INNATE IMMUNITY

It all ends in tears

New research in *Antimicrobial Agents and Chemotherapy* presents evidence that tear lipocalin (TL) could contribute to innate immunity by scavenging siderophores and thereby inhibiting bacterial and fungal growth.

The cornea acts as a physical barrier to protect the rest of the eye. As the cornea contains no blood vessels, nutrients are provided by tear fluid, which also contains a variety of small molecules with antibacterial functions, including human tear lysozyme. TL is another component of human tear fluid, but until now its exact function has been unknown.

TL is a member of a large protein family known as the lipocalins, secreted molecules that are found in a wide variety of species and which are known to bind small hydrophobic molecules. It had previously been suggested that TL binding to these molecules could have a general protective function. In this work, Fluckinger *et al.* wanted to have a more detailed look at the function of TL.

Fluckinger *et al.* first analysed the binding of TL to different siderophores using a competitive displacement assay. There are two main groups of bacterial siderophores: the catecholates such as *Escherichia coli* enterobactin and the hydroxamates such as *Streptomyces* deferroxamine. Both these groups were analysed, along with fungal siderophores including *Aspergillus nidulans* triacetylfusarinine C. The results showed that TL bound with high affinity to all the siderophores tested with the exception of *Pseudomonas aeruginosa* pyoverdine — the authors suggest that this reflects the fact that pyoverdine has an additional chromophore residue that precludes binding in the TL active site.

Fluckinger *et al.* went on to look at the effects of TL on bacterial and fungal growth. Under non-iron-limiting conditions, the presence of TL had no effect on *E. coli* growth. However, under iron-limiting conditions, the presence of TL severely inhibited

PROTOZOAN PARASITES

Ordered domains key to *Entamoeba* virulence

A report in the latest issue of *Infection and Immunity* presents evidence that not only are lipid rafts present in the membrane of the protozoan parasite *Entamoeba histolytica* but they also contribute to the virulence of this pathogen.

The view of the plasma membrane as a homogeneous structure was dispelled long ago with the 'fluid mosaic' concept, and further modified more recently with the discovery of lipid rafts — tightly ordered, cholesterol- and glycosphingolipid-enriched microdomains, which, although the methods for their detection remain controversial, have been shown to be involved in many vital cellular functions, including signal transduction, adhesion and secretion. *E. histolytica* is the causative agent of amoebic dysentery and estimates suggest that up to 100,000 people die each year and some 50 million people are symptomatically infected, almost all in developing countries. After ingestion of cysts in contaminated food or water, *E. histolytica* excystation occurs in the small intestine, releasing trophozoites that migrate to the large intestine, adhere to the colonic mucus mainly through the interaction of a galactose/*N*-acetyl galactosamine (Gal/GalNAc)-specific lectin with host glycoconjugates and then obtain nutrients via the endocytic pathway.

To investigate whether the *E. histolytica* membrane contained raft-like domains, Laughlin *et al.* stained trophozoites with two lipid analogues — one ($DiIC_{16}$) that preferentially partitions into ordered domains and another (FAST-DiI) that partitions into more fluid domains. The staining pattern indicated that tightly ordered domains were indeed present. To aid further interpretation of the staining pattern, the experiment was repeated but the trophozoites were first treated with raft-disrupting agents that remove (MBCD) or sequester (Filipin) cholesterol from the membrane. This treatment affected $DiIC_{16}$ but not FAST-DiI binding, suggesting that $DiIC_{16}$ co-localized with cholesterol-rich, raft-like domains.

To investigate the physiological function of the rafts, Laughlin *et al.* went on to look at the effects of raft-disrupting agents on pinocytosis (the uptake of fluids or solutes), secretion and adhesion to host cells. MBCD treatment significantly inhibited fluid-phase pinocytosis but had no effect on the secretion of cysteine proteases, suggesting that raft-like domains are involved in pinocytosis but that the secretion of cysteine proteases is raft independent. MBCD treatment also inhibited the adhesion of trophozoites to a mammalian cell monolayer and further work revealed that the Gal/GalNAc-specific lectin that has an important role in *E. histolytica* adhesion is enriched in cholesterol-containing membrane fractions; these results suggest that the adhesion of *E. histolytica* to host cells is also raft dependent.

This work is the first report of the presence of lipid rafts in the *E. histolytica* membrane. The authors hope that future analysis of these regions will provide a further insight into *E. histolytica* pathogenesis.

Sheilagh Clarkson