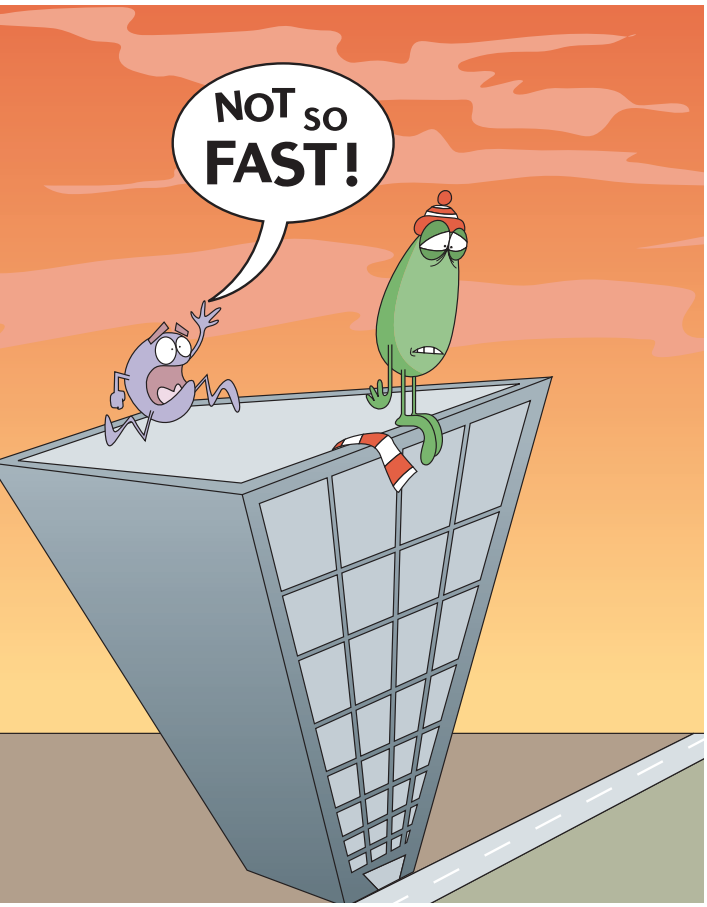
VIRAL PATHOGENESIS

Live and let live

In the fight against viral infection, host cells can undergo apoptosis prior to viral maturation. This antiviral response curtails the spread of infection to new host cells by preventing the release of viral progeny. A study just published in *PLoS Biology* sheds new light on how one virus, the Epstein–Barr virus (EBV), fights for control of the apoptotic pathways of infected cells, allowing the virus to control the lifespan of the host to complete its replication cycle.



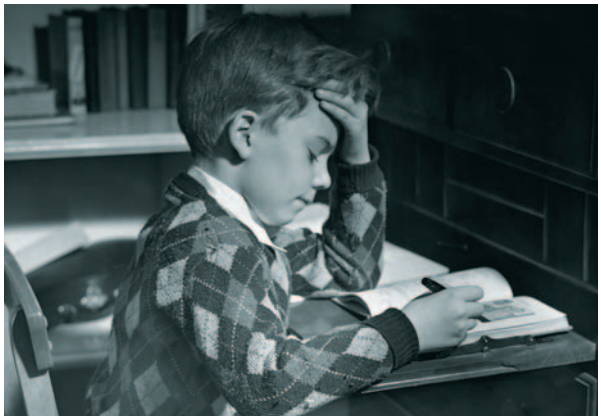
EBV, a tumour-causing herpes virus, targets B cells and establishes a latent infection, a state characterized by the absence of viral replication and the maintenance of the viral genome in the infected cell. During this process, 11 viral genes are expressed that are involved in the establishment of the latent phase of the EBV life cycle. Expression of these genes also contributes directly to B-cell transformation and proliferation, a defining characteristic of EBV infection. As well as the 11 'latency' genes, the virus encodes more than 80 additional genes, including two homologues of cellular *Bcl-2* (*vBcl-2*) which, in other viruses, have been shown to block apoptosis and the subsequent premature death of the host cell. In this study, Markus Altmann and Wolfgang Hammerschmidt investigated the role of these two genes — *BHRF1* and *BALF1* — in the initiation and maintenance of latent EBV infection. The authors constructed mutant virus in which both genes were inactivated. Primary resting B cells infected with the mutant virus did not enter the cell cycle and underwent immediate apoptosis, inhibiting the ability of the virus to establish latent infection.

