Immunological approaches and therapy in burns (Review)

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Abstract. Burns have become an important public health problem in the last two decades, with just over a quarter of a million deaths annually. Major burns are accompanied by a strong inflammatory response, which will most often lead to systemic response inflammatory syndrome, followed by sepsis and finally induce multiple organ failure. The main mechanism involved in wound healing after burns is the inflammatory process, characterized by the recruitment of myeloid and T cells and by the involvement of numerous cytokines, chemokines, complement fractions, as well as various growth factors. Inflammasomes, protein-based cytosolic complexes, activated during metabolic stress or infection, play a role in modulating and improving the defense capacity of the innate immune system. Nucleotide-binding domain and leucine-rich repeat protein 3 (NLRP3) inflammasome has been studied predominantly and several hypotheses have been issued. Restoring the balance between the pro-inflammatory response and the anti-inflammatory activity is the key element to effective therapy in burns. Severe burns require nutritional support and pharmacotherapy not only for burn area but for different pathological complications of burn injury. In-depth research is required to find new ways to modulate the defense capacity, to prevent the complications of abnormal immune response and to treat burn injuries efficiently.

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Contents

1. Introduction

Skin is one of the largest organs of the body that performs multiple functions including immunological, neurosensory and metabolic, water homeostasis and thermoregulation. The main role of the skin is to serve as a protective barrier against environmental aggression. When this barrier is damaged, the pathogens infiltrate the body that may result in infections (1).

An injury is described as a disruption of tissue continuity following trauma and is considered to be cured when the wound or inflamed area returns to the normal state. Dermal wound repair is a complex process, which involves a systematic progression of the phases that establish the integrity of the damaged tissues, involving different mechanisms, such as coagulation, inflammation, synthesis and matrix deposition, angiogenesis, fibroplasia, epithelialization, contraction and remodeling (2).

A burn can be defined as tissue destruction, caused by a variety of agents, such as heat, chemicals, electricity, and radiation. The presence of a burn on the skin causes loss of its protective function. Burns are some of the most common and devastating forms of injury. According to the World Health Organization, ~300,000 deaths are estimated to occur annually due to burns (3).

Each type of burn determines a wound healing response consisting of three evolutive periods: Inflammation, proliferation and remodeling. The response begins with the release of

histamine, free radicals and inflammatory cytokines, which induce vasodilation and tissue edema. This brings neutrophils and monocytes to the site of the lesion, which in turn provides chemotactic signals that recruit macrophages (4,5).

Wound repair depends on the process of neoangiogenesis, activation of the local immune response and the presence of both epidermal and fibroblast growth factors. Fibroblasts and other cells fill the space shaped by the lesion, along with new blood vessels and the extracellular matrix to form the granulation tissue, over which the keratinocytes will migrate, to restore the skin integrity (2,6).

The use of modern high-resolution techniques, such as reflectance confocal microscopy (RCM) may be a valuable tool in the assessing of the tissue morphological changes during the burn wound healing (7). At a depth of up to 250 μm, RCM enables a non-invasive evaluation of cutaneous tissue (8) including cutaneous blood flow changes (9).

In burns where the area of dermis and epidermis affected is enlarged, the repair process is much more complex. Here, the number of cells and matrices supporting the restoration of the skin is often reduced, or can even be missing, depending on the depth and severity of the lesion. This fact is responsible for the slow healing process, and scar development (10).

Deciphering the involvement of the immune system and the roles of specific immune cells in the evolution of tissue lesions in burns is important for restoring the immune balance, for finding new possibilities to treat these lesions, with efficient wound healing and for preventing the onset of complications.

The tissue lesion during burn causes a strong inflammatory response, which leads to the disturbance of immune function. Reestablishing the equilibrium between the pro-inflammatory response and the anti-inflammatory activity is the key element to effective therapy in burns. Comprehensive research is required to discover new ways to modulate the defense capacity, to prevent the complications of abnormal immune response and to treat efficiently the burn injuries.

The present review summarized the current data from the literature on the role of immune mechanisms in response to burn and on the main therapeutic interventions.

