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Perturbed mononuclear phagocyte system in severely burned and septic patients

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Abstract

Burn is one of the most common and devastating forms of trauma. Major burn injury disturbs the immune system, resulting in marked alterations in bone marrow hematopoiesis and a progressive suppression of the immune response, which are thought to contribute to increased susceptibility to secondary infections and the development of sepsis. Immunosuppression in severely burned and septic patients leads to high morbidity and mortality in these patients. Mononuclear Phagocytes System (MPS) is a critical component of the innate immune response and play key roles in burn immunity. These phagocytes are the first cellular responders to severe burn injury after acute disruption of the skin barrier. They are not only able to internalize and digest bacteria and dead cells and scavenge toxic compounds produced by metabolism, but also able to initiate an adaptive immune response. Severe burn and sepsis profoundly inhibit the functions of DC, monocyte and macrophage. Adoptive transfer of MPS or stem cells to severely burned and septic patients that aim to restore MPS function is promising. A better understanding of the roles played by MPS in the pathophysiology of severe burn and sepsis will guarantee a more rational and effective immunotherapy of severely burned and septic patients.

Keywords

Macrophage; Monocyte; Dendritic cells; Trauma; Hematopoiesis; LPS; Apoptosis

Introduction

Burn injuries are an unrecognized global health care problem, with millions of people getting burned and over 300,000 death each year throughout the world. Burns covering more than 30% TBSA are associated with stress, inflammation, hypermetabolism and catabolism that lead to profound morbidity and mortality. Despite the advances in burn and critical care medicine have been made, sepsis and multiple organ failure are still the major causes of death in burned patients. In severely burned patients (over 40% TBSA), about 75% of deaths result from sepsis, regardless of improvements in antimicrobial therapies. Burn injury disturbs the immune system, resulting in a progressive immunosuppression that is thought to contribute to the development of sepsis.

Phagocytes are an essential component of innate immunity. These innate immune cells are the first cellular responders to burn injury after acute disruption of a large part of the skin barrier, hence are vital to host defense. Cell types of phagocytes including monocytes (and their mature form, tissue macrophages), dendritic cells, and neutrophils are professional phagocytes, that are not only able to internalize and digest bacteria and other dead cells and

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scavenge toxic compounds produced by metabolism, but also can indirectly kill bacteria, parasites, and viruses by producing inflammatory mediators. The pattern recognition receptors, such as TLR, expressed either on their surface or intracellular compartment can recognize pathogens or non-pathogens through binding to pathogen-associated molecular patterns and endogenous stress signals termed danger-associated molecular patterns. Once stimulated, these cells should rapidly and robustly produce pro-inflammatory cytokines. Following the migration of circulating monocytes into the burn site, these phagocyte cells function by controlling the infection through bacterial uptake and destruction of the pathogen as well as production of soluble factors that initiate activation and recruitment of additional immune cells to the sites of injury. Concurrently, antigen-presenting cells are activated upon contact with antigen protein and begin to migrate toward the lymph node and undergo maturational changes including enhanced expression of MHC, CD80, CD86 and CD40, decreased antigen uptake ability and increased antigen processing and presentation. In the lymph node, activated DCs and their interaction with T cells and B cells can initiate and shape the adaptive immune response. Initially, during the first several days post-burn, immunologic response to severe burn injury is pro-inflammatory, later it becomes predominately anti-inflammatory in an effort to maintain homeostasis and restore normal physiology.

In this review, we intend to provide a short survey of basic biology and function of MPS with a focus on recent advances in how and why this system is perturbed in severely burned patients. The potential therapeutic strategies to prevent immune suppression in the severe burn patients are also briefly reviewed. Since a high percentage of death cases in burn patients result from sepsis and some similarities between the immune deficiency resulted from severe thermal injury and sepsis-induced immunosuppression, disturbed MPS in sepsis will also be included in this review.

II Mononuclear phagocyte system

Monocytes, macrophages and DCs are a group of professional phagocytes that play a crucial role in maintaining immune homeostasis and mounting an immune response against infection (Table 1). Phagocytes were first discovered more than 100 years (1882) ago by Russian-born evolutionary biologist Elias Metchnikoff who was interested in the phagocytic capacity of cells. Through a continuing investigation stretching over more than a century, we now know that these phagocytes are key components of the innate immunity and play crucial roles in both innate and adaptive immune responses in vertebrates. Given that monocytes, macrophage and DCs have a common origin in hematopoietic stem cells in bone marrow and similar functions such as phagocytosis and migration, they are grouped as Mononuclear Phagocyte System. The initiating concept of MPS only includes macrophage, monocytes and their bone marrow precursors. Although initially described in 1868 by Langerhans, DC did not join MPS until 2002. Neutrophils are an important member of innate cellular immune system. Given MPS and neutrophils clearly share several functionalities, including avid phagocytosis, similar kinetic behavior under inflammatory/infectious conditions, and antimicrobial and immunomodulatory activities, despite being grouped into MPS until 1995, currently neutrophils and MPS are commonly viewed as distinct lineages. Therefore, recent advances of neutrophils associated with burn injury will not be included in this review.

