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The Role of the Inflammatory Response in Burn Injury

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Abstract

Burns are characterised by significant local swelling and redness around the site of injury, indicative of acute inflammation. Whilst the inflammatory response is fundamental to the healing process, triggering a cascade of cytokines and growth factors to protect against the risk of infection, it is clear that prolonged inflammation can be detrimental and lead to scarring and fibrosis. Severe burns may display chronic, persistent inflammation long after the initial burn injury and may even result in multiple organ failure (MOF) due to systemic inflammatory response syndrome (SIRS). Excessive inflammation in the early stages of healing has been identified as a causative factor in the formation of scars which can be disfiguring, functionally restrictive and may require revisionary surgeries. Therefore, it is imperative that inflammation is effectively managed following burn injuries in order to optimise the benefits it provides whilst actively preventing the complications of inflammation including SIRS, multiple organ failure (MOF) and the development of scarring and fibrosis. Reviewing the current knowledge about the role of the inflammatory response in burns and the treatments available for the management of inflammation during wound healing, highlights the importance of continued research into understanding and developing new approaches to regulate inflammatory responses post-burn injuries.

Keywords: burns, inflammation, systemic inflammatory response syndrome, scarring, fibrosis

1. Introduction

In assessing the role of inflammation in burn injuries, it is important to first recognise differences in the pathophysiology of burns. Unlike other wounds, burns consist of three zones of injury, initially described by Jackson in the British Journal of Surgery in 1953. These are the zone of coagulation, the zone of stasis and the zone of hyperaemia [1] (**Figure 1**).
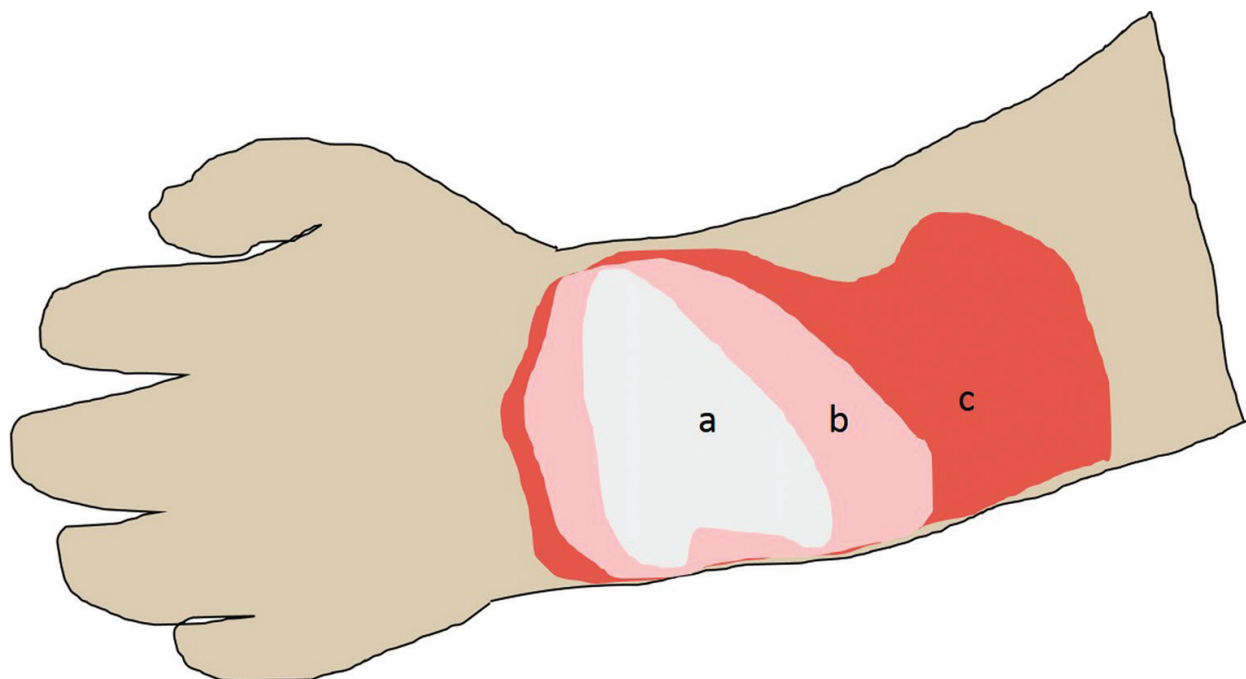
Figure 1. Scald burn in a child showing the Jackson's three zones of damage. (a) Zone of coagulation, (b) zone of stasis and (c) zone of hyperemia. Reproduced from [2].

