Pyroptosis Pathway Expression in Combat-Related Extremity Wounds

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INTRODUCTION

Combat-related extremity wounds represent one of the most prevalent injury class during Operation Iraqi Freedom and Operation Enduring Freedom. Blast injuries were frequent mechanisms of these traumas, and the kinetic nature of these injuries results in extensive tissue damage and gross contamination.[1] Despite a regimented schedule of serial debridement every 48-72 hours followed by delayed wound closure (DWC), approximately 20% of these wounds fail, requiring further intervention after delayed closure was attempted.[1]

Poor wound outcomes have been linked to high bioburden, particularly select gram-negative species and elevated levels of pro-inflammatory cytokines.[1,2] We propose that pyroptosis, a pro-inflammatory form of programmed cell death, plays a central role in these failures.

Pyroptosis acts as a cell-intrinsic defense mechanism against a variety of pathogen-associated molecular patterns (PAMPs), particularly those associated with intracellular bacteria. The canonical pyroptosis pathway is initiated by pattern recognition receptors (PRRs) such as NOD (nucleotide-binding oligomerization domain), LRR (leucine-rich repeat), and pyrin domain-containing protein 3 (NLRP3), which respond to bacteria, viruses, and particulate stimuli.[3] Upon activation, NLRP3 promotes assembly of the inflammasome, leading to activation of caspase-1 and subsequent processing of pro-interleukin-1 beta (pro-IL-1β) and pro-interleukin-18 (pro-IL-18) into their mature forms (Figure 1).[4] The non-canonical pyroptosis pathway involves caspase-4 and caspase-5, which are activated by cytosolic lipopolysaccharide (LPS) and can also promote NLRP3 inflammasome activation. Both pathways converge on activation of gasdermin D (GSDMD). The N-terminal fragment of GSDMD forms 15–20 nm pores in the plasma membrane, permitting cytokine release and the influx of water and sodium ions (Na^+). This process leads to osmotic swelling, cell lysis, and amplification of the inflammatory response.[3]

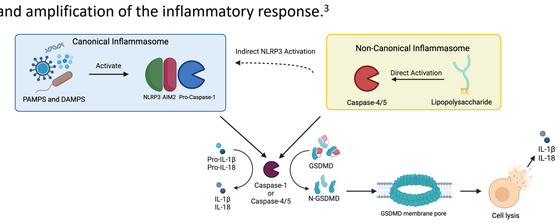
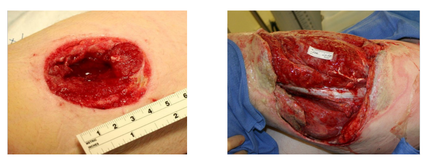


FIGURE 1. Schematic Overview of pyroptosis signaling pathways. Created with BioRender.com

METHODS

We analyzed 41 patients with 64 combat-related extremity wounds sustained during Operation Iraqi Freedom and Operation Enduring Freedom. Wound images, tissue biopsies, and serum samples were collected at each debridement until DWC. Wound images were analyzed for visual signs of infection or non-viable tissue (Figure 2). Tissue RNA sequencing and serum protein profiling were performed using Luminex platforms. Differential expression was analyzed using DESeq2, pathway enrichment with gene set enrichment analysis, and tissue-serum associations with Kendall rank correlations. Proportions were compared with Fisher's exact test, and false discovery rate corrections accounted for multiple testing.



No visual signs of infection or non-viable tissue

Visual signs of infection and non-viable tissue

FIGURE 2. Visual analysis of wound images obtained during serial debridements. Wounds were categorized based on visible signs of infection (purulence or erythema) and non-viable tissue (necrosis or eschar).

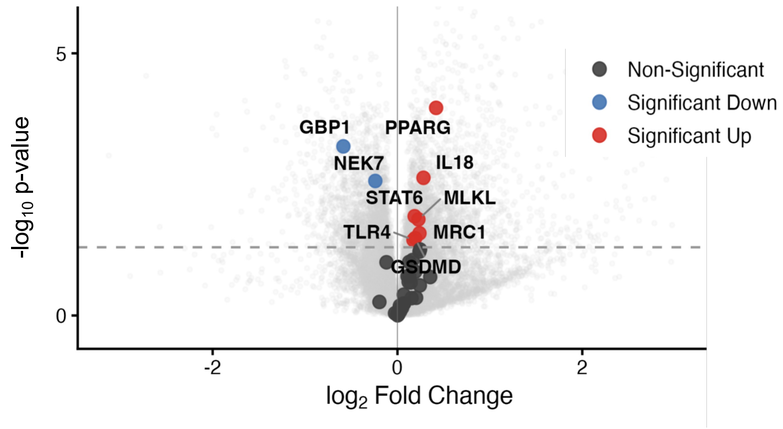


FIGURE 3. Expression of pyroptosis pathway genes across all wound types. Analysis includes both wounds that displayed visible signs of infection or non-viable tissues and those that did not. GBP1=guanylate binding protein 1 (lipopolysaccharide sensor). MLKL=mixed linkage domain kinase like protein (activator of NLRP3, crosstalk with necroptosis). MRC1=mannose receptor C type 1 (macrophage receptor).