Monocyte, macrophage and dendritic cells originate from the same progenitors in bone marrow. Cells in bone marrow that are committed to the mononuclear phagocyte lineage are the macrophage and DC progenitor, at which point, erythroid, megakaryocyte, lymphoid and granulocyte fates have been precluded. MDPs give rise to monocytes and common DC progenitors. Whereas monocytes circulate in the blood, bone marrow and spleen, do not

proliferate in a steady state, but can transform into macrophage upon migration into tissues or differentiate into DCs in an inflammatory state. CDPs strictly give rise to DC lineage, plasmacytoid DCs and pre-DCs, which subsequently give rise to DC. During this process, transcription factors, such as PU.1, MafB, KLF4 and c-Maf, play a very important role in deciding the fate of MPS. For example, PU.1 is an essential factor required for macrophage-versus DC-choice of monocytes. High expression of PU.1 is required for monocytes differentiation to DC. C-Maf and MafB are macrophage-inducing factors. In addition to transcriptional factors, cytokines or growth factors such as Flt3L, GM-CSF, M-CSF, IL-4, are also essential for the differentiation of MPS and generation of the subtypes of macrophage and DCs. For example, whereas exposure to GM-CSF and IL-4 induces differentiation of human and mouse monocytes into DCs, exposure to M-CSF induces monocytes to differentiate into macrophages. Combination of IFN- γ and M-CSF induces the differentiation of M1-like macrophages, whereas addition of IL-4 to M-CSF induces the differentiation of M2-like macrophages.

It is increasingly clear that monocytes, macrophages and DCs are not homogenous populations. Mouse blood monocytes express CD115, CD11b and low levels of F4/80 and can be divided into two subsets based on the expression of Ly6C, CCR2, CD62L and CX3CR1; Ly6C^{hi}CX3CR1^{lo}CCR2⁺CD62L⁺ and Ly6C^{lo}CX3CR1^{hi}CCR2⁻CD62L⁻ monocytes. The former cell type is the main subset of CD115⁺ monocytes in the blood. These monocytes can be selectively recruited to inflamed tissues, where they produce high levels of TNF- α and IL-1 that potentially fuel the inflammation or exacerbate tissue damage. Therefore, murine Ly6C⁺ monocytes were termed inflammatory monocytes. In contrast, bone marrow-resident monocytes express high levels of CX3CR1 and lack the expression of Ly6C, CCR2 and L-selectin (CD62L). Ly6C⁻ resident monocytes are involved in the renewal of resident macrophages and dendritic cells. The third subset of monocytes is the patrolling Ly6C⁻ monocytes that are responsible for the very early inflammatory response and supposed to differentiate into alternatively activated macrophage during infection. Accordingly, based on monocyte-specific marker CD14 and the activation marker CD16 as well as chemokine receptor CCR2, human monocyte can be divided into three subsets, CD14(+)CD16(-)CCR2(+) (Mon1), CD14(+)CD16(+)CCR2(+) (Mon2) and CD14(low)CD16(+)CCR2(-) (Mon3) subsets.

Macrophage heterogeneity is classified based on tissue location, such as Kupffer cells (macrophages in liver), microglia (macrophages in the central nervous system), dermal macrophages, spleen and lymph nodes (sinus histiocytes), lungs (alveolar macrophages). However, based on their functions and cytokine secretion, they can be generally divided into two types: CAM (or M1) or AAM (or M2). Upon activation by IFN- γ and LPS in vitro, macrophages increase their production of pro-inflammatory cytokines and expression of MHC II molecules and CD86, which lead to increased antigen presentation and cellular immunity. In contrast, alternative activation of macrophages by IL-4 and IL-13 will lead to increased tissue repair and humoral immunity. M2 can be subdivided further into three subsets such as M2a, M2b and M2c under different stimuli.

