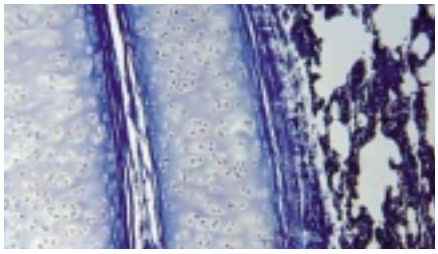
News in brief

Targets and mechanisms

A barrier to breathing



A simple yet effective repair mechanism in airway barrier cells has been discovered scientists at the University of Iowa (UI; http://www.uiowa.edu) [1]. The study shows that by placing a messenger molecule and a receiver molecule on either side of the barrier the cells have a repair mechanism that is activated when the barrier is breached.

Certain airway diseases, such as asthma, cystic fibrosis and smoking-associated bronchitis, impair the airway barrier. This recent study has shown that, under these conditions, messenger molecules might not be well segregated from their receptors, which consequently might be abnormally activated. 'If everything is healthy, the message never gets to its receptor because the barrier keeps them apart, ' said Paola Vermeer, lead author of the study. If, however, a disease weakens the barrier so the message gets to the receptor when it should not, the repair mechanism could be activated inappropriately.

The ligand that is produced by human airway epithelia is the growth factor heregulin and its receptors are erbB2, erbB3 and erbB4. Immediately after a mechanical injury is initiated, heregulin-α activates erbB2 in cells near the wound, which quickens damage repair. This ligand–receptor separation could be crucial for cell repair and these results could also have implications in disease processes.

Michael Welsh, a Professor at UI, said: 'If this mechanism is disrupted in disease, then these findings might suggest targets for therapeutic intervention. It might be possible to interfere with the message or its receptor to break the line of communication.' He added that the results of this study could also be relevant to other biological systems, such as cancer and developmental processes.

1 Vermeer, P.D. *et al.* (2003) Segregation of receptor and ligand regulates activation of epithelial growth factor receptor. *Nature* 422, 322–326

Cilia dysfunction linked to renal failure

Tissue-specific knockout mice have provided invaluable evidence for abnormalities in renal cell cilia formation and function being the possible cause of polycystic kidney disease (PKD) [2], the most common genetic cause of renal failure in humans.

Researchers at University of Texas Southwestern Medical Center (UTMSC; http://www3.utsouthwestern.edu/) inactivated gene KIF3a (a subunit of kinesin-II that is essential for cilia formation) in only the tubular epithelial cells of kidneys, and the resulting mice were viable, but with cysts beginning to develop in the kidney already at day five after birth, and with renal failure by day 21. The dissected cysts had similar characteristics to those found in PKD.

Fangming Lin at UTMSC said: 'Once we understand the mechanism, we will have the target to prevent or slow the cyst formation'. Essentially, this means that a potential treatment for the one in every 500 people who suffer with PKD, could lie ahead.

2 Lin, F. *et al*. (2003) Kidney-specific inactivation of the KIF3A subunit of kinesin inhibits renal ciliogenesis and produces polycystic kidney disease. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.0836980100 (http://www.pnas.org)

Good news for asthma sufferers

Natural killer (NK) T cells have been identified by researchers at Stanford University Medical Center (http://www.mednews.stanford.edu) as being absolutely crucial in the regulation and development of asthma [3].

Mice deficient in $V_{\alpha}14i$ NK T cells showed no sign of allergen-induced airway hyperreactivity (AHR), such as wheezing or shortness of breath. When compatible NK T cells producing interleukin (IL)-4 and IL-13 were injected into mice a similar mouse model of asthma [Ja281(–/–) mice, which lacked the invariant T-cell receptor of NK T cells], AHR was restored to that shown by wild-type controls – even before exposure to antigens.

Administration of IL-13 (which directly affects smooth muscles and glands in the lungs) to Cd1d-deficient mice induced the same asthmatic symptoms as the control group, indicating that the NK T cell-deficient mice have weaker lungs than normal mice.

Immune response, Th2, is confined to the upper airways and causes symptoms of allergies, such as sneezing, but alone is not sufficient to induce asthma. Significantly, NK T cell-deficient mice produced normal Th2-biased responses when immunized at non-mucosal sites, but mice were not asthmatic.

NK T cells play a major regulatory role in autoimmune diseases. People without asthma may have fewer proactive NK T cells in the lung. Dale Umetsu at Stanford University Medical Center concluded 'if these NK T cells are increased in numbers or have increased potency, one way to treat or prevent the disease would be to remove these cells'.

3 Akbari, O. *et al.* (2003) Essential role of NK T cells producing IL-4 and IL-13 in the development of allergen-induced airway hyperreactivity. *Nat. Med.* 10.1038/nm851 (http://www.nature.com)

Lung cancer linked to overactive hedgehog

Small-cell lung cancer (SCLC) is highly aggressive and frequently lethal. It is difficult to treat surgically and, often despite initial success with chemotherapy, most patients relapse. However, new insights into how SCLC develops now suggest how this cancer might be treated more effectively [4].

