News in brief

Targets and mechanisms

Neutrophil factor targets bacterial virulence proteins

Scientists have discovered a mechanism for combating virulent bacteria that could form the basis for a new generation of antibiotics. Researchers at the Max Planck Institute for Infection Biology (Berlin, Germany), have discovered a key defence protein produced by neutrophils in response to bacterial infections.

Neutrophils enclose bacteria in compartments called vacuoles that release degradative enzymes that destroy the bacteria, enabling the body to clear mild infections or small wounds. However, some bacteria have highly evolved defences that are much more difficult to overcome. An example of this is *Shigella*, which expresses proteins called virulence factors that enable the bacteria to invade any cell and escape from vacuoles. This should mean that, if *Shigella* can outsmart white blood cells, infection should always be lethal, and yet most people recover from *Shigella* infection.

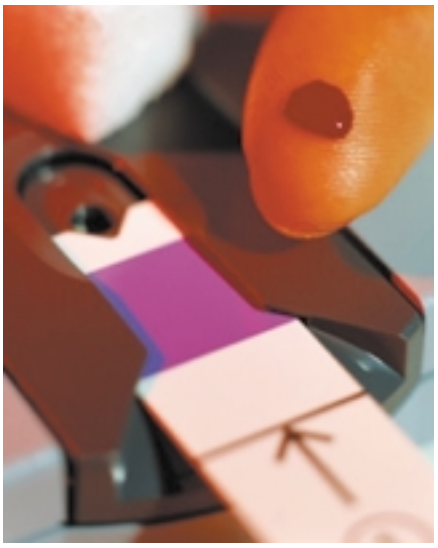
Weinrauch and Zychlinsky reasoned that neutrophils might play a significant role in the defence against this pathogen. Neutrophils, unlike other types of cells, can prevent *Shigella* from escaping their vacuoles, but of further interest was the discovery that neutrophils produce an enzyme called neutrophil elastase (NE) that is able to degrade *Shigella* virulence factors at a much lower concentration than that needed to degrade other bacterial proteins [1]. Neutrophils therefore retain the bacteria within the vacuole for long enough for other antibacterial defences to be mobilized.

NE also degrades the virulence factors of *Salmonella* and *Yersinia*, the etiological agents of typhoid fever and bubonic plague, respectively.

NE is the first neutrophil factor to target bacterial virulence factors, and these findings could aid the development of a new generation of antibiotics that focus specifically on virulence factors of disease-causing bacteria.

1 Weinrauch, Y. *et al.* (2002) Neutrophil elastase targets virulence factors of enterobacteria. *Nature* 417, 91–94

Autoimmune diabetes development arrested by vitamin D_3 analogue



A synthetic analogue of the active form of vitamin D_3 (1α,25-dihydroxyvitamin D_3) has been used to halt the development of type 1 diabetes mellitus (T1DM) in non-obese diabetic (NOD) mice, the first time that such an analogue has been used successfully in this way [2].

T1DM is a chronic, progressive autoimmune disease that results in the destruction of insulin-producing pancreatic β cells, and which can be inhibited by vitamin D_3 at nonhypercalcaemic doses. The NOD mouse model closely resembles the pathogenesis of T1DM in humans and so is a useful model with which to study the disease.

Researchers from BioXell (Milan, Italy) treated adult NOD mice with a synthetic analogue of vitamin D_3 and found that the production of cytokines (interleukin-12 and interferon-γ) that contribute to insulinitis was decreased, and the development of T1DM was inhibited, without any toxic side effects. The arrest of the progression of the disease was accompanied by an increase in the number of $CD4^+CD25^+$ regulatory T cells in the pancreatic lymph nodes. These cells are able to inhibit the T-cell response to pancreatic autoantigen insulinoma-associated protein 2 and can also delay the transfer of disease by pathogenic $CD4^+CD25^-$ cells.

There is an unmet need for drugs for T1DM, and existing vitamin D_3 analogues have only inhibited autoimmune diseases in NOD mice when combined with strong immunosuppressive agents, such as cyclosporin A. The results from this work suggest that this non-toxic analogue might also inhibit the progression of disease in patients who are pre-diabetic or newly diagnosed with T1DM.

