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Burn wounds in the young versus the aged patient display differential immunological responses

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ABSTRACT

Background: Individuals in the geriatric age range are more prone than younger individuals to convert their partial thickness thermal burns into full thickness injuries. We hypothesized that this often observed clinical phenomenon is strongly related to differential local injury responses mediated by the immune system.

Materials & methods: Skin samples from areas with partial thickness thermal burns were obtained during routine excision and grafting procedures between post burn days 2–6. Tissue samples were grouped by age ranges with young patients defined as <30 years of age or aged patients defined as >65. Formalin fixed samples were used to confirm depth of burn injury and companion sections were homogenized for multiplex analysis using a Luminex platform. Immunohistochemical staining was used to quantify total macrophage numbers as well as the M1 and M2 subpopulations.

Results: Our analysis includes samples derived from 11 young subjects (mean age=23) and 3 aged subjects (mean age=79.2). Our initial survey of analytes examined 31 cytokines/chemokines. Twelve were excluded from consideration as they were present in concentrations either above or below the optimal detection range. Two analytes emerged as candidate molecules with significant differences between the young and the aged patient responses to burn injury. EGF levels were on average 21.69 pg/ml in young vs 14.87 pg/ml in aged ($p=0.032$). RANTES/CCL5 levels were on average 14.86 pg/ml in young vs 4.26 pg/ml in aged ($p=0.026$). Elevated macrophage numbers were present within wounds of younger patients compared to the old ($p<0.01$), with a higher concentration of the M1 type in the elderly ($p>0.05$).

Conclusion: Our study has identified at least 2 well known cytokines, CCL5 (RANTES) and EGF, which are differentially regulated in response to burn injury by young versus aged burn victims. Evidence suggests that a proinflammatory environment can explain the high conversion rate from partial to full thickness burns. Our data suggest the need for future studies at the point of injury (cutaneous targets) that may be modulated by post burn release of cytokines/chemokines.

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1. Introduction

The geriatric patient population currently comprises about 14% of the admissions to Burn Centers in the United States and Canada with expectations that this percentage of the populations is expected to grow considerably. This currently translates into over 40,000 burn admissions annually [1]. Burn injury in the elderly has long been associated with predictably higher mortality rate as evidenced by both the Baux and Revised Baux Scoring tool [2]. At present, the multi-focal causes for these differential responses are not fully understood. While advanced age is associated with inherent changes in both innate and adaptive immune responses, burn injury is well recognized as evoking an immune dysfunction following systemic release of proinflammatory cytokines, chemokines and acute-phase reactants [3,4]. The systemic immunologic response after burn injury has been investigated [5], but a scarcity of literature exists concerning the immunologic response within the healing burn tissue itself. Clinically, notable differences exist between wound healing responses of the young and the aged; particularly the elder population that has a more limited capacity to rescue deep partial thickness burns.

Wound healing is a process composed with different stages such as: hemostasis, inflammation, proliferation, and remodeling. Focusing in the proliferative stage is very well known that aged individuals delayed re-epithelization, collagen synthesis, and angiogenesis [6]. Nishio et al. evidenced a defectuous inflammatory stage in aged mice with neutrophil depletion, translated into wound healing delay; however, the same effect is not seen in young mice [7]. The phenomena of scarless fetal wound healing is postulated to result from the lack if inflammation within the wound [8]. These findings suggest that variations in inflammatory chemokine/cytokine profiles at different ages may be the etiology for different outcomes in the young and aged populations. When examining cytokine profiles in wounds, elevated Monocyte Chemoattractant Protein (MCP-1) has been observed in the wounds of aged mice, while most other chemokines declined [9,10].

Similar studies have not been conducted in the burn literature. It is known that the elderly are a susceptible population to burns, and their outcomes are often dismal [11]. Elderly patients have slower wound healing, longer hospital stays, and greater mortality than their younger counterparts [12]. In our clinical experience, we have frequently noticed a delay in wound healing in the elderly and have noted a propensity for these patients to convert deep partial thickness burns to full thickness, while young patients have a general tendency to heal their partial thickness burns without progression to full-thickness tissue loss.

Interestingly, we have also noticed clinically how the older burned patients tend to scar less than the younger patients. An imbalance between proliferative stages of wound healing and apoptosis could be the root of the cause. Marcus et al. found in experiments with young rabbits compared to old, an increased Scar Elevation Index (SEI) and a higher proliferative cell nuclear antigen index. No difference was found when comparing Apoptotic indexes in both groups [13]. Targeting and shortening the proliferative phase of wound healing can be a gateway for modulating scarring in our younger patients.

While the difference in burns responses between the young and elderly has been corroborated in animal studies [14], the etiology for worse outcomes in the elderly human population has remained elusive to date but is thought to relate to natural aging, which is associated with impaired immune function and elevated pro-inflammatory cytokines [15]. The systemic response to burns in aged individuals has been investigated [4,5,16–18], but the differences in local wound response across various age groups remains to be explored.

We hypothesize differences in the immunologic response within burn wounds of the young and the aged that account for the limited abilities of elder patients to mount an effective cutaneous response to burn injury. In this report, we identify differences between the immune response within the burn wounds of young and aged patients. Discovery of the differential cutaneous responses within the burn wound has the potential to serve as the foundation for specialized treatment strategies aimed at improving outcomes in the aged burned population.

2. Methods

2.1. Study recruitment

Our study was approved by the Vanderbilt University Institutional Review Board. Informed consent was obtained for all collections of normally discarded burn tissue. Our inclusion criteria were males or females under 30 years of age (young) and greater than 65 years of age (aged), who were in patients at the Vanderbilt University Medical Center Regional Burn Center and who required excision in the operating room between post burn day (PBD) 2–6. Burns were typically deep second degree or third degree thermal burns. Exclusion criteria included chemical or electrical burns, patients with superficial burns not requiring operative debridement, or those patients unable to provide consent.

2.2. Sample acquisition

During operative burn excision, a partial thickness section of skin was obtained from the wound edges. All samples were collected from peripheral areas of the injury that were excised as part of the normal operative procedure. No additional skin was removed from any patient as a result of their participation in this study and areas of full thickness burn injury were excluded from this study. These pieces of marginal skin that had the visual appearance of partial thickness injury were grossly dissected into sections roughly 2.5 cm by 2.5 cm and preserved in 10% neutral buffered formalin. Adjacent companion samples of burn tissue were immediately flash frozen in liquid nitrogen. Frozen pieces were stored at −80 °C until ready for protein extraction and formalin fixed tissues were processed for paraffin embedding.

2.3. Histological assessment and protein extraction

Formalin fixed paraffin embedded (FFPE) tissues were sectioned at 5 μm, stained via Gomori's Trichrome, and examined under the microscope. Depth of burn injury and suitability of sample inclusion was confirmed. Samples which contained

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