

**DETERMINATION OF ANAPLASMA MARGINALE MAJOR SURFACE PROTEIN 2
HYPERVARIABLE (MSP2 HV) REGION CD4⁺ T-CELL EPITOPES AND VARIANT
EPITOPE EFFECT ON T-CELL PROLIFERATION**

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Anaplasma marginale persists in cattle by means of antigenic variation of at least one of the major surface proteins, MSP2. In a previous experiment using 30-mer synthetic peptides that overlapped by 20 aa, MHC class II-restricted T-cell epitopes within the immunodominant areas of MSP2 hypervariable region were identified. To further define one of the specific epitopes, T-lymphocyte proliferation assays were done on cow #61 T cell lines using 9 to 20 amino acid (aa) length peptides. The exact epitope was identified as a 10 amino acid peptide, GDELSKKVCG, which is contained in the HV 3, 4 and 5, 30-mer peptides previously tested. To further test the immunostimulatory nature of the identified epitope, an interferon-gamma assay was conducted on the supernatants from a representative proliferation assay. Interferon-gamma levels were elevated in supernatants from T cells stimulated with the identified 30-mer HV MSP2 epitope peptides, including the 10 aa epitope, designated HV 3/4b.

In order to test the effect of antigenic variation on T-cell recognition and proliferation, two variant HV 3/4 peptides contained within the original immunogen were synthesized and tested. These peptides, designated HV3/4m (GEKVSQNVCG) and HV 3/4n (GQTVSQQVCG) were not recognized and T-cell proliferation did not occur in the presence of these peptides.

Antigenic variation within T-cell epitopes of MSP2 seems to be a successful strategy for evading the established primary response. Proposed mechanisms to explain the lack of T cell responsiveness to variant epitopes are i] escape from MHC class II

presentation, ii] the possibility that variants are of such low numbers in the immunogen that an immune response is not detectable, and iii] anergy of primed T-cells may be occurring in response to the variant peptide.