Inactivation of the *vBcl-2* genes also prevented the virus from transforming B cells. Analysis of *BHRF1* and *BALF1* gene expression by RT-PCR revealed that both genes were maximally expressed in the initials stages of infection but were neither expressed nor required once latent infection had been established. Taken together, these results show that the early and transient expression of the two *vBcl-2* genes prevents the EBV-infected B cells from undergoing apoptosis. By keeping their host cells alive during the early stages of infection, EBV is able to activate and express the other latency genes that allow the virus to persist and begin the process that ultimately leads to cellular transformation and EBV-associated B-cell lymphomas.

The findings presented in this study are the first to demonstrate a direct role for vBcl-2 proteins in latent viral infection. Interesting questions for future investigation include the role of vBcl-2 homologues in other viruses that establish latent infection as part of their life cycle. Future studies can also begin to explore the molecular mechanisms that regulate expression of *BHRF1* and *BALF1*, knowledge that could potentially lead to a new generation of antiviral strategies.

David O'Connell

ORIGINAL RESEARCH PAPER Altmann, M. & Hammerschmidt, W. Epstein–Barr virus provides a new paradigm: a requirement for immediate inhibition of apoptosis. *PLoS Biol.* **3**, e404 (2005)
FURTHER READING Benedict, C.A. *et al.* To kill or be killed: viral evasion of apoptosis. *Nature Immunol.* **3**, 1013–1018 (2002)
WEB SITE
Wolfgang Hammerschmidt's laboratory: http://www3.gsf.de/GENV



TECHNIQUES & APPLICATIONS

Biofilms: you do the maths!

Mathematical modelling can be used to predict the effectiveness of different treatments for biofilms, and the latest in a series of models was presented by Joao Xavier and colleagues in a recent issue of *Microbiology*.

The matrix of extracellular polymeric substances (EPSs) that embeds the bacterial cells in a biofilm is primarily responsible for biofilm surface attachment. One as-yet relatively unexplored method of biofilm removal involves promoting their detachment from surfaces. In this paper, Xavier *et al.* present a mathematical feasibility study that uses mathematical modelling to assess the prospects for biofilm-control strategies that are based on promoting detachment by compromising the integrity of the EPS matrix.

A three-dimensional representation of the biofilm was created using a technique known as individual-based modelling, and the effects of different treatment scenarios with detachment-promoting agents (DPAs) were then analysed to determine which characteristics of the DPA were important in achieving detachment. Only a few of the simulations produced a clean surface, with many simulations showing that a thin layer of the biofilm was extremely difficult to remove, and it is the removal of this last fraction that constitutes most of the overall removal time.

This work is the latest in a series of mathematical approaches to the problems that biofilms pose, and it highlights the utility of theoretical studies. The challenge now is for the modellers and microbiologists to get together and translate these mathematical results into practical applications.

Sheilagh Molloy

ORIGINAL RESEARCH PAPER Xavier, J. B. *et al.* Biofilm-control strategies based on enzymic disruption of the extracellular polymeric substance matrix — a modelling study. *Microbiology* **151**, 3817–3832 (2005)
FURTHER READING Xavier, J. B., Picioreanu, C. & van Loosdrecht, M. C. M. A framework for multidimensional modelling of activity and structure of multispecies biofilms. *Environ. Microbiol.* **7**, 1085–1103 (2005) | Hall-Stoodley, L., Costerton, J. W. & Stoodley, P. Bacterial biofilms: from the natural environment to infectious diseases. *Nature Rev. Microbiol.* **2**, 95–108 (2004)

VIROLOGY

Promoting silence

Herpesviruses are characterized by their ability to establish latent infections from which the virus can reactivate and cause recurrent disease. The transition from productive (lytic) to latent infection in herpes simplex virus (HSV) is associated with marked shutdown of the herpesvirus genome — during lytic infection more than 80 gene products are expressed, whereas during latent infection only the latency-associated transcript gene (*LAT*) is highly expressed. Now, David Knipe and colleagues argue that *LAT* represses HSV gene expression by promoting the assembly of heterochromatin on viral lytic-gene promoters.