Since their identification as APCs in 1973 by Steinman and Cohn, DCs have been one of the hottest immunology areas of research. DCs bridge the innate and adaptive immune system. DCs are not homogenous populations either. DCs can be divided into different subsets based on their location, migratory pathways and specific markers. Generally, they can be divided into two major populations. One is classical or common DCs (also can be grouped into nonlymphoid tissue migratory and lymphoid tissue-resident DCs), the other is pDCs, which are also called natural interferon-secreting cells. The main functions of migratory and resident DCs are the maintenance of self-tolerance and the induction of specific immune response against invading pathogens, whereas the main function of pDC is to respond to

viral antigens with massive production of type I interferon (IFN- α). Nonlymphoid tissue DCs can be distinguished by tissue where they are located. For example, DCs in the epidermis are called LCs and DCs in the dermal are called DDCs, which can migrate to the lymph node upon collecting the antigens from the peripheral and then present these antigens to T cells in the LN. LCs can be distinguished by high CD1a expression. In the dermis, CD1a⁺ CD11c⁺ and CD1a⁻ CD11c⁺ DCs are present. CD1a⁺ human DCs are the primary immunostimulatory subsets with high levels of IL-12 secretion. Based on CD103 expression (a ligand of the cell adhesion molecule E-cadherin), DCs in other non-lymphoid tissues also can be divided into CD103⁺DC and CD103⁻DC, whose functions are not fully understood. Lymphoid-tissue-resident DCs, such as thymic cDCs and splenic cDCs, do not migrate through the lymph and their functions and their life-histories are restricted to one lymphoid organ. They can be further divided into three subsets: CD4⁻CD8⁺DCs, CD4⁺CD8⁻DCs and CD4⁻CD8⁻DCs. Of the three splenic DC subsets, CD8⁺ DC are the most efficient at cross-presentation of cellular, soluble, or latex bead-associated antigens.

Mononuclear phagocytes promote both innate and adaptive immune responses and, as such, are essential for the maintenance immune homeostasis and against the infection. The mononuclear phagocyte system has an important role in defense against microorganisms either by direct clearance by phagocytosis or indirectly by cytokine secretion. Monocytes help in replenishing dendritic cells and macrophages in normal conditions and in tackling inflammation of any kind. Macrophages remove senescent erythrocytes, leukocytes, and megakaryocytes by phagocytosis and digestion. Both M ϕ and DCs can present antigens to T cells, whereas DCs are the most potent antigen-presenting cells and the only APCs that can activate the naïve T cells. Once activation is triggered, the activated mononuclear phagocytes system should be disposed of in a controlled and effective way. Otherwise, their activation left unchecked could do significant harm to already inflamed tissue. Therefore, MPS is not always beneficial to host, they have very serious side effects at some time. In severely burned or septic patients, overwhelming systemic inflammation to infection or severe trauma (called SIRS) is mostly mediated by the cytokines secreted by MPS. In addition, MPS-mediated chronic low-grade inflammation is the main mechanism of many chronic diseases such as diabetes, cardiovascular disease.

III Perturbed MPS in Severely Burned Patients

Burn injury is an extreme trauma that induces a profound and altered immune response. The current concept of the immune response to severe trauma is viewed as an early systemic inflammatory response followed by a compensatory anti-inflammatory response and in the end, the suppression of adaptive immunity. Recently, a new genomic study of patients with severe trauma and burn injury shows that those human immune responses to the severe trauma occur rapidly and simultaneously instead of one after another. No matter what the model is, the final outcome of severe blunt trauma and burn injury usually is the global suppression of both innate immune response and adaptive immunity. In regards to the MPS, myeloid commitment shifting toward monocytopoiesis, hyporesponsive monocytes, dysfunctional macrophage and DC depletion are the hallmark of disturbed immune response post-burn (Table 2). Most of therapeutic studies are experimental treatment based on animal models. It needs to be stressed here that due to the complexity of the pathophysiology of severe burn and sepsis, demonstrated benefits from the experimental treatments in animal models rarely translate into a successful treatment for human patients.

DC depletion in severely burned patients

Immature DCs originate in the bone marrow and migrate throughout the body. In the normal state, the immature DCs will capture antigens upon recognizing the invading pathogens or other foreign bodies. At this point, the primary function of the immature DC is to capture

antigens. Once captured, antigens are processed and migrate to the lymph nodes where they stimulate CD4⁺ T helper cells or CD8⁺ cytotoxic T cells, by MHC class II and MHC class I presentation pathway respectively. In addition to antigen capture, processing and presentation, a key component of DCs is the presence of co-stimulatory molecules, which are critical to the activation of T cells and for the proper homing of DCs before and after antigen capture. Upon appropriate stimulation, DCs undergo further maturation and migrate to secondary lymphoid tissues, where they present antigens to T cells and induce an immune response. Any factors that interfere with the process of DC maturation and activation will lead to DC dysfunction.

