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Research Interests

Toxoplasma gondii is a protozoan parasite that causes severe congenital disease in newborn infants, and life-threatening disease in people whose immune systems have been weakened by immunosuppressants, age or AIDS. The tissue destruction associated with toxoplasmosis is in large part due to repeated cycles of host cell invasion, parasite multiplication and host cell lysis. A better understanding of the mechanisms of host cell invasion by *T. gondii* is therefore important to the development of new approaches to the treatment of the disease it causes. *T. gondii* is also a powerful model for studying invasive mechanisms in related, but less experimentally tractable, protozoan parasites within the Phylum Apicomplexa, including *Plasmodium* (the causative agent of malaria) and *Cryptosporidium*.

Our research is directed towards identifying *T. gondii* proteins that play a role in host cell invasion and determining what these proteins do. We have studied in detail the function of one specific protein, TgAMA1, and we have developed more general, small-molecule-based approaches to identifying previously uncharacterized invasion-related proteins.

TgAMA1. Using a combination of photoaffinity labeling (Gilk *et al*, 2006) and targeted monoclonal antibody screening (Ward and Carey, 1999), we identified and characterized several novel secreted and surface proteins of *T. gondii* (Carey *et al*, 2000, 2004; Gaskins *et al*, 2004; Gilk *et al*, 2006). One of these proteins, TgAMA1 (Donahue *et al*, 2000), was of particular interest to us. It is a member of a family of proteins that we now know is widely conserved in apicomplexan parasites. Considerable indirect evidence had accumulated suggesting a role for *Plasmodium* AMA1 in invasion, and *Plasmodium* AMA1 was - and remains - a leading malaria vaccine candidate. Nonetheless, despite almost two decades of intense interest in AMA1 family proteins, little was known about their function. This was in part because AMA1 is an essential gene; attempts to disrupt AMA1 for functional studies had been uniformly unsuccessful.

Using a recently developed system for conditional gene expression in *T. gondii*, we generated a parasite line in which the expression of TgAMA1 could be experimentally controlled (Mital *et al*, 2005). A decrease in TgAMA1 expression in these conditional knockout parasites causes a dramatic decrease in their invasiveness, providing direct evidence that TgAMA1 plays a critical role in invasion. Further phenotypic analysis of the mutants suggested that attachment of *T. gondii* to host cells occurs in two distinct stages, the second of which requires TgAMA1 and is involved in regulating secretion from the rhoptries, apical secretory organelles that function in invasion (Mital *et al*, 2005). TgAMA1 also appears to play an important role in forming the junction between the parasite and the host cell, through which the parasite physically pulls itself during invasion (Alexander *et al*, 2005).

TgAMA1 is stored in apical secretory organelles known as the micronemes, and released onto the parasite surface during invasion. There, it is proteolytically cleaved within its transmembrane domain and “shed” from the parasite. The shedding of microneme proteins during invasion is a common phenomenon in apicomplexan parasites, but its functional significance remains unknown. The intramembrane cleavage site of TgAMA1 was recently determined; we are using this information, together with our TgAMA1 conditional knockout parasites, to determine the functional consequences of mutations in the transmembrane domain that disrupt TgAMA1 cleavage and shedding.

Small-molecule-based approaches. In contrast to reverse genetic approaches, such as the

one just described for TgAMA1, forward genetic approaches have the advantage of being assumption-free, *i.e.*, they require no preconceived ideas about what gene products are important. Unfortunately, standard forward genetic approaches to studying invasion in haploid, obligate intracellular parasites such as *T. gondii* are problematic, since the disruption of any gene essential for invasion will likely be lethal. The generation of conditional knockouts/knockdowns offers one potential way around this problem; we have developed an alternative approach, in which large collections of structurally diverse small molecules are screened for compounds that affect invasion, and then used as probes to identify the relevant invasion-associated gene products.

In one such screen, 12,160 small molecules were assayed, resulting in the identification of 24 novel, non-cytotoxic inhibitors of *T. gondii* invasion (Carey *et al*, 2004). Unexpectedly, the screen also identified six small molecules that dramatically *enhance* invasion. Secondary assays demonstrated that the different inhibitors/enhancers perturb different aspects of invasion, including gliding motility, cytoskeletal rearrangement, and microneme secretion. Some have similar effects on other apicomplexan parasites, suggesting that they target conserved component(s) of the apicomplexan invasion machinery.

The small molecules we have identified in this and other screens represent a powerful new set of tools for studying invasion, and we are currently exploring specific hypotheses regarding their mechanisms of action. We are using biochemical, synthetic and molecular genetic techniques to identify their target(s) (*e.g.* Haraldsen *et al*, 2007) and to determine the roles that these target molecules play in the process of invasion. Target identification is typically the most difficult aspect of a small-molecule approach (Ward *et al*, 2002), but is facilitated in our case by the recently released sequence of the *T. gondii* genome, the molecular genetic tools available in *T. gondii* and the extent to which we are integrating biological experiments and synthetic chemistry (in collaboration with Dr. Nick Westwood's group at the University of St. Andrews). In addition to providing new probes for studying the mechanisms of host cell invasion, this work has clear and exciting drug development implications for *T. gondii*, and perhaps for other apicomplexan parasites as well.

http://www.uvm.edu/microbiology/research_ward.htm?id=23