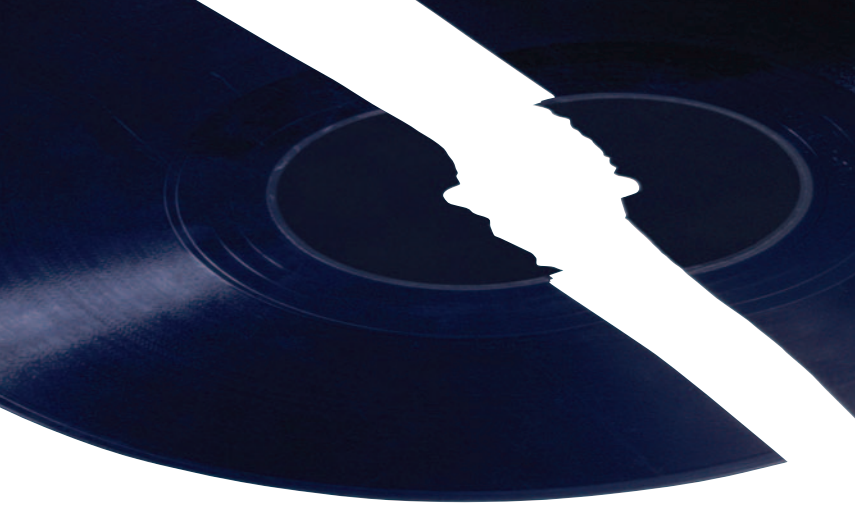
The authors used chromatin immunoprecipitation assays to study the assembly of chromatin on HSV lytic genes during infection of murine trigeminal ganglia. Initially, during lytic infection, low levels of histone H3 were associated with viral DNA, which is consistent with findings that the viral genome is relatively nucleosome-free during productive infection. But as latent infection was established, the genome became increasingly chromatinized. Consistently, during latent infection, lytic-gene promoters showed a higher level of association with heterochromatin (marked by histone H3 methylated at lysine 9) compared with euchromatin (marked by histone H3 methylated at lysine 4). Using LAT^- HSV, Wang *et al.* showed that expression of *LAT* significantly increased the amount of heterochromatin and decreased the amount of euchromatin associated with most lytic-gene promoters. This suggests that *LAT* facilitates shutdown of the HSV genome by promoting the assembly of inaccessible chromatin on viral DNA.

ANTI-INFECTIVES

Effective protection

Two recent *Nature* papers report promising results on the use of microbicides to prevent sexually transmitted infections.

In a paper published online on November 23, Judy Lieberman and her colleagues from Harvard Medical School describe how they used RNA interference to develop a microbicide that disrupts both infection and replication of herpes simplex virus 2 (HSV-2) in mice. Small interfering RNAs (siRNAs) that target two viral genes, *UL27* and *UL29*, which encode envelope glycoprotein B and a DNA-binding protein, respectively, suppressed viral replication *in vitro* in NIH3T3 and Vero cells. Vaginal instillation of these siRNAs was found to protect mice from vaginal challenge with a lethal dose of HSV-2. This protective effect occurred whether the siRNAs were administered before or after infection and, although under certain circumstances siRNAs can induce the interferon pathway and trigger inflammation, in this study treatment of mice with the siRNAs did not induce an inflammatory response.



The authors acknowledge that this accumulation of heterochromatin on the lytic-gene promoters might be an indirect process that takes place on loci silenced by a mechanism unrelated to chromatin. Nevertheless, the proposal that *LAT* directly affects viral chromatin structure is appealing. Double-stranded RNA has been shown to target the formation of heterochromatin through the RNA interference machinery. As yet, *LAT* has not been shown to encode protein products, and therefore the authors contend that *LAT* RNA might form small interfering RNAs that could induce heterochromatin formation on lytic-gene promoters.

