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Review Article

The biology of burn injury

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Abstract: Burn injury is a complex traumatic event with various local and systemic effects, affecting several organ systems beyond the skin. The pathophysiology of the burn patient shows the full spectrum of the complexity of inflammatory response reactions. In the acute phase, inflammation mechanism may have negative effects because of capillary leak, the propagation of inhalation injury and the development of multiple organ failure. Attempts to mediate these processes remain a central subject of burn care research. Conversely, inflammation is a necessary prologue and component in the later-stage processes of wound healing. In this review, we are attempting to present the current science of burn wound pathophysiology and wound healing. We also describe the evolution of innovative strategies for burn management.

Key words: apoptosis – burn injury – wound healing – zone of stasis

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Burn injury

Burn trauma represents a type of injury that can be caused by heat, freezing, electricity, chemicals, radiation or friction. Burn injuries are highly variable in terms of the tissue affected, the severity and resultant complications. Muscle, bone, vascular, dermal and epidermal tissue can all be damaged with subsequent pain because of profound injury to nerves. Depending on the location affected and burn depth, a burn victim may experience a wide number of potentially fatal complications including shock, infection, electrolyte imbalances and respiratory failure. Beyond physical complications, burns can also result in severe psychological and emotional distress because of long-term hospitalization, scarring and deformity (1).

Background and incidence

The majority of burns are a result of flames with 55%, followed by scald burns with 40%. Flame burns are often associated with inhalational injury and other concomitant trauma. Mild burns occur with an annual rate of 600/100 000 inhabitants, severe burns occur with a rate of 5/100 000 inhabitants. The age of the patients influences the cause of trauma significantly. In children, the majority (70%) are scald burns because of hyperactive behaviour and contact with hot liquids. In adolescent and young adults, the primary cause of burns is improper handling of fire and flammable liquids. In adults, flame burns rank first, 1/3 of them being accidents at work (2).

Classification

Burn wound depths are internationally classified in the degree I–III (Table 1). The following figures present a histological overview as well as clinical pictures of various burn wound depths (Fig. 1).

The depth of burn wound evolves over time especially with partial thickness wounds. Wounds that start as superficial partial or deep partial burns may progress to deep partial or deep burns over period of 2–4 days after burn injury. As evidenced by histologic studies, burn injury is a dynamic process that peaks at about 3 days. Necrosis in the zone of stasis has been thought to be responsible for this progression. Recently, apoptosis has been recognized to be present in the zone of stasis and may contribute to the wound progression (3). Because of this unique pathophysiology, patients with partial thickness burn wounds need to be evaluated for depth of the wound periodically. As a rule, partial thickness burns that are predicted not to heal by 3 weeks should be excised and grafted. A potential new promising tool in the evaluation of indeterminate burn wound depth could be an innovative multispectral optical system, which enables a parallel acquisition of spectrally filtered images and allows to depict burn degrees (4).

Table 1. Description of clinical characteristics of burn wounds of various depth

Degree/depth	Aetiology	Layer of skin involved	Appearance	Pain	Healing time
Superficial I°	Sun exposure, hot liquids with low viscosity and short exposure	Epidermis only	Pink to red, moist, no blisters	Moderate-Severe	3–7 days
Superficial partial IIa°	Hot liquids, chemical burns with weak acid or alkali, flash	Superficial (papillary) dermis	Blister, red, moist, intact epidermal appendages, blanches of pressure	Severe	1–3 weeks, long-term pigment changes may occur
Deep partial IIb°	Flame, chemical, electrical, hot liquids with high viscosity	Deeper layer (reticular) dermis	Dry, white, non-blanching, loss of all epidermal appendages	Minimal	3–6 weeks, with scars
Deep III°	Flame, electrical, chemical, blast, self immolation	Full thickness of skin and in to the subcutaneous fat or deeper	Leathery, dry, white or red with thrombosed vessels	No	Does not heal by primary intention, requires skin graft

