PART I

The role of complement in the acute phase response after burns
Review: The role of complement in the acute phase response after burns

Korkmaz HJ, Krijnen PAJ, Ulrich MMW, de Jong E, van Zuijlen PPM, Niessen HWM

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Severe burns induce a complex systemic inflammatory response characterized by a typical prolonged acute phase response (APR) that starts approximately 4–8h after-burn and persists for months up to a year after the initial burn trauma. During this APR, acute phase proteins (APPs), including C-reactive protein (CRP) and complement (e.g. C3, C4 and C5) are released in the blood, resulting amongst others, in the recruitment and migration of inflammatory cells. Although the APR is necessary for proper wound healing, a prolonged APR can induce local tissue damage, hamper the healing process and cause negative systemic effects in several organs, including the heart, lungs, kidney and the central nervous system. In this review, we will discuss the role of the APR in burns with a specific focus on complement.
INTRODUCTION

Severe burns induce a massive inflammatory response, both systemic and locally in the burn wound. This inflammatory response not only affects the healing process of the burn wound, but also has effects systemically. In addition, increased burn size leads to increased morbidity and mortality of burn patients due to especially increased inflammatory response [1]. As part of the acute phase response (APR), the complement system has been shown to negatively affect the local pathophysiology of the burn wound, but also to have negative systemic effects in burned patients [2–4]. It is therefore of utmost importance to get better insights in the role of the APR and the complement system after burns. The skin is composed of an outer epidermal layer, that functions as a barrier to bacterial and environmental toxins and an inner dermal layer, which provides e.g. temperature regulation, blood supply and strength and suppleness of the skin [5]. Prolonged exposure to temperatures higher than 40°C, as occurring in burn wounds, causes denaturation of proteins and loss of plasma membrane integrity [6]. This process is very rapid and may only take a second when exposed to temperatures higher than 60°C. Post-burn, necrosis occurs at the center of the injury and becomes progressively less severe at the periphery [6]. Tissue loss is rapidly followed by activation of inflammatory mediators, via the APR (4–8h post-burn) [7–10]. The complement system is a central part of the APR [11]. It is composed of several proteins that interact in three different enzymatic cascades: the classical, lectin and alternative pathways (figure 1).

Activation of the classical pathway is mediated by the formation of the C1 complex, consisting of C1q, C1s and C1r at the targets surface. The lectin pathway is mediated by the mannose-binding lectin-associated serine proteases (MASPs), MASP-1/MASP-2 together with a collectin (usually mannose-binding lectin, MBL) or ficolin at the mannose residues of pathogens. Subsequently proteases C1s and MASP-2 bound in these complexes cleave the circulating complement components C2 and C4, releasing C4b and C2a, which form a C3-convertase. In relation with burns, it has been shown that MBL plays an important role in the first-line host defence against infectious agents after burn. MBL initiates the lectin pathway and acts as an opsonin [12,13]. In the alternative pathway, C3b, derived from spontaneous hydrolysisation of C3, together with factor B from the blood forms an alternative C3-convertase (C3bBb) on the target surface [14]. All three pathways converge at the point of C3, which is cleaved by C3-convertase and forms C3a and C3b. The C3 cleavage-product C3b formed in either the classical or lectin pathway, may initiate the alternative pathway as well. Thereby, the alternative pathway functions as an amplification loop for the other two activation pathways. Binding of C3b to the C3-convertase, forms a C5-convertase on the target surface, which in turn cleaves phase C5 in C5a and C5b. The complement cascades end when C5b forms the membrane attack complex (MAC), together with C6, C7, C8 and C9. MAC forms a pore in the membrane, which triggers the lysis of the targeted host cells or pathogens. When C3 and C5 are cleaved, the respective anaphylatoxins C3a and C5a are released [15,16]. These
have a wide variety of pro-inflammatory properties, including the recruitment and activation of inflammatory cells. Burn wounds induce a prolonged inflammatory response with excessive complement activation, which not only negatively affects the healing process of the burn wound, but additionally exerts systemic effects in different organs. An important factor herein is the APR, in which complement is playing a central role. It is known that acute phase proteins (APPs) are elevated up to months after burn, both locally and in the blood [17–19]. The exact reason for prolonged persistence of the APR after burn is still unclear, however increased complement levels were shown to be related to the severity of burn trauma and to the clinical outcome. The initial aim of the APR is to restore homeostasis. However, a sustained or exaggerated APR has been shown to be life threatening [9,10,20–27]. In this review we discuss this post-burn APR, including its local and systemic pathophysiologic effects, in more detail.
Figure 1. Complement activation pathways. The complement system is activated through the classical, lectin, or alternative pathways that converge at the central molecule of the complement system: C3. MBL: mannose-binding lectin; MASP-1/2: mannose-binding lectin-associated serine protease-1/2; C1inh: C1-esterase inhibitor.
POST-BURN ALTERATION OF APPs IN BLOOD