The establishment of a quiescent form of HSV allows the virus to remain hidden from the host immune system and prevents the induction of apoptosis in infected cells. Understanding the mechanism of latency could therefore prove crucial in the development of effective therapies.

Shannon Amoils

ORIGINAL RESEARCH PAPER Wang, Q-Y. *et al.* Herpesviral latency-associated transcript gene promotes assembly of heterochromatin on viral lytic-gene promoters in latent infection. *Proc. Natl Acad. Sci. USA* **102**, 16055–16059 (2005)
WEB SITE
David Knipe's laboratory: http://knipelab.med.harvard.edu

Importantly, the amount of siRNA required to protect mice would have a realistic cost if translated to humans. As the most common causative agent of genital ulcer disease worldwide, HSV has an important role in HIV infection. Development of therapies that block HSV-2 infection in humans could therefore also help curb the spread of HIV. Lieberman and co-workers are currently working to see whether their approach might also work for HIV.

Focussing further on HIV, in the November 3 issue of *Nature*, John P. Moore, Ronald S. Veazey and co-workers report that vaginally delivered microbicides can protect female rhesus macaque monkeys against infection with simian–human immunodeficiency virus (SHIV), an SIV–HIV hybrid that is used because HIV does not infect Old World monkeys. Three compounds, each using a different mechanism to block the viral entry into cells, were tested. These compounds were found to inhibit infection of macaque and human peripheral blood mononuclear cells, and of cervical tissue explants. *In vivo*, each compound was found to significantly protect female rhesus macaques against SHIV infection, and using a combination of several microbicides proved even mor e efficient. This study represents the first succesful testing of combination microbicides in a primate model.

Motivated by the results of this last study, Merck and Bristol-Myers Squibb have given away rights to two key compounds, so that they can be developed into gels that protect against HIV.

Annie Tremp

ORIGINAL RESEARCH PAPERS Palliser, D. *et al.* An siRNA-based microbicide protects mice from lethal herpes simplex virus 2 infection. *Nature* (doi: 10.1038/nature04263) | Veazey, R. S. *et al.* Protection of macaques from vaginal SHIV challenge by vaginally delivered inhibitors of virus–cell fusion. *Nature* **438**, 99–102 (2005)
FURTHER READING Shattock, R. J. & Moore, J. P. Inhibiting sexual transmission of HIV-1 infection. *Nature Rev. Microbiol.* **1**, 25–34 (2004)
WEB SITES
Judy Lieberman's laboratory: http://www.cbrinstitute.org/lieberman
John P. Moore's homepage: http://www.med.cornell.edu/research/jpmoore

IN BRIEF

VIROLOGY

Domain III from class II fusion proteins functions as a dominant-negative inhibitor of virus membrane fusion

Liao, M. & Kielian, M. *J. Cell. Biol.* **171**, 111–120 (2005)

New data published recently in the *Journal of Cell Biology* have revealed that exogenous domain III can be used as a dominant-negative inhibitor of the membrane-fusion reactions of alphaviruses and flaviviruses, which both possess class II fusion proteins. During the fusion reaction between the host-cell membrane and the virus membrane, which is derived from the host-cell membrane during virus budding, class II fusion proteins undergo a refolding reaction during which they trimerize and form a hairpin-like structure, with domain III folded back towards the trimer tip. The addition of exogenous domain III prevents this foldback reaction and the subsequent mixing of the host and virus lipid bilayers. In addition to providing a useful research tool for further analysis of class II virus fusion, this study also uncovered the existence of a key interaction that could be a target for antivirals.

