

Immunity to the bovine apicomplexan parasite *Theileria parva* is associated with MHC-I restricted CD8<sup>+</sup> T cell responses directed against the intralymphocytic schizont stage of the parasite. A number of schizont-stage antigens that are targets of CD8<sup>+</sup> T cell responses from immune animals have been identified but an effective delivery strategy that consistently induces protective CD8<sup>+</sup> T cell responses remains to be developed. This study aimed to determine whether fusing Tat, a cell penetrating peptide (CPP) from HIV-1 TAT, to a CD8<sup>+</sup> T cell target antigen from *T. parva* (Tp2) enhances the cytosolic delivery and subsequent stimulation of bovine CD8<sup>+</sup> T cell responses in vitro. Using IFN-gamma ELISpot and cytotoxicity assays, it was demonstrated that recombinant Tat-Tp2 fusion protein possessed a superior ability to access MHC-I processing and presentation pathway and to stimulate CD8<sup>+</sup> T cell responses compared to recombinant Tp2 protein. Exposure of APC to Tat-Tp2 protein for only 30 min was sufficient for protein uptake and stimulation of CD8<sup>+</sup> T cells. This work describes for the first time the utility of a CPP to enhance MHC-I presentation in a veterinary species and supports the evaluation of CPP fusion proteins in the induction of CD8<sup>+</sup> T cell responses in vivo.