The primary site of injury, classified as the zone of coagulation, is the site of the most damage and will rapidly undergo necrosis. Outside of this zone, is the zone of stasis that is characterised by reduced blood flow or ischemia and further out, the zone of hyperaemia where microvasculature is not damaged but displays increased blood flow and significant inflammation [2]. If inflammation and vascularisation are not quickly returned to normal, the zone of stasis may also undergo necrosis meaning that the size of the wound may in fact enlarge over time [3]. Thus, the direction of injury in burns is predominantly horizontal as opposed to the vertical injury of an incisional wound [4]. Whilst burn injuries differ from other wound types in that they are sterile at the time of injury, rapid blistering and necrosis of the injured tissue soon opens the wound up to pathogens and the risk of infection [2]. Burns wounds are often larger than other types of wounds, particularly those arising from scalds or exposure to flame and burn injuries covering greater than 20% of the total body area can quickly lead to burn shock due to widespread oedema and fluid loss [5]. The immune status of the patient is also altered following severe burn injuries further contributing to the risk of infection [6]. Thus, infections can quickly overcome a patient if not effectively controlled. It may be for this reason that the inflammatory response in burns is so intense. Moreover, the activity of the immune cells is often dictated by the specific signals encountered within the microenvironment at the site of inflammation or injury [7]. So understanding the factors which alter the protein pathways which are altered in burn wounds is pivotal in the development of therapies to restore balance to the immune response in burn patients and support effective healing of the wound.

2. Inflammation and the healing cascade

Burn injuries initially present with local swelling (oedema) and redness (erythema) around the site of injury (**Figure 1**). More severe, second or third degree burns, which affect more than the superficial epidermis, are characterised by greater levels of oedema and erythema, alongside the formation of blisters and inflammation [2]. This inflammation is indicative of the active immune response which is an integral part of the wound healing cascade, however, it can be significantly elevated in severe burn patients [5, 8]. Although the source of the injury may differ, the phases of wound healing are generally similar and can be described as phases of haemostasis and inflammation, proliferation and remodelling [9] (**Figure 2**). These interrelated and overlapping phases normally progress over a matter of days or weeks to effectively heal a wound, although the timings are often different between types of wounds [10]. In acute wounds, the inflammatory phase lasts for the first 5–7 days, however, severe burns may display chronic, persistent inflammation long after the initial tissue damage and may even result in multiple organ failure (MOF) due to systemic inflammatory response syndrome (SIRS) [11, 12]. Moreover, dysregulation of the inflammatory response and the subsequent progression through the phases of healing are associated with sub-optimal wound outcomes and excessive inflammation can lead to large, thick and restrictive scars [13, 14]. Thus, understanding the interaction between the early inflammatory phase and the later proliferative and remodelling phases of healing are important for understanding the particular complications which may arise following burn injury.
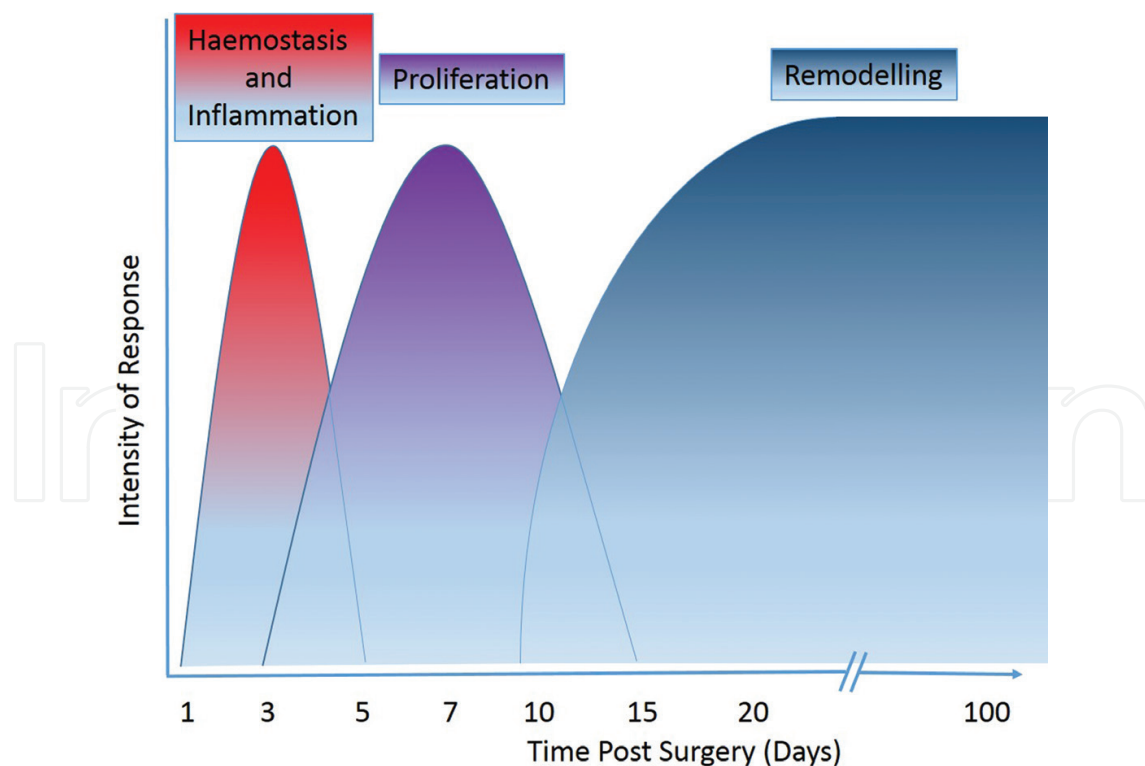

Figure 2. Schematic diagram of the three phases of the healing cascade.

