Local and systemic roles for bovine gamma-delta T cell subsets during the early immune response to *Mycobacterium avium* subspecies *paratuberculosis* (Map) infection

Brandon Lee Plattner, Elise Huffman, Jesse M Hostetter

*Iowa State University, USA*

The role of gamma-delta (gd) T lymphocytes during the immune response to infectious agents continues to be explored. Elevated numbers of gd-positive cells are found as intra-epithelial lymphocytes at mucosal surfaces and in peripheral blood of some species. Peripheral blood gdT cells from both naïve and previously exposed individuals (cattle and humans) proliferate robustly and secrete cytokines when stimulated with mycobacterial antigens, suggesting a role for gdT cells during early host defense against mycobacterial disease. A significant question that remains unanswered is whether peripheral blood studies accurately reflect what occurs *in vivo* at the infection site. While gdT cells have been shown to be recruited to early mycobacterial infection sites *in vivo*, their functional role remains unclear; this is largely due to the difficulty in obtaining sufficient numbers of gdT cells from infection sites for analysis. Our hypothesis is that bovine gdT cells have subset-specific roles during the initial host response to Map infection in young calves. In this study, we investigated our hypothesis using a novel subcutaneous biopolymer gel matrix-based assay developed in our laboratory to characterize the *in vivo* cellular and cytokine responses to Map infection in both naïve and vaccinated calves. To accomplish this objective, we used multi-color flow cytometry and enzyme-linked immunosorbent assay (ELISA) to characterize surface marker expression and cytokine secretion from cells recruited directly to Map infection sites. Further, we compared our findings with gdT cell responses from peripheral blood cells during Map infection. Our results suggest that bovine gdT cell subsets are differentially recruited to early mycobacterial infection sites. Further, we show that bovine gdT cell subsets recruited to infection sites differentially secrete cytokines. Our data suggests that this initial response by gdT cell subsets to mycobacterial infection may play an important role during development of the subsequent immune response.