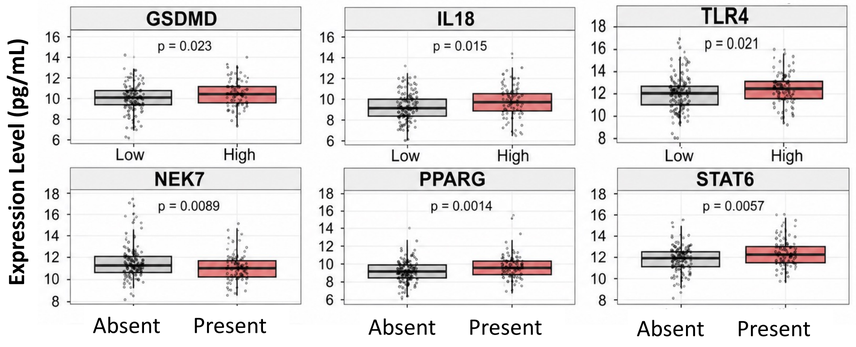


FIGURE 4. Transcriptional expression of pyroptosis related genes and cytokines across wound subtypes. "Absent" and "Present" denote whether visible signs of infection or non-viable tissue were observed in the wound image. GSDMD = gasdermin D (common pyroptosis execution protein). IL-18 = interleukin 18 (pro-inflammatory cytokine). TLR4 = Toll-like receptor 4 (pattern recognition receptor for lipopolysaccharide). NEK7 = NIMA related kinase 7 (canonical pyroptosis inflammasome activator). PPARG = peroxisome proliferator-activated receptor gamma (inflammasome suppressor). STAT6 = signal transducer and activator of transcription 6 (inflammasome suppressor).

RESULTS

Twenty-seven patients (66%) with 39 wounds (61%) developed visual signs of non-viable tissue and infection. Among these wounds, 27 (69%) achieved successful DWC and 12 (31%) failed. In contrast, 14 patients (34%) with 25 wounds (39%) showed minimal or absent signs of infection or non-viable tissue, and all achieved successful DWC. Wounds with signs of infection or non-viable tissue had a greater risk of closure failure (12/39 vs. 0/25; p=0.002).

Though analysis of the combined wound dataset showed no significant change to the expression levels of pyroptosis-promoting gene gasdermin D, subtype analysis of wounds with visual signs of non-viable tissue or infection showed upregulation of gasdermin D and cytokine IL-18 (Figures 3, 4). Additionally, wounds with these visible signs showed upregulation of resolution markers STAT6 and PPAR-γ. Canonical pyroptosis gene NEK7 and non-canonical pyroptosis gene GBP1 were downregulated in wounds with visible signs of non-viable tissue or infection. Local IL-18 expression in these wounds was positively correlated with circulating IP-10 and hepatocyte growth factor (HGF), while STAT6 was positively correlated with IL-6, and HGF (Figure 5).

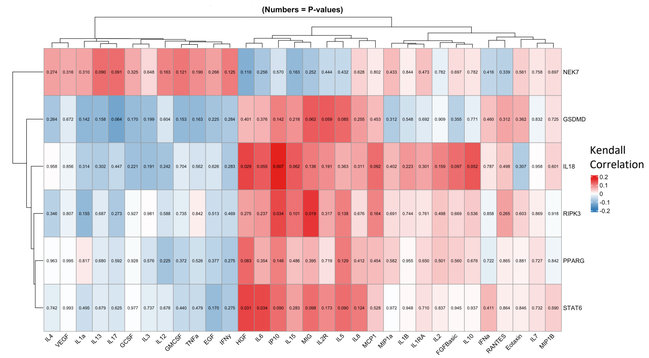


FIGURE 5. Correlation of pyroptosis genes to systemic signals. Kendall correlations were used to map the association of expression of selected pyroptosis genes (vertical axis) to systemic cytokines and signaling molecules (horizontal axis). Notable positive correlations include STAT6 with HGF (hepatocyte growth factor, r=0.23), IL-6 (r=0.21) and IL-18 with HGF (r=0.23), IP-10 (interferon gamma induced protein 10 r=0.27).

CONCLUSION

Combat-related extremity wounds pose a unique surgical challenge due to high-energy trauma, gross contamination, and treatment under austere conditions.

Transcriptional analysis of these wounds reveals increased expression of pyroptosis-related gene gasdermin D and cytokine IL-18. It is unclear currently if canonical or non-canonical pyroptosis predominates as NEK7 (canonical) and GBP-1 (non-canonical) genes are both downregulated. IL-18 expression was correlated with circulating IP-10 and HGF, which suggests a systemic response to this local inflammation. Resolution markers STAT6 and PPAR-γ are also upregulated, suggesting high levels of turnover in wounds that display visible signs of infection and non-viable tissue.

Pyroptosis-related genes and cytokines may provide mechanistic insight into the pathophysiology driving delayed wound closure failure. These molecular profiles could be utilized as predictive biomarkers to model the timing of successful delayed wound closure.

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