

Loss of antigen-specific CD4⁺ T cells during *Anaplasma marginale* challenge in cattle

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Anaplasma marginale is a rickettsial pathogen of cattle that causes morbidity, mortality, and persistent infection. How the rickettsia maintain persistence in the immunocompetent host is poorly understood. Previously, cattle immunized with an immunodominant surface protein of *A. marginale* developed strong antigen-specific CD4⁺ T lymphocyte responses that following challenge, rapidly disappeared in peripheral blood and remained undetectable throughout persistent infection. To further characterize this loss of infection-induced antigen-specific response, cattle were immunized with a DNA vaccine encoding a conserved 30-mer surface epitope (F2-5B) of *A. marginale* and challenged with live organisms. Upon challenge, F2-5B-specific peripheral blood CD4⁺ T-lymphocyte proliferation and IFN- γ secretion again disappeared at peak rickettsemia and remained undetectable until necropsy at 9 weeks to 12 weeks post-challenge. Bovine MHC class II DRB3*1101 tetramers were used to detect the frequency of F2-5B-specific CD4⁺ T cells in PBMC before and during challenge and from lymphoid organs at death. The frequency of F2-5B-specific CD4⁺ T lymphocytes sharply decreased in peripheral blood just before peak rickettsemia and remained low until death. F2-5B-specific T-lymphocytes were not detected with tetramers in lymphoid organs at the time of necropsy, showing that antigen-specific T cells were not sequestered in these tissues. Rapid loss of *A. marginale*-specific CD4⁺ T lymphocytes at the peak of antigenic stimulation during infection is consistent with activation induced cell death and represents an important mechanism by which *A. marginale* controls the host immune response to achieve persistence.