2. Burns and host immune response

Immediately after a burn, hepatic cells (hepatocytes, hepatic stellate cells, Kupffer cells, epithelial cells from the bile ducts or sinusoids), along with dendritic cells (DCs) and macrophages are involved in the synthesis of numerous immune cells within the innate immunity, such as cytokines, chemokines, adipocytokines, as well as catecholamines, cortisol and reactive oxygen species (ROS), which mediate both the local inflammatory response and systemic inflammation (11-13).

A neurogenic reaction based on the participation of the capsaicin-sensitive sensory nerve endings, mediating the release of substance P and the histamine discharge through the mast cell degranulation, is responsive for the local hyperalgesia (14,15).

Mechanisms of innate immunity are the first to react and recognize microbial pathogen-associated molecular patterns (PAMP) and danger-associated molecular patterns (DAMPs), by the instrumentality of pattern recognition receptors (PRRs), structures whose functions in cell signal mediation are well studied. As known thus far, the PRR category includes several receptors: Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptor (NLRs), retinoic-acid inducible gene I (RIG)-I-like receptors (RLRs) and C-type lectin receptors (CLRs) (16).

Natural killer (NK) and natural killer T (NKT) cells are the first cells of the innate immunity to react (17). These cells are activated by type I interferon (IFN) consisting of 13 subtypes [interferon-α (IFN-α), interferon-β (IFN-β) and interferon-ω (IFN-ω)] and type III interferons [including 3 members, interleukin-29 (IL-29) or interferon-λ1 (IFN-λ1), IL-28A or IFN-λ2 and IL-28B or IFN-λ3] (18). Once activated, these cells induce the synthesis of type II interferons, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α), within the innate immune response mechanism (19).

NK cells are involved in the immediate response (within the innate immunity) by having a cytolytic activity through the release of cytotoxic granules, which will attach to the infected cells and induce programmed cell death (20).

In addition to NK cells, myeloid cells (macrophages, neutrophils, and DCs) are similarly involved in the immediate immune response. These cells express receptors on the surface and will activate when stimulated by various PAMPs (21,22).

The inflammatory process plays a key role in the healing of burn injuries, due to the myeloid cells and various subpopulations of T cells, that are recruited at the lesion site. The myeloid cells, such as neutrophils, monocytes, mast cells and macrophages are recruited from the circulatory flow, their function is regulated by some cytokines and several growth factors, which stimulate the activity of fibroblasts and keratinocytes in the proliferation and remodeling phases during the burn healing processes (23,24).

It is known that in the skin and epithelial tissues, the subpopulation of γ-δ T-cells (γδ T-cells), which express γδ T-cell receptors (γδ TCR), predominates. Studies have shown that the patients with severe systemic inflammatory response syndrome (SIRS) presented increased circulating concentrations of γδ T-cells, a hypothesis also confirmed in studies in experimental induced burn mice (25-27).

Also, in other studies, the concept that γδ T-cells play a key role in regulating infiltration, inflammation and healing of burn wounds, has been proposed. Thus, it was observed that besides the infiltration with myeloid and myeloid-derived suppressor cells, at the place of the burn, the increase of the number of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), as well as of some chemokines [macrophage inflammatory proteins-1α and -1β (MIP-1α, MIP-1β), monocyte chemoattractant protein-1 (MCP-1)], was also highlighted (28,29).

Numerous clinical research studies in animals have shown that in cases of severe burn injuries a major disturbance of the intestinal mechanical barrier occurs. These major injuries are induced by a multitude of factors/mediators, acting through direct or indirect mechanisms (30-34). Finnerty *et al* (34), observed that after extensive burn injuries, considerable amounts of pro-inflammatory cytokines are released into circulation. These pro-inflammatory cytokines, such as IFN-γ, TNF-α, IL-1β, IL-4, IL-6, IL-13, IL-17, cause increased intestinal permeability, besides affecting the intestinal barrier.

Activated T cells proliferate and differentiate into effector or memory T cells. After activation, $CD4^+$ T-cell subset become engaged in mediating the adaptive immune response by producing T helper type 1 (Th1) cytokines, pro-inflammatory cytokines (IL-2, IFN-γ, TNF-α) involved in the cell-mediated immune response. $CD4^+$ T-cells also secrete T helper type 2 (Th2) cytokines and anti-inflammatory cytokines (IL-4, IL-10, IL-13) which modulate the humoral immune response. It has previously been established that the T cells can self-regulate their activity through the synthesis of IL-10 and the transforming growth factor-β (TGF-β), which inhibit T cell proliferation and cytokine production, either directly or via other cytokines (35-37).

