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# The Role of Dendritic Cells as a Predictor of Sepsis in Burnt Patients

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## ABSTRACT

Severe burn injury imposes a high risk for the development of opportunistic infections and associated complications that can delay wound healing, prolong hospitalization, lead to sepsis and increase mortality. Numerous immunological alterations induced by burn injury impair innate and acquired immunities and decrease the ability of the patients to control and eliminate infections. Dendritic cells (DCs) are potent antigen-presenting cells and play a key role in activation of T cells; they are essential in coordinating the host response to pathogens. In the present study the percentage of LIN-DR + DCs in the blood of patients suffering from non septic and septic burn wounds and healthy subjects was determined at day 1,3,7,10 & 14 post-burn using the flowcytometry. There was a highly significant reduction in the percentage of DCs in both septic and non septic burn patients when compared with the percentage of DCs in the blood of healthy subjects in day one post-burn, but there was no significant difference in the percentage of DCs in the blood of septic and non septic burn patients. The percentage increased to reach the normal level on day 3 in the blood of non septic burn patients. In contrast, septic burn patients showed a significant decrease in DCs% at day 3,7,10 & 14 when compared with non septic burn patients and healthy control. These data suggest that DCs% decreased early after burn injury and sepsis could be the cause of continuous reduction in the percentage of DCs and the immunosuppression after burn injury.

## INTRODUCTION

Severely burned patients are susceptible to infections with opportunistic organisms due to altered immune response and frequent wound contamination **[10]**. Loss of protective barrier provided by the skin is primarily responsible for susceptibility to infections after burns. Numerous immunological alterations induced by burn injury impair innate and acquired immunities and decrease the ability of burn patients to control and eliminate infections. In both burn patients and animal models, natural killer (NK) cell, neutrophil, macrophage and T cell functions are impaired **[8,9,12]**. Dendritic cells (DCs) are highly efficient antigen presenting cells (APS) that initiate and direct immune responses **[1,6]**. They are produced in the bone marrow, circulate via the blood and enter the body tissues and mucosal surfaces to act as immune sentenils. When tissues are damaged by trauma, infection or are altered by malignant transformation, inflammatory cytokines and other cellular products are released. These stimuli, whether from tissues (endogenous) or from microbial products (exogenous), activate resident DCs and also promote recruitment of circulating DCs to the site **[5]**. DCs actively capture and process antigens and then migrate via the lymphatics to draining lymph nodes. Here they present processed antigens to T and B lymphocytes to initiate primary or simulate secondary (memory) immune response. As such, they play a central role in immune surveillance, an essential part of the defense and protective mechanism against infection **[15]**. A study by Toliver-Kinsky et al. **[14]** points to the importance of dendritic cells in resistance of burn wound to infection. Although a number of defects in the immune system have been identified in sepsis, few studies have examined the effect of burn and sepsis on DCs, which is the purpose of the present study that monitor the changes in the DCs counts in patients with non septic and septic burn wounds.

## MATERIAL AND METHODS

Twenty six patients were included in the present study, 14 patients suffering from non septic burn wounds (8 males & 6 females) and 12 patients suffering from septic burn wounds (7 males & 5 females) with age ranged between 14 and 55 years. Ten healthy subjects with matching age groups (7 males & 3 females) were used as control. Venous blood samples were collected into heparinized test tubes from patients with septic and non septic burns at 1,3,7,10 & 14 post-burn days. A single blood sample was collected from each healthy control subject. Blood samples were processed for flowcytometry to study the percentage of DCs.

*Antibodies:*

In the present study the monoclonal antibodies (Mo Ab) used for staining DCs was combined to

prepare a lineage cocktail with flourescein isothiocynate (FITC) - labeled anti-CD3, anti-CD4, anti-CD19, anti-CD56 Mo Ab and Phycoerythrin (PE) labeled anti-HLD-DR Mo Ab (all from Deckman Coulter Company).

*Flowcytometry analysis:*

Three-colour flowcytometry analysis was performed using Coulter Epics XL flowcytometry, Coulter Corporation, Miami, Florida 33196, USA.

