Published in final edited form as:

Vet J. 2008 January ; 175(1): 10–11. doi:10.1016/j.tvjl.2007.02.026.

Unraveling the immune regulatory mechanisms imposed by *Anaplasma*

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The paper by Zerai Woldehiwet, published in this issue of *The Veterinary Journal*, reviews mechanisms of immune evasion and immune suppression by the mammalian pathogen, *Anaplasma phagocytophilum*, which primarily invades and replicates within neutrophils. *A. phagocytophilum* infection causes a generalized immune suppression in sheep characterized by neutropenia, lymphopenia, and susceptibility to various concurrent bacterial infections resulting from impaired neutrophil and lymphocyte function (Woldehiwet, 2007). The related cattle pathogen, *A. marginale*, which invades and replicates within erythrocytes, causes anemia, abortion in pregnant heifers, and weight loss, can, during acute infection, reach levels of 10^9 infected erythrocytes per milliliter of blood (Palmer et al., 1999). Following resolution of acute infection, both pathogens establish persistence, which for *A. marginale* infected cattle, can be of lifelong duration.

Both pathogens evade the immune response through antigenic variation of surface coat proteins, including major surface protein (MSP) 2 and MSP3 in *A. marginale*, and the related MSP2/P44 in *A. phagocytophilum* (Palmer et al., 2006). The immunological consequences of this antigenic variation have been documented for *A. marginale*. During persistent infection, organisms express sequentially variant B cell and T cell epitopes in MSP2 that evade effector IgG2 and CD4⁺ T cell recognition (French et al., 1999; Brown et al., 2003).

In contrast to the generalized immune suppression observed during *A. phagocytophilum* infection of sheep, infection of cattle with *A. marginale* does not result in altered numbers of circulating leukocytes, including lymphocytes, and generalized immune suppression has not been reported. However, immunization models using *A. marginale* MSPs have recently revealed infection-induced regulation of a strong pre-challenge CD4⁺ T cell response. In the first model, cattle were hyperimmunized by subcutaneous inoculation of gel-purified native MSP2 in Th1 adjuvants and challenged 6 months later with organisms expressing the same major MSP2 variant that was present in the immunogen (Abbott et al., 2005). The predicted protection against infection with organisms expressing this major MSP2 variant was not realized; rather the lack of protection against anemia and rickettsemia was accompanied by a dramatic loss in MSP2-specific CD4⁺ T cell responses. The robust pre-challenge levels of CD4⁺ T cell proliferation and IFN- γ secreting cells in response to *A. marginale*, native MSP2, or immunogenic MSP2 peptides in both conserved and hypervariable regions were reduced to background levels just prior to peak rickettsemia. The loss of response to epitopes conserved in all MSP2 variant proteins indicated that the loss of the MSP2-specific response was not due to emergence of new antigenic variants. Importantly, antigen-specific responses were never

detected in animals monitored periodically for a year after infection. In contrast, responses to the unrelated *Clostridium* antigen used to vaccinate the calves prior to the initiation of the experiment remained normal.

Although the mechanism of this infection-induced specific immune suppression is not yet known, functional suppressor cells in the peripheral blood of non-responding animals were not detected, the frequencies of circulating CD25⁺ CD4⁺ lymphocytes during the course of infection did not vary significantly, and the production of IL-10 and TGF- β by peripheral blood lymphocytes in response to antigen did not increase following infection. These results suggest the sudden loss of MSP2-specific T cell responses in the peripheral blood was not simply due to an increase in regulatory T cells in peripheral blood in response to infection (Abbott et al., 2005). However, the spleens were not evaluated for functional suppressor T cells, which if present, could explain these results, as the spleen is important for removing infected erythrocytes during hemoparasite infections.

In a second immunization model, cattle were inoculated intradermally with a DNA construct encoding a T cell epitope (F2-5) and B cell epitopes (the N-terminal repeats) from the conserved MSP1a, boosted once with recombinant protein (Mwangi et al., 2007) and subsequently challenged months later with *A. marginale*. As observed for MSP2, the strong peripheral blood CD4⁺ T cell responses measured after immunization and before challenge disappeared just prior to peak rickettsemia (W. C. Brown, unpublished data). The loss of response was again specific for *A. marginale*, and *Clostridium*-specific responses remained intact. This experiment confirmed that the infection-induced loss of response was not restricted to the variable MSP2 antigen, but occurred using a different immunization protocol and a conserved protein epitope. Furthermore, for both studies, *Anaplasma*-specific T cell responses were not detected in peripheral blood lymphocytes from control sham immunized animals following challenge (Abbott et al., 2005; W. C. Brown, unpublished data).

