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Abstract 22

Tissue-specific patterns of caspase-1 and cytokines in excisional wounds are altered by shock in rat skin and muscle

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Objectives: Skin and muscle wounds often lead to significant inflammation in the affected tissue. The primary mechanism by which inflammation is initiated, sustained, and terminated is cytokine-mediated immune signaling, but this can be altered by cardiogenic shock. The complexity and context sensitivity of immune signaling in general stymied a clear understanding of these signaling dynamics. We hypothesized that advanced numerical and biological function analysis methods would help elucidate the inflammatory response to skin and muscle wounds in rats, both with and without concomitant shock.

Methods: We studied 2 experimental groups: wound only ("wound group") and wound with cardiogenic shock ("shock group"). In the "wound group," 4 Lewis rats were anesthetized and an excision biopsy was taken from the lateral aspect of the thigh on one of the hind limbs in each of the rats. In the "shock group," 4 Lewis rats were sacrificed, and excision biopsy (wound) was carried out 15 to 30 seconds after cessation of heartbeat. Skin and muscle tissue was then separated and assayed for total protein content by BCA assay, and the inflammation biomarkers interferon-γ, interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-18, monocyte chemotactic protein (MCP-1), growth-related oncogene/KC, tumor necrosis factor α, and granulocyte-macrophage colony-stimulating factor were assayed by Luminex (Luminex Corp, Austin, TX). Caspase-1, which drives activation of the NLRP-3 inflammasome, was assessed by Western blot assay. Statistical and computational analyses of cytokine network profiles (1-way balanced analysis of variance, unpaired 1-tailed heteroscedastic *t* test, principal components analysis, and confirmatory factor analysis) were performed in Matlab (The MathWorks, Inc, Natick, MA).

Results: Granulocyte-macrophage colony-stimulating factor was elevated in the wound group ($P < .05$), whereas IL-4, IL-12p70, interferon-γ, and IL-18 were elevated in the shock group ($P < .05$). Interleukin-1α and IL-18 were more elevated in skin vs muscle ($P < .05$), which was suggestive of inflammasome activation in the skin. MCP-1, IL-1β, IL-2, IL-6, IL-10, IL-4, IL-12p70, and tumor necrosis factor α ($P < .05$) were also differentially higher in skin vs muscle. Immunoblotting revealed caspase-1 activation in skin but not muscle. Notably, IL-1α and IL-18, along with caspase-1, were greatly elevated in the skin after cardiogenic shock ($P < .05$). Principal components analysis suggested that more than 95% of observed variance could be explained by 2 principal components in the skin and muscle and suggested distinct groups of inflammatory mediators induced in the "wound group" vs the "shock group". Interleukin-18 and IL-1α were primary contributors to the first principal component, whereas IL-6, MCP-1, growth-related oncogene/KC, and IL-1β were primary contributors to the second principal component. Principal components analysis results were reinforced by confirmatory factor analysis.

Conclusions: Caspase-1 and the NLRP-3 inflammasome appear to be key factors in determining the type and severity of the inflammatory response to excisional wounding, especially in the presence of shock. Activated caspase-1 is associated with defined, compartmentalized patterns of cytokine production that may be discerned via data-driven modeling.

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Abstract 23

Global sensitivity analysis of endotoxin-induced acute inflammatory responses predicts the multimodal dependence of global tissue damage both on host IL-6 responses and endotoxin dose

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Introduction: Acute inflammatory responses to various stimuli involve complex nonlinear interactions among inflammatory cells and their products. We previously developed a nonlinear ordinary differential equation model to explain the dynamics of endotoxin (lipopolysaccharide, or LPS)-induced acute inflammation and associated whole-animal damage/dysfunction (a proxy for the health of the organism, whose likely molecular correlate is damage-associated molecular pattern molecules) [1]. That model includes the inflammatory mediators tumor necrosis factor α, interleukin (IL)-6, IL-10, and nitric oxide (NO) and was calibrated in part on LPS doses of 3, 6, and 12 mg/kg in C57Bl/6 mice. In the current work, we analyzed the major determinants of the resulting tissue damage using a global sensitivity approach.

Methods: The precise inflammatory role of IL-6 and its use as a biomarker or therapeutic target have been the source of much debate, presumably due to the complex proinflammatory and anti-inflammatory effects of this cytokine. Therefore, we chose to investigate the sensitivity of the area under the IL-6 curve (AUC_{IL6}) and the area under the damage curve (AUC_D) to the 51 rate parameters of the ordinary differential equation model for different levels of simulated LPS challenge (1-15 mg/kg). Owing to the complex nonlinear interactions between the rate parameters, we chose a variance-based global sensitivity approach. Specifically, we chose the Random Sampling High Dimensional Model representation developed by Rabitz et al to reduce the computational cost associated with Monte Carlo sampling of the parameter space [2]. Monte Carlo samples