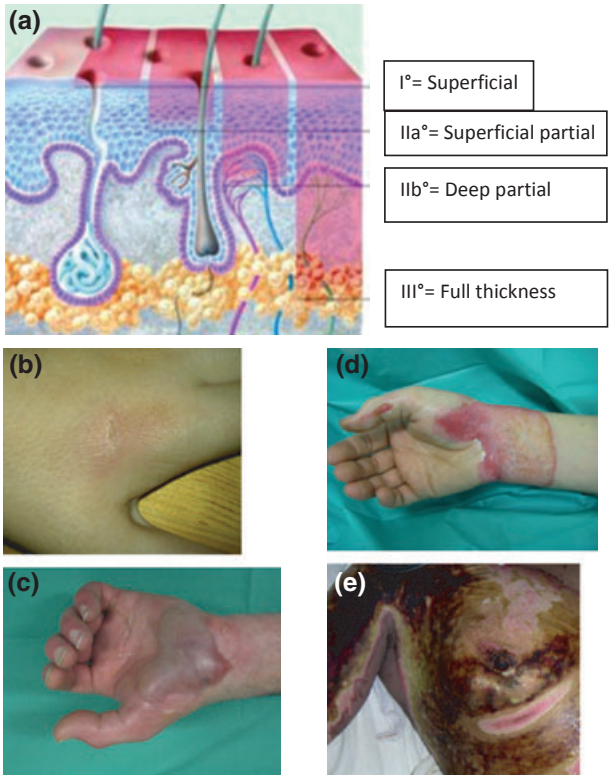

Figure 1. Classification of burn wound depth. (a) histologic overview, (b–e) clinical examples of burn degrees (b) superficial = I°, (c) superficial dermal = IIa°, (d) deep partial = IIb, (e) full thickness = III.

Burn wound extent

Universally, Wallace's rule of nine is used for rapid estimation of the extent of burn wounds (Fig. 2) (5).

While it allows accurate estimation in adults, following rule of nine may result in inaccurate estimation in children under the age of 15. Head amounts to more than 9% of body surface in children. In contrast, the lower extremities account for less than 9% body surface. The Lund-Browder chart provides accurate estimation of extent of burn wounds in paediatric patients (6).

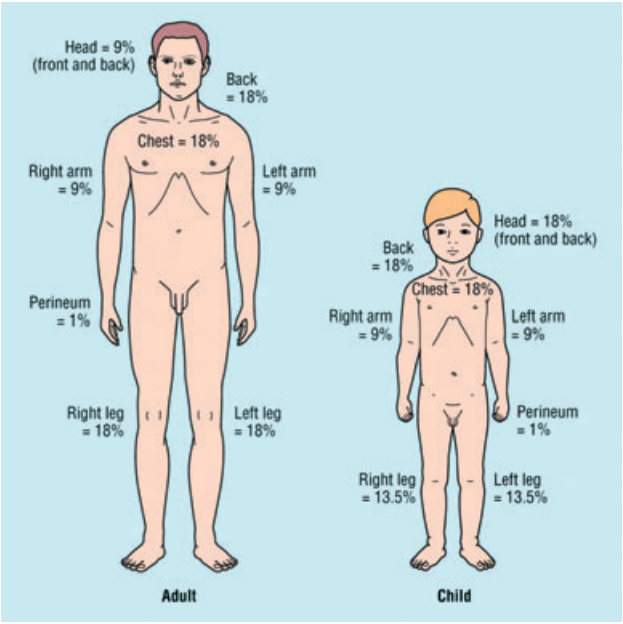

Figure 2. Clinical evaluation of burn wound extent (Wallace's rule of nine).

Pathophysiology

Local effects of burns

Prolonged exposure to temperatures higher than 40°C leads to denaturation of proteins and finally loss of their plasma membrane integrity. This process is rapid and may only take a second when exposed to temperatures higher than 60°C, i.e. flame burns. The local changes result in the clinical picture of coagulation necrosis. Temperature and duration of contact have a synergistic effect as described later (Table 2).

Table 2. Relationship of duration of temperature exposure and occurence of full thickness burn [modified to Moritz and Henriquez(7)]

Temperature in °C	Duration of exposure in sec
45.0	3600
54.4	30
60.0	10
69.0	1

Initial topical cooling immediately after burn maximizes epithelization and decreases scarring. Room temperature water (15°C) is equal to cooled water (2°C) and superior to ice water in terms of early re-epithelization and also late scarring (8).Through molecular structural alterations, toxic metabolites as well as antigens and immunomodulatory agents are released resulting in burn shock pathophysiologic effects. The local mediators released are histamine, serotonin, bradykinin, nitric oxide, oxygen-free radicals and products of eicosanoid acid cascade (prostaglandin, thromboxane), TNF, interleukins. Histamine is most likely to be the mediator most responsible for the early phase of increased microvascular permeability seen immediately after burn. Histamine causes large endothelial gaps to transiently form as a result of the contraction of venular endothelial cells. Studies demonstrated that the pathogenesis of burn oedema in the skin appears to be related to the interaction of histamine with xanthin oxidase and oxygen radicals (9).

