouse cardiac regeneration is restricted to early stages of postnatal life where the balance of innate to adaptive immune cells may be poised towards regeneration. In the injured adult mouse liver, specific macrophage subsets improve repair while other bone marrow derived cells can exacerbate injury. Other studies using genetically diverse mice have shown enhanced regeneration in certain strains, restricted to specific tissues. This enhanced repair is linked with expression of genes such as Insulin-like Growth Factor- 1 (IGF-1) and activin (Act 1), that both play important roles in shaping the immune system. Immune cells are now appreciated to have powerful influences on critical cell types required for regeneration success. The winning recipe for tissue regeneration is likely to be found ultimately by identifying the genetic elements and specific cell populations that limit or allow intrinsic potential. This will be essential for developing therapeutic strategies for tissue regeneration in humans.

Keywords

Innate immunity; regeneration; wound healing; inflammation; fibrosis

1. Regenerative medicine and cellular immunity are inextricably linked

The goal of regenerative medicine is to restore tissues and whole organ systems back to a normal or highly functional state. Whereas traditional approaches have used biomaterials and stem cells either alone or in combination, many recent therapies are now focusing on immunomodulation [1]. The discovery of endogenous resident progenitor cell populations in wide range of mammalian tissues is likely to focus future interventions on encouraging these cells to participate in adult regeneration [2-4]. In any therapeutic intervention, there are important immune related considerations to be taken into account, as the inflammatory landscape and profile of immune cells can directly or indirectly determine the potential for progenitor cell expansion, survival and integration. It is clear that delivering regenerative therapies will rely on mastering the complex inflammatory network of cells regulating tissue and wound homeostasis that “prepare the ground” for subsequent tissue repair. (Reviewed previously in [5]).

Historically, the benefit and harm of the inflammatory response associated with tissue injury has been debated since Cornelius Celsus first coined the term “inflammation” in the 1st century AD, from the Latin *inflammare* (to set on fire). Two centuries later, Galen developed a humoral model of inflammation where invading blood cells and pus were viewed as beneficial [6]. In 1871, Virchow challenged the benefit of inflammation and promoted the idea that inflammation and the associated immune cell recruitment was inherently pathological [7]. The *Age of Enlightenment* and beyond saw huge advances in microscopy and a growing awareness that immune responses following injury involve a complex and overlapping sequence of cellular events involving both cells recruited from blood and local sources [8]. Current thinking has landed on the notion that although the immune response to injury is biased in protecting the host from infection, the inflammatory process is far from perfect in balancing the needs of the host for efficient restoration of tissue architecture. In addition, some aspects of inflammation in humans may be geared to prioritize healing that reduces pathogen risk, rather than promoting scar-free repair, and restoration of function. In order for regenerative medicine to improve wound-healing quality and extend the range and of tissues that can be repaired, we need to understand which components can act as an impediment within a clinical setting, and which can be harnessed to act to actively promote regeneration.

2. The immune system: Balancing host defense with repair quality

A primary function of the immune system is host defense and the war within the body against foreign invaders can be lost in minutes. Without a functional immune system, bacteria in the gastrointestinal and respiratory system will breach the mucosal barrier, gaining access to the blood and other tissues, leading to fatal outcomes [9]. When tissues sustain a traumatic injury with exposure to environmental microorganisms, the risk of pathogen invasion is equally perilous. Selective pressure on warm-blooded vertebrates has evolved a wound closure process that prevents the access of potential pathogens to the systemic circulation by quarantining microorganisms within the injured tissue. This program includes the robust recruitment of a range of specialized cells to disinfect the local wound site by both sensing and destroying pathogens, and also managing the wound healing

response to restore function. Given the importance of host defense as a selective pressure, it is rational to speculate that this is the single most important driver of selective pressure in wound repair.

3. Cellular response to injury and cross-regulating cell-to-cell networks

Wounding causes a complex cascade of extracellular inflammatory signaling that recruits local and blood derived leukocytes to the injured tissue (reviewed in [10-12]). Innate immune cells play an early and dominant role in directing wound repair along with providing a potent source of cytokines and lipid mediators that shape the inflammatory microenvironment. Local endothelial or mesenchymal cells such as fibroblasts amplify the inflammatory signaling cascade, but in turn, are regulated by both innate and adaptive immune cells [13].

