

Short Communication

Burn Wound Exudate: Depth of Burn Predicts Cellular Recruitment

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Abstract

Previous research has demonstrated a correlation between burn injury and systemic cytokine response. Moreover, conflicting studies have identified burn wound fluid as being rich in either pro- or anti-inflammatory cytokines that may serve to modulate the healing process. We hypothesized that the cellular makeup of burn wound exudate correlates to the depth of burn insult. To test this, we collected blister fluid from five patients with superficial partial-thickness or deep partial-thickness burns admitted to our burn ICU. The cellular composition of the fluid was compared to that of whole blood from a healthy patient using flow cytometry. Any additional blister fluid was assayed for various cytokine expressions. The cellular constituency of superficial partial-thickness burn wound fluid appears to be predominated by lymphocytes whereas deep partial-thickness burn fluid is mostly made up of monocytes and granulocytes. Additionally, fluid from these deeper burns has a concentration of hepatocyte growth factor that was at least two times that seen in the fluid collected from the more superficial burns. Our data suggests a correlation between the cellular makeup of burn wound exudate and the depth of burn injury that may eventually serve as a surrogate measure of burn depth. Furthermore, these findings may offer insight into the possible mechanisms that lead to spontaneous healing more superficial wounds versus the less predictable nature and clinical course seen with the deeper partial-thickness burns.

ABBREVIATIONS

DPT: Deep Partial-Thickness; FGF: Fibroblast Growth Factor; HGF: Hepatocyte Growth Factor; SPT: Superficial Partial-Thickness; TGF- β : Transforming Growth Factor-Beta; VEGF: Vascular Endothelial Growth Factor

INTRODUCTION

Burn blister fluid and wound exudate are naturally occurring sequelae after burn insults that result in superficial-partial thickness (SPT) and deep partial-thickness (DPT) burns. It is known that burn injuries elicit an immediate inflammatory reaction that includes the release of a multitude of cytokines, signaling factors and differing cellular components from the burn wound. The burn wound fluid released by the dermis is rich in these inflammatory mediators which recruit and activate various cells that participate in wound healing and host defense against infectious bacteria. Numerous studies have previously examined the relationship between systemic cytokine release after burn injury as a mechanism of inflammation, and specifically, systemic inflammatory response syndrome. However, recent evidence would suggest that cytokine levels at the burn wound interface are a more fastidious reflection of the burn itself [1].

In clinical practice, superficial-partial thickness burns usually do not require medical attention and tend to heal without

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intervention. Conversely, deep partial-thickness burns may not heal without surgical debridement and are accompanied with an unpredictable clinical course on initial presentation. Based on these findings, we sought to examine the burn fluid from both SPT and DPT burns to determine if there is a correlation between the severity of the burn insult, and the constituency of the burn wound exudate.

MATERIALS AND METHODS

This study was conducted in accordance with the regulations of the University of California, Irvine Institutional Review Board (IRB #2013-9294). Five consecutive patients who presented to the hospital within 24 hours of sustaining 5% or less total body surface area thermal burns were admitted to the ICU and identified as having fluid-containing burn blisters. Six to 12 hours after presentation, informed consent was obtained and burn exudate was extracted under sterile technique using a large-bore syringe. All samples were passed through a 100 μ m strainer and analyzed via C6 Accuri flow cytometer (BD Biosciences, San Jose, CA) within 24 hours of collection; peripheral blood mononuclear cells from a whole blood sample of a single healthy patient was used as a control. Gating for each cell population was set based on the forward and side scatter patterns of the known cell types as previously described [2]. Any remaining burn exudate was stored at -80°C until further analysis. After surgical debridement,

the burn wounds for each patient were classified based on depth and extent of injury. The remaining burn fluid samples were assayed using a Quansys Biosciences ELISA multiplex array system (Logan, UT) for human fibroblast growth factor (FGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF- β). Any patients with significant comorbidities (e.g. diabetes, congestive heart failure, illicit drug abuse) were excluded from our study.

