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.

Typical Swelling Curve

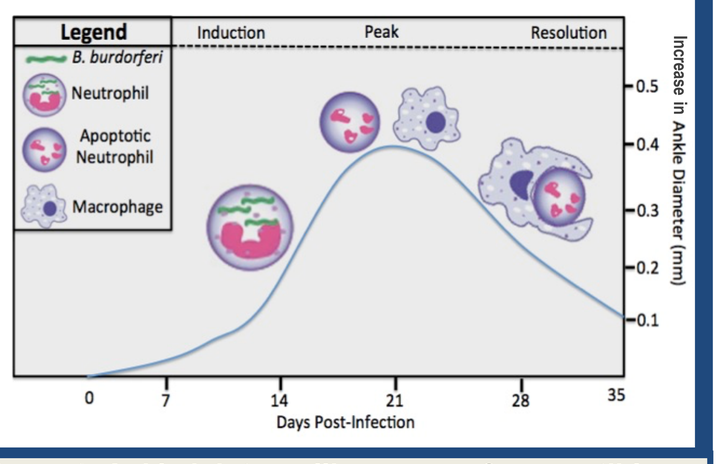

Figure 1: Ankle joint swelling curve of susceptible C3H mice. This pattern of inflammation can be observed over time after an infection with *B. burgdorferi*.

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 re-plated 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 collected the following day. ELISA testing for IL-10 and IL-6 was preformed and data was analyzed.

Production of IL-6

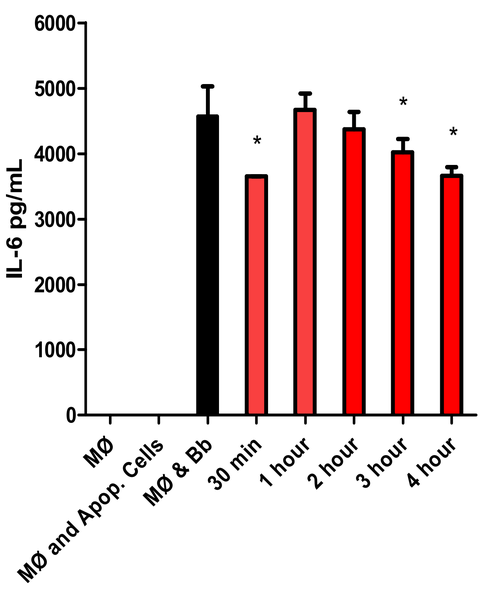

Figure 2: Production of IL-6 from macrophage culture supernatant. Time allotment between apoptotic treatment and *Bb*. infection. Treatment with apoptotic cells prior to infection causes a decrease in the amount of IL-6 produced.

Macrophage Cytokine Production

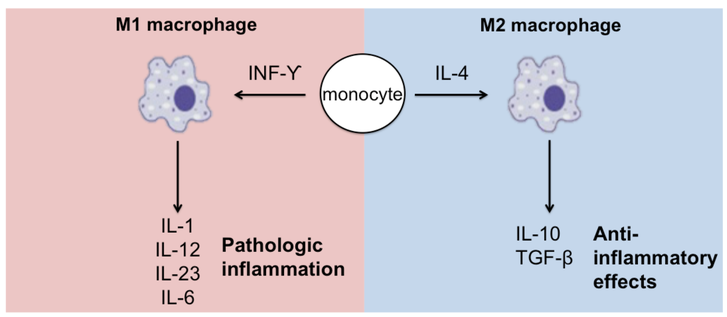

Figure 3: Production of pro-inflammatory and anti-inflammatory cytokines produced by classically activated (M1) macrophages and alternatively activated (M2) macrophages.

Production of IL-10

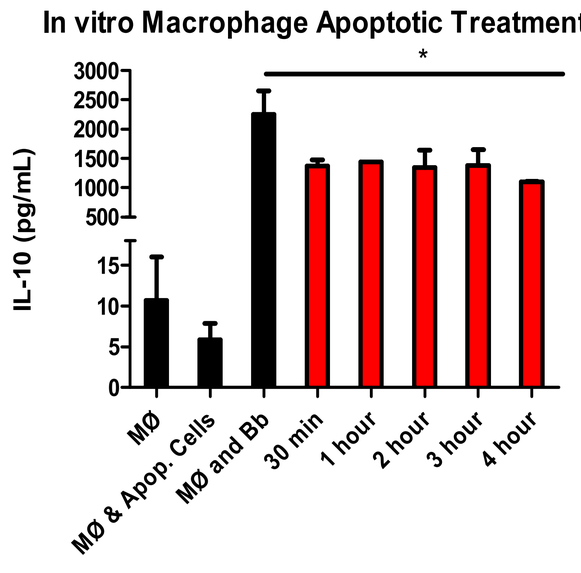

Figure 4: Production of IL-10 measured from macrophage culture supernatant. Treated samples produced less cytokine than the untreated control. There was no significant difference between IL-10 levels of timed increment samples.

Ankle Swelling Curve

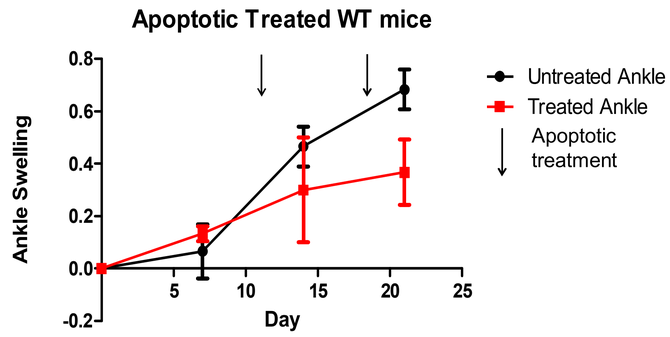

Figure 5: A representation of three repeated *in vivo* experiments, where C3H wildtype mice were given intraarticular injections of $1x10^6$ apoptotic cells on day 11, day 18 and sacrificed on day 21 of infection with *B. burgdorferi*.

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 those 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 B_4, and TGF-β from supernatant.
2. Repeat experiment testing different concentrations of apoptotic cells given to the macrophages.
3. Repeat *in vitro* experiment with different order of infection and treatment. Macrophages will be infected with *B. burgdorferi* and then treated with apoptotic cells.
4. Perform *in vivo* study treating mice with apoptotic cells before infecting them with *B. burgdorferi*.

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