This important observation suggests that in spite of high levels of rickettsemia and *A. marginale* specific IgG antibody titers, antigen-specific T cell responses are difficult to detect in peripheral blood. Examination of splenic lymphocyte responses should be conducted to determine whether *Anaplasma*-specific lymphocytes (effector cells or regulatory T cells) sequester there. In fact, one study performed by Gale et al. (1996) showed *A. marginale*-induced lymphocyte proliferation in spleens of persistently infected cattle at time points when peripheral blood responses were not detected. A caveat to these results is that negative control antigens were not included in these assays.

Intracellular *A. phagocytophilum* and *A. marginale* pose unique problems for vaccine development. In spite of very different intracellular niches, both bacteria achieve persistent infection, which is critical for tick transmission to susceptible individuals. Contributing to the ability to persist are extensive antigenic variation in their immunodominant surface proteins MSP2 (and MSP3 for *A. marginale*) and evasion the immune response, and modulation of the host immune response. *A. phagocytophilum* induces a generalized immune suppression attributed to lymphocytopenia, neutropenia and thrombocytopenia, whereas *A. marginale* infection suppresses a previously elicited *A. marginale* MSP-specific T cell response and in naïve animals infection fails to stimulate consistently detectable peripheral T lymphocyte responses. Unraveling the immune regulatory mechanisms imposed by *Anaplasma* is an important and exciting area of investigation.

References

- Abbott JR, Palmer GH, Kegerreis KA, Hetrick P, Howard CJ, Hope JC, Brown WC. Rapid and long-term disappearance of CD4⁺ T-lymphocyte responses specific for *Anaplasma marginale* major surface

protein-2 (MSP2) in MSP2 vaccinates following challenge with live *A. marginale*. Journal of Immunology 2005;174:6702–6715.

Brown WC, Brayton KA, Styer CM, Palmer GH. The hypervariable region of *Anaplasma marginale* major surface protein 2 (MSP2) contains multiple immunodominant CD4⁺ T lymphocyte epitopes that elicit variant-specific proliferative and IFN responses in MSP2 vaccinates. Journal of Immunology 2003;170:3790–3798.

French D, Brown WC, Palmer GH. Emergence of *Anaplasma marginale* antigenic variants during persistent rickettsemia. Infection and Immunity 1999;67:5834–5840. [PubMed: 10531237]

Gale KR, Gartside MG, Dimmock CM, Zakrzewski H, Leatch G. Peripheral blood lymphocyte proliferative responses in cattle infected or vaccinated with *Anaplasma marginale*. Parasitology Research 1996;82:551–562. [PubMed: 8832738]

Mwangi W, Brown WC, Splitter GA, Davies CJ, Howard CJ, Hope JC, Aida Y, Zhuang Y, Hunter B, Palmer GH. A DNA vaccine construct incorporating intercellular trafficking and intracellular targeting motifs effectively primes and induces memory B and T cell responses in outbred animals. Clinical and Vaccine Immunology. 2007In press

Palmer GH, Futse JE, Knowles DP Jr, Brayton KA. Insights into mechanisms of bacterial antigenic variation derived from the complete genome sequence of *Anaplasma marginale*. Annals of the New York Academy of Sciences 2006;1078:15–25. [PubMed: 17114676]

Palmer GH, Rurangirwa FR, Kocan KM, Brown WC. Molecular basis for vaccine development against the ehrlichial pathogen *Anaplasma marginale*. Parasitology Today 1999;15:281–286. [PubMed: 10377531]

Woldehiwet Z. Immune evasion and immunosuppression by *Anaplasma phagocytophilum*, the causative agent of tick-borne fever of ruminants and human granulocytic anaplasmosis. The Veterinary Journal, Insert doi number. 2007