The local changes in burn wounds are classified by *Jackson* into three zones (Fig. 3) (10).

The zone of coagulation at the central focus of injury is generally thought to consist of devitalized tissue. The most peripheral zone is termed the zone of hyperaemia, characterized by vasodilation, inflammatory changes without structural damage. Between these zones, an intermediate region of indeterminate prognosis arises which is termed the zone of stasis (10). The zone of stasis is often best identified in mid to deep dermal burns and represents a region of vascular stasis and ischaemia. From a clinical perspective, it is this region which poses some of the greatest challenges for the burn team. This tissue has the potential to heal or alternatively to progress to a full thickness lesion. Clinically this ischaemic area can only be salvaged, if revascularization is achieved within a few days. Otherwise the irreversible tissue death is inevitable. The phenomenon of ischaemia-reperfusion events in the zone of stasis has been described in the past (11). This zone is exposed to oxidative stress resulting from reperfusion injury, particularly after sustaining major partial thickness burns. Reperfusion injury results in predominantly apoptotic cellular death. The apoptosis in the zone of stasis may contribute to progressive tissue loss. Previous studies showed a reduction in the apoptotic rate after inhibition of inducible NO synthase in partial thickness burn wound (12).

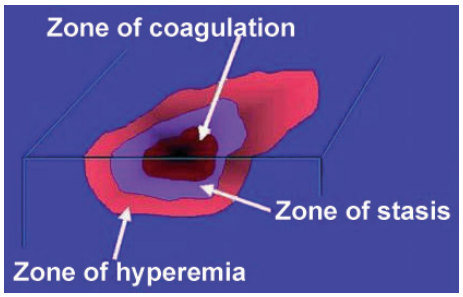

Figure 3. Zones of burn injury.

Wound healing mechanisms after burn injury

The epidermis, because it is derived from ectoderm, is capable of regenerative healing. Deep dermal burns heal slowly, if at all, and depend to a large degree on the migration of keratinocytes from surrounding uninjured skin. This is also the mechanism whereby the interstices of meshed split-thickness skin grafts are filled in by keratinocytes that migrate from the skin bridges. The biology of epidermal renewal is currently an area of intense research. Translation into a useful technology that will improve wound healing and outcomes may soon be a reality (13).

Major burn injury is followed by an enormous inflammatory response. While it is transient systemically, burn wound may suffer from the effects of acute influx of inflammatory mediators and growth factors for a prolonged time. The burn wound contains a variety of cell types including platelets, neutrophils, lymphocytes, macrophages and fibroblasts, whose activity is regulated by a complex interplay of multiple cytokines as well as host neuroendocrine mechanisms. The principal molecular regulators controlling the evolution of the burn wound include vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and transforming growth factor-ß (TGF-ß). Transforming growth factor (TGF)-beta is essential for the activation and proliferation of fibroblasts during the initial stage of wound healing. Sustained activity of TGF-beta is associated with hypertrophic scars and wound contraction leading to disfigurement and deformity (14). In experimental models, use of neutralizing antibodies against TGF-beta has shown reduction in scar formation without adversely affecting wound healing (14). New burn wound management strategies would involve reducing the extent of inflammatory response related to acute injury and regulating fibroblast–myofibroblast activity to reduce scarring and contracture without affecting wound healing and strength of wound repair.

Another recent study concluded that fibrocytes are present in abundance in acute burn wounds, and these cells

appear to be involved in the local response to burn wound and may correlate with the later development of hypertrophic burn wound scarring. This might be very important in the future to predict those who will develop hypertrophic scar (15).

A potential promising new approach for wound healing and treatment involves the use of stem cells for skin regeneration. Investigators showed that collagen–glycosaminoglycan constructs seeded with mesenchymal stem cells improve healing and keratinization, decreased wound contracture and improves vascularization. It may lead to the development of skin substitutes from the source of pleuripotent cells (16).

Agents, either systemic or topical applied are used to improve wound healing of burn wounds in several studies. The application of a novel nitric oxide (NO)-containing topical gel improved re-epithelization associated with the increased abundance of fibroblasts and inflammatory cells (17).