Clinical studies have demonstrated a significant reduction of circulating DCs (including mDC and pDC) in the peripheral blood of burn patients [1]. One of these studies compared DC frequency in burn patients with 46.2% TBSA burn (ranging between 23–68%) and volunteers and found that although about 1.7% of PBMCs from control subjects were composed of mDCs, it was only 0.53% of PBMCs in burn patients [1]. Besides alterations in DC frequency, burn patients also have decreased expression of HLA-DR and CD11c. However, there were no significant changes in neither HLA-DR nor CD11c expressions in CD45⁺ leukocytes within the first week after burn, but were down-regulated from weeks 1 to 2 after burn and remained lower until 4 weeks in comparison to controls [1]. Our own data show that DC is reduced or depleted in severely burned patients and there is higher expression of MafB in mDCs (CD11c⁺HLA-DR⁺), pDC (CD123⁺HLA-DR⁺) and monocytes (CD14⁺HLA-DR⁺) compared to healthy controls (unpublished data).

In addition to the peripheral blood, DCs in the skin from patients (TBSA 15–70%) are significantly lower compared to healthy subjects and non-burned skin from burned patients. Those DCs express less HLA-DR and TLR-4. Since TLR4 is important for DC sensing LPS and recognizing of bacteria. Therefore, it is not only the numbers of skin DCs from the burn patients are reduced but also their functions are impaired. In addition, DC's T cell stimulatory function is also impaired. An *ex vivo* burn injury model to study DC function in burned patients observed a strong decrease in the T cell stimulatory function of both LCs and DCs after burn injury. However, no differences were observed in the migration capacity of both LCs and dermal DCs in an *ex vitro* culture of burned skin, nor in the expression of co-stimulatory molecules. It has been reported that disturbed lipid metabolism could bias the differentiation of CD1a⁺ and CD1a[−] monocyte-derived dendritic cells [2]. Circulating monocytes from septic patients differentiate rapidly and overwhelmingly into CD1a[−]DCs that induced a 3-fold increase in regulatory T cells (Foxp3⁺ T cells) [3].

Animal models of severe burn also showed the same trend as patients in DC frequency and functions, whereas models with 20–25% TBSA are far more controversial. Using a mouse model of 25% TBSA, one study examined the effects of burn injury on splenic cDC and pDC responses to TLR9 activation. They found that splenic cDCs' cytokine production profile in response to TLR9 activation was anti-inflammatory dominant, with high production of IL-10. CD4⁺ T cells activated by these cDCs were defective in producing Th1 and Th17 cytokines. On the contrary, in another study using mice burn model with 25% TBSA, the author did not find marked changes of DC function and number change at day 1 and 7 of burn. Burn did not change the relative frequencies of lymphoid, myeloid or pDCs and surface molecules such as CD40, CD80 and CD86. In addition, the authors failed to find the suppressed reactivity of these DCs to the stimulations of TLR2, 4 and 9. The antigen presentation ability was unchanged compared to sham mice as well. Therefore, these findings in models of 20–25% TBSA suggest that DC frequency and functions are associated with the severity of burn injury. DC depletion and dysfunction have been consistently observed in major burn patients or burn models (>25% TBSA), whereas in

minor and moderate burn injury the function status of DC function varies from one lab to another.

DC depletion is not a result of bone marrow failure. In fact, LSK cell pool (cells negative for lineage markers [Lin] and positive for Sca1 and c-Kit) is expanded due to the greater demand for immune cells for the immune response in a sepsis model [4]. A study conducted by Howell and colleagues showed that Lin-IL-7R-cKit+FcγRhiCD34+GMP was significantly increased on day 5 and 7 post burn in a mouse burn model. However, severe sepsis induces the reduction of bone marrow multipotential progenitors, CMPs and GMPs, and defective myeloid differentiation [5]. Burn injury impairs DC differentiation in GMPs [6]. Although it has not been known whether HSC senses infection directly, it does respond to LPS [5]. Both LPS signaling and reduction in bone marrow cellularity alone can induce the expansion of bone marrow hematopoietic stem and progenitor cells. The emergency myelopoietic response to severe trauma redirects myeloid cell differentiation in severely burned and septic patients, but the differentiation fails in time in the chronically inflamed patients.

