IN THE NEWS

West Nile virus

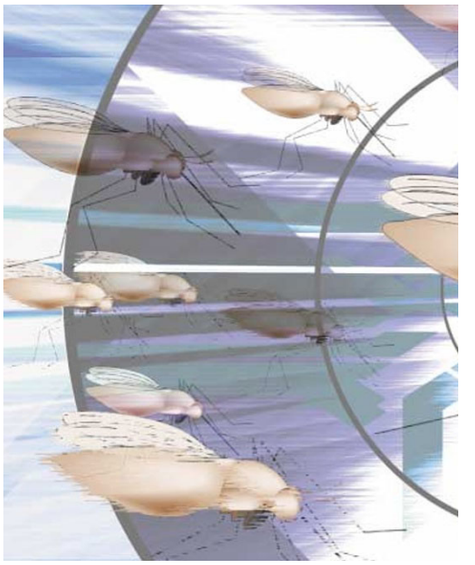
This month has seen reports of the sixth death this year in Colorado from the mosquito-borne disease West Nile virus, and it seems that cases will reach record levels in 2003. West Nile virus, which is common in Africa, arrived in New York in 1999, and has since spread across the continent. As Mark Loeb, Associate Professor of Clinical Epidemiology and Biostatistics at the University of McMaster, said, "There's more and more evidence telling us that West Nile is here to stay" (*The Globe and Mail*). Worryingly, although there have been no cases of the disease in the United Kingdom so far, a recent survey of British birds showed that "an unexpectedly high proportion" contained antibodies specific for the virus (*BBC News*).

So, the publication (in *Proceedings of the National Academy of Sciences*) of a successful vaccine study in mice is welcome news. The new DNA vaccine contains a replication-defective, harmless relative of West Nile virus known as Kunjin virus. The authors suggest that this "may provide ... an effective vaccination strategy against further outbreaks" (*New Scientist*). However, others have raised concerns that this weakened virus might be "more virulent than we believe it is at the moment" (*New Scientist*). The new vaccine joins two other candidates — a hybrid of yellow fever and West Nile virus, and a hybrid of dengue virus and West Nile virus. None of these vaccines has yet been tested in humans, so as Diane Griffin of Johns Hopkins School of Public Health points out, "it's good to have other candidates in the wings" (*Science Now*).

Kirsty Minton

VACCINES

New targets for malaria vaccines identified



More effective malaria vaccines could now be developed thanks to a new approach for mining the genomic sequence of *Plasmodium falciparum*, which has led to the identification of new antigens with enhanced immunogenicity.

It is possible to generate protection against malaria by immunization with sporozoites (the infectious form of *P. falciparum* injected by the mosquito) that have been attenuated by radiation, so justifying the search for a malaria vaccine. However, the antigens mediating this protective immunity induced by vaccination with the whole organism are unknown. It is unlikely that a vaccine directed against a single antigen will be protective, so multivalent vaccines that combine antigens expressed at different stages of the parasite life cycle have been developed.

The search for further parasite antigens has been aided by the availability of the genomic sequence of *P. falciparum*, as well as the elucidation of the *P. falciparum* proteome. Here, Doolan *et al.* combine bioinformatic epitope predictions and *in vitro* cellular assays to identify new malaria target antigens.

First, multidimensional protein identification technology was used to identify 27 open-reading frames that encode antigens that are potentially expressed by the sporozoite and intra-hepatic stages of the parasite life cycle. Of these, antigens with predicted HLA-binding capacities were tested, together with four previously