2.1. Haemostasis and inflammation

Immediately following burn injury, haemostasis and coagulation occurs through the formation of a blood clot of platelets and cross-linked fibrin and fibronectin to quickly prevent excessive fluid loss from the wound site [15, 16]. Injured vasculature rapidly constricts to stem blood flow from the open vessels and later vasodilate to facilitate the entrance of blood cells to the wound site needed in the inflammatory phase [9]. Whilst burn wounds exhibit less blood loss than incisional wounds due to heat-induced tissue coagulation, there is still significant damage to the vasculature, with vasoconstriction extending out from the initial injury zone and into the zone of stasis [17]. Moreover, these early stages following burn injury may be complicated by continued damage due to the process of necrosis leading to a significant delay in healing [3, 4]. In all wounds though, key immune cells are recruited to the wound site by signals released from degranulating platelets within the injured tissue [18]. Within hours, the early inflammatory stage begins with the influx of immune cells to the wound site. These cells are responsible for protection from infections, clearance of necrotic and damaged tissue from the wound site and the stimulation of the cells required for repair of the wound during the proliferative and remodelling phases of wound healing [19]. Activated platelets aggregating at the ends of damaged vessels also release growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor-beta 1 (TGF-β1), to initiate fibroblasts and mesenchymal cells migration from surrounding the wound tissue which will be required for the formation of new extracellular matrix and dermal tissue during the proliferative phase of wound healing [9, 11].

2.2. Proliferation

During the proliferative phase of healing, cells of the epidermis and dermis, the keratinocytes and fibroblasts, proliferate and migrate into the wound site to form the neo-epidermis, restoring barrier function and produce new extracellular matrix which will reconstitute the damaged dermis following injury [19, 20]. The fibroblasts migrate along the fibrin-fibronectin plug into the wound site where they synthesise collagen and elastin and begin remaking the extracellular matrix (ECM) [19]. Whilst fibroblasts migrate into the wound site and form granulation tissue and the new dermal layer, keratinocytes crawl across the provisional matrix for re-epithelialization of the wound to occur [20]. Also during this time, angiogenesis, stimulated by factors released during the inflammatory phase, sees the formation of new blood vessels within the healing tissue [14]. This phase proceeds quickly to heal vertical injuries such as those arising from an incision, or superficial burns which affect only the epidermis, due to the availability of new epidermal cells from residual intact skin appendages residing within the undamaged dermis [4]. However, deep dermal burns heal much slower because of the loss of these skin appendages and reepithelialisation, which can only occur from the edges of the wound, does not begin until the progression of necrosis is halted [21]. Endothelial cells which form new capillary sprouts also interact with the ECM within the wound site, initially producing a dense microvascular network and later, as the levels of collagen increase, reduce the number of blood vessels leaving the resultant tissue with vascularisation levels similar to that of the original tissue [22, 23].

2.3. Remodelling

During the final, remodelling phase of healing, newly formed ECM deposited by fibroblasts is reformed and contracted by myofibroblasts, a process which continues long after the wound appears to be healed and the process of re-epithelialisation is complete [24]. Growth factors released during the inflammatory phase are key to the differentiation of fibroblasts into myo-fibroblasts [25] and it is the exertion of force by these differentiated, α-smooth muscle actin (α-SMA) positive fibroblasts, upon collagen fibres in the ECM which narrows the margins of the wound assisting wound closure [23, 24]. It is during this final phase of wound healing that the scar tissue is formed. Again, in burn wounds, the remodelling phase is significantly extended, often leading to the formation of hypertrophic scarring and contracture [2]. Initially, fibroblasts deposit type III collagen in the wound, however, as the tissue matures this is replaced by collagen I and collagen fibres are cross-linked to increase the tensile strength of the tissue [26]. A family of matrix metalloproteinases (MMPs), degrade collagen and other ECM components and have key roles in many of the phases of wound healing [27]. Once an equilibrium between collagen deposition and degradation is reached, the scar is considered mature although the organisation of the collagen fibres may continue to be remodelled for many years [13]. Along with this ECM remodelling, apoptosis of immune cells, endothelial cells, keratinocytes, fibroblasts and myofibroblasts and their subsequent clearance from the wound determines the final appearance of the healed tissue [28, 29]. Under normal healing conditions, myofibroblasts will undergo apoptosis and leave the wound site once re-epithelialisation has completed. However, in burn wounds this fails to occur with increased numbers of myofibroblasts observed within the dermis of the scar and fibrosis or pathological contracture ensues [29, 30]. Excessive matrix deposition combined with reduced remodelling are the hallmarks of fibrotic healing, such as is observed in hypertrophic scarring which often occurs following severe burns [26].

In addition to the important role of the inflammatory response in triggering the proliferative and remodelling phases, dysregulation of the inflammatory phase can result in excessive scar formation [10]. The inflammatory response therefore, is clearly fundamental to successful healing of the burn injury. Understanding the immune response, the roles of the specific immune cells and the cytokines and chemokines expressed by these cells within the healing wound is therefore key to understanding how to treat burn injuries and avoid complications due to dysregulation in the inflammatory response.

