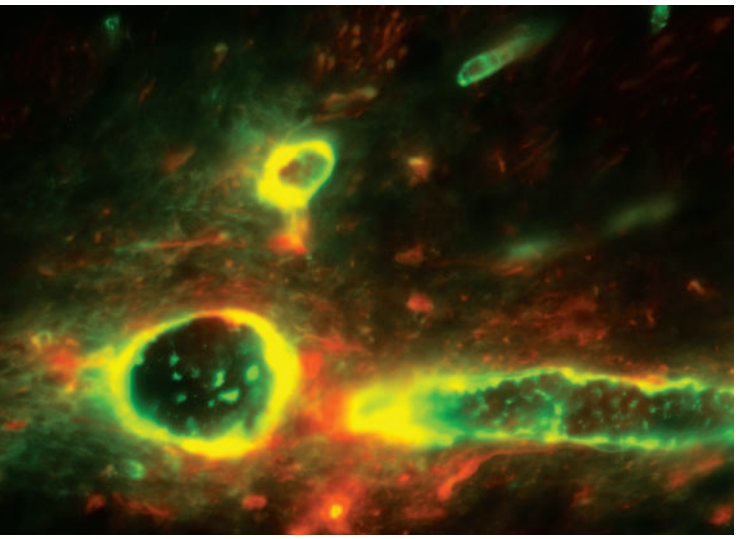
INFECTIOUS DISEASE

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) Macmillan Magazines Ltd.

A new report in *Nature Medicine* has revealed that recognition of West Nile virus (WNV) by Toll-like receptor 3 (TLR3) is the main factor that allows this virus to cross the blood–brain barrier and cause lethal encephalitis.

WNV mainly infects birds and mosquitoes; however, humans and horses can also become infected. In humans, infection is generally asymptomatic, but in elderly and immunocompromised individuals, 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 single-stranded RNA. In this study, researchers investigated the role of TLR3 in the detection of WNV, using a mouse model of WNV encephalitis.

Initial investigations showed that, after intraperitoneal challenge with a lethal dose of WNV, *Tlr3*$^{-/-}$ mice were more resistant to infection than wild-type mice. Quantitative PCR assays showed that, after 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 amounts of inflammatory cytokines in wild-type mice 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 than in 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 experiments 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 has a role in WNV entry to the brain. This was confirmed by comparing the permeability of the blood–brain

B CELLS

Memory B cells — or not?

About 40% of the B cells in human peripheral blood are CD27^{+} and have hypermutated variable regions in their immunoglobulin receptors, and as such, they are classified as memory B cells. However, a proportion of these CD27^{+} cells are IgM^{+} and have not undergone class switching. Weller and colleagues previously suggested that these cells could be a subset that is distinct from the classical germinal-centre-derived memory B-cell subset. The present study shows that the peripheral IgM^{+}IgD^{+}CD27^{+} B cells correspond to splenic marginal zone (MZ) B cells, which control T-cell-independent responses.

First, the authors used phenotypic analysis to show that the memory IgM^{+} B cells in the blood were similar to those in the splenic MZ; both cell types were found to be IgM^{hi}IgD^{low}CD1c^{hi}CD21^{hi}. Second, gene-expression profiling showed that the two cell types expressed a similar pattern of genes, which differed from the genes expressed by naive or class-switched memory B cells. Third, the authors used spectratyping analysis of the CDR3 (complementarity-determining region 3) of the immunoglobulin receptor to establish a relationship between the cell types. This technique enabled the authors to track the development of cells with specific V(D)J rearrangements after immunization with a T-cell-independent antigen. The results showed that a particular B-cell clone was detected in both the blood and the splenic B-cell population after vaccination with pneumococcal and meningococcal polysaccharides, indicating that IgM^{+} B cells recirculate between the blood and the splenic MZ. When the authors studied children, they found IgM^{+} B cells with hypermutated receptors in children of a very young age, before the splenic MZ has matured, indicating that these cells can develop and mutate before being able to respond to T-cell-independent antigens. Moreover, asplenic children have mutated IgM^{+} B cells, indicating that this population can mature in extra-splenic sites.

Previous work from the same authors showed that patients with hyper-IgM syndrome who have a defect in CD40 ligand do not have germinal centres and cannot generate class-switched memory B cells, but they can generate IgM^{+} memory B cells. On the basis of these studies, the authors propose that there might be two different programmes for B-cell development — one that depends on germinal-centre formation and leads to 'true' hypermutated memory B cells in response to antigen stimulation, and another in which a diverse set of B cells is generated by hypermutation early in life. It seems that these IgM^{+} 'memory' B cells are not really memory cells at all but, instead, function as innate-like cells, producing IgM at a time when the adaptive immune response is still developing.