The systemic effects of burns

The systemic pathophysiologic changes following thermal injuries affect multiple organs and body systems leading to clinical manifestations including shock, intestinal alterations, respiratory and renal failure, immunosuppression and others. Major thermal injury is associated with extreme hypermetabolism and catabolism being the principal metabolic manifestations after successful resuscitation from the shock phase of the burn injury. The metabolic response in burn patients is biphasic wherein the initial ebb phase is followed by a hypermetabolic and catabolic flow phase of injury (18). The increased oxygen consumption/metabolic rate is in part fuelled by an evaporative heat loss from wounds of trauma victims, but likely also by a direct central effect of inflammation upon the hypothalamus (18). Recent advances in the comprehension of underlying mechanisms of systemic complications have uncovered part of cellular and molecular processes, which are involved in triggering complication of thermal injuries.

Pathophysiologic changes that occur upon severe thermal injuries involve the cardiovascular system (myocardial depression, oedema formation, hypovolaemia), pulmonary (local vasoconstriction, oedema), gastrointestinal (impairment of gastrointestinal motility and absorption, splancnic vasoconstriction, loss of mucosal barrier function with bacterial translocation, increased intragastric pH), haematopoietic (anaemia, immunodepression) and renal (splancnic vasoconstriction). All these changes lead to important clinical syndromes such as shock, respiratory insufficiency and acute respiratory distress syndrome (ARDS), paralytic ileus, sepsis and renal failure. This complicated situation involves a variety of pathological events which will condition the clinical outcome of burned patients.

Gut/digestive system

The digestive system is impaired by neutrophil influx with extravasation in the lamina propria (postburn day 1), increased intestinal myeloperoxidase (postburn day 3), decreased epithelial cell proliferation, migration and E-cadherin expression (postburn day 3), increased E. faecalis bacterial translocation (postburn day 3) (19) and massive apoptosis and moderate necrosis (20). Such situation is dominated by two major events: first of all, by the oxidative stress secondary to hypoperfusion or delayed perfusion. Secondly, by TNF-alpha (α) production induced by macrophages primed by gamma delta ($\gamma\delta$) T cell after a thermal injury (21). It is concluded that delayed resuscitation is responsible for mucosal apoptosis, and that oxygen-free radicals generated during the process of ischaemia-reperfusion injury also have a negative effect on mucosal cells (22). Ischaemia triggers oxidative stress leading to the generation of molecular mediators that ultimately will cause two types of cellular damage: necrosis and apoptosis. These molecular mediators consist of mucosal or monocyte-macrophage-derived radical oxygen synthase (ROS) and NO synthase (NOS) activity that will lead to H_2O_2 and NO production, which are toxic to the enterocytes (23). Inhibition of inducible NOS reduced the apoptotic rate in the gut. Also the changes in gut mucosal homeostasis after severe burn are affected by the activation of TNF-α-TNF receptor interaction (24). $\gamma\delta$ T cells were associated with increased TNF-α expression and gut epithelial turnover in the small bowel after severe burn (25).

Leucocytes and lymphoid organs

Thermal injury-associated specific immune deficiency occurs despite indicators of systemic activation of the lymphoid compartment. Severely injured trauma patients usually experience T-cell depletion but only a subset also develops T-cell anergy (26). Interestingly, Leptin showed a positive protective effect against apoptosis (27). Also a direct relationship between nitric oxide (NO) and apoptosis occurrence was demonstrated. NO had an influence on the synthesis of immunoregulatory and proinflammatory cytokines, such as interferon gamma (IFN-δ), interleukin-2 (IL-2), interleukin-6 (IL-6) and tumor necrosis factor (TNF) (28). Another study demonstrated that NO produces cytostatic, apoptotic and necrotic effects on activated T cells. Early immune suppression (3 days after burn injury) stimulates CD8(+) T-cell late (14 d) hyperresponsiveness. These data could have broad clinical implications for allogenic skin grafting and rejection (29).

Heat shock proteins (HSPs) were reported to protect cells against a variety of environmental stresses. Major burns were shown to cause long term, enhanced expression of HSPs in neutrophils along with increased oxidative activity and decelerated apoptosis. The enhanced expression

of HSPs could regulate the oxidative stress response and the lifespan of neutrophils in burn patients (30).