3. Key immune cells and protein expression in the healing burn wound

Key immune cells are required to present quickly to the burn injury. Whilst some are dermal resident cells, the majority are recruited from the circulation and crawl out of the vasculature into the site of injury [25]. The main inflammatory cells responsible for promoting burn injury repair are mast cells, neutrophils, monocytes and macrophages, whose activities are mediated by a number of the growth factors and signalling proteins (or cytokines) responsible for directing the progression through the healing cascade that are secreted by the immune cells themselves [10]. These ensure both the correct localisation of the required cells within the

injured tissue and stimulate the cells to proliferate or differentiate as required to heal the burn. Initially, chemokines, a subset of cytokines, which induce chemotaxis, are expressed following strict spatial and temporal patterns to ensure correct phase-specific recruitment and trafficking of immune cells to the wound site [31]. The expression of growth factors and cytokines further stimulate these immune cells, and other wound active cells, such as the fibroblast and keratinocyte, to carry the out the processes required for burn injury repair [11].

3.1. Mast cells

Burn injury stimulates mast cell degranulation [32, 33] and causes almost instantaneous secretion of histamine and cytokines by tissue resident mast cells [34]. Mast cells are tissue resident cells which play a role in both innate and acquired immunity, through the production histamine, to mediate allergic reaction and are responsible for hypersensitivity reactions [35]. However, these cells are also the first responders following tissue injury, promoting healing during the inflammatory phase where they release cytokines and chemokines, including not only histamine, but also tumour necrosis factor-α (TNF-α), prostaglandins, interleukin (IL)-1, and IL-6, to increase vascular permeability and recruit neutrophils and monocytes to the wound site when stimulated by heat or mechanical trauma [35, 36]. As well as releasing the pro-inflammatory cytokines, mast cells release proteases, such as chymase, which stimulates fibroblasts to migrate into the wound and is associated with fibrosis via its role in stimulating the expression and conversion of TGF-β1 and MMP-9 to their active forms [37]. Mast cells are also found to produce reactive oxygen species (ROS), which despite being an important stimulant of wound healing, excessive or prolonged production of ROS is detrimental to repair. Indeed, the microvascular injury characteristic of burn injuries is likely caused by ROS [32]. In addition to being an important catalyst for wound repair, mast cells may be further recruited to the wound site later in the healing cascade, migrating in from nearby connective tissue or differentiating from circulating basophils [11, 35] and producing tissue plasminogen activator and its antagonist, plasminogen activator inhibitor-1, as well as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and PDGF to modulate both the clotting response and remodelling of the ECM [37]. Likewise, they can release the anti-inflammatory protein IL-10 which helps to dampen an excessive immune response [38]. Thus, mast cells help to fine tune wound healing, depending on temporal and local concentration of the cytokines released by them within the healing tissue.

3.2. Neutrophils

Burn wounds are characterised by persistently high numbers of neutrophils, however, studies have shown that neutrophil chemotaxis is impaired following burn injury, with a reduced directional migration speed, impaired phagocytic function and reduced bactericidal capacity [39–42]. Neutrophils also help clear devitalised tissue through the production of MMPs, collagenase and elastase, clearing the way for the formation of new ECM [8]. These cells also express pro-inflammatory cytokines TNF-α, IL-1β and IL-6 which provide further signals to more neutrophils and other immune cells, such as the monocytes and macrophages, to collect at the wound site [8].

A number of mechanisms are employed by neutrophils to clear infectious agents which include phagolysosomes, the release of free radicals such as ROS and antimicrobial proteases to damage cell membranes and by trapping microbes within nets of histone and DNA [8, 11].

3.3. Monocytes and macrophages

Monocytes and macrophages are often thought as the most important of the immune cells, with important roles in tissue repair and particular alterations in activity following burn injury [43]. Monocytes are a subset of white blood cells which are rapidly recruited to the site of infection or injury from the circulatory system [44]. Here, they may transiently persist as monocytes secreting pro-inflammatory and angiogenic factors, or differentiate into macrophages to support tissue resident macrophages at the wound site to begin clearing the site of extracellular debris, such as damaged matrix, along with fibrin, spent platelets and neutrophils [7, 11]. Both monocytes and macrophages can have pro- or anti-inflammatory effects, often beginning as pro-inflammatory mediators [8]. Initially, most monocytes and macrophages present within the wound site exert pro-inflammatory effects, with an M1 phenotype, and transition to an anti-inflammatory or M2 phenotype later to resolve inflammation, stimulating angiogenesis and progression through the healing cascade [8, 44]. M1 macrophages are the main source of the pro-inflammatory mediators of prostaglandin E2, reactive oxygen and nitrogen intermediates, TNFα, IL-1, IL-6 and IL-8 within the wound to amplify the immune response [8, 43]. M2 macrophages, however, produce an IL-1 receptor antagonist to regulate this response and further produce PDGF, TGF-β1 and FGF to stimulate ECM production and angiogenesis within the newly formed tissue [8, 31, 43]. In burns, there is are significant differences in macrophage populations, with both an initial increase M1 activity, but also a concomitant increase in M2 signalling later in the inflammatory phase [8, 43].