A key pathophysiological event in sepsis is the widespread apoptosis of immune cells. Systemic apoptosis is considered to be the cause of DC depletion in sepsis. Tinsley et al. found that the remarkable expansion in FDCs early in sepsis followed by their profound depletion by 48-hours after onset of sepsis. Both FDCs and IDCs undergo caspase-3 mediated apoptosis. Interestingly, research using CLP sepsis model found that macrophage and DC depletion was inhibited by either overexpression of the antiapoptotic protein, Bcl-2, or deletion of the proapoptotic protein, Bim. Using mice overexpressing Bcl-2 in selected hematopoietic cells, overexpression of Bcl-2 confers protection during sepsis to the splenic “immature” and “monocyte-like” macrophage and splenic DCs [7]. Therefore, modulation of the apoptosis pathway might be a novel therapy for sepsis through blocking sepsis-induced depletion of DC and macrophage.

DC depletion and dysfunction can also be attributed to oxidative stress. Reactive oxygen species are known inducers of both apoptosis and necrosis under inflammatory conditions and have been proposed as common stimulators of cell death. In a sepsis model, increased ROS generation was found to lead to rapid depletion of FDCs from septic spleen. These results suggest that sepsis along with a concomitant release of TNF-α and ROS might regulate the process of cell death from an initial apoptotic mode to a secondary necrotic one at later time points [8].

TLR-dependent signaling also plays a major role in modulating the maturation process and survival of DC in septic models. Studies in a septic mouse model using TLR2(-/-), TLR4(-/-), and TLR2/TLR4(-/-) mice found that TLR2 and TLR4 are involved in the mechanisms leading to depletion of spleen DC. By 24-hours post CLP, a decrease in the relative percentage of CD11c^{high} splenic DC occurred in wild-type mice but was prevented in all knockout mice. In wild-type mice, sepsis dramatically affected both CD11c⁺CD8α⁺ DC and CD11c⁺CD8α⁻ DC [9].

Due to their pivotal role in regulating innate and acquired immune response, DCs are the promising candidates for therapeutic manipulation. The advantage of using DCs to treat immune system dysfunction in severely burned or septic patients is their ability to comprehensively regulate immune system function [10]. One of the strategies is the adoptive transfer of either naïve DCs or gene modified DCs to CLP mice [11]. This resulted in significant improvements in the survival rates and pulmonary immunosuppression. An alternative way to restore DC immunocompetence is using GM-CSF. Treatment using GM-CSF is a safe and effective measure to restore mHLA-DR expression and cytokine release in

patients with severe sepsis/septic shock and sepsis-associated immunosuppression. GM-CSF treatment improved the course of disease severity in a multicenter clinical trial. It also indicated that GM-CSF therapy in sepsis is safe and effective for restoring monocytic immunocompetence. GM-CSF also can be used in the form of controlled slowly released from chitosan gel system for burn wound healing. Realistically speaking, all of these treatments are at very early stage and there is a long way ahead. Successful treatment is largely dependent on our understanding of burn pathophysiology.

Monocyte hyposponsiveness

Early studies showed both aberrant monocytosis and abnormal monocyte functions in burn patients over 30 years ago. By simple morphological analysis and differential cell counts, they found there are more monocytes in the circulation in patients with burns (severity range from 20% TBSA partial-thickness to 91% TBSA with 10% full thickness). Another early study showed that thermal injury induced dysfunctional monocytes with abnormal chemotaxis. Using a modified Boyden chemotactic chamber and human C5a and a bacterial chemotactic factor (b-CTX) as chemotactic factors, Altman et al. compared the chemotaxis of monocyte from burn patients and healthy control. Patients with burns of ~20% of TBSA had normal monocyte chemotaxis, while monocytes from patients with burns 20% had depressed chemotaxis. This defect was most pronounced in patients with burns 40% TBSA. These findings demonstrate that monocyte chemotaxis is markedly impaired in severely burned patients and is associated with burn severity. More recently, these findings are supported by a study showing that burn injury induces diminished expression of CD47 expression on monocytes in humans [12]. CD47 is a membrane protein, which is involved in the increase in intracellular calcium concentration that occurs upon cell adhesion to extracellular matrix [13]. Reduced CD47 on monocyte following burn injury may be one mechanism of abnormal monocyte chemotaxis.

Clinical study with severe burn patients (>30% TBSA) showed that expression of HLA-DR on circulating monocytes was decreased in every patient at days 2–3 after burn. But from days 4–6, this expression increased in patients who survived whereas it remained low in non-survivors. Patients who were going to develop secondary septic shock exhibited significantly lower HLA-DR expression on monocyte as early as days 7–10 after burn. In addition, the duration and the intensity of this decrease were associated with severe septic complications and pejorative outcome [14]. Decreased HLA-DR expression and cytokine production can be observed at day 28 post septic patients [15].