Wound repair in mammals results in the formation of an extensive acellular extracellular matrix (ECM), often termed a scar, which is very different to the original tissue (reviewed in [14]). Mesenchymal cell types such as fibroblasts and myofibroblasts largely control the production of ECM components and granulation tissue. Although these cells play an important role in stabilizing the wound bed and sealing the tissue from the external environment, their prolific ECM synthesis presents a significant clinical problem as fibrosis and scar production have profound effects on tissue function and integrity. The myofibroblast is particularly problematic in many tissue-healing contexts and is the major focus of many clinical efforts to reduce scarring outcomes and pathologies [15-17]. Many cell types have been implicated in the production of myofibroblast-like cells, including platelets, pericytes, endothelial cells, epithelial, smooth muscle and fibrocyte populations [18,19]. The production of myofibroblasts is a process very sensitive to a wide range of innate immune cells and their influences on the local inflammatory microenvironment. (See Fig. 1.)

Interestingly, and of critical clinical importance, mesenchymal stem cells (MSCs) isolated from either bone marrow, amniotic fluid or placenta, exhibit profound anti-inflammatory and immunoregulatory effects on a range of innate and adaptive immune cells. (See Fig.1.) MSC do not constitutively exert their immune modulating properties but are primed by inflammatory mediators released from immune cells and inflamed tissue [20]. Fibroblasts and mesenchymal/stromal stem cells appear phenotypically indistinguishable using many immunophenotyping panels [21]. The paucity of specific fibroblast markers makes it difficult to appreciate how similar these cells really are. Sophisticated gene expression analysis can detect molecular differences, however these are lost upon culturing *in vitro* [22,23]. MSCs primarily have immunosuppressive roles while fibroblasts can show both pro- and anti-inflammatory roles [24-26]. As MSCs, fibroblasts and myofibroblasts may represent different states of differentiation, other immune cell inputs may play the deciding role in determining the maturation of MSCs and fibrotic potential.

4. The cellular origins of innate immune cells can confer functional diversity

Numerous studies attempting to assign positive or negative roles for immune cells in repair and regeneration have been confounded by the lack of tools or experimental design addressing phenotypically diverse roles of individual subpopulations. Although this may be a general problem for assessing various immune cell types, the role of macrophages in scar formation serves as a useful illustrative example of how both the specific wound environment and developmental history of macrophages may explain some of the conflicting roles for “macrophages” within the alternative wound models and tissues described in the literature [27].

Different tissues may exert contrasting influences on macrophage function by providing wound-specific cues in the form of inflammatory factors and tissue ECM. The specific microenvironment within the wound site can directly influence their differentiation into specific phenotypic states that can either enhance or limit fibrosis. The observation that phagocytosis of debris in itself can have significant effects on macrophage phenotype and function adds weight to this notion [28,29]. There is now a great deal of supporting evidence *in vivo* and *in vitro* for this idea but the potential for phenotypically restricted monocyte precursors is still debated [30,31].

In addition to the influences of the wound environment, an added complexity has emerged, whereby the embryonic origin of the macrophage may impose limitations on macrophage function and intrinsic differences in potential [27,32-35]. It is now appreciated that macrophages within a wound site have several distinct origins, serving as an illustrative example of what may be true for several other immune cell types.

Injury associated macrophages are not a homogeneous population, but are in fact a grouping of cells with similar functions and phenotypes. Tissue resident macrophages are self-renewing cells that provide homeostatic functions and can act as early responders to tissue injury or infection. After a tissue insult, bone marrow (BM) derived monocytes are recruited from the blood and differentiate into macrophages within the wound. These macrophages appear functionally similar to tissue resident macrophages but are educated differently from the long-lived tissue-resident macrophages originally seeded from the fetal liver. It is still unclear to which extent resident or recruited cells mediate the distinct functions of macrophage subpopulations. It is also unclear the capacity of these cells to undergo reversible phenotypic switching *in situ* or influence the phenotype of each other.