Morphologic changes were described in the spleen (31). After 2 h from thermal injury, numerous B lymphocytes accumulated in the markedly expanded marginal zone of the splenic white pulp. After 5 h, B lymphocytes were present in the marginal zone as well as in the lymphoid sheath, and follicles were markedly decreased in number with an increase in tingible bodies and tingible body macrophages. After 12 h, the splenic white pulp became atrophic with the appearance of a small number of large blastic cells and mitotic figures. After 24 h, the splenic white pulp was still atrophic with a decrease in the number of lymphocytes, especially B lymphocytes. After 48 h, the lymph follicles were slightly enlarged and a small germinal centre occasionally appeared. A recovery in T-cell number was observed only after 48 h. The percentage of CD4+ and CD8+ T cells in the spleen remained altered for 10 days after thermal injury (31).

Muscle

The skeletal muscle weakness that usually occurs after thermal injuries often causes hypoventilation and dependence on respirators, a condition that increases morbidity and mortality. Patients with severe burns [total body surface area (TBSA) of >30%)] had weaker muscle tonus even years after the trauma, suggesting either an inability to fully recover or an insufficient rehabilitation (32). Morphologic changes present in muscles following distant thermal injuries include mitochondrial alterations (33) and intracellular lipid accumulation (34). The endocrine status is also markedly altered with a sustained increase in proinflammatory 'stress' hormones such as cortisol, other glucocorticoids and catecholamines by the adrenal medulla and cortex. These hormones exert a catabolic effect and the intensity depends upon the percentage of TBSA involved (35). Furthermore, a variety of inflammatory circulating factors have been implicated including prostaglandins, IL-1, IL-6 and TNF-α (36–39). Also the effect of surface cooling, which is frequently used after burn injuries to reduce tissue damage, on striated muscle and its apoptotic rate after thermal burns was studied. It was concluded that the protective effect of surface cooling on traumatized tissues was because of an attenuation of the microvascular inflammatory response and associated response with an inhibition of the apoptosis process (40).

Liver

The liver furnishes the metabolic substrates to the organism and it also mounts part of the inflammatory response. Studies have demonstrated that many of the metabolic perturbations of burns and sepsis may be because of inflammatory cytokines and these activate specific transcription of liver genes including classic acute phase response markers, complement, kinin, clotting and fibrinolytic protein systems (41). Hepatomegaly is a common finding at autopsy in severely burned patients. Large intrahepatocytic fat droplets within hepatocytes and cholestasis were important contributors to hepatomegaly. Other common histologic findings included congestion, centrilobular necrosis and cholestasis (42).

Heart

A depression of cardiac output immediately follows burn injury. As hypovolaemia ensues, diminished plasma volume and reduced venous return then contribute to a continued cardiac output. However, even when plasma volumes are restored and both arterial pressure and urinary output are normalized, a persistent reduction in cardiac output remains. Many studies found that, during burn traumas, TNF-α, IL-1β and IL-6 secretion are secreted by cardiomyocytes, and all these cytokines were correlated with an increased cardiac dysfunction (43–45). Major burn injury also alters the flux of calcium ions between the sarcoplasmic reticulum and the cytoplasm (46). Lipopolysaccharide (LPS) has been clearly demonstrated to induce cardiac apoptosis in many studies (47). Endotoxin induces a TNF-α-dependent apoptotic cascade in the myocardium by caspases activation, which contributes to the development of cardiac dysfunction (48). Furthermore, LPS is a potent inducer of inducible NO synthase (iNOS) in cardiac myocytes activating the apoptotic pathway (49).

Lungs

The lungs appear to be particularly susceptible to oedema formation, regardless of whether the burn injury is accompanied by an inhalation injury. Within the first 24 h after a severe burn, nearly all patients develop generalized oedema which is related to the size of the burn and the timing, composition and amount of fluid resuscitation (50). Early after large burns, there is a pronounced increase in pulmonary vascular resistance and pulmonary wedge pressures that derive from a general vasoconstriction of the microcirculation. Hypoproteinemia is also a main factor in the development of pulmonary oedema (51). In fact, this results from the loss of plasma proteins from the burn wound and the intravenous infusion of large volumes of crystalloids fluids during the first hours of resuscitation. In addition to reducing plasma oncotic pressure, hypoproteinaemia appears to alter the intestinal matrix in a way that facilitates the movement of fluid across the capillary endothelium (52). The physiologic consequences of such injuries include impaired gas exchange and reduced airway compliance. Furthermore, the morbidity of severe cutaneous burn injuries is increased when accompanied by smoke inhalation, a

stimulus for the inflammatory response and ARDS (53). Histologic alterations of lungs in burns injury include intestinal oedema, hyaline membrane formation and neutrophils sequestration within the lung (54). The increase in pulmonary microvascular permeability is probably mediated by neutrophils activations and TNF-α production (55). Complement, neutrophils and oxygen-derived free radicals all appear to be intimately involved in the pathogenesis of burn-induced acute lung injury. Complement activation generates the potent anaphylatoxin C5a, resulting in neutrophil superoxide and hydrogen peroxide release, enhanced chemotaxis and neutrophil–endothelial cell adherence (56). The lung is also an important source of TNF-α release after severe burns. TNF- α, IL-6 and IL-8 are present within the bronchoalveolar lavage fluid of patients 48 h after burns and probably contribute to the increased microvasculature permeability (57).