When considering the circulating numbers of monocytes in human septic patients, there are some inconsistencies between studies. It has been observed that there is a significant increase in the circulating numbers of CD14brightCD16– and CD14brightCD16+ monocytes in sepsis [15]. But, CD14dimCD16+ monocytes were significantly decreased in adult male patients (age 59 ± 9.7 years) but not in neonates and children with sepsis. This may reflect age-related differences in the differentiation and/or survival of CD16+ monocytes.

Animal model of severe burn and sepsis also showed the increased inflammatory monocytes (CD11b+ LY6C++) in the compartments of bone marrow, blood and spleen at days 8 post-burn in mice receiving scald burn, but no significant changes in resident monocytes (CD11b + LY6C+) were observed [16]. The observation of myelopoietic shift toward monocytopoiesis in burn patients is substantiated by the findings in burn and septic animals. Changes in bone marrow myelopoiesis occur at 72-hours following thermal injury and sepsis and not at earlier time points of 24- and 48-hours. In addition to induce circulating monocytosis, burn injury also induces a concomitant granulocytopenia in the peripheral blood as well. Colony-forming assays demonstrated an increase in the growth potential of monocyte progenitors and a significant decrease in granulocyte progenitors after burn and

burn sepsis. Flow cytometric analysis of early (ER-MP12, also named as CD31) and late (ER-MP20, also named as Ly6c) monocyte progenitors showed an increase in monocyte lineage growth in burn sepsis [17].

Lineage commitment and differentiation of bone marrow progenitors toward monocytes is dysregulated in severely burned and septic patients. GMPs are the progenitors for granulocytes and monocytes. However, due to the higher expression of transcription factors like MafB and receptor for M-CSF, GMPs is skewed to favor monocyte commitment. A burn and trauma research group from Loyola University (Maywood, IL, US) has shown that experimental thermal injury with sepsis is associated with both increased monocytopoiesis and increased nerve-stimulated release of bone marrow norepinephrine. Further studies from the same lab found that β -adrenergic receptors on bone marrow monocyte progenitors are changed by conditions of burn sepsis. Therefore, the observed enhanced monocyte production in sepsis is mediated in part by sympathetic activation. More information about catecholamines and the differentiation and functions of MPS will be addressed in the following section [18].

Cell-based therapy has targeted monocyte hyporesponsiveness. In a mouse model with peritoneal sepsis induced by gram-negative bacteria, the therapeutic effects of intravenous bone marrow-derived human MSC were examined. They found that MSC treatment markedly increased the bacterial phagocytosis of monocytes [19]. Propranolol restores the tumor necrosis factor- α response of circulating inflammatory monocytes after burn injury and sepsis. Moreover, stem cell therapy has become a potential novel therapy for burn wound healing. Most of the times, a severely burned patient has immune dysfunctions of both monocyte and DC. As such, the future studies should explore how to 'correct' both immune dysfunctions at the same time.

Macrophage dysfunction

Macrophage's activities are more complicated in severely burned and septic patients. Severe thermal injuries induce an initial systemic inflammatory response that is followed by a compensatory anti-inflammatory phase. Due to the large number of macrophages in the tissues and the major cell source of cytokines during injuries, hyperactive macrophages are essential for SIRS during the first phase of the injury. However, during the anti-inflammatory or sepsis phase, the macrophage dysfunction is a key element of the global post-burn immunosuppression.

Macrophage hyperactivity with increased production of mediators, such as TNF, IL-6, IL-1 and PG-E₂, is markedly enhanced following thermal injury. Macrophages are the major producers of these pro-inflammatory mediators in burn patients. The activation of a pro-inflammatory cascade after burn injury appears to be important in the development of subsequent immune dysfunction, susceptibility to sepsis and multiple organ failure. Severity of burn determines M ϕ 's activity. Cohen et al. showed that cytokine secretion by macrophage was associated with severity of burn injury and development of sepsis. Whereas, mild burn injury led to higher levels of TNF- α , IL-1, and IL-6, severe injury and sepsis attenuated this response significantly. Both bone marrow progenitor-derived macrophages and peritoneal macrophages have the same cytokine responses to similar microenvironments. Their results suggest that the same progenitor-derived macrophages essentially became hyporesponsive with the increased severity of burn and septic challenge [20].

HMGB1 and TLR ligand signaling contribute to the increased cytokine secretion of macrophage after severe burn. HMGB1 is a nuclear protein that may be released actively from monocytes and macrophages or passively from necrotic or damaged cells. The

HMGB1 levels are increased upon patients' admission in parallel with IL-10 increase and highly associated with following induction of IL-8 and IL-6 [21]. In a 20% full thickness burn rodent model, Cairns et al. examined the cytokine secretion profile of purified splenic macrophage in response to TLR2 ligand peptidoglycan or TLR4 ligand LPS. Burn injury resulted in a steady accumulation in the periphery of CD11b+F4/80+ macrophages, which was attributed to their resistance to TLR-induced cell death. Macrophages purified early after burn injury upregulated TLR2 and TLR4, followed by a decrease in their expression late after burn injury. Although the authors failed to find increased cytokine secretion at 3 days post burn, at 14 days the macrophage secreted more IL-10 and less TNF- α [22].

