DIFFERENTIAL ACUTE PHASE PROTEIN RESPONSE INDUCED IN MICE EXPERIMENTALLY INFECTED WITH *Brucella microti* AND THE REFERENCE STRAIN *Brucella suis 1330*

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The aim of this study was to use the acute phase protein response of haptoglobin, serum amyloid A (SAA), α1-glycoprotein acid (AAG), hemopexin, inter-α-trypsin inhibitor heavy chain 4 (ITIH4), C-reactive protein (CRP), α1-cysteine proteinase inhibitor (IPC) and transferrin to quantify the systemic reaction of mice infected with *B.microti* or *B.suis 1330*. A particular objective was to determine the differences of the systemic acute phase response between the two type of *Brucella* infections

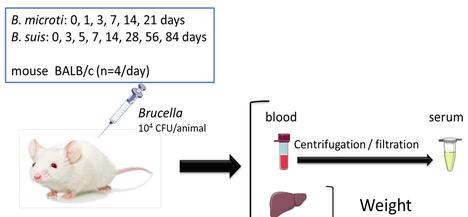
MATERIALS AND METHODS

Kinetics of infection. BALB/c mice were infected intraperitoneally with 10^4 CFU of *B. microti* and *B.suis 1330. S*pleen and livers were weighed and harvested for bacterial counts at 1, 3, 7, 14 and 21 days (*B. microti*) and 3, 5, 7, 14, 28, 56 and 84 (*B.suis*) days post infection.

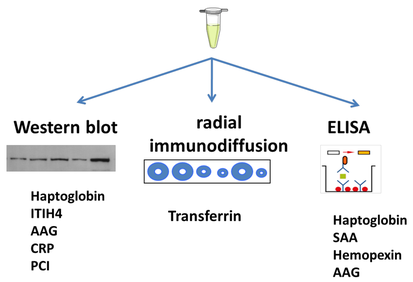
Acute phase proteins (APPs). APPs were measured in the mice serum. SAA and hemopexin were assayed by ELISA; ITIH4, CRP, PCI by western blot and probed with the respective polyclonal rabbit anti-protein antibody. Haptoglobin and AAG were measured by both methods, ELISA and western blot and transferrin was assayed by radial immunodiffusion.

Data are represented as Mean ± SEM * p<0.05, **p<0.01

Experimental infection with *B. microti* and *B. suis*



Quantification of Acute Phase Proteins



INFECTION RESULTS

Mice were unable to remove *B.suis* at 84 days while *B. microti* was eliminated from spleen and liver on day 21 which corresponds to a chronic or acute infection, respectively. The evolution of cfu and weight of spleen and liver were also delayed in *B.suis* infection.

B. microti cfu maximum was reached at day and later on, at day 7, it was reached by *B. suis* in both, liver and spleen. *B.microti* infection also showed a maximum of weight of spleen and liver earlier (7 day) than *B.suis* infection (14 day) (Figure 1).

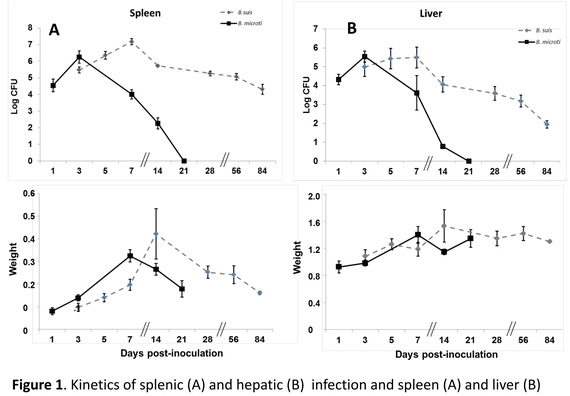


Figure 1. Kinetics of splenic (A) and hepatic (B) infection and spleen (A) and liver (B) weight (g) in mice inoculated intraperitoneally with 10^4cfu *B. microti* (solid line) and *B.suis* 1330 (dotted line) at each time-point.

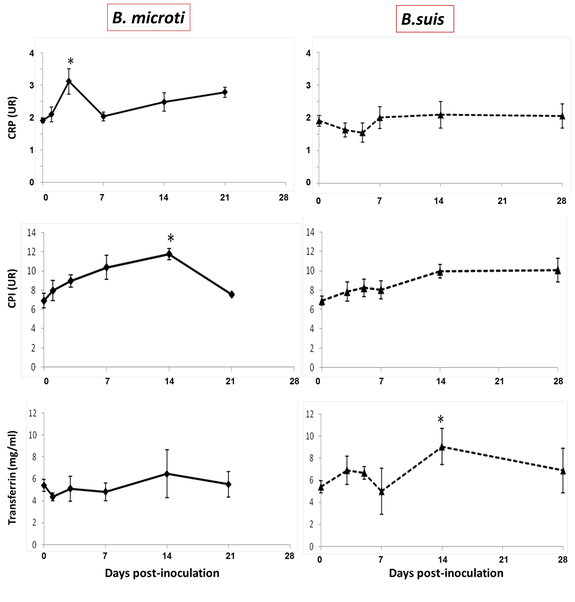


Figure 4. Concentration of CRP, CPI and transferrin in serum of mice inoculated intraperitoneally with 10^4cfu *B. microti* (solid line) and *B.suis* 1330 (dotted line) at each time-point

ACUTE PHASE PROTEINS RESULTS

Acute phase proteins were quantified from sera of normal and infected mice by Brucella. Maximum values of APPs concentration were higher in animals infected by *B. microti* than in animals infected by *B. suis* .