3.4. DAMPs and PAMPs

Some of the earliest signalling pathways to facilitate the wound repair process are triggered by the presence of damage-associated molecular patterns (DAMPs) within the injured tissue. These DAMPs include small molecules, such as ATP, adenosine and bioactive lipids leaked from damaged cells [11]. Studies have shown that a range of DAMPs are significantly elevated immediately following burn injury, due to the significant necrosis induced by burns, which in turn contributes to the excessive monocyte/macrophage activation characteristic of burns [45]. Where infectious agents are also introduced into the wound, these DAMPs are also accompanied by pathogen-associated molecular patterns (PAMPs) such as peptides cleaved from bacterial proteins [7, 11]. Thus, the inflammatory response stimulated by DAMPs found following sterile tissue damage, such as the initial burn insult, can be further exacerbated by PAMPs in infected wounds [7]. PAMPs stimulate the nuclear factor kappa-B (NF-κB) and interferon (IFN) pathways leading to significant upregulation of the cellular immune response to defend against microbial infection [8]. The DAMPs and PAMPs are quickly joined by histamines released from degranulating mast cells, and the growth factors PDGF and TGF-β1 released from platelets [11]. Ischemic injury, such as is present following burn injury, can stimulate additional effects due to the hypoxic environment and

reactive oxygen species [7]. Together, these powerful signals trigger the active immune response and result in a strong presence of neutrophils and M1 macrophages and a heightened cellular immune response within the burn wound. In particular, the expression of TNF-α, IL-1, IL-6 and IL-8 by these cells is fundamental to the amplification of the immune response, via the activation of NF-κB and attraction of increasing numbers of immune cells into the wound site [8].

3.5. Complement activation

Burn injury causes systemic upregulation of complement and C-reactive proteins (CRP), enhancing the risk of SIRS and also negatively affecting the healing of the burn [42]. The complement cascades play a key role in the acute phase of the immune response. Part of the innate immune response, the complement system is made up of a number of complement (C) proteins which enhance the ability to fight infection by both directly and indirectly attacking microbes and clearing debris [46]. The complement system is dynamically involved with the cellular immune response, operating via different pathways including the classical, lectin and alternate pathways or via properdin and thrombin, triggered by antibodies expressed on apoptotic cells or microbes, by distinct carbohydrate and lipid residues on injured cells and by DAMPs and PAMPs in the wound site [37, 47–49]. Whilst the different pathways are employed, all complement cascades act to lyse microbes via the formation of C5b and C9 into the membrane attack complex and converge to produce C3a which together with C5 attracts inflammatory cells and promotes phagocytosis and clearance of damaged cells by macrophages [42, 47]. Degranulation of platelets activates the C5 protein [50]. C5, along with C3, in turn stimulate mast cells and thus the complement cascade can modulate mast cell involvement in the resolution of the blood clot and ECM remodelling [37]. Following burn injury, CRP and C3d levels are increased within the local wound site and this increase persists long after the initial injury. The increase in complement is also associated with increased number of macrophages and neutrophils, indicating that the local immune response in burns persists locally much longer than other types of acute wounds [42]. Indeed, it appears that the entire immune system are dynamically involved in the process of burn wound healing.

4. Duality of the immune response in burns patients

The two phases of the inflammatory response are of particular concern following burn injury due to the profound systemic effects upon the patient. Rather than remaining a local response, the initial enhanced pro-inflammatory phase may lead to multiple organ failure and even death as a result of the systemic inflammatory response. Additionally, this can then be followed by an anti-inflammatory phase which often leads to complications for the immuno-compromised patient [12]. The greatest difficulty for the treatment of burns lies in the paradox that an active immune response is required for the progression of wound healing and the control of infection, and yet following the excessive initial pro-inflammatory response, increasing the risk of infection due to immune suppression [12, 51, 52]. Thus, the regulation of the intensity and length of the pro-inflammatory and subsequent anti-inflammatory response is of particular importance in burn wound management.

4.1. Systemic inflammatory response syndrome

Excessive immune activation can lead to a systemic inflammatory response syndrome (SIRS) culminating in distant tissue damage and multiple organ dysfunction with the very great risk of death. When pro-inflammatory cytokines, produced during the local immune response that promote the vascular permeability needed for immune cell infiltration, are released into the circulation, they may attack the integrity of distant blood vessels, allowing blood to flood end organs leading to organ failure [51]. Within hours, the increased capillary permeability can lead to hypovolemic shock due to massive fluid loss and requires immediate fluid resuscitation to prevent death [6]. Moreover, intestinal permeability which occurs following severe burns may itself be the source of the inflammatory signalling that causes distant tissue damage [12, 53]. Excessive neutrophilic inflammation is an early hallmark of SIRS but there is significant involvement of the macrophage during the initial phase of the inflammatory response, particularly mediated by their production of pro-inflammatory cytokines TNFα, and IL-6 [43, 52]. As with other severe injuries such as femoral fraction with blood loss greater than 40%, the macrophages in burns appear hyperactive with increased capacity for the production of pro-inflammatory mediators. Indeed, elevated systemic levels of IL-6, IL-8, reactive nitrogen intermediates and prostaglandins are detected in burns patients, all of which mediate both local and distant tissue damage [43]. IL-6 has been shown to be quickly upregulated in the plasma of burns patients, peaking within 6 hours post burn [54]. The levels of IL-6 have been shown to be proportional to the size of the burn and persistently high levels of IL-6 post burn injury may be indicative of both the severity of the burn and likelihood of mortality [54, 55]. Therefore at this time, reducing the severity of the immune response is of critical importance, which needs to be managed carefully before it switches to a profoundly anti-inflammatory phase which itself can have significant side effects.