After burn trauma, the APR results in the alteration of acute phase protein (APP) levels in the blood (figure 2). The APR starts when interleukin-6 (IL-6) is produced by Kupffer cells in the liver and then induces the secretion of APPs including C-reactive protein (CRP) and complement [17,26,28–33]. Several studies in both burned humans as well as in animal burn models have shown a post-burn increase of the pro-inflammatory CRP and complement (C3, C3a and C5a) levels in the blood, up to months after the initial trauma (figure 2) [33–38]. It also was found that both CRP and C3 blood levels did correlate with the severity of the burn trauma, i.e. to the area (% Total Body Surface Area (TBSA)) and depth of the burn [38–42]. In addition, it has been shown that the age of patients also influenced the APR and clinical outcome post-burn. In elderly patients, significantly higher C3a blood levels were found, coinciding with significantly more thrombotic blood vessels in deep dermal tissue and delayed wound healing [43]. Indeed it is known that activated platelets can initiate activation of the complement system and formation of the C5b-9 complex, but also the other way around [44]. The APP CRP can activate the complement system via the classical pathway [11], which in turn can cause inflammatory cell attraction via the release of anaphylatoxins C3a and C5a, and also via an increase in vascular permeability by these anaphylatoxins (table 1) [36,45–48]. Remarkably, prior to the long-term increase, complement C3 and C3a levels were found to decrease during the first days after-burn (figure 2) [17,35]. This phenomenon was explained by mechanical leakage of complement due to increased vascular permeability [47,49]. Several other APPs, such as transferrin (TRF), apolipoprotein- A1 (Apo-A1), pre-albumin (PAB) and retinol-binding protein (RBP), were found to be decreased in the blood for up to 2 months post-burn in severely burned pediatric patients (figure 2) [17,33]. Whether this decrease was also caused by mechanical leakage due to increased vascular permeability post-burn is not known, however in contrast to complement these APPs did not increase over time and remained decreased up to months after the initial burn trauma. Moreover, whether mechanical leakage initially also results in lower blood levels of other APPs, including CRP, is not known yet.

TRF is an iron-binding blood plasma glycoprotein, that can bind iron released from bacteria and as such impedes bacterial survival (table 1). The long-term burn-induced decrease in TRF theoretically could result in iron overload facilitating bacterial infection [17,33]. Apo-A1, a major protein component of high density lipoprotein (HDL), has anticoagulant effects by preventing platelet plug formation and inhibiting platelet activation (table 1). Therefore, the long-term burn-induced decrease in blood Apo-A1 levels may lead to an increased risk of thrombosis [50]. The tryptophan-rich protein PAB is synthesized in hepatocytes in the liver and is a blood marker for the nutritional status of patients (table 1) [51–53]. The long-term decrease in PAB blood levels post-burn may be indicative for malnutrition after burn trauma [17,33]. Finally, RBP, a carrier for the antioxidant vitamin A (retinol), which stimulates epithelial
formation and a deficiency can impede wound healing (table 1) [54–57], was significantly decreased immediately after-burn, persisting for up to 2 weeks, where after RBP levels increased in time again up to 6 months post-burn (figure 2) [17,33,37].

Several studies in both human and animal models have also found changes in the blood levels of anti-inflammatory APPs following thermal injury. For instance, C1-esterase inhibitor (C1inh), an APP that circulates in the blood, can prevent the cleavage of C4 and C2 and thereby inhibit complement activation (see figure 1 and table 1) [11,14,58]. In patients with burn wounds reduced C1inh blood levels were found, especially in the first week post-burn (figure 2), which coincided with edema formation [59]. Indeed, it is known that in addition to complement inhibition, C1inh can reduce vascular permeability [60]. This may explain edema formation at reduced circulating C1inh levels. Furthermore, blood levels of Haptoglobin (Hp), a major hemoglobin (Hb)-binding protein that inhibits T-lymphocytes (table 1) [61], were significantly elevated in burned mice and rats for up to weeks post-burn [36,62]. While in burned children both Hp and serum a1-Acid glycoprotein (a1-AGP), an inhibitor of complement activation that also causes aggregation of platelets (table 1) [63,64], were increased immediately upon burn and remained significantly elevated for up to 3 months post-burn (figure 2) [17,33,37].