The hedgehog pathway, so called because disruption of hedgehog

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HIV targets and mechanisms

Our antibodies could be driving HIV escape

New research now shows that human antibodies, crucial in the defence of the body against invading pathogens, might actually drive HIV to mutate and escape detection by the immune system.

Researchers at the University of California (http://www.ucsd.edu/) have characterized neutralizing antibody responses directed at circulating autologous HIV in plasma using a sophisticated new recombinant virus assay developed by ViroLogic (http://www.virologic.com/) [7]. HIV was cloned from the blood plasma of HIV patients and genetically combined with a gene that makes luciferase. The glowing enzyme helped the researchers to track HIV replication.

Patients infected with HIV rapidly developed a strong antibody response against the virus. However, the same antibodies that recognize and disable HIV seem to force its ongoing evolution into new strains that can avoid the antibody response and continue to replicate. 'The neutralizing antibodies are exerting a very strong selective pressure on the virus and the virus is continually mutating to avoid it', says Douglas D. Richman, lead author of the study.

The AIDS virus has been described as a 'genetic moving target' because of its frustrating ability to rapidly mutate and escape the immune system; dozens of strains can develop within the same person. Although ~60 potential HIV vaccines have been studied since 1987, non were found to be both safe and effective. It is therefore hoped that these results could be key in continuing efforts to develop an effective AIDS vaccine.

7 Richman, D.D. *et al.* (2003) Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.0630530100 (http://www.pnas.org)

HIV plays cat-and-mouse

Researchers at the Howard Hughes Medical Institute, University of Alabama (http://www.hhmi.org/) have discovered one way in which HIV evades the immune system [8]. HIV-1 (a common strain of the virus that causes AIDS) uses a strategy not seen before in other viruses to escape attack by antibodies, which are prime weapons of the immune system used against invading viruses and bacteria.

Neutralizing antibodies (NAbs) are a principal component of an effective human immune response to many pathogens, however, their role in HIV-1 infection is unclear. This new study aims to increase our understanding of this role by looking at the plasma from patients with acute HIV infection. Autologous NAb was detected as early as 52 days after the detection of HIV-specific antibodies. The viral inhibitory activity of NAb resulted in complete replacement of neutralization-sensitive virus by successive populations of resistant virus, with escape virus containing mutations in the *env* gene, which primarily involved changes in *N*-linked glycosylation.

Viruses typically vary the protein sequence or epitope of the envelope that acts as a docking station for antibodies, thus preventing antibodies from targeting the virus for destruction. HIV-1, however, continuously changes the arrangement of large sugar molecules studded across its gp120/41 protein coat. This causes obstruction of the antibody docking regions. George Shaw, lead researcher in the study, said, 'These changes in glycan molecules prevent the binding of neutralizing antibodies to the virus surface through steric inhibition, thereby enabling the virus to avoid antibody-mediated elimination'.

The immune system therefore does try to fight HIV, but the glycan shield often mutates at a faster rate than the immune system can keep up. Although HIV is resourceful, there is still hope for the development of an effective vaccine to protect currently uninfected but at-risk individuals. 'If uninfected patients were vaccinated against HIV-1 with an appropriate immunogen, then neutralizing antibodies in this setting could conceivably have a far greater impact,' says Shaw.

8 Wei, X. *et al.* (2003) Antibody neutralization and escape by HIV-1. *Nature* 422, 307–312

'Forming' a new approach

New crystal forms of the HIV drug Ritonavir could lead to improvements in therapy and highlight the need to screen for alternative crystal forms [9]. Researchers at TransForm Pharmaceuticals (http://www.transformpharma.com/) have extended the number of known forms of Ritonavir using high-throughput technology.

Ritonavir (also called Norvirr) is a protease inhibitor that has been used in the treatment of HIV for several years. It received much attention in 1998 when an unexpected polymorph of the drug was discovered during the manufacturing process. This alternative crystalline form, which had different solubility properties, was potentially hazardous, as all clinical trials had been performed on the original form. Abbott, who marketed the drug, had to halt production until a more reliable formulation could be established. On top of the health risks associated with such a hitch, had it gone unnoticed, there is also the danger of rival companies bypassing intellectual property by exploiting an alternative polymorph of a drug.

Cases like this highlight the importance of investigating the possible forms and formulations a drug can take. TransForm Pharmaceuticals specialize in such analysis and have taken a fresh look at Ritonavir. Using their proprietary technology, the company found several new forms of the drug, including one previously unknown polymorph. In addition, they developed reproducible methods of preparing both the new and old forms. Over 2000 opportunity for developing even better drugs or for improving marketed products.'