4.2. Anti-inflammatory response syndrome

Within just a few days of the severe burn, the immune response may become significantly depressed resulting in an anti-inflammatory response syndrome (AIRS). TGF-β1 expression initially peaks 1 day post burn injury and it is likely to have a pro-inflammatory role stimulating the migration of monocytes, neutrophils and fibroblasts into the wound [43, 56]. However, TGF-β1 is also anti-inflammatory and a second peak in TGF-β1 is again detected 1–3 weeks post burn injury [56]. Elevated levels of serum TGF-β1 correlate with the post-burn immunosuppression which is fundamental to the progression of systemic infection or sepsis [43]. Severe burn injuries also display increased anti-inflammatory cytokine IL-4 and IL-10 expression, which inhibit M1 macrophages but stimulate M2 macrophages [8, 57]. Serum levels of the anti-inflammatory cytokine IL-10 peak in burns within 2.5 days following burn injury where increased IL-10 levels is associated with reduced resistance to infection [56, 58]. Indeed, high levels of serum IL-10 at 3 days post burn may be a useful clinical marker of increased risk of mortality in septic burns [58]. During this time, the focus of clinical management is the clearance of infection and supporting the immune response rather than continuing to dampen the response in the hopes of protecting from further tissue damage. It is clear then that dysregulation of the inflammatory response poses great risk to mortality and morbidity, however, the immune response following burn injury may pose one further threat, even when

wounds heal without the complications of chronic inflammation leading to SIRS or AIRS. This is due to the fact that excessive inflammation in the early stages of healing has been identified as a causative factor in the formation of scarring and fibrosis.

5. Chronic inflammation and burn scar formation

Burn injuries are often characterised by debilitating hypertrophic scarring, often requiring revisionary surgery (**Figure 3**). In children, scar formation following burn injury is of particular concerns, as the growing child will be restricted by non-elastic scars, which when occurring over moving joints can become functionally restrictive [59]. This is due to an excessive synthesis and deposition of ECM alongside the reduced degradation and remodelling of tissue, leading to the dense formation of collagen in long bundles rather than the normal basket weave formation [60]. Scars are also characterised by an absence of skin appendages such as hair follicles, sweat glands and nerves, which results in functional deficiency, loss of ability to regulate body temperature and absence of sensation [61]. Due to the potentially large areas which may be affected by severe scarring following burn injury, the ability to reduce scar formation is of critical importance.

The link between the increased inflammatory response and the formation of scars is well established [61, 62]. It is clear that prolonged and/or excessive inflammation in the early stages of burn injury leads to excessive fibrosis and scarring [63]. In particular, the numbers of macrophages found within a wound at specific times of the healing cascade are associated with the level of fibrosis and scar formation observed [10]. Likewise, elevated TGF-β1 signalling is directly associated with increased collagen deposition and fibrosis within the healed wound

Figure 3. Post-burn hypertrophic scar on anterior chest wall. Reproduced from [59].

as well as myofibroblast over-activation and contracture formation [29, 30, 64]. As described earlier, both the pro-inflammatory macrophage phenotype and elevated TGF-β1 signalling seen in burn wounds contribute to the systemic complication of the immune response as well as this additional role in the formation of hypertrophic scars. There is clearly a very great need for the development of treatments which can control inflammation in burn wounds, to reduce the risk of SIRS and prevent excess scar formation, whilst maintaining the ability to fight infection.

6. Treatments to control inflammation in burns

6.1. Traditional clinical management

Although minor burns heal quickly with little intervention, more severe burns require specialised clinical care to prevent hypovolemic shock, curb inflammation whilst protecting the patient from infection [6, 12]. Fluid resuscitation is often the first step in treating the severe burns patient and research generally centres on determining the optimal fluid volume to avoid cardiac and pulmonary complication, however, the effect of the fluid resuscitation strategy may also impact the inflammatory response [6]. For example, the lactate in a once preferred resuscitation fluid actually stimulates the inflammatory response, negatively affecting the prognosis for burns patients [53]. But the addition of the soluble polysaccharide, glucan phosphate to resuscitation fluid may prove useful as it has been seen to reduce pro-inflammatory cytokine expression post burn whilst increasing resistance to infection by *Pseudomonas aeruginosa* in mice [65]. The nutritional support given to the patient may also modulate the immune system [5]. Indeed, the amounts and specific types of carbohydrates and fats consumed can greatly alter the immune status. It is important to acknowledge then the impact of the dietary changes upon the immune response and investigate how different enteral feeding strategies may impact the immune profile of the patient [5, 66]. For example, a combination of arginine and Omega-3 fatty acid supplementation could be a useful addition due to the initial findings of positive effects on wound healing rates and resistance to infection, but it is not yet known if these will translate into burns patients [67]. A recent clinical trial found that supplementing with isolated soy protein, with or without fish oil was able to decrease the inflammatory response and improve wound healing in burn patients, but it is not yet known which specific compounds in the soy protein are responsible for this action [68]. Interestingly, the topical application of fatty acids isolated from various animals and plants have also been shown to have a positive effect upon burn wound healing and dampening excessive inflammation post burn injury [69], which supports the use of dietary fats in treating burns. However, other adjunct therapies which modulate the immune response have also become the focus of much research into curbing inflammation to reduce mortality and scar formation following burns.