TECHNIQUES & APPLICATIONS

Real-time imaging of type III secretion: *Salmonella* SipA injection into host cells

Schlumberger, M. C. *et al. Proc. Natl Acad. Sci. USA* **102**, 12548–12553 (2005)

Secretion of type III effectors into host cells in real time

Enninga, J. *et al. Nature Methods* **2**, 959–965 (2005)

Many Gram-negative pathogens use type III secretion systems to inject bacterial virulence factors, or effector proteins, into host cells. Two recent papers report the development of new techniques based on time-lapse microscopy to monitor this process *in vivo*. Schlumberger *et al.* exploit the fact that most secreted effector proteins require the action of secretion chaperones for effective delivery into host cells. Their strategy detects the arrival of a bacterial effector into host cells indirectly by adding a green fluorescent protein (GFP) tag to the secretion chaperone. Enninga *et al.*, by contrast, have developed a technique that can directly assess the secretion of bacterial effectors after contact with host cells by adding a fluorescein-based biarsenical (FlAsH) label to bacterial effectors that have been tagged with a tetracysteine motif (4Cys).

BACTERIAL TRANSCRIPTION

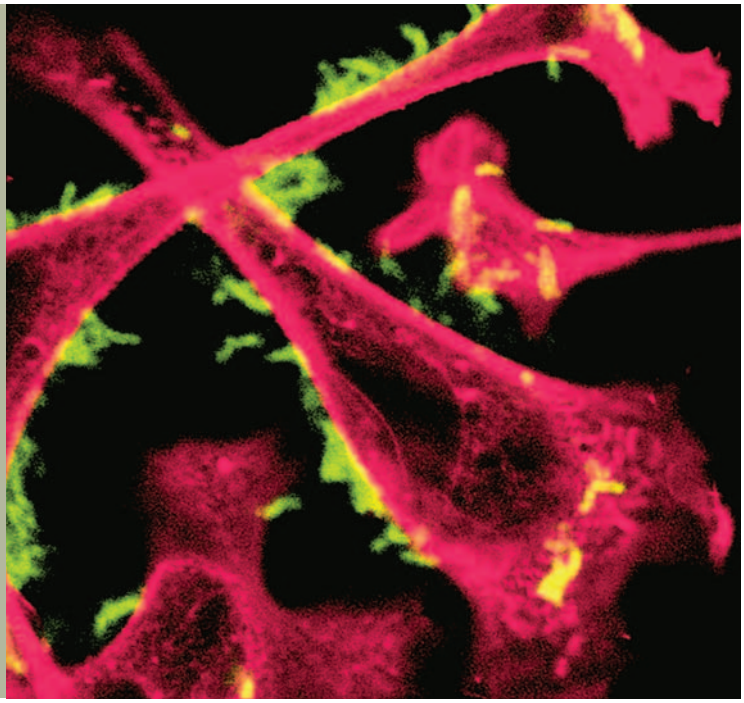
Structures of the bacterial ribosome at 3.5 Å resolution

Schuwirth, B. S. *et al. Science* **310**, 827–834 (2005)

In prokaryotes, protein synthesis is carried out by the 70S ribosome, which comprises a 1:1 complex of the large (50S) and small (30S) ribosomal subunits. Atomic-resolution structures of the 50S and 30S subunits from *Haloarcula marismortui* and *Deinococcus radiodurans*, respectively, were solved a few years ago, and the structure of the 70S ribosome of *Thermus thermophilus* is available at 5.5 Å resolution. However, efforts to obtain crystals of the intact 70S ribosome from *Escherichia coli*, the 'model prokaryote', had been unsuccessful. Now, in a landmark paper, two structures of the intact 70S ribosome from *Escherichia coli* at 3.5 Å resolution are reported in *Science* and have yielded a significant amount of new detailed information.

BACTERIAL PATHOGENESIS

Shaping a hummingbird...



► The image shows *H. pylori*-infected AGS cells with the typical hummingbird/scattering appearance. The yellow particles are *H. pylori*, whereas the cells are coloured red. Image courtesy of Chihiro Sasakawa, University of Tokyo, Japan.

The *Helicobacter pylori* virulence factor CagA is implicated in the pathogenesis of gastric disorders such as chronic gastritis, peptic ulcers and gastric cancer. CagA is translocated into *H. pylori*-infected cells, where it interacts with cellular scaffolding and signalling proteins, causing host-cell elongation and dispersal, and giving rise to the so-called 'scattering/hummingbird' phenotype. Despite the recognition of CagA-dependent epithelial disruption and the elucidation of several of its molecular interactions, the mechanism by which CagA contributes to gastric neoplasia is still unclear. Two new studies now enhance our understanding of CagA-induced cellular transformation.