Therapeutic strategies/clinical treatment

The main therapeutic factors are as follows:

1. Surgical intervention (early excision/skin grafting).
2. Volume therapy.
3. Therapy of sepsis and multi organ failure.
4. Nutrition.
5. Rehabilitation.

A main column of effective burn treatment represents the early surgical intervention. Increasingly aggressive surgical approaches with early tangential excision and sufficient wound closure probably represent the most significant change in recent years, leading to improvement in mortality rates of burn victims at a substantially lower cost. Children particularly have benefited from timely and extensive surgical intervention (58). Early excision of the devitalized tissue appears to reduce the local and systemic effects of mediators released from burned tissue, thus reducing the progressive pathophysiologic derangements. Tangential excision removes necrotic tissue while preserving as much of the underlying viable tissue as possible. A number of different instruments can be used to perform tangential excision. The Rosenberg knife, Goulian knife, Watson knife and Versajet water dissector (59) are all used around the world.

For secondary procedures, recent studies investigated the role of fat injection (lipofilling) into scars to improve function and appearance. They reported the clinical appearance and subjective patient satisfaction 6 month after the procedure suggested considerable improvement in the mimic features, skin texture and thickness. Histologic examination showed patterns of new collagen deposition, local hypervascularity and dermal hyperplasia in the context of new tissue, with high correspondence to the original (60).

For the emergency medical treatment, the initial care provided involves special attention to the airway to ensure that it is patent and that oxygenation, ventilation and circulation are not compromised. A delay in fluid resuscitation increases the incidence of renal failure and mortality. Several volume restoration formulae are being used, the most common is Parkland formula: 4 ml of lactated Ringer's solution per kilogram of body weight per percentage TBSA burn during the first 24 h (61). The first half is administered over the first 8-h postinjury, with the remaining half divided equally over the next 16 h. This formula is only a guide, and other indices of adequate resuscitation must be taken into consideration. Interestingly, recent results from Parkland Medical Center in Dallas, Texas (the home of the Parkland formula) showed that their patients were receiving approximately 6 ml/kg/% TBSA burned, which was in excess of their formula (62). They concluded that the Parkland formula only represents a resuscitation ''starting'' point, and that urine output should drive later volumes.

Future perspectives

Extended burn injuries cause systemic modification of the patient's clinical conditions that usually worsen the prognosis. Hypovolaemia, respiratory insufficiency and shock are only a few of such changes. The pathophysiologic mechanisms that underlie them are complex and involve almost all organs and body systems. Apoptotic processes following burn injury have been described increasingly. Future potential therapeutic targets which need to be addressed are the clinical significance of this systemic phenomenon and the development of promising antiapoptotic drugs with the intriguing possibility to block the 'systemic apoptotic response' and its pathophysiologic effects. For instance, specific therapies could derive from the experimental application of selective antiapoptotic drugs i.e. selective inhibitors of death receptors, caspases or the proteasome complex.

Another potential pathway of new therapeutic options after thermal injuries is gene expression profiling, which has inspired new hope for finding genes involved in complications resulting from burn injury. Therefore, genetic dissection of burn injury should be carried out in a global context. A similar investigative approach is the understanding of Pseudomonas aeruginosa burn wound infections by profiling gene expression. Pseudomonas represents a key opportunistic pathogen causing severe acute and chronic nosocomial infections. It is prevalent in burn wounds and generally multi-drug resistant. Understanding the genetic programs underlying infection is essential to develop highly needed new strategies for prevention and therapy. Future efforts should focus on the identification of direct virulent factors and elucidation of their mode of action. These new data sets obtained from global transcriptional profiling could be essential for the development of new targets and options for the prevention and intervention of burn wound infections.

In summary, in the last decade, major effort is made to establish new strategies to improve clinical outcome of burned patients. In our opinion, the key points of future research should be infection control, especially against multi-resistent bacteria, new wound healing pathways and genetic dissection of the burn injury.

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