In conclusion, the APR is activated in the blood for an extended period of time after burn. Especially persistently increased levels of pro-inflammatory CRP and complement for up to 12 months post-burn, could result in adverse effects in burn patients. In addition, decreased levels of PAB, TRF and APO-A1 as found early post-burn could increase the risk of e.g. bacterial infection (TRF) and vascular thrombosis (APO-A1), which could also negatively affect the clinical outcome of the burn wound patient.
Figure 2. An overview of post-burn alteration of APPs in serum. Acute phase proteins are significantly altered for up to months post-burn. Serum retinol binding protein (RBP) [17,33,37]; a1- acid glycoprotein (a1-AGP) [17,33,37]; haptoglobin (Hp) [17,33,37]; C-reactive protein (CRP) [17,33,37,40,41]; complement C3 (C3) [17,33,34,37,38]; C1-esterase inhibitor (C1inh) [58]; transferrin (TRF) [17,33]; apolipoprotein A1 (Apo-A1) [17,33] and pre-albumin (PAB) [17,33].

Table 1 – Acute phase proteins and their function that may be involved in (burn) wound healing processes.

<table>
<thead>
<tr>
<th>APP</th>
<th>Function that may be involved in (burn wound healing processes)</th>
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<tbody>
<tr>
<td>CRP</td>
<td>Pro-inflammation (Activator of the complement classical pathway by binding damaged host tissue and microorganisms)</td>
</tr>
<tr>
<td>C3/C3a</td>
<td>Pro-inflammation C3 activation results in a cascade of non-enzymatic protein cleavage (e.g. C3a) and can stimulate inflammatory cells</td>
</tr>
<tr>
<td>TRF</td>
<td>Impedes bacterial survival</td>
</tr>
<tr>
<td>Apo-A1</td>
<td>Anti-coagulation Prevents platelet plug formation and inhibits platelet activation</td>
</tr>
<tr>
<td>PAB</td>
<td>Marker for nutritional status Malnutrition causes slow wound healing</td>
</tr>
<tr>
<td>RBP</td>
<td>Carrier of antioxidant Vitamin A (retinol), which stimulates epithelial formation (Vitamin A deficiency can impede wound healing)</td>
</tr>
<tr>
<td>C1inh</td>
<td>Anti-inflammation Inhibits complement activation</td>
</tr>
<tr>
<td>Hp</td>
<td>T-lymphocyte inhibition</td>
</tr>
<tr>
<td>a1-AGP</td>
<td>Platelet aggregation and complement inhibition</td>
</tr>
</tbody>
</table>

Summary: Illustration of the acute phase proteins and their function that may be involved in (burn) wound healing processes. CRP: C-reactive protein; C3/C3a: complement C3/C3a; TRF: transferrin; Apo-A1: apolipoprotein A1; PAB: pre-albumin; RBP: retinol binding protein; C1inh: C1-esterase inhibitor; Hp: haptoglobin; a1-AGP: a1-Acid glycoprotein.
THE LOCAL PATHOPHYSIOLOGIC EFFECTS OF COMPLEMENT IN SKIN BURNS

The post-burn APR has local pathophysiological effects. In non-thermal cutaneous wound healing, the complement cascade has been shown to promote wound healing. Complement C3 and C5 have hemostatic, antibacterial, and pro-inflammatory effects that have been shown to also accelerate wound healing [11,65]. A burn wound also induces a local inflammatory response, with a central role for complement, which is initially important in the process of wound healing. It causes clearance of cellular debris and protects the wound against microbial agents [66]. In burn wounds it was shown that during the early stage of burns (up to 12 days post-burn), topical application of a-gal liposomes, resulted in an antibody response to a-gal that activated the chemotactic factors C3a and C5a, induced rapid recruitment and activation of neutrophils and macrophages and accelerated wound healing in mice [67,68]. In “normal” wound healing this inflammatory response is resolved in a few days (0–5 days), where after tissue proliferation (3–21 days) and remodelling (>21 days) takes place [69]. However in the burn wound, enhanced levels of C3d have been shown in the burn wound until 46 days after burn trauma, coinciding with an increase in CRP and the number of neutrophils, implicating a persistent ongoing acute inflammation locally in the burn wound [19]. The prolonged local acute inflammatory reaction, can have detrimental effects in wound healing, resulting in endothelial damage (e.g. increased vascular permeability, vascular thrombosis), delayed wound closure, excessive wound contraction and finally hypertrophic scarring, that all have been described in burn wounds (figure 3) [11,19,38,43]. Intravenous injection of soluble human recombinant CR1 (sCR1), an inhibitor of both classical and alternative pathway by blocking C3 and C5 convertase activity [70], resulted in decreased dermal vascular permeability and reduced recruitment of neutrophils in burn wound biopsies up to 4h post-burn in rats as compared to control rats [71]. While, in burned pigs, daily application of C1inh up to 96h reduced edema formation and reduced inflammation induced tissue destruction of the skin [58,72–74]. This in part may be the result of C1inh preventing capillary leakage and as such protecting the dermal microcirculation in the acute phase of thermal injury [73,74]. Moreover, C1inh did protect burn wounds in pigs against secondary ischemic effects and thus the progression of tissue damage into deeper dermal areas [72,74].

