Does sepsis-induced immunoparalysis cause variable immune responses in the canine model?

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Introduction

Sepsis is a form of systemic inflammatory response syndrome (SIRS) that occurs secondary to infection and affects approximately 5 percent of dogs in the intensive care unit (ICU). Sepsis is difficult to treat and has a mortality rate as high as 70%. Sepsis was previously believed to be a hyperinflammatory response, however, recently a variable immune response (i.e., hyperdynamic, hypodynamic, or a combination) has been recognized in people. Sepsis can result in death due to overzealous inflammation and tissue damage or a prolonged hypoinflammatory stage resulting in opportunistic secondary infections, termed "immunoparalysis".

Little is known about the immune response during sepsis or SIRS in dogs. The purpose of this study is to observe dogs in the ICU and to test their immune responses to see if dogs are similar to people.

Hypothesis

We hypothesize that there will be variability in immune response in a population of dogs with sepsis and SIRS, with some having an increased immune response and others having immunoparalysis.

Materials and Methods

Sample collection

- Whole blood samples from 9 adult dogs in the ICU with either sepsis or SIRS were used for each assay.

Leukocyte Phagocytosis

- Samples were incubated with FITC-labeled *Escherichia coli* or a negative control solution for 10 minutes at 37°C.
- Phagocytic activity was measured via flow cytometry.

Leukocyte Oxidative Burst

- Samples were incubated with negative control solution, unlabeled opsonized *E. coli* bacteria or phorbol 12-myristate 13-acetate (PMA) for 10 minutes at 37°C. Dihydrorhodamine (DHR) was added as a fluoregenic substrate to determine the extent of oxidative burst.
- Samples were analyzed via flow cytometry.

TLR4 and HLADR Expression on PMNs

- Peripheral mononuclear cells (PBMCs) were isolated.
- The following samples were incubated for two hours at 37°C:
  - Control (unstained cells)
  - Unstimulated
    - Stained with either TLR4-PE or HLADR-FITC, or both
  - Stimulated with LPS
    - Stained with both TLR4-PE and HLADR-FITC

Cytokine Production

- After stimulation, the blood was stimulated with PBS (control), LPS, LTA, and PG and incubated for 24 hours at 37°C.
- Supernatant TNF, IL-6, and IL-10 will be measured using a canine-specific bead-based multiplex cytokine assay.

Phagocytosis and Respiratory Burst

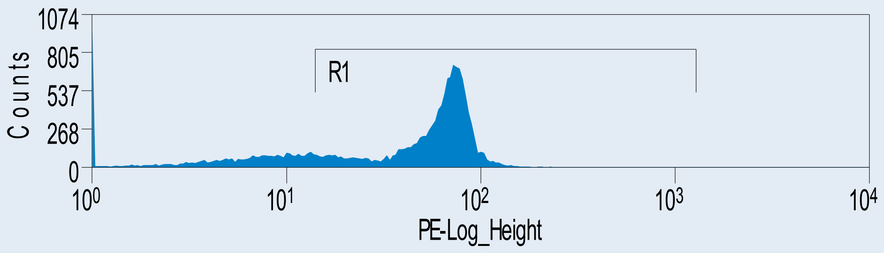

Figure 1. Gating for DNA positive cells.
Cells staining positive for propidium iodide (PI) DNA stain were isolated based on fluorescence (R1).

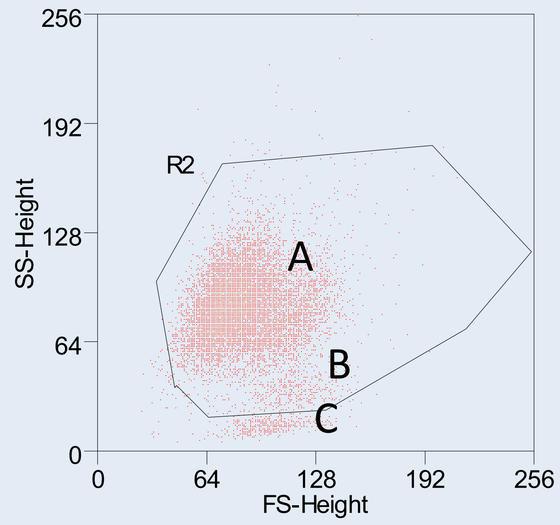

Figure 2. Gating for DNA positive cells.
Cell populations were separated based on size (FS – forward scatter) and complexity (SS – side scatter). The cell populations were isolated into neutrophils (A), monocytes (B), and lymphocytes (C). Neutrophils and monocytes were included in the gate to evaluate phagocytosis and oxidative burst.