Suzuki *et al*. show that phosphorylated CagA activates downstream host cell-signalling pathways by binding to the Crk adaptor proteins. In gastric epithelial cells infected with *H. pylori*, Crk–CagA interaction was required for cell–cell dissociation and for the development of the hummingbird phenotype. Crk–CagA signalling activated the Ras–Raf–MEK/ERK pathway, which promotes cell

BACTERIAL PATHOGENESIS

Pulling the *Yersinia* needle from the haystack

Two recent publications have provided a detailed look at the function of a *Yersinia* virulence factor in type III secretion and host immunomodulation.

Type III secretion in the yersiniae involves a secretory apparatus — the injectisome — and the secreted *Yersinia* outer proteins, or Yops. The injectisome itself comprises a basal body that is embedded in the bacterial membrane and a protruding 'needle', which contains the *Yersinia* protein YscF. In addition to YscF, three other *Yersinia* proteins facilitate type III secretion without being secreted into host cells: YopB and YopD, which form the translocation pore, and LcrV (the V antigen), which until now has had an undefined role in the secretion apparatus. Now, reporting in a recent issue of *Science*, Catherine Mueller, Petr Broz and colleagues provide evidence that LcrV is localized in a distinct structure at the tip of the injectisome needle.

Analysis of the surface of *Yersinia enterocolitica* E40 by transmission electron microscopy revealed that the needle tip was capped by a well-defined structure. Careful purification of the needle complex revealed that YscF was not the only structural component and showed that LcrV was also present. Deletion of *lcrV* resulted in the formation of needles lacking the needle tip complex, and this defect could be complemented by LcrV. Immunoelectron microscopy confirmed that LcrV forms a specific structure at the tip of the *Yersinia* injectisome needle. In a hypothetical model, the authors propose that LcrV

> “LcrV is localized in a distinct structure at the tip of the injectisome needle”

proliferation and growth, and induced actin reorganization by activating Rac1, Wave and the Arp2/3 complex.

The dissociation of *H. pylori*-infected cells was due in part to the disruption of adherens junctions. Immunofluorescence analyses showed that, in infected cells, the adhesion molecules E-cadherin and β-catenin translocated from adherens junctions into the cytoplasm. Furthermore, β-catenin crossed into the nucleus in these cells. Previous studies have shown that nuclear β-catenin coactivates the transcriptional regulator T-cell factor, which upregulates the expression of various oncogenes. So the presence of nuclear β-catenin in *H. pylori*-infected cells is significant.

In another study, Bagnoli and colleagues expressed different domains of CagA in polarized epithelial cells, and analysed CagA's effects on epithelial junctions, cell morphology, polarity and migration. Using confocal immunofluorescence microscopy and live-cell imaging, they found that cells expressing CagA lost many of their epithelial characteristics, including the integrity of the apical junctions and the ability to maintain apicobasal polarity. CagA-expressing cells also extended invasive pseudopodia that penetrated the basement membrane, and migrated away from their neighbours. The authors note that these changes resemble those seen in the epithelial-to-mesenchyme transition (EMT), a morphological event that takes place during normal embryogenesis, but that also heralds the progression of cancer.

Interestingly, both the Ras/MAPK pathway and β-catenin have been linked to the induction of EMTs, which implies that the signalling pathways induced by CagA might provoke oncogenesis by disrupting cellular differentiation programmes.

Shannon Amoils

ORIGINAL RESEARCH PAPERS Suzuki, M. *et al.* Interaction of CagA with Crk plays an important role in *Helicobacter pylori*-induced loss of gastric epithelial cell adhesion. *J. Exp. Med.* **202**, 1235–1247 (2005) | Bagnoli, F., Buti, L., Tompkins, L., Covacci, A. & Amieva, M.R. *Helicobacter pylori* CagA induces a transition from polarized to invasive phenotypes in MDCK cells. *Proc. Natl Acad. Sci. USA* **102**, 16399–16344 (2005)

functions as an 'assembly platform' that allows the translocation pore to be formed.