APOPTOSIS

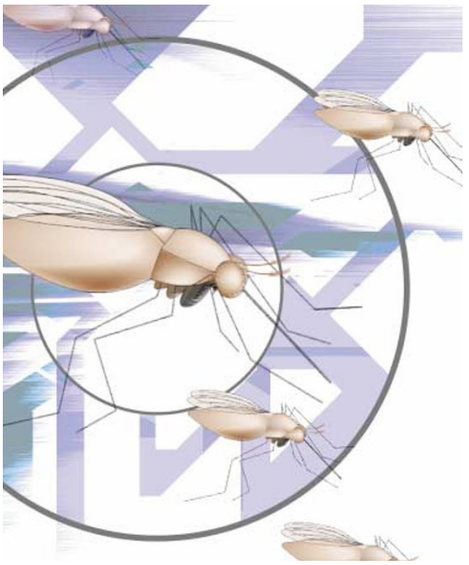
Lifesaver

How the pro-apoptotic molecules BAK and BAX — which are potentially lethal — are maintained in an inactive, monomeric conformation in viable cells is poorly understood. However, recent structural insights into the monomeric BAX molecule have provided a possible mechanism for its inactive status. And now, reporting in *Science*, Stanley Korsmeyer and colleagues have identified a protein — voltage-dependent anion channel 2 (VDAC2) — that keeps BAK in check.

BAK and BAX are required for mitochondrial apoptosis — 'BH3-only' members of the BCL2 family respond to death signals and subsequently trigger the activation of BAK and BAX, which leads to mitochondrial membrane permeabilization and the release of cytochrome *c*. This then initiates the caspase cascade.

To investigate whether BAK interacts with another mitochondrial protein that regulates its activity, Korsmeyer and colleagues used protein crosslinkers to identify a candidate protein (X) that complexes with BAK in purified mitochondria or whole cells. This BAK–X complex was lost when mitochondria were treated with the BH3-only protein BID or when cells were treated with death stimuli. By testing various BH1- and BH3-domain mutants of both BID and BAK, the authors concluded that X interacts with the BAK pocket that is formed by the BH1, BH2 and BH3 domains and can be displaced, directly or indirectly, by BH3-only molecules.

Protein X was identified as VDAC2, a low-abundance isoform of the VDAC outer-mitochondrial-membrane porin. VDAC2 was further implicated when the authors found that VDAC2-deficient embryonic stem cells lacked the BAK–X complex, which



identified *P. falciparum* antigens, for reactivity with peripheral-blood mononuclear cells from volunteers who had been immunized with radiation-attenuated *P. falciparum* sporozoites or from control volunteers. Sixteen of the proteins were recognized as antigens by the volunteers immunized with irradiated sporozoites, but not by the controls. The immune response directed against some of the newly identified antigens was higher that that observed for the previously identified *P. falciparum*-derived protein antigens, indicating that these might be better candidates for vaccine development.

The approach used here shows how complex the immune response to *P. falciparum* is, and also validates this approach for the identification of new target antigens, which could be used in multiantigen vaccines for malaria.

Jenny Buckland

References and links

ORIGINAL RESEARCH PAPER Doolan, D. L. *et al*. Identification of *Plasmodium falciparum* antigens by antigenic analysis of genomic and proteomic data. *Proc. Natl Acad. Sci. USA* 28 July 2003 (DOI: 10.1073/pnas.1633254100)

FURTHER READING Good, M. F. Towards a blood-stage vaccine for malaria: are we following all the leads? *Nature Rev. Immunol.* **2**, 117–125 (2001)

was seen when VDAC2 was re-expressed in these cells. Using haemagglutinin (HA)-tagged VDAC2, endogenous BAK, but not BAX, was coprecipitated. Similarly, HA-tagged BAK, but not BAX, coprecipitated endogenous VDAC2.

BAK in its active, oligomeric conformation is more susceptible to proteolysis than the inactive form, and the absence of VDAC2 increased its susceptibility. So, VDAC2 interacts with BAK, but not BAX, and regulates its conformation.

Next, Korsmeyer and co-workers set out to determine how VDAC2 modulates BAK-dependent apoptosis. Is VDAC2 an inhibitor of BAK-mediated apoptosis, or does it function as a pro-apoptotic factor itself when released from BAK? VDAC2 expression in *BAX*$^{-/-}$ cells inhibited apoptosis, but had no effect in *BAK*$^{-/-}$ cells, which argues against the latter possibility. Also, expression of VDAC2 inhibited BID-induced apoptosis of *BAX*$^{-/-}$ cells, but not *BAK*$^{-/-}$ cells, which indicates that VDAC2 negatively regulates BAK-dependent apoptosis.