2 Gregoria, S. *et al.* (2002) A 1α,25-dihydroxyvitamin D_3 analog enhances regulatory T-cells and arrests autoimmune diabetes in NOD mice. *Diabetes* 51, 1367–1374

Lung cancer tumour suppressor genes are candidates for gene therapy

Researchers at the University of Texas M.D. Anderson Cancer Center (Houston, TX, USA) and University of Texas Southwestern Medical Center (Dallas, TX, USA) have identified three tumour suppressor genes on human chromosome 3 that dramatically reduce lung cancer growth in mice. Phase I clinical trials for one of these genes could begin within six months [3].

John Minna and colleagues from UT Southwestern identified eight possible candidate tumour suppressor genes from a set of 20 within a 120 kb crucial tumour homozygous deletion region (found in breast and lung cancers). In collaboration with Jack Roth from the UT M.D. Anderson Cancer Center, six of these genes were introduced into human lung cancer cells using his group's adenovirus-mediated gene transfer technology. Forced expression of wildtype *FUS1*, *101F6* and *NPRL2* in H1299 and A549 cells (which are deficient in the 3p21.3 120-kb region), significantly inhibited tumour-cell growth by induction of apoptosis and alteration of the cell cycle, with no effect in control cells.

Intratumoural injection of adenovirus constructs or systemic injection of protamine-complexed adenovirus vectors containing the 3p genes significantly suppressed the growth of H1299 and A549 tumour xenografts and inhibited A549 experimental lung metastases in nude (*nu/nu*) mice. Gene transfer was achieved successfully, delivering enough DNA to cure the mice with no toxicity.

These results demonstrate, along with earlier data, that multiple contiguous genes in the 3p21.3 chromosomal region might exhibit tumour suppressor activity *in vitro* and *in vivo*. One of the genes, *FUS1*,

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will be administered to patients in a lipid capsule that binds to tumours, in a clinical trial that is expected to start within the next six months.

'The discovery of a region of the chromosome, as opposed to a single gene, that contributes to cancer development is novel,' said Roth. 'This is the earliest genetic change yet identified in lung cancer, a change that takes place in normal-looking tissue.' These findings could lead to earlier detection, diagnosis, prevention and treatment of lung cancer.

3 Ji, L. *et al.* (2002) Expression of several genes in the human chromosome 3p21.3 homozygous deletion region by an adenovirus vector results in tumor suppressor activities *in vitro* and *in vivo*. *Cancer Res.* 62, 2715–2720

Miscellaneous

Early warning sign for cancer drug cardiotoxicity

Researchers at the University of Milan (Italy) [4] have shown that elevated levels of troponin I (TnI), a cardiac peptide, could be an early warning sign for heart failure. The team measured the blood plasma levels of TnI in 211 breast cancer patients before and after chemotherapy and then assessed the left ventricular ejection fraction (LVEF) 1–12 months after the completion of treatment with anthracyclines.

Researchers found a strong correlation between elevated TnI levels and a decrease in left ventricular function. Daniela Cardinale, lead author of the study, said that TnI was thus demonstrated as a risk marker for future LVEF reduction. 'The innovative aspect of this new marker is that it gives us information long before functional impairment can be detected with other techniques.' She continued, 'The possibility of identifying patients who will develop late myocardial function depression is a golden opportunity for both oncologists and cardiologists.'

4 Cardinale, D. *et al.* (2002) Myocardial injury revealed by plasma troponin I in breast cancer treated with high-dose chemotherapy. *Ann. Oncol.* 13, 710–715

Brain atlas could help diagnose brain abnormalities

A new electronic atlas of the brain could help doctors to diagnose abnormalities that are too subtle to be obvious on traditional brain scans. Using only a laptop computer, the Dynamic Brain Atlas project, developed at King's College (University of London, UK), could enable doctors to compare, and even overlay, a conventional scan of a patient's brain with a computer-generated scan that is typical for a patient of that gender, age and past medical history.

The high accuracy of the tool could help radiologists to analyze subtle brain abnormalities in patients, which might have previously been missed by looking at a normal scan, and could lead to more accurate diagnosis of psychiatric conditions and dementia.

Jo Hajnal, from the MRC Clinical Sciences Centre, Imperial College (London, UK) comments that: 'This technology could have huge implications for patients for whom conventional diagnosis fails,' but warns, 'although routine use of these tools in district general hospitals is a long way off.'