The concentration of haptoglobin and SAA was risen from 0 to 1.4 in B. microti sera and 0.76 mg/ml for B. suis sera, respectively. Increases of hemopexin, AAG and ITIH4 were around (3.5x), (11x) and (3x), respectively, during infection by *B.microti*. These increases were lower during infection by *B.suis*: from 0 to 0.6 mg/ml for haptoglobin and SAA; (5x) for AAG and (1.5x) for ITIH4. Hemopexin was the only APP that reached a slightly higher maximum of concentration during infection by *B.suis* (4x) than by *B.microti* (3.5x) (Figures 2 and 3).

The maximum APPs serum concentration caused by Brucella was reached before by *B. microti* than by *B. suis* infection. *B.microti* infection induced a peak serum concentration of haptoglobin, SAA and ITIH4 on day 3 and hemopexin and AAG on day 7 while the peak of *B.suis* was at day 7 for SAA and ITIH4 and at day 14 for haptoglobin, hemopexin and AAG (Figures 2 and 3).

In sera of mice infected with *B. microti* a significant increase (1.5x) of CRP and (2x) CPI was observed at days 3 and 14 of infection, respectively. By contrast, increase in transferrin concentration (1.7x) happened only in *B. suis* infection at day 14 (Figure 4).

CONCLUSIONS

APP response followed the clinical symptoms of the outcome of the disease and could constitute a rapid tool for detecting early infection processes.

The most important APPs in *Brucella* infections were haptoglobin, SAA , AAG, ITIH4 and hemopexin.

Virulence differences between *Brucella* strains have been reflected in a different APP response from the host. *B. microti* was the most virulent infection and caused an increase in these APPs earlier and more pronounced than *B. suis*, which is consistent with situations of stress or inflammation.

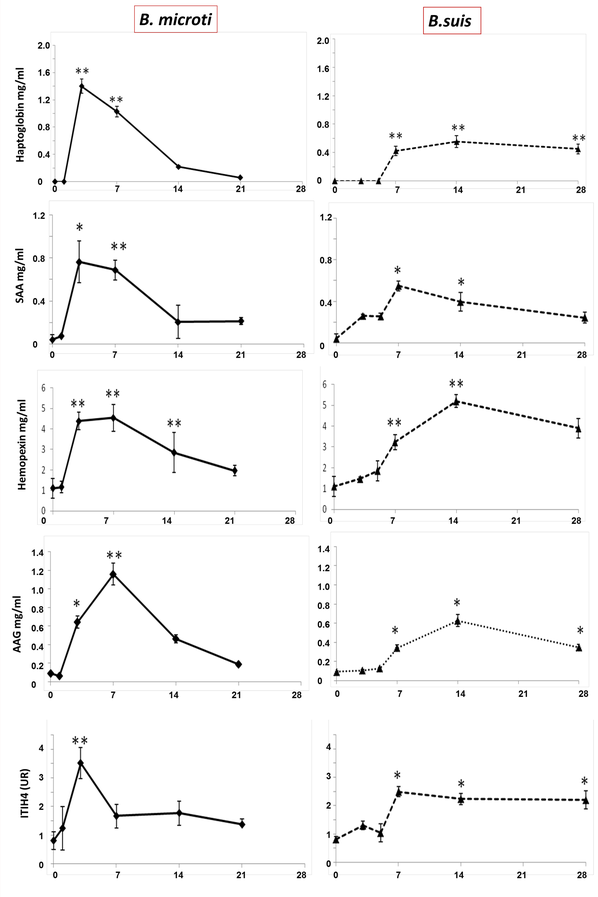


Figure 2. Concentration of Haptoglobin, SAA, hemopexin, AAG and ITIH4 in serum of mice inoculated intraperitoneally with 10^4cfu *B. microti* (solid line) and *B.suis* 1330 (dotted line) at each time-point

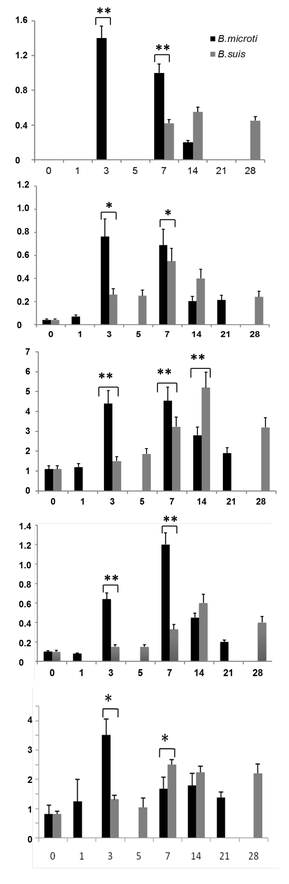


Figure 3. Comparison of the evolution of the acute phase response between mice infected intraperitoneally with 10^4cfu *B. microti* (black) and *B.suis* 1330 (grey) at each time-point (from Figure 2).

REFERENCES and acknowledgements

1. Jiménez de Bagüés MP et al. J. Infect. Dis. 2010; 202 (1):3-10. 2. Alonso-Fauste I et al. J.Proteomics 2012; 75: 3015-3030

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