As previous studies focused on the short-term (i.e. up to the first 96h post-burn) effects of complement inhibitors on wound healing, we determined the effects of daily intravenously administered C1inh during 14 days post-burn in dorsal full-thickness burn wounds in rats [20]. We found that C1inh reduced the amount of granulation tissue and the infiltration of macrophages in the burn wound, and at the same time enhanced re-epithelialization. This coincided with a nonsignificant decrease in the presence of C3d and C4a in the dermis of burn wounds. In addition, in a burn model of C4-knockout mice, burn wound depth and neutrophilic granulocyte migration were found to be significantly reduced up to
10 days post-burn. Even more, these burn wounds healed without contracture, scar formation or hair loss, in comparison to wild type mice [75].

In summary, the complement system has been shown to play an important role in the local wound healing post-burn, however the prolonged activation of complement can induce local tissue damage and hamper the healing process. The application of a complement inhibitor (e.g. C1inh, sCR1) showed favorable effects in wound healing post-burn. This implicates that although complement is first beneficial for wound healing on the one hand, a prolonged and out of control complement activity may cause further damage like the progression of tissue damage into deeper dermal areas.

Figure 3. An overview of the complement activation after (burn) injury. Summarizing illustration of complement activation after burn. Burn to the skin causes excessive complement activation both locally in the skin and systemically in the blood (interrupted arrow) after 4–8h up to months post-burn. Locally in the skin this provides persistent inflammation, after >5 days up to months post-burn, which eventually results in impaired wound healing and further damage (e.g. progression into deeper dermal areas, increased vascular permeability, vascular thrombosis). Systemically, prolonged elevated complement induces several systemic adverse effects in other organs (e.g. heart, CNS, lungs).
THE SYSTEMIC EFFECTS OF COMPLEMENT IN OTHER ORGANS POST-BURN

In patients with severe burn wounds, systemic effects are induced in which complement plays an important role (table 2) [2,37,49,76–78]. This will be discussed in the next paragraph.

Several studies demonstrated cardiac dysfunction occurring after thermal injury, coinciding with increasing secretion of complement in blood [79–81]. In both mice and rats with burns up to 60% TBSA, cardiac inflammation, cardiac tissue injury and myocardial contractile depression were shown up to 8 days post-burn [82–84], which were positively correlated with the percentage TBSA burn [82]. In a rat burn wound model with burns of 1.5% of TBSA, we showed that daily systemic administration of C1inh during 14 days post-burn already reduced cardiac macrophage infiltration and facilitated the transition to the anti-inflammatory subtype of macrophages, in both atria and ventricles of the heart [20]. In rats with burns up to 60% of TBSA, increased complement (C5) activity was linked to cardiac contractile dysfunction and decreased left ventricular pressure in the early post-burn period up to 48h. This resulted in declined cardiac output (CO) and stroke volume, and increased systemic vascular resistance (SVR), heart rate and mean arterial pressure (MAP) [85–88].

In both rats and sheep, with burns of up to 30% of TBSA, burns have been shown to induce systemic injuries in the lungs too. During the first hours up to 48h post-burn, complement (CS) activity coincided with ICAM-1 upregulation, vascular permeability, neutrophil accumulation and decreased lung compliance [89–91].

