Apoptosis in neutrophils and lymphocytes in septic and critically-ill dogs

Veterinary Research Scholars Program
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Background

Sepsis is a leading cause of mortality in people and dogs. Traditionally, sepsis was thought to be due to a severe pro-inflammatory response causing tissue damage and ultimately death. New insights indicate that the pathophysiology of sepsis is more complex as both pro-inflammatory and anti-inflammatory responses occur early in sepsis and result in morbidity and mortality. Apoptosis is an important mechanism for regulating immune cell clearance during sepsis. Failure of neutrophil apoptosis results in inappropriate neutrophilic inflammation and tissue damage during sepsis in people. Concurrently, in people with sepsis, lymphocytes apoptosis results in immunosuppression and secondary infections. Similar pathology is suspected in dogs, but this has not been studied previously.

Hypothesis

We hypothesized that dogs with sepsis would have reduced spontaneous neutrophil apoptosis and increased lymphocyte apoptosis compared to dogs with other forms of critical illness or healthy dogs. We also hypothesized that cytokines, stress hormones and pathogen associated molecular pattern motifs (PAMPs) are involved with apoptosis signaling.

Methods

- Dogs were selected from the intensive care unit and assigned to the sepsis or critically ill groups based on diagnosis.
- Healthy dogs were recruited from students and faculty at MU CVM for controls.
- Additional samples of healthy dog blood were incubated with various cytokines, stress hormones and pathogen associated molecular pattern motifs (see figure 1.) to investigate the mechanism of sepsis-induced alterations in apoptosis.

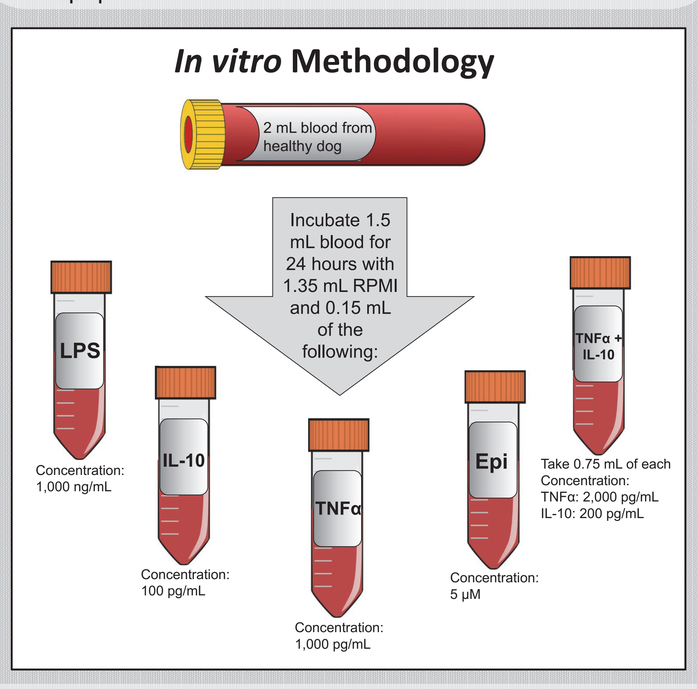

Figure 1. *In vitro* methodology.

- 2mL of whole blood was mixed 1:1 with complete RPMI for 24 hours. Next, the red blood cells were lysed and the leukocytes collected for assessment of apoptosis.
- Neutrophil and lymphocyte apoptosis was assessed with an annexin V–FITC and PI kit which has been validated for the dog. Leukocytes were incubated with annexin V–FITC and PI for 15 minutes. Binding buffers were added, and then flow cytometric analysis was performed. A minimum of 10,000 events was recorded per sample.
- Cells were applied to a forward scatter versus side scatter plot to identify and gate the neutrophils and lymphocyte population on the basis of their size and granularity (see figure 2.). Each cell type was then analyzed for fluorescence, with cells that were positive for FITC identified as apoptotic cells (see figure 3.). Early and late apoptotic cells were grouped together.

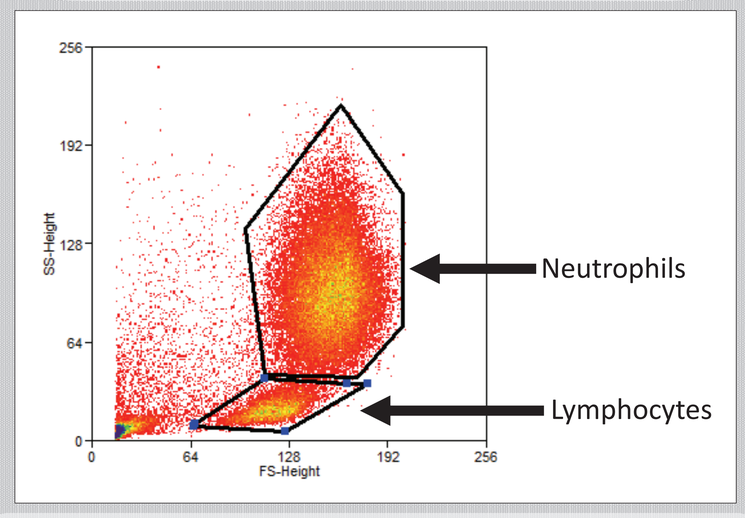

Figure 2. Using results from flow cytometry, cell populations were separated based on size (FS - forward scatter) and complexity (SS – side scatter). Neutrophils and lymphocytes were gated to determine percentage of apoptosis.