Medigene delay clinical trials of Extomoxir

Medigene (Martinsried, Germany) has prematurely terminated Phase II clinical trials of its therapeutic drug candidate for congestive heart failure, Extomoxir, after side effects were seen in a small number of patients.

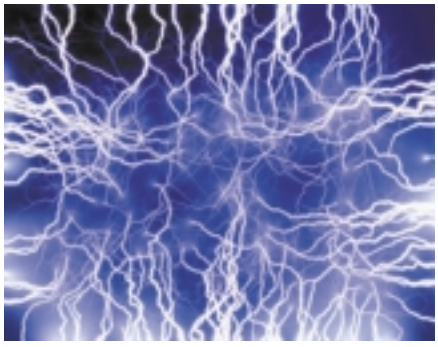
The company is now preparing for the development of a lower dose formulation; recent preclinical results indicate that the drug could be efficacious at a lower dosage than tried so far. This might improve patient tolerability and reduce production costs.

The trial, in which 155 patients so far have received 40 or 80 mg day^{-1} of Etomoxir or a placebo for 12 weeks, will be extended by approximately one year.

Electricity can drive nanoscale drug delivery

A new computer model has shown that the addition of a small electric current is sufficient to drive fluid through tubes only a few nanometers in diameter [5].

Although nanotechnology is a promising concept for drug delivery, researchers have previously had difficulty pumping fluid through the tiny passages in such devices, but Terry Conlisk, Professor of Mechanical



Engineering at Ohio State University (Columbus, OH, USA) believes that using a small electrical current could be the solution.

The model has been favourably compared with wet-lab experiments carried out at iMEDD (Columbus, OH, USA), in which engineers were able to flush almost 0.5 nl of saline per minute through a 7 nm channel. This offers great potential for the design of electricity driven drug-delivery technology, in which implanted devices would deliver small amounts of fluid to specific tissues or locations, such as within a tumour.

'The basic principle has been around for a long time,' said Conlisk. 'However, no other projects have involved the same combination of theoretical and experimental work,' he says. Charges as small as one volt can overcome flow resistance by repelling the ions in a fluid and forcing it down a tube, but in practice the current required would depend on the size of the implantable device and the amount of drug that had to be dispensed. However, the currents are too small to be dangerous to a patient and would not cause a build-up of heat, even if run continuously.

5 Conlisk, T. *et al.* (2002) Mass transfer and flow in electrically charged micro- and nanochannels. *Anal. Chem.* 74, 2139–2150

Drug portfolio and R&D pipeline key to satisfying investors

Companies will have to increase the size and quality of their drug portfolios if they are to satisfy the double-digit revenue growth demanded by their investors, says a new Datamonitor (London, UK) report. A traditional reliance on the revenue generated by blockbuster drugs (those with annual global sales of more than US$1 billion) will not match this because sales of existing and potential blockbusters are only expected to grow at 4.3% annually for the period 2000–2008. Similarly, the previous protection of

blockbuster patents through litigation has limited success and cheaper equivalent formulations will unavoidably arrive on the market.

Companies are thus advised to pursue other strategies to maintain growth such as increasing therapeutic or promotional expertise in certain disease markets. In-licensing strong products will be easier for companies with attractive R&D technologies or promotional strengths, which highlights them as 'partners of choice'. Keeping a balance between pricing and promotional investment will be key to deciding the future success of lifecycle management initiatives.

Diabetic fruit flies

Researchers have engineered fruit flies that have a condition similar to human type 1 diabetes [6]. A team at Stanford University Medical Center (Stanford, CA, USA) believe that, although the evolutionary step between *Drosophila* and humans is great, these diabetic flies could help elucidate how insulin-releasing cells develop. This could lead to the replacement of these lost cells in diabetic patients.

In diabetes mellitus, the insulin-producing pancreatic cells are destroyed by the immune system; this causes sugar to accumulate in the blood, which damages the kidneys, blood vessels, nerves and eyes. Type 1 diabetes patients must inject insulin to survive. A potential cure is the use of stem cells to generate replacements for these damaged cells. To ensure that these cells develop as pancreatic cells, it is essential that researchers have knowledge of their normal development.