100U of whole blood or cell suspension containing up to 1 x 10 6 leucocytic cells completed to 1ml by ammonium chloride lyzing solution (NH4cl 8gm, EDTA 1gm and KH2po4 0.1gm) in a tube at room temperature for 10-20 minutes. Then centrifugation for 5min at 400g, the supernatant is discarded and the pellet is washed with phosphate buffer solution (PBS) 2-3 times to obtain white pellet which is resuspended in 1ml buffer. After lysing and washing techniques, each tube was stained with a cocktail of antibodies to identify lineage –ve cells (LIN-cells), which lack CD3, CD19, CD14 and CD56 markers but HLA-DR+ and those are the dendritic cells population. Isotypic control was used to define the negatively stained cells. The gating strategy used to identify and quantify LIN-DR+ cells was as follows: Cells in lymphocyte-monocyte light scatter gate were evaluated for expression of lineage markers LIN-FITC and DR-PE.

DC percentages are expressed as a percentage of total mononuclear cells.

## RESULTS

*The patients were classified as septic on the basis of one of the following signs and symptoms:*

- Positive blood culture for bacteria.
- Hyperthermia.
- Impaired mental status.
- Hemodynamic instability.

There was an observable difference in the TBSA between the two burn groups. The occurrence of sepsis was observed among the patients with large TBSA.

Table (1) shows the percentage of dendritic cells (DC%) in the blood of patients suffering from non septic and septic burns compared to healthy control at the indicated times. At day 1 there was a highly significant decrease in the DC% in non septic and septic patients compared to control (10.35±1.09, 10.33±0.98 and 15.28±2.99 respectively), but there was no significant difference of DC% between non septic and septic burn patients. In day 3 post-burn the DC% increased in the group of patients with non septic burns to reach that of the control. In contrast, septic burn patients showed a significant decrease in DC% at days 3,7,10 & 14 when compared with non septic burn patients and healthy control (Table 2 & Fig. 1).

Fig. (1): Flowcytometric of DCs percentage in post burn patients.

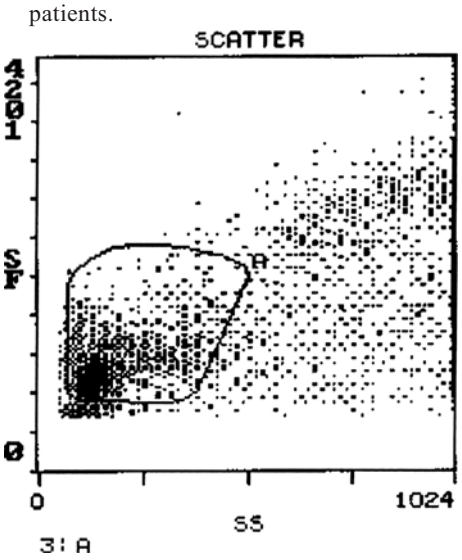


Fig. (1-A): In plot 1, forward and side scatter of mononuclear cells is represented with region (A) drawn around the lymphocyte and monocyte.

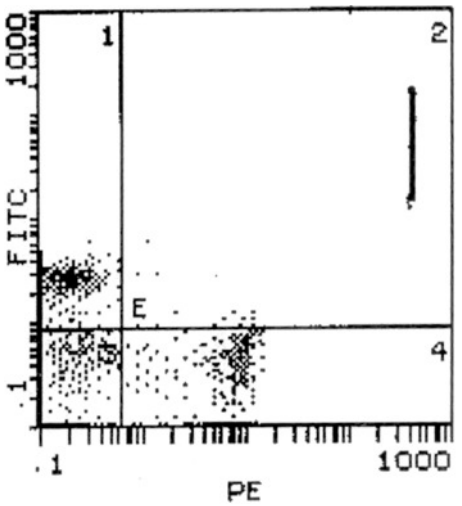


Fig. (1-B): In plot 2, LIN –ve, HLA +ve DCs is represented in the lower right quadrant (quadrant 4).