Post-burn liver failure is generally characterized by edema in the liver. However, the liver is also a crucial organ in the APR, by generating the majority of the APPs after severe trauma, such as burn trauma [92–94]. The role of complement in liver failure after burn has not been studied yet. Increased CRP levels in the kidney were found in mice with burns of 10% of TBSA. It was shown that this CRP deposition coincided with severe renal tubular damage in the kidney [78]. Complement however was not analyzed in this study. Nevertheless, evidence from several studies shows that the complement system is involved in the pathogenesis of several liver [95], and renal disease [96], indicating that these organs may be intrinsically susceptible to complement-mediated injury. Complement activation in the liver and kidney can be explained by an impaired or inadequate control of the complement system by the body’s endogenous complement regulator proteins [96,97]. Therefore, it can be hypothesized that the complement system is involved in post-burn liver and renal disease [17,18], as the complement system is prolonged (out of control) elevated after burn too (especially in the blood).

Finally, in rats with burns of 30% of TBSA, burn-induced cerebral inflammation and edema were found. Furthermore, in hippocampal area, neuronal death coincided with C5a upregulation in the hippocampus [98]. Such as for the liver and kidney, in non-burn pathologies it has been shown that complement is
involved in diseases of the brain [99,100]. Therefore, it is not inconceivable that the complement system is involved in post-burn diseases in the brain too.

In summary, massive prolonged APR after severe burn trauma not only negatively affects the healing process locally of the burn wound, but additionally induces, partly life threatening systemic effects in several other organs, which may be related to complement [101]. However, most of these systemic effects of complement activation have been studied in animals and need to be confirmed in humans. In addition, the role of complement in other organs, that are also systemically affected post-burn e.g. stomach, small intestine, colon and skeletal muscle, has still to be studied.

### Table 2 – Post-burn systemic effects in various organ systems and the possible role of complement.

<table>
<thead>
<tr>
<th>Organ (systems)</th>
<th>Systemic post-burn effects</th>
<th>Possible role of complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>▪ Sarcomere contractility</td>
<td>C5a</td>
</tr>
<tr>
<td></td>
<td>▪ Inflammation</td>
<td>C3b, C4a</td>
</tr>
<tr>
<td>CNS</td>
<td>▪ BBB dysfunction</td>
<td>C1q, C3a, C5a</td>
</tr>
<tr>
<td></td>
<td>▪ Neuronal death</td>
<td>G2</td>
</tr>
<tr>
<td></td>
<td>▪ PMN recruitment</td>
<td>C5a</td>
</tr>
<tr>
<td></td>
<td>▪ Cerebral edema</td>
<td>C5a</td>
</tr>
<tr>
<td>Lungs</td>
<td>▪ PMN recruitment</td>
<td>C5a</td>
</tr>
<tr>
<td>Kidney</td>
<td>▪ Renal tubular damage</td>
<td>CRP</td>
</tr>
</tbody>
</table>

**FUTURE DIRECTIONS OF RESEARCH**

Several studies already described beneficial effects of complement inhibition after burn. In rats, sCR1 resulted in decreased vascular permeability and inflammatory cell infiltrates [70]. Furthermore, C1inh application reduced edema formation, inflammation induced tissue destruction, reduced the amount of granulation tissue and enhanced re-epithelialization in the skin in both rats [20] and pigs [72–74]. However, these beneficial effects of complement inhibition have been studied only in animals and therefore need to be confirmed in humans. In addition, the pre-clinical studies that made use of models close to humans, the studies in pigs, only focused on the short-term (i.e. up to the first 96h post-burn) effects of complement inhibition. Therefore it is necessary to unravel the long-term effects of complement inhibition, as it is known that complement is elevated for up to months after the initial burn trauma [17–19].

**CONCLUSION**

In conclusion, severe burn trauma is characterized by a prolonged APR, which starts approximately after 4–8h post-burn and persists for up to months after the initial burn trauma. Even though the APR is initially necessary for wound healing on the one hand, a prolonged (out of control) APR may cause
further damage. Especially, persistently increased levels of pro-inflammatory APPs like CRP and complement could result in adverse effects in burn patients, including increased vascular permeability and vascular thrombosis. In addition, persistently decreased levels of APPs like PAB, TRF, APO-A1 and RBP as found in the early period post-burn could point to e.g. decreased bacterial infection (TRF) and vascular thrombosis (APO-A1) after burn trauma.

The sustained APR not only negatively affects the local healing process of the burn wound, but has also a negative effect systemically, which may be related to complement.

Several studies already described beneficial effects of complement inhibition (e.g. C1inh, sCR1), on both local wound healing and systemic inflammation in other organs post-burn. Therefore complement inhibition might be an important starting point for therapeutic intervention in burn wound patients related to both local and systemic effects.

REFERENCES