Eric Rulifson, a postdoctoral fellow in developmental biology and lead author of the paper, said: 'The idea is that the more you know about normal development, the better chance you have to make stem cells develop into insulin-producing cells.'

Roel Nusse, Professor of Developmental Biology and co-author of the study said that this is where the fruit fly excels: 'There are many examples of functions that are conserved between flies and humans.' Rulifson explained, 'If the fly cells are using the same molecules and genes as humans, then there's a good chance that much of the pathway of development is conserved.'

The team engineered the diabetic flies by first identifying insulin-producing cells (IPCs) in the brain; these were then destroyed in fly larvae. The resultant flies were slower to develop, smaller and had higher blood-sugar levels than normal flies; these levels were comparable with those in human diabetes. These IPCs were found to release insulin into the circulatory system of the fly, which led to the proposal that IPCs and the β-cells of the human pancreas are analogous and could have evolved from a common ancestor. Rulifson said that these similarities between the hormonal systems of the fly and humans are 'the tip of the iceberg'.

6 Rulifson, E.J. *et al.* (2002) Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. *Science* 296, 1118–1120

Potential new treatment for diabetes and Alzheimer's

Researchers at University College London (UK) have developed a drug that could be used as a treatment for type 2 diabetes and Alzheimers' disease (AD) [7]. The drug targets the plasma protein, serum amyloid P component (SAP), which itself binds to fibrils in all amyloid deposits, contributing to the pathogenesis of amyloidosis.

Amyloidosis is a disorder where proteins accumulate as abnormal fibres that lead to tissue and organ damage. Amyloid deposits in the brain are associated with AD, and those in the pancreas are associated with type 2 diabetes.

The team, working with Roche, developed a new drug, CPHPC, which prevents SAP binding to amyloid deposits and thus managed to remove the SAP protein from blood plasma. The drug crosslinks and dimerizes SAP, which is then rapidly cleared by the liver.

The research is supported by the Medical Research Council (MRC; London, UK) and is the culmination of 25 years of research. Mark Pepys, lead author of the study, said: 'Our experimental studies identified SAP as a key suspect in the development of amyloidosis and we aimed to find a drug that could block its effects.' He added, '[The drug] produces a complete knockout of the single protein in the blood that we targeted.'

Pepys continued, 'Although amyloid deposits are closely associated with AD and mature onset diabetes, it is not known whether they actually cause these diseases. Our work offers real hope for systemic amyloidosis, which until now has been difficult and dangerous to treat.' This could provide a therapeutic approach to treat AD and type 2 diabetes; the drug could be used to remove SAP from deposits in the brain and the first clinical studies in patients with AD are due to begin shortly.

7 Pepys, M.B. *et al.* (2002) Targeted pharmacological depletion of serum amyloid P component for treatment of human amyloidosis. *Nature* 417, 254–259

Link found between mitochondrial enzyme and cancer gene

The cell-growth promoting cancer gene *c-myc* has been shown to control activation of the mitochondrial enzyme PRDX3 [8],which is overexpressed in breast cancer. This finding could enable researchers to inhibit tumor growth by limiting the expression of PRDX3.

The PRDX3 encodes a mitochondrial protein of the peroxiredoxin family, which remove peroxides within the cell. Using a method called chromatin immunoprecipitation analysis to span the entire PRDX3 sequence, researchers at Johns Hopkins University School of Medicine (Baltimore, MD, USA) identified a region to which Myc preferentially binds. They showed that PRDX3 is required for Myc-mediated proliferation, transformation and apoptosis after glucose withdrawal.

Chi Dang, senior author of the study, and colleagues studied different levels of PRDX3 activation in rat and human cancer cell lines in which Myc is activated. When the PRDX3 enzyme gene is switched off, tumor growth ceased, whereas when the enzyme was switched on the tumours grew rapidly.

'Think of PRDX3 as a light bulb and c-myc as the light switch,' said Dang, 'in this case, we've removed the light bulb rendering the switch powerless.' he said. 'Now our challenge is to find out in which cancers this pathway is most important and what drugs may do the job [of switching it off].' Dang said.

8 Wonsey, D.R.. *et al.* (2002) The c-myc target gene *PRDX3* is required for mitochondrial homeostasis and neoplastic transformation. *Proc. Natl. Acad. Sci. U. S. A.* 99, 6649–6654

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