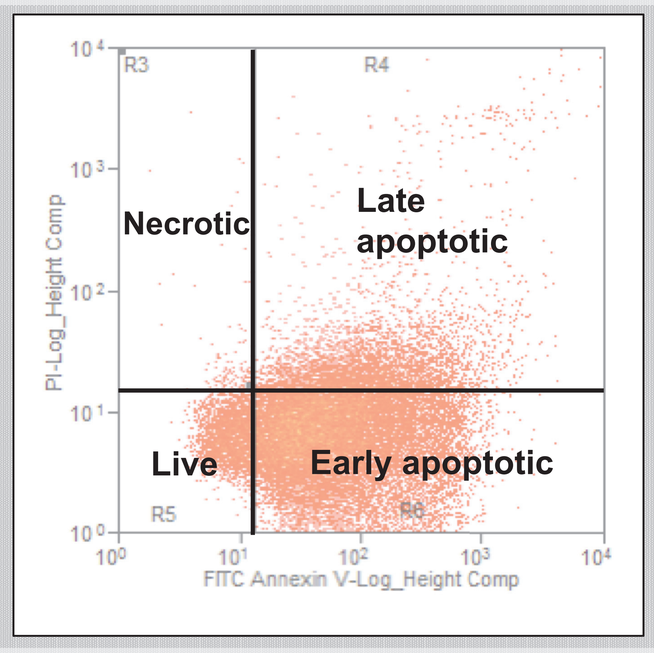

Figure 3. Analysis of fluorescence using plot of propidium iodine (PI) versus annexin V-FTC. FITC annexin V binds to phosphatidyl serine located on the inner leaflet of the plasma membrane which is flipped outward during early apoptosis. PI is not permiable in live cells and binds to DNA in necrotic and dead cells. Presence of FITC annexin V was measured as a determinant of early and late apoptotic cells, which were grouped together.

Results

Eight sick dogs have been enrolled to date. Dogs were 3-18 years of age. Two were intact males, four were castrated males, one was an intact female and one was a spayed female. Breeds were mixed breed (n=3), Labrador retriever, Doberman pincher, English cocker spaniel, bullmastiff and French bulldog. Diagnoses included acute kidney failure, leptospirosis infection (n=2), left sided congestive heart failure, septic shock, intestinal foreign body, abdominal mass and gastroenteritis (n=2).

Six healthy dogs have been enrolled to date. Dogs were 2-7 years of age. One was an intact male, three were castrated males and two were spayed females. Breeds were husky (n=2), Bernese mountain dog, Australian shepherd, pug and mixed breed.

Results are preliminary at this time and data collection is ongoing. For preliminary evaluation critically ill dogs and dogs with sepsis were combined into to form the sick group. There was no difference in lymphocyte apoptosis between the healthy dogs and the sick dogs (figure 4). However, neutrophil apoptosis was significantly reduced in the sick group compared to the healthy dogs. Evaluation of the effect of cytokines, stress hormones and pathogen associated molecular pattern motifs on sepsis-induced alterations in apoptosis are pending.

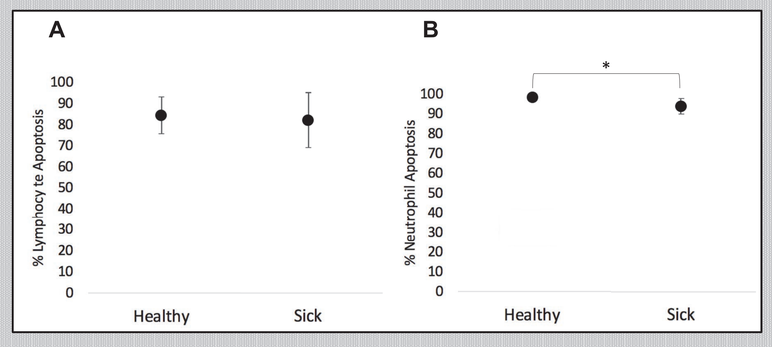

Figure 4. Comparison of mean±SD percentage of peripheral blood lymphocytes (A) or neutrophils (B) undergoing spontaneous apoptosis between healthy and sick dogs. *P=*0.0135*.

Conclusion

Lymphocyte apoptosis does not significantly increase in sick dogs. Neutrophil apoptosis, however, is decreased in sick dogs compared to healthy dogs. These preliminary data indicate that dogs are similar to people in that illness reduces spontaneous neutrophil apoptosis but may differ in that illness does not appear to increase lymphocyte apoptosis. Additional samples need to be collected to confirm these results.

Acknowledgments

Student support was generously funded by Mizzou Advantage Initiative in One Health / One Medicine. Special thank you to Dr. DeClue and the Comparative Internal Medicine Laboratory for their support and guidance.