6.2. New research for the modulation of the immune system

A number of avenues for modulating the immune system have so far been investigated which may prove useful in the treatment of burns. These include inhibiting the activation of the immune response, preventing the recruitment and activity of immune cells, interrupting the signalling pathways involved in inflammation and enhancing the resolution of the inflammatory phase.

6.2.1. Inhibition of the activation of the immune response

Curbing inflammation in burns may be achieved at the outset by inhibiting complement activation. Treatment with a C1 inhibitor in a porcine model of burn injury was found to attenuate inflammatory tissue destruction post burn [70, 71] and reduction of C4 in knockout mice prevented hypertrophic scarring following burn injury [72]. Alternatively, blocking immune cell activation using antibodies to block cytokines such as TNFα and IL-1β have been shown to be effective in vitro [52] and local reduction of TNFα using a hyaluronic acid conjugated TNFα antibody reduced necrotic burn progression in a rat model [73]. It has also been suggested that developing therapeutics to prevent NF-κB activation with antioxidants or an agent to block intracellular processes involved in its activation may be a better approach, and there is a current search for a highly specific compound [52]. Stabilisation of mast cells using cromolyn solution prevents both the rise in levels of plasma histamine and xanthine oxidase following thermal injury in rats preventing the initiation of the immune cascade and reducing the severity of the burn [34]. Treatment with pentoxifylline (PTX) immediately following burn injury in mice was found to reduce intestinal permeability and lung injury by reducing levels of intestinal IL-6 and limiting the increase in pro-inflammatory cytokine levels and inflammatory cell activation which may be useful in the prevention of SIRS [53]. Rather than preventing the activation of the immune cells though, one may also aim to reduce their numbers within the wound site.

6.2.2. Prevention of immune cell recruitment and activity

Early studies in mice to deplete numbers of specific immune cells have shown that whilst no one immune cell is critical to wound healing, altering the recruitment or activity of these cells, provided no overt infection pre-exists, may be beneficial to the repair process and indeed may lessen the scarring observed [11]. Studies which specifically depleted macrophage numbers prior to wounding have shown that although wound closure is delayed, the wounds heal with reduced fibrosis and scar formation [74, 75]. Indeed, the targeted depletion of macrophages from the early inflammatory stage (days 0–5) of healing results in minimised scarring. Although these mice showed a reduced rate of epithelialisation during the inflammatory stage, once the mice were allowed to produce new macrophages, wound closure was rapid compared to control animals and with greatly reduced scarring [76]. It has been suggested that reducing the ability of immune cells to receive chemotactic signals may also be useful in modulating the immune response to facilitate better healing [11]. Blocking the activity of chymase released by mast cells using a specific chymase inhibitor TY51469 was effective in supressing neutrophil accumulation, reducing TGF-β1 expression and the extent of pulmonary fibrosis in mice [77] and may be a candidate therapeutic for reducing fibrosis and scarring following severe burns. Indeed, mice which lack mouse mast cell protease (mMCP)-4, the functional equivalent to human chymase, showed a much reduced level of injury following a second-degree scald burn [78].

6.2.3. Interruption of immune signalling pathways

Pharmacological agents which themselves reduce TGF-β1 signalling may also prove useful for reducing fibrosis following burn injury [11]. Alternatively, applying an antibody to interrupt the