Elaine Bell

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FURTHER READING Lopes-Carvalho, T. & Kearney, J. F. Development and selection of marginal zone B cells. *Immunol. Rev.* **197**, 192–205 (2004).

barrier in wild-type and *Tlr3*$^{-/-}$ mice: after infection with WNV or stimulation with the viral mimic poly I:C (polyinosinic–polycytidylic acid), 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 indicating that signalling through tumour-necrosis-factor receptor 1 downstream of TLR3 promotes WNV entry to the brain.

Sporadic outbreaks of WNV infection of humans have become increasingly common in the past five years, particularly in North America and Europe. This new work identifying TLR3 as the receptor that allows WNV to enter the brain can hopefully be exploited for the development of new therapeutics.

Sheilagh Molloy, Associate Editor,
Nature Reviews Microbiology

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MAST CELLS

Tempering toxicity

A recent report in *Nature* describes a new biological function for mast cells: limiting the toxicity and pathology associated with endothelin-1 (ET1).

ET1 is a 21-amino-acid peptide with potent vasoconstrictor activity, and it is released by vascular endothelial cells during sepsis and other pathological processes, such as asthma and atherosclerosis. Previous *in vitro* studies have shown that ET1 induces the activation of mast cells, which triggers the release of proteases that degrade it; however, the involvement of mast cells in the regulation of ET1-associated pathology *in vivo* is unknown. So, to assess their role in ET1-induced pathology, Maurer *et al.* gave an intraperitoneal injection of ET1 to wild-type and mast-cell-deficient mice. All mast-cell-deficient mice developed severe hypothermia and diarrhoea, and most died within 3 hours of the injection. By contrast, ET1 injection had no effect on wild-type mice. Restoration of mast cells to the peritoneal cavity of the mutant mice, by adoptive transfer of wild-type mast cells, completely protected the mice from ET1-induced morbidity and mortality.

Using an inhibitor of mast-cell degranulation, BAPTA-AM, the authors next assessed whether degranulation is required for the reduction of ET1-induced toxicity *in vivo*. When wild-type cells were treated with BAPTA-AM before transfer to mast-cell-deficient mice, the protective effect of the transferred cells was greatly reduced. Further studies identified that chymase, which is released on mast-cell degranulation, is involved in mediating the protective effect by degrading ET1, as wild-type mice that were pretreated with a chymase inhibitor developed hypothermia and diarrhoea after injection of ET1 and had increased amounts of ET1 in the peritoneal cavity compared with untreated mice.

To further explore the mechanism of ET1-induced mast-cell activation, the authors tested the ability of ET1-receptor antagonists to alter the susceptibility of wild-type mice to ET1-induced pathology. Wild-type mice pretreated with an antagonist selective for the ET1 receptor ET_A and then injected with ET1 developed marked hypothermia and diarrhoea, although not to the extent seen in mast-cell-deficient mice, indicating that ET_A has an important role in mediating mast-cell activation. Accordingly, mast-cell-deficient mice that were reconstituted with mast cells that lack expression of ET_A were not protected from ET1-induced hypothermia and diarrhoea, and some mice died. Moreover, in the peritoneal cavity of these mice, mast-cell degranulation was reduced, and ET1 levels were increased compared with those of mice that were reconstituted with wild-type cells, indicating that mast cells limit ET1-associated pathology *in vivo* by reducing the concentration of the peptide.

Finally, the authors used the caecal ligation and puncture (CLP) model of acute bacterial peritonitis to assess the biological significance of mast-cell-mediated protection from ET1-induced pathology. As expected, the mortality that occurred within 90 hours of CLP was greater in mast-cell-deficient mice than in wild-type mice. However, reconstitution of mast-cell-deficient mice with wild-type cells, but not with ET_A-deficient cells, increased their survival to levels that were similar to those of wild-type mice, indicating that protective mast-cell function in the CLP model involves ET1- and ET_A-dependent mechanisms.

In this study, the authors describe a novel role for mast cells in tempering the toxicity that is induced by endogenous mediators, and they speculate that, in future, mast-cell-dependent mechanisms might also be found to regulate the toxicity of other compounds.

Lucy Bird

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