In addition to its role in type III secretion, which has now been clearly delineated, LcrV is also known to be involved in modulating the host immune response. In another recent paper, Andreas Sing, Dagmar Reithmeier-Rost and colleagues present experimental evidence confirming that LcrV is involved in Toll-like receptor 2 (TLR2)-dependent interleukin-10 (IL-10) induction in the *Y. enterocolitica* O:8 strain. Sequence comparison between LcrV and PcrV, the *Pseudomonas aeruginosa* homologue, had previously pinpointed the TLR2-activating region to N-terminal residues 31–57. The authors used reverse genetics to construct a mutagenized LcrV derivative selectively defective in TLR2 signalling. Analysis of the effects of this mutant strain in mice that were wild-type, TLR2$^{-/-}$ or IL-10$^{-/-}$ showed that the LcrV-mediated TLR2-dependent induction of IL-10 is important for *Yersinia* virulence and that a single residue — lysine 42 — is crucial for this effect. The authors add that the N-terminal TLR2-activating domain of LcrV might be more important in the virulence of *Y. enterocolitica* O:8 strains than in the virulence of *Yersinia pestis* and *Yersinia pseudotuberculosis*. The results from both papers are consistent with recent results from Susan Straley's group showing that anti-LcrV antibodies had a protective effect in a mouse model of plague.

Sheilagh Molloy

ORIGINAL RESEARCH PAPERS Mueller, C. A. *et al.* The V-antigen of *Yersinia* forms a distinct structure at the tip of injectisome needles. *Science* **310**, 674–676 (2005) | Sing, A. *et al.* A hypervariable N-terminal region of *Yersinia* LcrV determines Toll-like receptor 2-mediated IL-10 induction and mouse virulence. *Proc. Natl Acad. Sci USA* **102**, 16049–16054 (2005) **FURTHER READING** Philipovskiy, A. V. *et al.* Antibody against V antigen prevents Yop-dependent growth of *Yersinia pestis*. *Infect. Immun.* **73**, 1532–1542 (2005) | Wren, B. The yersiniae — a model genus to study the rapid evolution of bacterial pathogens. *Nature Rev. Microbiol.* **1**, 55–64 (2003)

PARASITOLOGY

Avoiding attraction

The helminth parasite *Schistosoma mansoni* causes chronic infections in humans, because it can evade host immune defences long-term. Such infections alter the immune response in a manner that prevents the development of various immune-mediated diseases, indicating that schistosomes produce immunomodulatory molecules. Now, *S. mansoni* eggs have been found to secrete a protein that binds certain chemokines and has potent anti-inflammatory activity.

Because certain viruses have been shown to produce chemokine-binding proteins (CKBPs) and because infection with *S. mansoni* affects the local recruitment of immune cells, Philip Smith and colleagues examined whether *S. mansoni* produces CKBPs. Secretions from live eggs (produced by adult worms, which reside in the intestinal blood vessels) were shown to bind the chemokines CXC-chemokine ligand 8 (CXCL8; also known as interleukin-8) and CC-chemokine ligand 3 (CCL3). A single protein was found to provide this activity, and the gene that encodes this protein was cloned. This novel CKBP was shown not only to bind specific chemokines but also to prevent these chemokines from interacting with their receptors, thereby inhibiting the migration and activation of leukocytes that express the cognate chemokine receptors (particularly neutrophils, but also macrophages and eosinophils).

This is the first report of a parasite that produces a CKBP. The authors showed that *S. mansoni* CKBP modulates the size and cellular content of the granuloma that forms around the eggs, allowing the eggs to be expelled and thereby maintaining the life cycle of the parasite. In addition, the anti-inflammatory activity of *S. mansoni* CKBP indicates that it could be used to treat acute inflammation in unrelated situations, and this possibility is supported by data from three *in vivo* models of acute inflammation.

Davina Dadley-Moore

ORIGINAL RESEARCH PAPER Smith, P. *et al. Schistosoma mansoni* secretes a chemokine binding protein with antiinflammatory activity. *J. Exp. Med.* **202**, 1319–1325 (2005)