Macrophages efferocytosis alters cytokine production in response to B. burgdorferi

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Abstract
Experimental Lyme arthritis caused by an infection with Borrelia burgdorferi presents an ideal model for the study of inflammation. Although progress has been made in the understanding of the disease progression and initial response to the Borrelia spirochete, comprehension of the mechanism that results in the resolution of inflammation is lacking. The purpose of the present study was to test whether treatment with apoptotic cells preceding an infection with B. burgdorferi results in the prevention of inflammation caused by macrophages. We expected that the apoptotic cells would cause the production of anti-inflammatory cytokines, such as IL-10 and lipoxins, and the down regulation of pro-inflammatory cytokines IL-12 and IL-6. In this study bone marrow macrophages were cultured from C3H wildtype mice. The macrophages were then treated with apoptotic Jurkat cells and activated with B. burgdorferi at timed increments following treatment. ELISA tests were performed to determine the pro-inflammatory and anti-inflammatory cytokines produced. The results would indicate that the uptake of apoptotic cells by macrophages inhibits inflammatory arthritis caused by B. burgdorferi. By identifying key components of inflammation, our research will enable a better understanding of the mechanism of inflammatory resolution and the components absent in the case of chronic inflammation.

Materials and Methods
Bone marrow macrophages from two C3H wildtype mice were harvested and cultured. Jurkat cells were also cultured. On the eighth day of culture, macrophages were collected, counted, and replated on a 24-well plate with 1x10^6 cells per well. Macrophages were incubated overnight at 37°C. Jurkat cells were treated with Fas antibody in order to induce apoptosis. Three controls were determined: isolated macrophages, macrophages with apoptotic cells, and macrophages with B. burgdorferi. All other macrophages were treated with apoptotic Jurkat cells at a 1:1 ratio. Following treatment, macrophages were activated with 1x10^7 B. burgdorferi at time increments of 30 minutes, 1 hour, 2 hours, 3 hours, and 4 hours. Cultures were incubated overnight at 37°C, and supernatant was harvested and cultured. Jurkat cells were then treated and supernatant was determined: isolated macrophages, macrophages with apoptotic cells, macrophages with B. burgdorferi and macrophages with apoptotic cells and B. burgdorferi. All other macrophages were treated with apoptotic Jurkat cells at a 1:1 ratio. Following treatment, macrophages were activated with 1x10^7 B. burgdorferi at time increments of 30 minutes, 1 hour, 2 hours, 3 hours, and 4 hours. Cultures were incubated overnight at 37°C, and supernatant was harvested and cultured. ELISA testing for IL-10 and IL-6 was performed and data was analyzed.

Results
Treatment with apoptotic cells suppressed IL-10 and IL-6 secretion from macrophages activated with B. burgdorferi. Both pro-inflammatory and anti-inflammatory cytokines were effected. Production of IL-10 from treated macrophages is not different between the time points. However, the amount of IL-6 produced by treated macrophages varied some based on the time that the infection was induced.

Conclusions
In vivo studies of apoptotic treatments of B. burgdorferi infected mice resulted in decreased swelling, however cytokine production did not support these results. This in vitro study reveals that the production of cytokines is suppressed when the macrophages are treated with apoptotic cells. IL-10 is produced both during pro-inflammatory and anti-inflammatory states, and IL-6 is typically produced during pro-inflammatory responses. Less IL-10 and IL-6 is produced by treated macrophages. This could mean that apoptotic treatments induce a more mild form of the infection; and that apoptotic cells suppress the macrophages ability to secrete cytokines. Treatment with apoptotic cells on macrophages infected with B. burgdorferi does not appear to be time-dependent.

Future Directions
1. Measure levels of cytokines MIP-1α, IL-12, lipoxin B4, and TGF-β from supernatant.
2. Repeat experiment testing different concentrations of apoptotic cells given to the macrophages.
3. Perform in vitro experiments where the treatment mice with apoptotic cells before infecting them with B. burgdorferi.

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