Some studies have reported correlations between the serum concentrations of some cytokines (IL-6, IL-8, IL-10) or MCP-1 with the size of the lesions at 24 or 48 h after the burn. It was also found that the serum concentrations of IL-10, even have a prognostic value, when measured in hospitalized patients, but also at 24-48 h after the burn (38). In animal studies, high levels of IL-10 were evidenced even 84 days after the burns occurred (39).

Another study direction consists of the inhibition of the immune defense activation by modulation of the activity of the complement system. Non-specific immune response activation occurs through the involvement of the C3 and C5 complement fractions, which increase the ability of defense by direct and indirect action on microbial agents and facilitate wound healing (40-43). Under burn conditions, systemic upregulation of the complement cascade and the C-reactive protein occurs, which increases the risk of generalized inflammation and delays wound healing (44-46). Experimental investigations have shown favorable effects of using a C1 fraction inhibitor, to limit tissue destruction in case of experimentally-induced burns in pigs (47). Other researches revealed that the treatment with an inhibitor of the C4 fraction prevented the development of hypertrophic scars in a burn model in mice (48).

Stress that is the result of burn injury causes disruptions of the immune system as a consequence of suppressing the cellular immunity (Th1 cell activity, which mediates pro-inflammatory processes) and stimulating the humoral immunity, involved in the anti-inflammatory response (35,49,50).

Recent research has shown that the granulocyte-colony stimulating factor (G-CSF), with stimulatory effects on defense capacity, is an essential element in modulating the immune response, with favorable effects in the evolution of burn wound healing (51). Clinical investigations evidenced that the administration of a recombinant glycoprotein of the granulocyte-macrophage colony-stimulating factor 2 (GM-CSF2), a drug approved for use by the Food and Drug Administration (FDA), has been associated with an increased percentage of healing and a higher survival rate in patients with septic burns (44,52). It was suggested that the effects of GM-CSF could be the effect of the restoration of macrophage and monocyte dysfunctions, as well as the increase of monocytes and neutrophils in the case of the burn injury (44).

At the same time, following mast cell degranulation histamine is released, thus stimulating the production of Th2 cells and IL-10, with consecutive vasodilation (36,53).

3. The involvement of inflammasomes in burn and host immune response

Studies performed over the last decade have brought to light the involvement of inflammasomes (multi-protein complexes) in innate immunity. As a caspase-activating complex, the inflammasomes are involved in the mediation and/or transmission of intracellular signals. Most notable is their ability to recognize pathogenic microorganisms with the added benefit of providing a warning sign in certain pathological conditions such as oxidative stress, insulin resistance or lipotoxicity (54). Once inflammasomes are stimulated, they can activate the pro-inflammatory cytokines, such as IL-1β and IL-18. These complexes, in which inflammasomes participate, often contain NLR molecules (22 different NLR proteins, being identified in humans) (16). Other elements may also be part of this complex, such as cysteine-dependent aspartate-directed protease-1 (caspase-1), cysteine-dependent aspartate-directed protease-5 (caspase-5), a pyrin and C-terminal caspase-recruitment domain (PYCARD) also called apoptosis-associated speck-like protein, containing a caspase recruitment domain (ASC) (55,56).

Cysteine-dependent aspartate-directed protease-1 (Caspase-1). The enzyme caspase-1, part of the cysteine protease family (synthesized as an inactive zymogen, pro-caspase-1) plays a vital role in the start-up of inflammatory reactions by allowing the transformation of cytokine precursors for IL-1β and IL-18 (pro-IL-1β and pro-IL-18) in functional molecules, mature and biologically active cytokines (55).

IL-1β and IL-18 help host in the defense against infection by generating Th1 and Th17 adaptive immune responses. For the most pro-inflammatory cytokines, the production is chiefly regulated at the transcriptional level. However, for the secretion of IL-1β and IL-18, an additional step is required. Initially, they are synthesized via TLR or RLR stimulation as inactive precursors, lacking a signaling peptide. Afterward, NLR-mediated inflammasome activation catalyzes the post-translational modification needed for their subsequent secretion and bioactivity (57,58).