Macrophage activity can be sensitized by endotoxin. During the assessment of burn insult effect on endotoxin shock, Enomoto et al. found that 50% mortality was observed in rats that were given 30% TBSA followed by an intravenous injection of a sublethal dose of LPS (5 mg/kg) 24-hours later. On the contrary, all control rats that only received LPS injection survived. Interestingly, the destruction of Kupffer cells by the use of gadolinium chloride completely prevented the mortality due to LPS in burn-injured rats, indicating that Kupffer cells are involved in this phenomenon. Moreover, mortality was completely prevented by gut sterilization with antibiotics. This study demonstrates that endotoxin released from permeabilized intestine exacerbate burn outcome by sensitizing Kupffer cells [23].

Macrophages are essential for burn wound healing. Attracted to the wound site by chemoattractants at the site of injury, monocytes from the bloodstream enter the area through blood vessel walls. Once they are in the wound site, monocytes mature into macrophages. In this process, MCP-1 is one of the mediators potentially important to the repair process. Elevated MCP-1 has been observed in both patients and animals. Macrophages, fibroblasts, endothelial cells, and keratinocytes can produce MCP-1 in response to inflammatory stimuli. Failure to produce MCP-1 in the burn site may delay the inflammatory response and wound healing. Less macrophage recruited into wound site in aged mice due to lower MCP-1 content might be the cause of delayed wound healing in aged subjects. Macrophage infiltration into the wound was observed as early as 4 days post injury. This influx of macrophage into wound resulted from preceded elevated MCP-1 in the wound site. TNF- α that is known to induce chemokine production was elevated systemically in burn mice. Elevated MCP-1 was associated to elevated TNF- α since administration *in vivo* with TNF- α neutralizing antibody reduced the dermal levels of MCP-1 [24].

Some early studies were conducted in burn models 2 or 3 decades ago. Loose et al. found that serum obtained from rats at 4 or 24 hours post-burn had suppressive effects on phagocytosis of macrophages [25]. Of these serum factors, PGE2 and COX-2 play an important role in the suppressed macrophage activity in severe burn and sepsis. PGE2 has long been recognized as a negative regulator of myelopoiesis and inhibits macrophage proliferation in *ex vivo* culture [26]. Macrophage isolated from C57BL/6 female mice with 25% TBSA full-thickness burn secreted higher levels of COX2 and PGE2 and lower level of IL-12. The COX-2 inhibitor NS-398 suppressed PGE2 production and normalized IL-12 production [27]. Overexpression of PD-1 on macrophage mediates the deterioration of macrophage function during severe sepsis, thereby providing a potential novel therapeutic target [28].

Macrophage can play a bad role for patients with burn injury. In normal unburned mice, abscess formed in the burn site surrounded with M1M ϕ is essential for host resistance against intradermal infection. However, dominant M2M ϕ in the burn site inhibited M ϕ conversion from resident M ϕ to M1M ϕ , thus in burned mice, M2M ϕ promoted sepsis development [29]. Our unpublished data show that the inflammasome pathway is activated in fat tissues from burned patients indicated by an increased caspase-1+ CD14+ macrophage

cells in stromal vascular fraction, which is hypothesized as a factor for insulin resistance in burned patients.

There are some experimental therapies targeting macrophage hyperactivity. O’Riordain explored the ways to modulate hyperactivity by using the combination of IL-1 beta and indomethacin sodium therapy. When utilized in burned mice, this treatment mitigated overproduction of cytokines and thus improved the survival rates [30]. Though promising, these outcomes of targeting proinflammatory mediators in animal models are rarely successful in human patients [31].

Strategies of restoring macrophage activity in severely burned and septic patients include GM-CSF and stem cells. Therapy of sepsis with IFN- γ /GM-CSF sufficiently restores the activity of macrophages, which are adequate for the development of a protective Th1-like immune response [32]. CAM (M1M ϕ) is a major effector in the first line of host antibacterial defense because of its secreted IL-12. The appearance of alternatively activated macrophages (M2M ϕ) in severely burned mice inhibits macrophage conversion from resident M ϕ to M1M ϕ by CCL17 and IL-10. PGE2 is a key mediator in the gram-negative sepsis-induced suppressed macrophage production. PGE2’s neutralizing antibody greatly reversed the inhibition of GM-CFC growth induced by burn, burn plus infection or endotoxin [33]. Adoptive transfer of CD34(+) cells during murine sepsis significantly improves late-sepsis survival by improved macrophage phagocytosis and resists lethal LPS-induced inflammatory shock by globally reduced levels of circulating inflammatory mediators [34].