action of a protein which acts upon the signalling pathway may prove beneficial. The protein Flightless I (Flii) has been shown to modulate TGF-β1 signalling in fibroblasts, with reduced TGF-β1 expression and collagen production in cells with reduced Flii expression [79]. Flii also directly affects the immune response, altering macrophage activation and cytokine production in vitro and increasing Flii expression in diabetic wounds is associated with increased NF-κB production and a prolonged inflammatory phase is observed in the healing of incisional wounds of mice [79–81]. The application of a Flii neutralising antibody in an animal model of the inflammatory skin condition, psoriasis, reduces pro-inflammatory cytokine expression and immune cell infiltration [82]. By treating mouse burn wounds with this antibody and reducing the expression of Flii, it was possible to decrease TGF-β1 levels and cause faster wound healing with less scar formation in mice [83] (**Figure 4**).
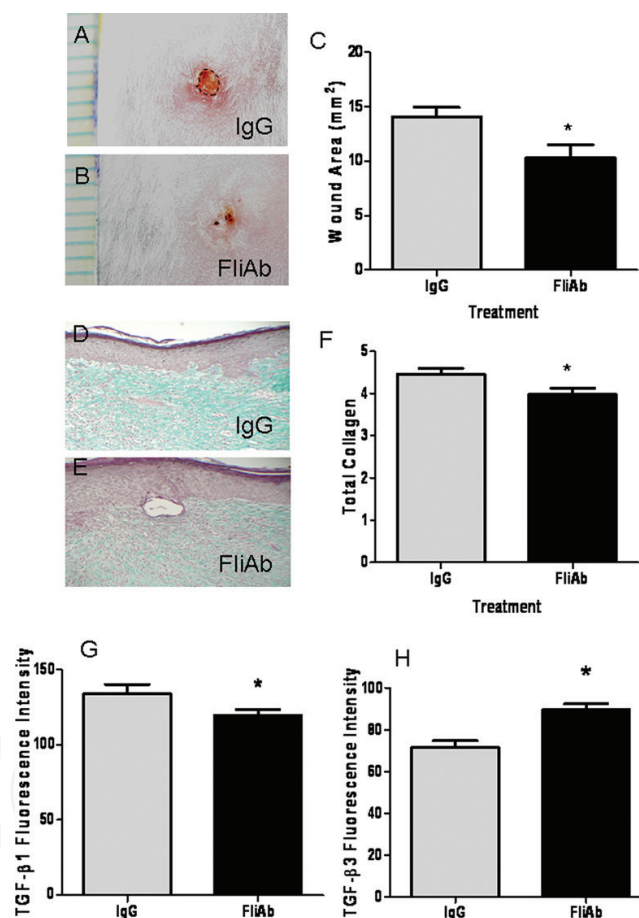

Figure 4. Flii neutralising antibody (FliAb) improves burn injury repair. Representative partial-thickness burn wounds treated with intradermal injection of (a) control IgG (50 μg mL⁻¹) or (b) FliAb (50 μg mL⁻¹) 14 days post-treatment. Dotted line indicates residual burn wound. (c) Graph showing surface wound area of partial-thickness burns treated with either IgG or FliAb post-burn injury. Representative images of (d) IgG and (e) FliAb-treated partial-thickness burns at day 14 post-injury stained with Masson's Trichrome. (f) Graph showing semi-qualitative assessment of total collagen levels within wound of IgG or FliAb-treated burn wounds. (g) Graph showing TGF-β1 fluorescence intensity after 14 days in burn wounds treated with IgG or FliAb post-burn injury. (h) Graph showing TGF-β3 fluorescence intensity after 14 days in burn wounds treated with IgG or FliAb post-burn injury. *Denotes significance P <0.05. Results represent mean ± SEM (n = 8). Reproduced from [83].

The potential therapy has been further investigated in a model of hypertrophic scarring, where it was found that application of Flii antibodies resulted in less fibrosis by reducing the number of myofibroblasts within the wound [84]. Further investigation into the use of neutralising antibodies to dampen inflammation and reduce fibrosis in human burns patients is clearly warranted.

6.2.4. Enhancement of the resolution of the inflammatory response

New therapies are also being developed to resolve post burn inflammation quickly and lead to better healing outcomes. Lipid mediators, such as the resolvins and lipoxins, which stimulate the resolution of the inflammatory phase, have become of particular interest in recent years [11]. Resolvin D2 has recently been found to restore burn neutrophil directionality and can reduce neutrophil numbers and minimise secondary necrosis in burns [85, 86]. The use of phototherapy has been shown to significantly reduce the number of immune cells within a rat burn wound, increasing angiogenesis and collagen deposition [87] and may herald a new area of research for the development of devices to treat burn wounds. Novel biomaterials are also being investigated in the treatment of burn injuries, not only to provide a provisional matrix or augment skin grafting, but are also being assessed for their ability to modulate the immune response [88]. Fibrin-based hydrogels delivered into pig burn wounds prevented contracture but also reduced immune cells within the hydrogel as well as reducing neutrophil and macrophage numbers within the in the surrounding granulation tissue on day 7 post burn [89]. It is expected that future therapies will aim to provide the dual roles of enhancing healing outcomes whilst preventing the excessive systemic inflammatory effects post burn.

7. Considerations and conclusions

Recent studies have revealed some difficulties in predicting and assessing the efficacy of therapies in treating burn wounds. For example, the drugs Atorvastatin and Losartan, originally developed to lower cholesterol levels, showed promise in reducing fibrosis and inflammation in a number of conditions. However, when applied to partial and full thickness burn wounds, it was found that whilst Atrovastatin improved healing of full thickness burns, Losartan was detrimental, but found to be beneficial when applied to partial thickness burns [90]. This highlights the complexities of the immune response and progression through the healing cascade in burn injury and demands that careful consideration be paid during the development of any new therapy to the specific use of a treatment. Moreover, it is not yet known if dampening the immune response by any of these approaches would result in a heightened risk of infection. Therefore investigations into the effect of local versus systemic delivery methods, a thorough understanding of the dose-response effect and confounding effects due to the severity of the injury itself, combined with a careful evaluation of timing of treatments is required. Nevertheless, research into methods, which modulate the immune response, to avoid the complications of a dysregulated immune response and the formation of excess scarring and fibrosis following severe burns remains of critical importance for the future developments of new therapeutic approaches for the treatment of burns.

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