Inflammasomes. Inflammasomes are protein-based cytosolic complexes that are switched on during metabolic stress or infection. By activating caspase-1, these factors can initiate the maturation of specific cytokines such as IL-1β, IL-18 or type I IFNs, to modulate and enhance the innate immune system defense capacity (59).

The complexes include NLR proteins. The NLR family consists of 22 proteins, of which most are commonly associated with inflammation: NLRP1, NLRP3, NLRP6, NLR family caspase activation and recruitment domain (CARD) containing protein 4 and 5 (NLRC4 and NLRC5), adaptor proteins (ASC), caspase-1 and the caspase-5 in some cases (60).

Inflammasomes are defined by their association with receptors, NLRs or RLRs: NLRP3 inflammasome, the absent in melanoma 2 (AIM2) inflammasome, RIG-I inflammasome, and γ interferon-inducible protein 16 (IFI16) inflammasome. The NLRP3, AIM2 and RIG-I inflammasomes are produced in response to viral infections (61).

Nucleotide-binding domain and leucine-rich repeat protein 3 (NLRP3) inflammasome. Of all the inflammasome, the NLRP3 complex is still the best studied. Viral (Hepatitis C virus, Influenza A virus, Respiratory syncytial virus, Vaccinia virus, Rabies virus, Herpes simplex virus 1, Varicella-Zoster virus), bacterial or fungal pathogens are typically regarded as NLRP3 agonists. Such stimuli are identified during an infection, which can lead to NLRP3 inflammasome formation (61).

Structurally, NLRP3 exhibits a tripartite arrangement consisting of N-terminal caspase-recruitment domain (CARD), a pyrin domain (PYD), a nucleotide-binding oligomerization domain (NACHT or NOD) and a C-terminal leucine-rich-repeats (LRRs) domain (tripartite arrangement from N terminus to C terminus is PYD-NACHT-LRRs) (62). This serves as a scaffolding platform, enabling the formation of the inflammatory complex. Once these active complexes are formed, they will trigger the activation of the caspase-1 enzyme that will convert pro-IL-1β and pro-IL-18 precursors into biologically active mature cytokines (59,63).

The activation of inflammasome NLRP3 is induced by bacterial ribonucleic acid, lipopolysaccharides (LPS) and by various other endotoxins. The activation can also be caused by endogenous means such as extracellular adenosine triphosphate (ATP), ROS, cholesterol/uric acid-based renal crystals and the DAMP biomolecules (58).

Feng *et al* (64), have shown that bacterial endotoxins, such as LPS, play an important role in disrupting the intestinal epithelial barrier after burn injury. It seems that LPS act by activating the NLRP3 inflammasome. To confirm this hypothesis, cell culture studies were performed (Caco-2 cell monolayer), which were treated for 24 h with the LPS. The LPS caused a considerable increase in Caco-2 cells of NLRP3, ASC, caspase-1, IL-1β, pro-IL-1β and pro-IL-18 (65).

It has been found that in severe burns, the formation of reactive oxygen species (ROS) and the production of free fatty acids increases (FFA) in circulation (66). Recent studies have hypothesized that NLRP3 inflammasome would play a key role during the acute phase after burns, an important period in patients' survival. Its expression is positively correlated with mortality (67). Vinaik *et al*, observed that NLRP3 inflammasome has an intense activity in the adipose tissue of the burned patients within the first 7 days after the burn (68).

The NLRP3 inflammasome activity is modulated by fatty acid synthase (Fasn), the main enzyme involved in lipid synthesis, a process that is activated in inflammatory processes. This inflammasome has an effect on the balance between lipolysis and lipogenesis. Thus, the deficiency of expression of NLRP3, at one hour post-burn, may influence lipid metabolism, in the sense of affecting *de novo* lipid synthesis and shifting the balance to lipolysis (68,69). It is also hypothesized that inhibition of NLRP3 action would decrease the inflammatory response in local tissues (68,70).