The developmental origins of diverse immune cell populations are mediated by the spatiotemporal development of the hematopoietic system. Adult hematopoiesis primarily occurs in the bone marrow (BM), however the site of immune cell development can occur at different sites, depending on developmental stage and cell type. This is exemplified by tissue macrophages. Microglia (resident macrophages of the brain) arise from yolk sac (YS) macrophages at 7.5 days post coitum (dpc), while most other tissues are populated by YS-derived erythro-myeloid progenitors that are not HSCs around 8.5 dpc [36,37]. Macrophage progenitors most likely transit through the fetal liver but may also migrate directly to some

target tissues [36,37]. Moreover, tissue macrophage populations resident within tissues may represent cells from diverse ontogenic backgrounds that change with age [33,38,39].

While tissue macrophages may have complex origins, other immune cell types are presently thought to develop from hematopoietic stem cells (HSCs) that contribute to all hematopoietic lineages. In mice, the first wave of hematopoiesis is observed in the yolk sac and the aorta—gonad—mesonephros (AGM) between E7.5 and E10.5 (reviewed in [40]). Activity is also detected in placenta, then in fetal liver, then spleen at E15 [41]. HSC activity is subsequently detected in BM from 16.5 dpc and maintained throughout adult life. A simplified current model of mouse immune cell ontogeny and its implications for wound macrophage heterogeneity is shown in Fig. 2.

Chemokines and adhesion molecules regulate the migration and relocation of HSC between hematopoietic organs during ontogeny, and play a pivotal role in directing the trafficking of adult immune cells [42]. Chemokine and adhesion molecule guidance systems (eg, CXCR4 and CXCL12) are used to guide cell migration during embryogenesis, immune cell trafficking and cancer metastasis [43,44]. These systems are evolutionarily conserved among vertebrates and can direct the migration of a range of cells including HSCs, leukocytes, endothelial cell progenitors, germ cells, neurons, cortical interneurons, lateral line primordium, cardiomyocytes, cancer cells, and a range of other cell types [45]. The expansion in adaptive immune cell complexity in higher vertebrates may have created a conflict between the migration required by the redevelopment phase in regeneration and adult immune cell trafficking. This could in part explain the inverse correlation between advanced adaptive immunity and regenerative capacity if adaptive immune cells are advantaged in their response to chemokine signals.

5. Regeneration in mammals is restricted to early developmental stages

As adults, humans show very limited regenerative capacity. Although some tissues can undergo physiological replacement of cells during an animal's life, only the liver has the ability to recover from major injury and restore normal architecture and function. Skeletal muscle can regenerate following significant tissue insults provided the basal lamina remains intact and does not undergo volumetric loss [46]. Other adult tissues are limited in their repair potential and in many cases undergo wound healing that results in scar formation and abnormal tissue reconstruction. Mouse and human embryos are capable of scar-free repair and regeneration but lose this potential shortly after birth with the exception of the oral mucosa [47-50].

Mouse models of injury have been very informative in the search for mammalian stages and tissues capable of regeneration. Studies interrogating the potential for scar-free repair in mouse hearts have revealed a temporal regenerative window that is lost somewhere between the 1st and 7th day of life [51]. During this period of development the mouse immune system undergoes significant changes in both innate and adaptive immunity [52]. Although the full spectrum of immune cell engagement has yet to be elucidated, macrophages are essential for regeneration in this model, promoting angiogenesis and cardiomyocyte proliferation [53]. In the adult heart, embryo-derived cardiac macrophages are progressively

replaced by bone marrow derived macrophages with aging [39], which may also limit the potential for repair [54].

The role of macrophages in regeneration is also important in other tissues such as liver. Administration of CSF-1 matured macrophages, but not precursor cells, into a CCl₄-induced model of liver injury model resulted in chemokine up-regulation, recruitment of endogenous macrophages, reduced inflammation, decreases in hepatic myofibroblasts and several clinical improvements including reduced scar formation [55], whereas unfractionated bone marrow exacerbated fibrosis. Human macrophages have also shown cross-species activity in this model [56]. Additional reports indicated that the timing of administration was important, as endogenous macrophages aided the resolution of fibrosis but infiltration by inflammatory monocyte/macrophages enhanced the severity of scar formation [57]. Collectively these studies provide valuable insights into the potential therapeutic possibilities for immune cells. Further work will be necessary to clarify the role of individual subpopulations and unmask some of the processes that lead to wound fate decisions.

