

## ***Neospora caninum* cyclophilin elicits interferon-gamma production**

**Wenbin Tuo, Raymond Fetterer, Mark Jenkins and J.P. Dubey**

Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Center, United States Department of Agriculture, Agricultural Research Service, Beltsville, Maryland 20705, USA

### **Abstract**

Interferon-gamma (IFN-gamma) response is necessary for the development of a host protective immunity during infection by intracellular parasites. Neosporosis which is caused by the intracellular protozoan parasite *Neospora caninum* is known to be fatal in IFN-gamma deficient hosts. However, the mechanism to elicit IFN-gamma by the invading parasite is unclear. This study has identified a microbial protein in the *N. caninum* tachyzoite, the *N. caninum* immunophilin, as a major component of the parasite responsible for inducing IFN-gamma production by bovine peripheral blood mononuclear cells (PBMC) and antigen-specific CD4<sup>+</sup> T cells. The immunophilin has high sequence homology (86%) with the *T. gondii* 18 kDa cyclophilin, and has a calculated molecular mass (Mr.) of 19.4 kDa. The Immunophilin is a secretory protein with a predicted signal peptide of 17 amino acids. The immunophilin has detectable peptidylprolyl isomerase activity and may not be N-glycosylated

although potential glycosylation sites are present. The immunophilin was detected in whole-cell *N. caninum* tachyzoite lysate (NcAg) and in culture supernatant of live *N. caninum* tachyzoite. In *N. caninum* tachyzoite culture supernatant, three immunophilin bands of 19, 22, and 24 kDa were identified, whereas only two bands of 19 and 24 kDa were detected in whole-cell tachyzoite lysate. NcAg induced high levels of IFN-gamma production by PBMC and CD4<sup>+</sup> T cells *in vitro*. IFN-gamma-inducing effect of NcAg was blocked by cyclosporin A (CsA), a specific ligand for immunophilin, in a dose-dependent fashion. Furthermore, CsA abolished immunophilin-induced IFN-gamma production by PBMC from naive cows and PBMC and CD4<sup>+</sup> T cells from immune cows, suggesting that IFN-gamma induced by immunophilin may be independent of antigen stimulation. These results indicate that the *N. caninum* tachyzoite naturally produces an IFN-gamma-inducing protein, immunophilin, which may in part mediate parasite survival as well as host protection.