Table (1): Patients clinical characteristics.

| Groups | TBSA (Burn) range | Age (years) range |
|---|---|---|
| Normal controls (no=10) | – | 16-50 ys |
| Non septic burn patients | 15-30% | 14-48 ys |
| Septic patients | 25-50% | 20-55 ys |

Table (2): Percentage of DC in burn patients.

| Day of study | Non septic burn patients (n=14) | Septic burn patients (n=12) | *p* |
|---|---|---|---|
| Day 1 | 10.35±1.09 | 10.33±0.98 | 0.97 |
| Day 3 | 15.95±1.08 | 9.38±0.69 | 0.0001 |
| Day 7 | 18.79±0.85 | 8.82±0.61 | 0.0001 |
| Day 10 | 15.37±4.17 | 8.94±0.79 | 0.0001 |
| Day 14 | 16.76±0.61 | 10.06±0.6 | 0.0001 |

Data are expressed as mean ± SD.
$p<0.05$ is significant.

## DISCUSSION

Patients with large burn injuries are susceptible to opportunistic infections due to impaired functions of multiple effector cells of innate immunity and acquired immunity, including macrophages, dendritic cells (DCs), natural killer (NK) cells and T cells. The vast majority of clinical and basic science research on the immune consequences of burn injury and sepsis conducted during last decades has focused mainly on the roles of macrophages, neutrophils and to lesser extent, conventional T lymphocytes. During recent years, however, it has become increasingly clear that some subsets of immune cells, innate regulatory lymphocytes in particular, are central to the process involved in both protective immunity and immunopathology. Recent reports have just begun to shed light on the critical roles of innate lymphocytes subsets, including natural killer T cells, natural killer cells, gamma-delta T cells and naturally occurring dendritic cells during immune response to burn injury and sepsis **[11]**.

In the present study we observed a significant drop in the percentage of DCs after burn injury when compared with control and remained lower in septic patients from day 1 to 14. However, in non septic burn patients the DCs count was significantly rising in day 3 and was even higher in day 7 when compared with control.

At day 14 the percentage of DCs did not differ from those in healthy controls. A significant depletion of dendritic cells in spleens of patients that died from sepsis-associated complications has been reported **[7]**. In an animal model of sepsis, there was a significant apoptosis associated depletion of DCs in the spleen **[13]**.

Host defence against pathogenic microorganisms require the coordinated actions of the innate and acquired immune system. However, dysregulation of the immune system occurs during severe sepsis, leading to rapid death due to the development of multiorgan failure or an increase in complications due to long term immunosuppression **[4]**. Considering that DCs are the major initiators of specific immune response to pathogens, the drop in their number might account for the immunosuppression associated with burn injury and more with sepsis. To address this question, the avaluation of DC percentage in the peripheral blood from burn patients might be the answer. Zhang et al. **[16]** investigated the characteristics of dendritic cells (DCs) in patients with severe burn, they reported a definite decline in their functions, which may partly contribute to infection after burn. In a study by Toliver-Kinsky et al. **[14]** the ability of the Fms-like tyrosine kinase-3 ligand (Flt3L), a hemopoietic cytokine that stimulates the production of dendritic cells was evaluated. Flt3L-enhanced dendritic cell production to increase the resistance of mice to a burn wound infection with pseudomonas aeroginosa, a common source of infections in burn patients that have impaired immunity and are susceptible to opportunistic micro-organisms. Treatment of mice with Flt3L for 5 days caused a significant increase in dendritic cell numbers in the spleen and significantly increased survival upon subsequent burn wound infection.

In another study the administration of Flt34 after burn injury lead to a decrease in overall leucocytic apoptosis (including those of dendritic cells) in response to infection **[3]**.

In the present work the overall loss of DCs during progression of sepsis may contribute to suppression of immune response and impair the host's capacity to resist and/or respond to microbial infection and lead to the development of sepsis.

Benjamin et al. **[2]** were capable of isolating dendritic cells in experimental animals and found that intrapulmonary transfer in these DCs in post-septic mice prevented fatal asperigillus infection.

Given their emerging importance and documented upstream regulatory capacities over macrophage, dendritic cells represent attractive new target for therapeutic intervention of the overall immune paralysis that occur with injury and sepsis.

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