RESULTS AND DISCUSSION

Each data point described below is represented as a mean \pm standard deviation unless otherwise specified. Two patients sustained wounds classified as SPT (2 females, 26-34 years old). Exudate extracted from these wounds was relatively devoid of monocytes ($1.57 \pm 1.5\%$) and granulocytes ($4.1 \pm 6.2\%$), with the majority of the cell population comprising smaller less granular lymphocytes ($14.8 \pm 3.6\%$) and platelets. The remaining three patients sustained wounds classified as DPT (2 females, 20-70 years old, 1 male, 30 years old). The burn fluid collected from these patients was much more abundant in granulocytes ($39.4 \pm 10.7\%$) and monocytes ($11.5 \pm 4.4\%$). The lymphocyte population closely mirrored that of the SPT burn group ($12.1 \pm 3.8\%$ - Figure 1). ELISA multiplex analysis of the burn fluid was only possible for two DPT thickness and one SPT burn fluid samples. The cytokine levels were very similar for all analytes except for HGF, which was more than two times greater in the DPT burn when compared to the SPT burn exudate (Figure 2).

Lymphocytes have been implicated as key regulators in dermal wound healing whose numbers decline as the wound

completes the healing process. Specifically, resident cytotoxic T cells are the most abundant cells at the wound interface, and soon after wounding [3]. Therefore, it is of little surprise that superficial-partial thickness burn wound fluid is primarily populated with these cytotoxic cells that serve as first-responders during this inflammatory phase of wound healing. The deep-partial thickness burn fluid contained a much greater proportion of granulocytes and monocytes, likely correlating with a greater amount of tissue damage and therefore, inflammatory response. Interestingly, of the cytokines studied, only HGF was elevated in the DPT burn fluid when compared to the SPT group. An early study in rats identified different forms of HGF produced by various organs in response to burn injury [4]. Additionally, HGF has been described as a potential keratinocyte autocrine signaler that is produced in response to local burn injury in humans [5]. Our study corroborates the findings that HGF may possibly serve as a biomarker for burn severity, and the study by Yamashita et al., suggests that it may also be an early indicator of organ dysfunction.

The small sample size and lack of comorbidity assessment are clear weaknesses to our findings. In the future, an optimal study would be to collect burn fluid from a larger population of burn victims and normalize the data based on age, sex, percentage of body surface area burned as well as medical comorbidities. One limitation is the collection of burn fluid from patients with partial thickness burns is often difficult if the epidermal layer of skin has denuded. In an effort to overcome this challenge, our group has developed a lightweight microfluidic patch that is worn by the burn victim, and attached to light negative pressure for the