Figure 3. Neutrophil and monocyte populations were determined to be either FITC negative (top) or FITC positive (bottom) depending upon whether or not they fluoresced within the first and third decades (R3). A FITC positive cell means that the cell phagocytosed FITC opsonized bacteria.

Leukocyte Apoptosis

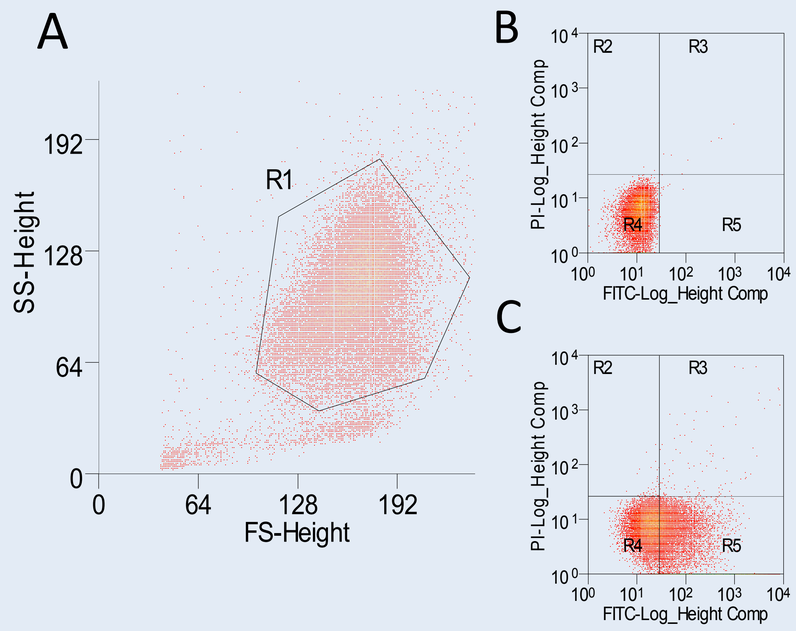

Figure 5. Graph A indicates that neutrophils and monocytes were included in the gate. Graph B is the unstained population. Graph C includes cells that have been stained with both PI and FITC Annexin V. FITC Annexin V is a phospholipid-binding protein used to determine the percentage of cells that are actively undergoing apoptosis, and PI distinguishes viable from nonviable cells. $PI^-/FITC^+$ cells are undergoing early apoptosis, while those that stain positive for both FITC and PI are undergoing late apoptosis.

TLR4 and HLADR Expression on PMNs

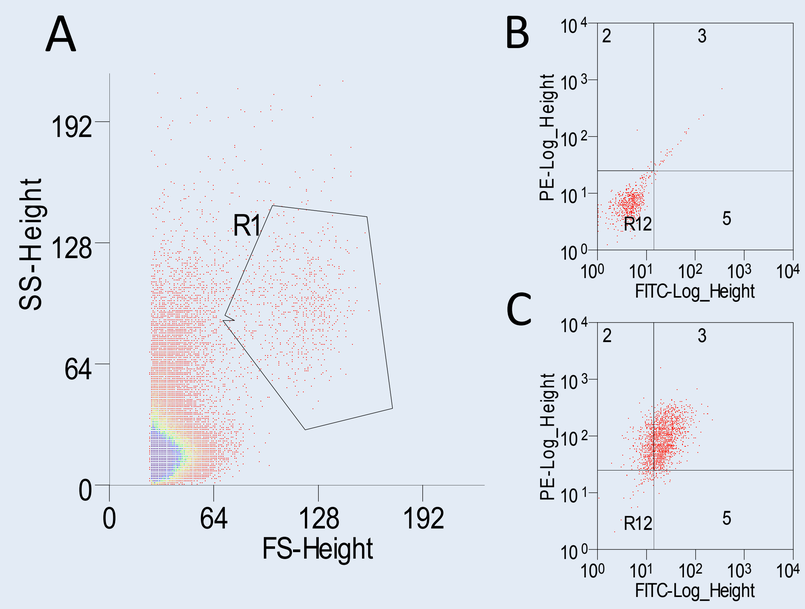

Figure 6. Graph A shows that neutrophils were included in the gate. Gate B shows the unstained population. Graph C shows cells stained with both FITC and PE and challenged with lipopolysaccharide (LPS).

Acknowledgments

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Conclusion

Results for this study are currently pending. More dogs will continue to be added to the study in order to achieve statistically significant results.