Cells deficient for VDAC2 were more sensitive to death stimuli than *VDAC1*$^{-/-}$ and *VDAC3*$^{-/-}$ cells, both of which had similar sensitivities to wild-type cells. By re-expressing VDAC2, the susceptibility to apoptosis of *VDAC2*$^{-/-}$ cells was returned to normal. So, VDAC2 has a physiological role that is distinct from the other VDAC isoforms.

When analysing the apoptosis phenotype of *VDAC2*$^{-/-}$ cells, the authors noted an increased loss of mitochondrial transmembrane potential and the accelerated release of cytochrome *c*, compared with wild-type cells. After treatment with death stimuli, *VDAC2*$^{-/-}$ cells showed caspase activity and BAK oligomerization earlier than wild-type cells.

The authors concluded that VDAC2 is a specific inhibitor of BAK-dependent mitochondrial apoptosis, which, when absent, causes increased susceptibility to apoptotic death.

Arianne Heinrichs, Editor,
Nature Reviews Molecular Cell Biology

References and links

ORIGINAL RESEARCH PAPER Cheng, E. H.-Y. *et al*. VDAC2 inhibits BAK activation and mitochondrial apoptosis. *Science* **301**, 513–517 (2003)

FURTHER READING Cory, S. & Adams, J. M. The BCL2 family: regulators of the cellular life-or-death switch. *Nature Rev. Cancer* **2**, 647–656 (2002)

IN BRIEF

B-CELL RESPONSES

BCL6 controls the expression of the B7-1/CD80 costimulatory receptor in germinal center B cells.

Niu, H. *et al*. *J. Exp. Med*. **198**, 211–221 (2003)

In this study, Dalla-Favera and colleagues looked at the targets of transcriptional repression by BCL6 to gain a better understanding of its involvement in germinal-centre (GC) formation. Previous studies have not been able to distinguish direct targets of BCL6 from secondary effects. This study shows that BCL6 directly inhibits transcription of the *CD80* gene by binding to its promoter enhancer and prevents the upregulation of expression of CD80 in response to CD40–CD40L interactions mediated by nuclear factor-κB (NF-κB). CD80 has a crucial role in T-cell–B-cell interactions required for GC development and T-cell-dependent antibody responses. Therefore, constitutive expression of BCL6 owing to a translocation might lead to lymphomagenesis by disrupting the normal GC differentiation pathway of B cells.

MONOCYTES

Blood monocytes consist of two principal subsets with distinct migratory properties.

Geissmann, F. *et al. Immunity* **19**, 71–82 (2003)

Two subsets of human blood monocytes that differ in their phenotypic and functional characteristics were identified several years ago, but it has proven difficult to characterize any such subsets in mice. Now, Geissmann *et al*. describe two mouse monocyte subsets with distinct chemokine-receptor and adhesion-molecule expression profiles, which correspond to the human subsets. CC-chemokine receptor 2 (CCR2)-positive monocytes are short-lived cells that are recruited to inflamed tissues, whereas the CCR2-negative subset is recruited to non-inflamed tissues and these cells might be the precursors of tissue-resident macrophages and dendritic cells. Both subsets have the capacity to develop into dendritic cells. Further characterization of monocyte subsets in mice should contribute to an increased understanding of the role of monocytes in human diseases.

SPLENIC ARCHITECTURE

A conduit system distributes chemokines and small blood-borne molecules through the splenic white pulp.

Nolte, M. A. *et al. J. Exp. Med.* **198**, 505–512 (2003)

The splenic white pulp is structurally similar to a lymph node, although the spleen receives antigen directly from the blood and not through afferent lymphatics. This paper shows that, similar to the lymph node, the spleen has a conduit system — a kind of structured transport system. The splenic conduit is a tubular network containing collagen fibres that are surrounded by reticular fibroblasts. The white pulp is known to restrict cellular movement to lymphocytes and dendritic cells, but this study shows that the conduit also limits the entry of large molecules. Locally produced chemokines were also found in the conduit system — CC-chemokine ligand 21 (CCL21) in the T-cell area and CXCL13 in the B-cell area.