Another important finding, at one hour post-burn, was an early synthesis of the Th1 pro-inflammatory cytokines (TNF-α and IFN-γ), that play a role in activating pro-inflammatory macrophages MIP-1α, MIP-1β, respectively. No increases in IL-6 and IL-1β or anti-inflammatory cytokines were observed. Corroborating the two conclusions, it can be stated that the lack of expression of the NLRP3 inflammasome after burn leads to increased chemotaxis and activity of macrophages (68).

4. Burns and the therapy

The therapy of a burn is difficult and complex, because it aims, first, to modulate the intensity and duration of the pro-inflammatory reaction and then of the anti-inflammatory response (45). Proper management goals are represented by the decrease of the inflammatory phase, shortening its evolution and, the prevention of fibrosis, restrictive scars, respectively the associated functional disturbances (71).

Scientific research has been focusing on mechanisms and microscopical changes from cutaneous inflammatory processes (72).

Over time, there have been multiple and varied experimental and clinical studies targeting different modalities of modulation of the immune system, to obtain promising results in the healing of burns.

The first studies focused mainly on reducing the inflammatory phase by using corticosteroids or by decreasing the level of pro-inflammatory mediators such as TNF-α, platelet activating factor, IL-1β (44). In this regard, it was found that the administration of anti-TNF-α antibodies, anti-IL-1β respectively, resulted in reduction of the necrosis extension in the burn wounds in rats (51,73,74).

An efficient modality of blocking the activation of the immune system is the inhibition of mast cell degranulation by the use of disodium chromoglycolic acid, which diminishes the severity of the experimentally-induced thermal burns in rats (71). Preventing the recruitment and activation of immune cells is also another possibility of influencing the activity of the immune system, under burn conditions (75).

Literature data highlight various other modalities of direct or indirect modulation of the defense capacity, to prevent and remove the immune suppression associated with burn injuries (24). One of the target elements of these immunomodulatory therapies is interleukin IL-7, which plays a key role in the activity of T cells and the maintaining of homeostasis (52,76,77).

Severe burns are associated with systemic metabolic disturbances and patients require nutritional support and pharmacotherapy. The diet should be rich in protein, amino acids, carbohydrate and glucose, and also, supplemented with arginine, glutamine and essential fatty acids (78). Up to 6,000 kcal/day is needed for adequate energy intake (79).

World Health Organization's recommendations as drug treatments for burn area are: i) Initial therapy - tetanus prophylaxis; cleansing with 0.25% (2.5 g/liter) chlorhexidine solution, 0.1% (1 g/liter) cetrimide solution, or another mild water-based antiseptic; silver sulfadiazine cream; no alcohol-based solutions should be used;ii) Daily therapy - systemic antibiotics for β-hemolytic Streptococcus wound infection or septicemia; topical antibiotics (alternating: silver nitrate, 0.5% aqueous solution; silver sulfadiazine, 1% miscible ointment; mafenide acetate, 11% in a miscible ointment) (79).

Severe burns determine a hypermetabolic response with harmful consequences including extensive catabolism, insulin resistance, inflammation, and supraphysiologic levels of catecholamines. Various facets of the post-burn pathophysiologic response have to be neutralized with alternative uses and combinations of drugs (80). Pharmacological intervention in treating different pathological complications of burn injury consists

of: insulin (to enhance muscle protein synthesis, quicken the healing time of skin graft donor sites, and to alleviate the loss of lean body mass); metformin (to reduce hyperglycemia); oxandrolone (to increase protein synthesis, diminish weight loss, and amend the skin graft donor sites' healing); propranolol (to reduce heart rate, skeletal muscle catabolism and thermogenesis); insulin-like growth factor and recombinant human growth hormone (to reduce muscle catabolism and to increase the immune function); glucagon-like-peptide 1 analogs (to improve insulin secretion) (78,81).

5. Conclusions

Burns cause traumatic injury, which provokes large disruptions in both local and systemic immune response, including repression of immune function. Disruption of the immune balance determines increased sensitivity of the body to infections, by translocation of bacteria or their products (from the intestinal level), development of sepsis that will eventually lead to systemic inflammatory response syndrome and multiple organ dysfunction syndromes in critically burn patients. All these aspects cause the patient with a burn injury to have a difficult and complex therapeutic behavior.

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Not applicable.

Patients consent for publication

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Competing interests

The authors declare that they have no competing interests.

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