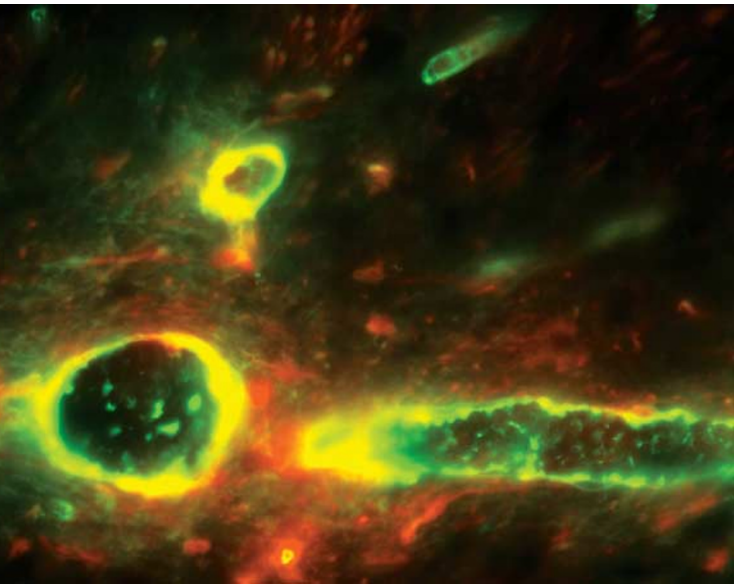
VIROLOGY

West Nile virus makes an entrance



Fluorescence micrograph showing breakdown of the blood–brain barrier in WNV-infected mice. Image kindly provided by T. Town © (2004) *Nature Medicine*.

A new report in *Nature Medicine* has revealed that recognition of West Nile virus (WNV) by Toll-like receptor 3 (Tlr3) is the major factor allowing this virus to cross the blood–brain barrier (BBB) and cause lethal encephalitis.

WNV is a single-stranded RNA (ssRNA) flavivirus with a life cycle that primarily involves birds and mosquitoes; however, humans and horses can also become infected. In humans, infection is generally asymptomatic, and if West Nile fever does develop then the illness is generally mild and self-limiting. In elderly and immunocompromised individuals, however, WNV infection can progress to severe neurological disease. The molecular details of the pathogenesis of severe disease are scarce. It is known that some TLRs can detect viral motifs such as ssRNA. In this study, researchers investigated the role of one of these TLRs, Tlr3, in the detection of WNV using a mouse model of WNV encephalitis.

Initial investigations showed that following intraperitoneal challenge with a lethal dose of WNV, *Tlr3*$^{-/-}$ mice were more resistant to infection than wild-type mice. Quantitative PCR assays showed that, following this challenge, the viral burden in the periphery (blood and spleen) was increased in *Tlr3*$^{-/-}$ mice compared with wild-type mice. Analysis of blood cytokine levels showed increased levels of inflammatory cytokines in wild-type, compared with *Tlr3*$^{-/-}$, mice early in infection. The analysis was then switched from the periphery to the brain. A comparison of the viral load in brain tissue found that in *Tlr3*$^{-/-}$ mice the levels of WNV RNA were significantly lower compared with wild-type mice at day 6 after infection. The inflammatory cytokine profiles indicated that the inflammatory reaction was markedly reduced in *Tlr3*$^{-/-}$ mice and immunofluorescence work showed that the numbers of activated microglia (brain macrophages) and infiltrating leukocytes were reduced in *Tlr3*$^{-/-}$ mice, indicating fewer neuropathological effects.

Collectively, these results strongly indicated that Tlr3 did have a role in

BACTERIAL PATHOGENESIS

Haem, sweet haem

It has been known for some time that *Haemophilus influenzae* has an absolute growth requirement for a porphyrin or haem source, but new research just published in *Microbiology* reveals that haem utilization by this pathogen is even more complex than previously supposed.

The only known niche exploited by *H. influenzae* are humans, where the microorganism causes a range of infections including meningitis and pneumonia. As *H. influenzae* lacks all of the biosynthetic enzymes that are required to produce the porphyrin ring, a precursor of haem, the microorganism has evolved a complex array of uptake mechanisms to acquire this essential nutrient from its host environment. Previous work has shown that *H. influenzae* can use these acquisition systems to access a variety of haem sources including haemoglobin, the haemoglobin–haptoglobin and haem–haemopexdin complexes, and free haem itself. Stull and colleagues add a new layer of complexity to this essential process by describing an *H. influenzae* protein, called haem-utilization protein (Hup), which is central to the microorganism's exploitation of multiple haem sources.

To identify new proteins used by *H. influenzae* to acquire haem, the authors used a haemoglobin affinity method by which a 100-kDa protein was isolated. N-terminal amino acid sequencing of this protein allowed identification of the corresponding gene, which was used to create a deletion mutant of *H. influenzae*. As anticipated, characterization of the Δ*hup* mutant strain revealed a reduced capacity to utilize haemoglobin *in vitro*. Furthermore, the mutant strain also demonstrated a reduced capacity to exploit haem, haem–haemopexin, haem–albumin and haemoglobin–haptoglobin, indicating a central role for the protein in haem acquisition by *H. influenzae*. The authors also investigated whether Hup contributes to the virulence of *H. influenzae* in an infant rat model of invasive disease and showed that, at least in this model, expression of Hup was not required for pathogenicity. The authors suggest that this finding could reflect the fact that bacterial haem-uptake mechanisms may be specific to the haem sources from the host natural species — it is not known whether *H. influenzae* can exploit sources of haem from the rat.