6. Regenerating vertebrates reveal essential ingredients

Amphibians and fish demonstrate repair pathways, not yet understood, that can inform regenerative medicine of the required ingredients and deliver new strategies not previously considered. The Italian priest, teacher and scientist, Lazzaro Spallanzani, first documented salamander limb regeneration in 1768 [58]. Since these early observations, the salamander has provided examples of regeneration in many other clinically relevant tissues including the heart, brain, spinal cord and ocular tissues (reviewed in [59-61]). The salamander's ability to avoid scarring and execute regenerative programs repeatedly throughout life continues to inspire the field of regenerative medicine, in hopes that that human tissues may one day be able to replicate these amazing feats.

Amphibian and fish immunity is linked with their regenerative potential (reviewed in [62,63]). In recent years, several studies have provided clues to what components of the immune system may be compatible or instructive for regeneration.

The process of limb regeneration in salamanders is dependent on macrophages that form an essential part of the innate immune system [64]. In the absence of phagocytic macrophages, fibrogenic pathways are activated during the wound-healing phase. Excess collagen production during this period is likely to derail tissue regeneration by disrupting critical signaling required for the activation of downstream developmental gene networks [65]. This is intriguing considering that salamanders appear highly resistant to pro-fibrotic drugs such as bleomycin [66], bringing up the possibility that macrophages play a dominant role in actively preventing fibrosis in this system.

The functional role of macrophages appears to vary at different stages of regeneration. In zebrafish, specific ablation of the *Mpeg*⁺ subset of macrophages during fin regeneration alters the inflammatory landscape, tail fin growth and key development genes that control patterning [67]. In the phase leading up to blastema formation, *Mpeg*⁺ macrophages are required to maintain the rate of proliferation. During the tissue outgrowth stage,

macrophages play a critical role in regulating patterning during regeneration. Using genetic ablation of *LysM⁺* myeloid cells, different roles for macrophages at distinct time periods during mouse wound healing have also been described [68]. These studies suggest that the timing of immune cell recruitment an important ingredient in regeneration. A role for macrophages in directing aspects of development has also been implicated by a number of studies in mouse [69-71].

Myeloid cells also regulate certain aspects of salamander lens regeneration within the eye. Salamander lens regeneration is a unique model where removal of the lens triggers de novo lens generation remotely and specifically in the dorsal iris (not ventral iris). In this process pigmented epithelial cells (PECs) lose their pigment granules in association with macrophages and trans-differentiate into new lens tissue [58,72]. The new lens tissue is then remodeled and connected back into a normal position tethered to the ciliary muscle. This process is particularly fascinating and differs from most other regeneration models, as the site of new lens formation is not directly injured prior to the initiation of regeneration [73]. What directly links lens removal with de novo lens formation from the dorsal iris, is still unclear. Recent proteomic analysis in this model implicates myeloid cells involvement in the initiation of regeneration. After lens removal, myeloid specific gene products such as myeloperoxidase (MPO) were specifically localized to the future site of regeneration on the dorsal iris, and not the ventral iris. This indicates that the myeloid response is specifically targeted to the site of regeneration in the absence of damaged PECs.

In a related model in which the lens is not removed but damaged by puncture, myeloid cells have also been implicated in triggering salamander lens regeneration [74]. Lens puncture induces an innate immune response targeted to the damaged lens. In these experiments, myeloid cells from injured animals were transplanted into naïve animals where they initiated ectopic lens regeneration from the dorsal iris resulting in two lenses. This induced regeneration was dependent on the spleen and could possibly rely on mobilizing other immune cells after antigen presentation (discussed in [75]). So far, lens regeneration appears to be the only reported example of its kind where regeneration of a tissue structure is initiated in an anatomically distinct location from the initial injury.

Salamanders and fish lack some of the more advanced functions of the adaptive immune system found in mammals and adult frogs (reviewed in [62]). However, immunosuppressive drugs such as Cyclosporin can inhibit limb regeneration in salamanders and this can be rescued upon treatment with Interleukin 2 (IL-2) [76]. IL-2 is a key cytokine regulating T lymphocyte differentiation into T regulatory cells or T effector cells. Although salamanders show poor T effector cell immunity and it is still unclear to what extent their regenerative response involves adaptive immune functions.

