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Neospora and neosporosis: achievement and perspectives in host and parasite cell biology

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Abstract

The protozoan parasite *Neospora caninum* is one of the most important abortifacient organisms in cattle worldwide. While most studies on host-parasite interactions to date have been focusing on the epidemiology, immunology and pathogenesis of neosporosis, we have been investigating the host-parasite relationship on the cellular level. In this context, three key events play a major role. First, unlike many other apicomplexan parasites, but in analogy to *Toxoplasma* gondii, *N. caninum* profits immensely from its ability to interact with, and invade, a large number of different host cell types. Secondly, *N. caninum* exploits its capability to respond to alterations in living conditions by converting into another stage (tachyzoite-to-bradyzoite or vice versa). Thirdly, this parasite has evolved mechanisms that modulate its host cells according to its own requirements. These three key events (host cell invasion – stage conversion – host cell modulation) represent potential targets for intervention.

With respect to host cell invasion, we have identified and characterized several *N. caninum* antigens, most notably associated with the surface and the micronemes, which play important roles as adhesins and promote tachyzoite-host cell attachment. For instance, we found that the initial contact between parasite and host cell surface membrane is mediated preferentially through chondroitinsulphate A (CSA), and the microneme antigens NcMIC1, NcMIC3 and NcMIC4 all bind to CSA. The fact that pepstatin inhibits tachyzoite host cell invasion suggests the involvement of aspartyl proteases in the invasion process. The identification of these proteases remains to be undertaken. Furthermore, many major components of the adhesion and invasion machinery of apicomplexan parasites are cysteine-rich and dependent on correct folding via disulfide bond formation. In this respect, we found clear evidence that tachyzoite host cell adhesion is mediated by surface-associated protein disulfide isomerase, an enzyme involved in formation, cleavage and isomerization of disulfide bridges. Some of the *N. caninum* antigens such NcSAG1, NcSRS2, NcMIC3 and NcMIC1, have been investigated as potential vaccine candidates in a mouse model, and especially NcMIC3, expressed as a histidine-tagged recombinant protein in *E. coli* and purified by affinity chromatography, has been shown to prevent cerebral infection in mice upon experimental challenge with *N. caninum* tachyzoites.

The establishment of the *Neospora* bradyzoite in vitro culture model, and the development of techniques for the enrichment of bradyzoites, has allowed us to investigate bradyzoite host cell invasion. We found that bradyzoites are much less invasive compared to tachyzoites, and, in contrast to tachyzoites, they depend on host cell surface sialic acid residues as receptors for host cell binding.

Once inside their host cells, *N. caninum* tachyzoites and bradyzoites reside within a vacuole, surrounded by a parasitophorous vacuole membrane (PVM) and they are thus separated from the host cytoplasm. It has been shown for other apicomplexa (e.g. *Toxoplasma*), that these parasites must have evolved a machinery to communicate with the host cell, and the same must be true for *Neospora*. For instance, these parasites interfere in mechanisms leading to either activation or inhibition of host cell apoptosis. This crosstalk between intracellular para-

site and host cell is most likely mediated through proteins associated with the PVM, or through parasite-derived molecules, which are either actively or passively transported through the PVM. In the future, the identification and characterization of such molecules involved in *Neospora*-host cell communication will be a major focus of research in this group.