VI Summary and concluding comments

Malfunctional mononuclear phagocyte system resulted from dysregulated bone marrow progenitor differentiation is a hallmark in severely burned and septic patients. Disturbed MPS is associated with high morbidity and mortality in these patients. However, the molecular mechanisms are far from being fully understood, that remains a major barrier for effective clinical treatment of these patients. Traditionally, the pathophysiology of severe burn and sepsis is viewed as the hypotheses of “mediators theory”. The main theory of the hypotheses is that cytokines released from immune cells circulate in the blood and induce the secondary distant organ inflammation and failure. Macrophages are the dominant cells in this process, which secrete a large amount of TNF- α and IL-1. Recently, accumulating studies show a different theory, called “the apoptosis theory”. Apoptosis of lymphocytes has been described in both patients and mouse models. Findings demonstrate that there are significantly different sensitivity to sepsis between monocytes and lymphocytes, where sera from septic patients lead to decreased apoptosis from CD14+ monocytes but increased apoptosis from CD4+ lymphocytes [35].

A more comprehensive understanding of the relationship between MPS and post-burn immune dysfunction will hopefully provide the basis for improving therapeutic regimens in the treatment of burn patients. Since human sepsis often is diagnosed too late to reduce the hyper-inflammation in the early phase, most of the immunotherapies are targeted to septic patients. Future investigation should find the early markers of immune dysfunction and provide immune intervention before it goes to sepsis.

Adoptive transfer of certain cell types of MPS into severely burned or septic patients still holds promise. Stem cell based therapies, such as MSC and HSC-based therapies are becoming increasingly attractive because of their ability to augment burn wound repair, attenuate tissue injury and reverse the dysfunctional MPS in severely burned and septic patients. Future studies should determine the proper sources of stem cells and focus on how to direct the transferred cells to the burn site and how to maintain their *in vivo* activity.

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Abbreviations

PBMC	Peripheral Blood Mononuclear Cells
MPS	Mononuclear Phagocytes System
GMP	Granulocyte-Monocyte progenitor
TBSA	Total Body Surface Area
MDP	Macrophage and DC progenitor
CDP	Common DC progenitors
DDCs	Dermal DCs
CAM	Classically Activated proinflammatory Macrophages
AAM	Alternatively Activated anti-inflammatory Macrophages
CLP	Cecal Ligation and Punctuation
FDC	Follicular Dendritic Cell
IDC	Interdigitating Dendritic Cell
ROS	Reactive Oxygen Species
HMGB1	High-Mobility Group Box protein 1
SIRS	Systemic Immune Response Syndrome

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Table 1

Comparison of members of MPS

	Monocyte (blood)	Macrophage (tissue)	DC
Half-life	1 day	months to years ^a	days ^b
Naïve T cell activation	No	No	Yes
Direct pathogen clearance	Yes	Yes	No
Scavenger	No	Yes	No
Major subtypes	Mon 1, Mon 2, Mon 3	M1, M2(a,b,c)	mDC, pDC
antigen presentation	+	++	++++

^a Activated macrophages and activated dendritic cells have a lifespan of only a few days

^b It varies with the locations where they reside. Dermal and epidermal DC are much longer than the myeloid- and the lymphoid-related subsets

Table 2

Disturbed MPS in severely burned and septic patients

Cells	Dysfunctions	Immunotherapy ^a	
DCs	1 Reduction or depletion of circulating DC	1	Adoptive transfer of naive or IL-10 gene transfected DCs
	2 Reduced expression of MHCII		
	3 Reduced expression of TLR4		
	4 Decrease of the T cell stimulatory function		
	5 Inducing more regulatory T cells		
Monocytes	1 Markedly impaired chemotaxis	1	i.v. administration of bone marrow-derived human MSC
	2 Increased number in the circulation		
	3 Decreased HLA-DR expression		
Macrophages	1 MΦ hyperactivity at the first phase	1	1. Anti-IL-β or anti-TNF-α
	2 MΦ dysfunction at the sepsis phase including:		
	1. inhibited cytokine secretion,		
	2. inhibited phagocytosis,		
	3. impaired migration		
		2	1. IFN-γ/GM-CSF
			2. Adoptive transfer of CD34+ cells
			3. Anti-PGE2

^aMost of them are experimental therapy on animal models.