Developing a Minimally Invasive Cell-Based Model to Predict Response to Major Trauma

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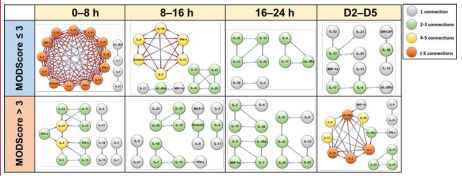
BACKGROUND

Physical trauma results in a systemic inflammatory response. Preliminary research in orthopedic trauma patients suggests that patients with similar demographics and severity of injury vary in their response to traumatic injury. Analysis of the immunological response post-injury showed a sustained pro-inflammatory response with delayed reparative cytokine expression in trauma sensitive patients, while the trauma tolerant patients had an early inflammatory expression with resolution by 72 hours post-injury.[1] Thus, we hypothesize that differential response to non-traumatic injury might serve as a predictive tool for the identification of trauma tolerant and sensitive patients prior to injury.



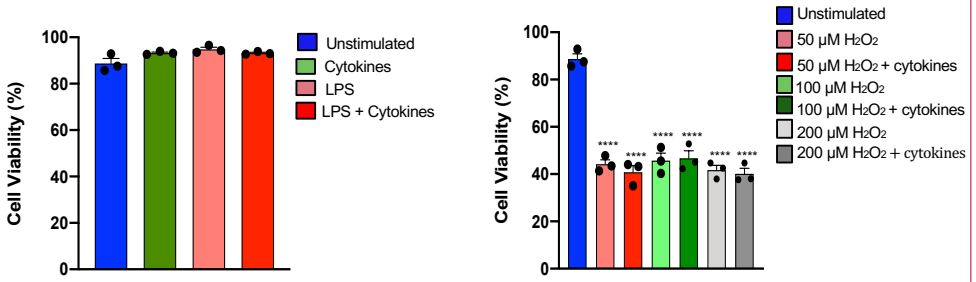
Main Goal of Study. Test whether immunological changes to inflammatory stimuli can predict tolerance or sensitivity to trauma using an-vitro cell-based assay.

METHODS

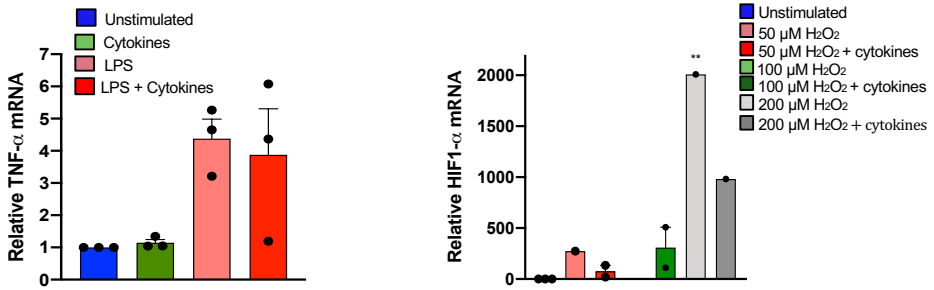
Splenocytes were isolated from naive C57BL/6 mice and subjected to biological trauma in vitro using LPS (100 ng/mL) or hypoxic trauma using hydrogen peroxide (50 μM, 100 μM, and 200 μM) with or without proinflammatory cytokines, IL-1β (1 ng/mL), IL-6 (200 ng/mL), IL-33 (150 ng/mL), and HMGB1 (10 ng/mL). Inflammation and hypoxia were assessed using HIF-1α, TNFα, and BAX expression respectively via qPCR 24 hours post-treatment. Cell death and pro-inflammatory cytokine production using multiplex analysis were used to measure outcomes.

RESULTS

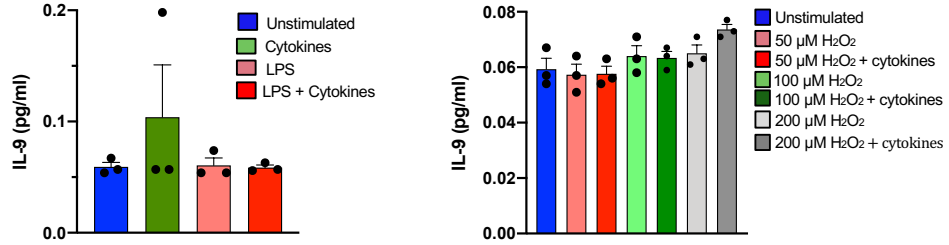
I. Hydrogen Peroxide (H_2O_2) Reduces Viability in Murine Splenocytes



II. LPS upregulates TNF-α, while H_2O_2 induces hypoxic inducible factor- 1α (HIF-1α)



III. Interleukin-9 (IL-9) production by splenocytes post- chemical and hypoxic trauma in vitro



CONCLUSIONS

These data suggest that:

1. Treatment with hydrogen peroxide promotes cell death
2. TNF-α mRNA production was increased post-LPS treatment group, indicating a pro-inflammatory response
3. Hydrogen peroxide also increases HIF-1α mRNA expression relative to control, confirming oxidative stress within the splenocytes
4. This in vitro cell-based assay can be used to assess immunologic response to inflammatory stimuli across the genetic variation of mouse strains

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REFERENCES

1. Namas, Rami A. et al. 201. A time window-based platform for the rapid stratification of blunt trauma patients into distinct outcome cohorts.