9 Morissette, S.L. *et al.* (2003) Elucidation of crystal form diversity of the HIV protease inhibitor ritonavir by high-throughput crystallization. *Proc. Natl. Acad. Sci. U. S. A.* 100, 2180–2184

signalling in flies seems to cause an excess of spiky hairs, is required for lung development. Previously discovered links between pathways that regulate development and cancer led researchers at Johns Hopkins University (http://www.jhu.edu/) to look at whether hedgehog signalling might also have a more sinister role in the lungs.

Neil Watkins and colleagues found that the hedgehog pathway was activated in airway epithelia during recovery from acute airway injury. The pathway was also unusually active in SCLC samples and in the majority of SCLC cell lines. 'We believe that chronic injury to the lungs by cigarette smoking re-activates genes in the hedgehog pathway to repair cell damage in the lining of the lungs. The ongoing and regular assault to the lungs by cigarettes causes the normally dormant pathway to be stuck in activation mode making too many new cells, ultimately resulting in cancer,' says Watkins.

The researchers went on to show that blocking hedgehog signalling inhibited the growth of SCLC cells *in vitro* and also that of SCLC cells grafted into mice. Their findings offer real hope that SCLC might be treated by pharmacological blockade of the hedgehog pathway.

4 Watkins, D.N. *et al.* (2003) Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. *Nature* 422, 313–317

Miscellaneous

MC1R variation gives redheads the edge

In a study that could change the way painkillers are prescribed in the future, a gene associated with red hair and fair skin – melanocortin-1 receptor (*Mc1r*) – might also be responsible for how females respond to painkillers [5]. Jeffrey Mogil and colleagues have demonstrated that *Mc1r* mediates κ-opioid analgesia in female mice only. This intriguing discovery suggests that individuals with variants of *Mc1r* red hair and fair skin might also display altered κ-opioid analgesia.

Previous research by the group at McGill University (http://www.mcgill.ca/) indicated a difference in pain tolerance between men and women. Moreover, their studies have shown that males and females feel pain using different pain pathways in the brain. Now, Mogil *et al.* have tested the effects of the κ-specific analgesic on mice: the typical sex differences in analgesic effects were seen in normal mice, but these differences disappeared in mutant mice with an inactive variation of *Mc1r* – analogous to the 'redhead' variation in humans.

Taking the study further, a clinically used κ analgesic – pentazocin – was tested on male and female humans with several *MC1R* variations. The *MC1R* variation did not effect the analgesic response in men, but caused a heightened response in redheaded, fair-skinned women. This suggests that *MC1R* modulates the κ-specific pain pathway only in females. Mogil explains, 'While we believe pain is the same in all women of all hair colours, our study shows women with red hair respond better to the pain-killing drug we tested than anyone else, including men'.

Mogil believes that pain is a disease in its own right and, thus, deserves to be studied as such. Research in Mogil's laboratory aims to find a better, more effective treatment of pain. In the future, prescription of painkillers could be tailored to the individual.

5 Mogil, J.S. *et al.* (2003) The melanocortin-1 receptor gene mediates female-specific mechanisms of analgesia in mice and humans. *Proc. Natl. Acad. Sci. U. S. A.* 100, 4867–4872

Cancer-drug resistance breakthrough

Researchers at the Whitehead Institute for Biomedical Research (http://www.wi.mit.edu/home.html) have found a way to identify genetic mutations that cause resistance to targeted anti-cancer drugs before treatment. This finding will help drug-development scientists and enable physicians to monitor patients for resistance problems before they occur [6].

The team, led by George Daley, have developed a screen to identify mutations that cause patients with chronic myeloid leukaemia (CML) to become resistant to Gleevec (known as Glivec® in the European Union), which targets the protein product of *BCR–ABL*. The *BCR–ABL* gene constantly mutates in CML patients and cells carrying certain mutations can resist Gleevec and continue to grow. 'We have found a way to discover those mutations experimentally,' said Daley.

Recombinant DNA methods were used to randomly mutate *BCR–ABL* to mimic potential mutations in CML patients. The mutated genes were transfected into mouse blood cells, which were then treated with Gleevec. Certain cells survived and the genes from these cells were sequenced. Fifteen mutations were identified that had previously been associated with Gleevec resistance; 97 new mutations were also identified. All mutations found previously in relapsing CML patients were found within the Bcr–Abl kinase domain, most around the drug-binding pocket; interestingly, however, many of the new mutations were located far from this domain, implying no direct involvement with drug binding.

Their findings could also lead to the development of new drugs that are effective despite these mutations. Daley and colleagues Robert Latek and Mohammed Azam mapped the 112 mutations and created a three-dimensional computer model of Bcr–Abl, which begins to illustrate how different parts of the protein interact to influence gene function. 'By creating a three-dimensional structural model,' Latek said, 'we're able to look at the actual site that we want the drug to bind to and design a drug customized for that binding site.'

6 Azam, M. *et al.* (2003) Mechanisms of autoinhibition and STI-571/Imatinib resistance revealed by mutagenesis of *BCR–ABL*. *Cell* 112, 831–843

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