In larval frogs, regeneration of tails appears critically sensitive to inflammatory signaling and is reported to be dependent on T regulatory cells to moderate the pro-inflammatory activity of myeloid cells [77]. As frogs approach metamorphic climax, massive changes occur in the diversity and activity of adaptive immune cell populations and regenerative capacity is diminished proportionally with these changes (reviewed in [62,78,79]). This

strong inverse correlation between regeneration and the sophistication of adaptive immunity may provide an important clue to the requirements for complete regeneration.

7. Evolution of the adaptive immune system as a barrier to regeneration?

The immune system of several highly regenerative organisms have largely been interpreted as relatively immune deficient due to the limited pro-inflammatory responses and poor adaptive immune cell diversity and responsiveness shown in these species. However, regenerative organisms such as the salamander and zebrafish live in environments with a diverse range of potential pathogens. It is also likely that these animals have evolved sophisticated innate immune strategies to deal with these challenges that reduce the dependency upon adaptive immunity. One advantage of relying on non-specific immunity is that it can react within a short time scale and does not depend on a previous encounter. This strategy must be efficient as over half of all living vertebrates are thought to be fishes. However, the evolution of warm-blooded vertebrates resulted in the development of highly specialized adaptive immunity that bought with it a range of added functions. This provided significant advantages in some aspects of immunity such as anti-viral responses but could have altered the dependency on or functions of other innate immune circuits [80].

Adult frogs however have diverged from their more ancestral amphibian counterparts and have developed an advanced adaptive immune system in adulthood that is very close to the sophistication and reactivity of humans and mice [79]. As adults, these animals show good protection from viruses that are extremely fatal to salamanders that lack robust adaptive immunity [81]. However, post-metamorphic frogs fail to regenerate after the adaptive immune system matures (reviewed in [62,82]). It is tempting to speculate that aspects of adaptive immunity have evolved at the cost of regeneration in both frogs and mammals. It is possible that regeneration requires the reversion of adult cells to more larval phenotypes that are “histo-incompatible” and thus would be rejected in adult tissue as foreign. In this case, some aspects of adaptive immunity may require disarming for successful regeneration to take place.

8. Uncovering genetic elements that regulate wound healing and regeneration

Mouse models of wound healing have been instrumental in shaping our understanding of various aspects of the biology that determine healing outcomes in humans and other vertebrates. The mouse has also been a powerful genetically tractable tool in modeling many human diseases. Genetic models of autoimmune diseases such as the Murphy Roths Large (MRL/Mpj) mouse show scarless healing in some tissues but not others and is dependent on the severity and type of insult (such as the inability to heal from burns). Improved repair or growth of ear skin, articular cartilage, skeletal muscle and digit tips have provided several insights into scar-less healing (reviewed in [83]). MRL/Mpj mice have an altered immune system and responses to injury, which may provide clues to some of the barriers to scar-less healing in humans. Despite the complexity of this phenotype, several related strains of mice show overlapping phenotypes and fine mapping of quantitative trait loci affecting ear tissue regeneration have revealed some important candidate genes that may equip these strains with

advanced scar-free repair in certain tissues [84,85]. Despite the susceptibility of MRL/MpJ mice to autoimmune disease it appears that the autoimmune and super-healing phenotypes do not genetically overlap [86,87].

The immune system is implicated in the genetic potential for regeneration. A screen of genetically diverse Collaborative Cross founder mouse strains has revealed a particular line (CAST/Ei) that had enhanced axon regeneration in models of spinal cord injury, optic nerve injury and stroke [88]. Genetic mapping of this trait implicated activin growth factor-hormone signaling as a potential modifier of this regeneration. Activins are now appreciated as key regulators of both innate and adaptive immunity [89]. IGF-1 is another important growth factor hormone that is known for its importance in wound repair and regeneration. This growth factor enhances muscle regeneration and is now understood to also alter innate immune cell recruitment and phenotype [90] along with activating FoxP3+ T regulatory cell production, which is also sufficient to suppress and reverse autoimmune disease progression [91]. This indicates a complex interaction between different immune cell compartments. Given an emerging framework for identifying genetic elements that enhance or limit regenerative potential exists, further studies will be highly informative for identifying additional elements that control regenerative capacity.