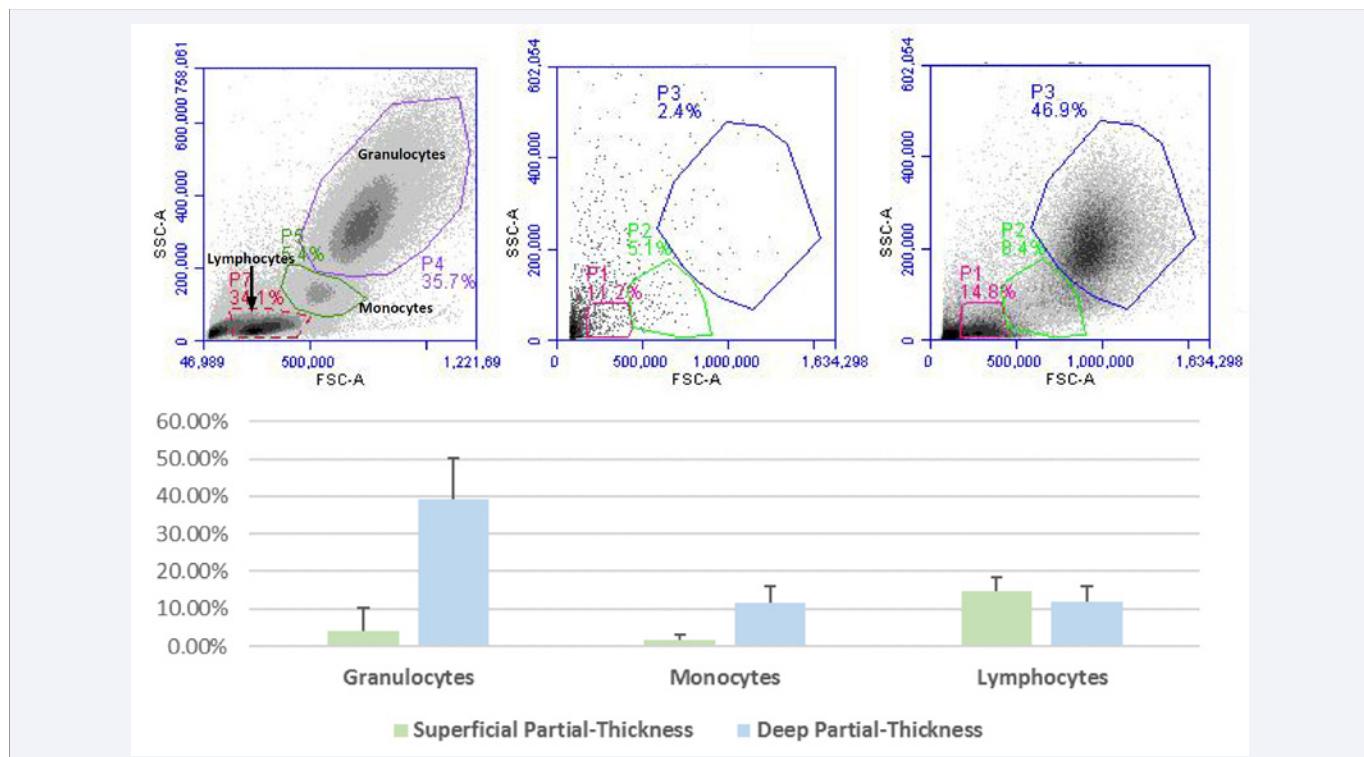


Figure 1 Top: Flow cytometry scatter pattern for cellular constituency for blood versus burn wound exudate. (Left) PBMCs from whole blood, (center) fluid from SPTburn patient, (right) fluid from DPT burn. Bottom: Bar graphs representing percentage of cell populations. DPT burn fluid has much greater population of granulocytes and monocytes compared to SPTburn fluid. Data is represented as mean \pm standard deviation.

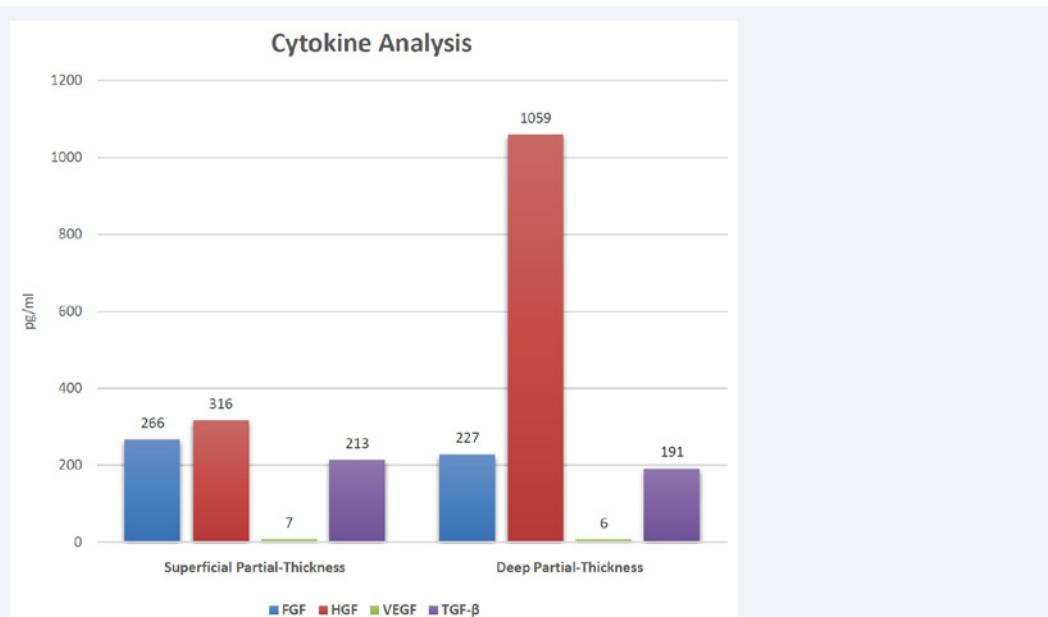


Figure 2 ELISA multiplex analysis of burn fluid cytokine content. HGF appears to be the only factor of those studied that is increased in DPT burns when compared to SPTburn fluid.

continuous college of exudate [6]. We hope that this will allow for the analysis of the burn microenvironment at various time points throughout the wound healing process.

CONCLUSION

Our data indicates a correlation between the cellular makeup of burn wound exudate and the depth of burn injury. Previous studies have shown conflicting evidence as to whether burn fluid possesses anti- or pro-inflammatory cytokines, but we portend that this depends on the depth of the wound. Superficial partial-thickness wounds tend to heal without surgical debridement, likely due to reduced pro-inflammatory cells. In contrast, deep partial-thickness burns usually require surgical debridement. It is possible that debridement and drainage of burn fluid in this setting rids the wound of this pro-inflammatory milieu, thus facilitating the healing process, however further studies are needed to validate these findings.

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