So, although the *in vivo* relevance of Hup remains to be established, the data presented in this study clearly suggests a central role for this protein in haem acquisition by *H. influenzae*. The authors hypothesize that Hup may be used in the extraction and internalization of haem from various haem-binding proteins in the host environment. Future work will investigate this hypothesis and clarify the role of Hup in the pathogenic mechanisms of *H. influenzae* disease.

David O'Connell

References and links

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FURTHER READING Genco, C. A. & Dixon, D. W. Emerging strategies in microbial haem capture. *Mol. Microbiol.* **39**, 1–11 (2001)
WEB SITE
The University of Oklahoma Health Sciences Center: http://www.ouhsc.edu/

WNV entry to the brain. This was confirmed by comparing the permeability of the BBB in wild-type and *Tlr3*$^{-/-}$ mice: following WNV infection or stimulation with the viral mimic poly (I:C), the permeability was increased in wild-type mice but not in *Tlr3*$^{-/-}$ mice. A further insight into the pathogenesis of severe disease was provided by results suggesting that TNF-α receptor 1 signalling downstream of Tlr3 promotes WNV entry into the brain.

Sporadic outbreaks of WNV infection of humans have become increasingly common over the past 5 years, particularly in North America and Europe. This new work identifying Tlr3 as the receptor allowing WNV to enter the brain can hopefully be exploited for the development of new therapeutics.

Sheilagh Molloy

References and links

ORIGINAL RESEARCH PAPER Wang, T. *et al.* Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. *Nature Med.* 21 Nov 2004 (doi:10.1038/nm1140)

A female sandfly (*Phlebotomus* species), which is the vector of *Leishmania major*. The females are blood suckers and transmit parasites to humans. Image courtesy of the WHO © (1975).

PARASITOLOGY

Stuck in the gut

Cutaneous leishmaniasis, caused by infection with *Leishmania major*, is the most common Old World leishmanial disease and is transmitted by the sandfly. To devise new methods of parasite control it is imperative to understand the biology of infection in both the host and the vector. In a recent report in *Cell*, Kamhawi *et al.* have identified the receptor that binds to and retains *L. major* in the insect midgut, so that it can complete its replication cycle and produce a transmissible infectious form, a crucial step in the life cycle.

L. major replicates as intracellular amastigotes in macrophages, but replication in the sandfly occurs extracellularly in the digestive tract. Amastigotes transform into procyclic promastigotes, which are not infectious but become attached to the sandfly midgut and differentiate to produce infectious swimming metacyclic promastigotes, which are transmitted to humans through insect bites. Procyclic promastigotes must be retained in the gut to complete the replication cycle and produce infectious metacyclic promastigotes. Kamhawi *et al.* show that a tandem-repeat galectin, which is expressed on the surface of sandfly midgut epithelial cells, functions to specifically bind the main surface receptor of *L. major* procyclic promastigotes and attach them to the insect midgut.

Galectins are a β-galactoside-binding family of lectins conserved from fungi to man that contain one or more carbohydrate-recognition domains (CRD) and function in immunity, homeostasis and embryogenesis. The main glycoconjugate on the surface of *L. major* promastigotes is lipophosphoglycan (LPG), which has sugar side chains including a poly-β-galactosyl epitope ($Gal\beta1\text{-}3_n$) and can bind to galectin-3 and galectin-9. Galectin-9 is thought to function as a surrogate macrophage receptor in humans.

Kamhawi *et al.* sequenced a midgut cDNA library from the *L. major* vector *Phlebotomus papatasi* and identified a galactose-binding protein named PpGalec. PpGalec is a tandem-repeat galectin with two CRDs separated by a linker and was specifically expressed in the midgut and upregulated in adult females. Moreover, PpGalec was only found in *P. papatasi* and its close relative *Phlebotomus duboscqi* (also an *L. major* vector) and was absent from other *Phlebotomus* species. Finally, only the procyclic (not the metacyclic) promastigotes bound to PpGalec.

Using confocal microscopy of insect midgut sections stained with PpGalec-specific antisera, expression of the galectin was localized to the lumenal midgut cells. Importantly, pre-treatment of dissected midguts with PpGalec-specific antisera reduced the number of parasites bound by 72%. Plus, by adding PpGalec-specific antisera to a bloodmeal containing *L. major* amastigotes, parasite retention in the sandfly was markedly reduced, confirming that PpGalec has an integral role in parasite survival in the vector.

During differentiation from procyclic to metacyclic promastigote forms, downregulation of the poly-galactosyl epitopes on LPG disrupts interactions between LPG and PpGalec and promotes release of the infectious parasites. Interactions with galectins could account for vector specificity and parasite survival within the vector.

Identifying the insect receptor for *L. major* LPG is a huge advance that might facilitate development of new control methods, such as transmission blocking vaccines, for *L. major*–sandfly and other *Leishmania* species–sand fly interactions.

Susan Jones

References and links

ORIGINAL RESEARCH PAPER Kamhawi, S. *et al.* A role for insect galectins in parasite survival. *Cell* **119**, 329–341 (2004)

WEB SITES

Jesus Valenzuela's laboratory:
http://www.niaid.nih.gov/dir/labs/LMVR/valenzuela.htm

David Sack's laboratory:
http://www.niaid.nih.gov/dir/labs/lpd/sacks.htm