9. Wound healing versus regeneration- finding true regenerative signals within the noise

The salamander limb model of regeneration has well characterized phenotypic stages that have allowed fine resolution of gene signatures throughout the regenerative process [92-97]. As to be expected, gene expression analysis comparing limb regeneration with lateral wounds have confirmed that both tissues share a common wound response phase but interestingly, a regeneration specific program can be detected by 24 hrs post injury. Notably, many salamander genes either lack true mammalian orthologues or are divergent enough to preclude accurate assignment in custom microarrays or RNAseq studies, such that the differences between wound healing and regeneration are likely to be underestimated. Fine temporal sampling showed that components of developmental signaling pathways were enriched within the early regenerating tissue not expected in simple wound healing [96]. Understanding how re-expression of developmental antigens is tolerated in the adult immune system is sure to inform the delivery of future therapeutic interventions.

Although many of the differences between wound healing and regeneration may not have been fully captured in the salamander, regeneration of rat muscle has provided important insights. Work done by the Sicard lab has provided evidence that signaling during wound repair and regeneration may prime immune cells differently [98]. These in vivo rat studies implanted either polyvinyl alcohol sponges, minced skeletal muscle or minced muscle lacking satellite cells attempting to model fibroblastic wound repair, regeneration and wound healing scenarios respectively [99]. Each of these interventions can evoke distinct inflammatory responses. If these experiments are representative they would indicate an important dialogue between endogenous tissue undergoing either repair or regeneration, with the immune system with relevance for regenerative engineering.

10. Tissue dependent mechanisms that govern regeneration pathways

One significant challenge ahead for understanding the molecular basis for regeneration is to define the distinct tissue-specific clusters or gene sets that may obscure any common regenerative program. It is still unclear if the regeneration program deployed in the amphibian limb overlaps significantly with that of the heart, spinal cord, skin or brain. The immune system shows many examples of tissue specificity where the inflammatory response to injury is markedly different between tissues [100]. It remains to be determined if the immune environment of certain tissues (i.e. cardiac vs skeletal muscle) provides limitations on regenerative potential. What is certain is that there is likely to be a range of important tissue-specific modifiers. Comparative analysis between tissues, and even different organisms, will in time provide some answers to these critical questions.

11. Conclusions

The identification of the essential ingredients required to make a regeneration circuit is getting much closer thanks to the sophistication of modern omics technologies and advances in live imaging and cell profiling. Although the prospect of regrowing an adult limb may not be a realistic short term goal, there are significant opportunities where harnessing the powerful influences of the immune system may allow regenerative medicine to improve clinical outcomes in a range of injury or disease contexts. Immune cells are now recognized as important regulators of regeneration success due to their powerful influence in shaping many contexts of repair and development. Dissecting the genetic elements that limit or allow intrinsic potential for repair is now possible in some animal models but genetic mechanism is still lacking in highly regenerative organisms like the zebrafish and salamander that continue to inspire. Significant challenges still lay ahead in identifying the multiple inputs driving a regeneration program. Despite these difficulties, the basic requirements for a regeneration permissive immune environment are beginning to emerge.

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Fig 1.

The complex regulatory network between immune cells, fibroblasts and mesenchymal stem cells (MSCs) can control myofibroblast differentiation, with important implications for fibrosis. Myofibroblasts can be formed from a range of precursor cells. Fibroblast-to-myofibroblast differentiation is promoted by a range of pro-fibrotic signals (red arrows) from various immune cells. Anti-fibrotic signals (green arrows) can also be delivered by a range of immune cells. MSCs can exert potent immunosuppressive activity (blue arrows) on a range of immune cells and can differentiate into fibroblasts under certain conditions. The sum of the regulatory network will determine the potential for myofibroblast induction and downstream fibrotic events.

Fig 2.

Macrophages attending injuries arise from myeloid progenitors with alternative developmental origins. A. The developmental origins of hematopoietic progenitors changes several times during development. B. Hematopoietic progenitors from several tissue sources can seed other tissue niches early in development. C. Fetal liver is a major source of myeloid progenitors that seed hematopoietic organs including the spleen and bone marrow along with non-hematopoietic organs throughout the body. Tissue resident macrophages of the brain serve as an exception as these cells are seeded directly from Yolk Sac progenitors. D. Most wound-associated macrophages arise through the activation of fetal liver derived self-renewing tissue macrophages and the recruitment of bone marrow derived monocytes that are differentiated and polarized in situ.