News in brief

Targets and Mechanisms

Pain relief without the side effects?

A compound has been designed that could achieve effective pain relief without the side effects, by avoiding the CNS. The compound, AM1241, seems to act on CB_2 cannabinoid receptors at peripheral sites with immune functions, independent of actions at CB_1, which are found in the brain and spinal cord [1].

Originally developed by Alexandros Makriyannis of the University of Connecticut (http://www.uconn.edu/), who is co-author of the report, AM1241 was found to reverse tactile and thermal hypersensitivity in rats, increasing their ability to withstand chronic pain that mirrored neuropathic pain states in humans.

Approximately one in eight Americans have some type of chronic pain, with treatment costing US$120–180 billion annually. However, many continue to experience pain despite receiving treatment. As Nora Volkow, Director of the National Institute on Drug Abuse (http://www.nida.nih.gov/) explains, 'a number of drugs used to treat pain have unpleasant side effects'; these side effects prevent administration of doses that would provide the most complete relief.

Although AM1241 has the potential to overcome these limitations, its mechanism-of-action is currently unknown; it might 'inhibit the release of chemicals like prostaglandins from immune cells that increase sensitivity to pain,' suggests Philip Malan, of the University of Arizona (http://www.arizona.edu/) and co-author of the report. Alternatively, AM1241 might work by triggering these cells to release substances that block the pain response. Whatever the case, the next step will be an assessment of the potential toxicity of the compound.

Although AM1241 is far from entering human clinical trials, these findings clearly advocate the potential use of CB_2 receptor-selective agonists for revolutionary treatment of human neuropathic pain.

1 Ibrahim, M.M., *et al.* (2003) Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.1834309100 (epub ahead of print; http://www.pnas.org/)

Rabbits sniff out link between copper and Alzheimer's

Recent rabbit models indicate that copper could be a major factor in the etiology of Alzheimer's disease (AD). The addition of trace amounts of copper to the drinking water of cholesterol-fed rabbits induced β-amyloid (Aβ) accumulation, including plaque-like structures in the brain, and significantly reduced the rabbits' ability to learn a difficult conditioning task [2].

Although copper is an essential nutrient, Larry Sparks of the Sun Health Research Institute (http://www.sunhealth.org/) and lead author of the report, believes that it somehow interferes with the body's ability to clear out the Aβ, adding, 'if there is no copper in the water then the Aβ is shuttled to the blood for clearance'. Cholesterol-fed rabbits are considered a good model for studying AD because they show at least 12 pathological markers of the disease.

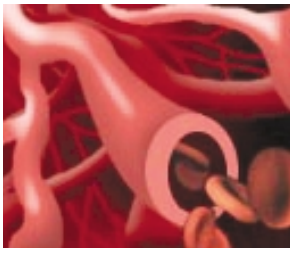
AD affects ~15 million people worldwide and is terminal, although certain treatments can prolong life expectancy. Disease onset is characterized by mild memory loss, with patients becoming increasingly senile and dependent on carers.

The US Environmental Protection Agency (http://www.epa.gov/) has a maximum contaminant level for copper in drinking water of 1.3 ppm. However, Sparks reports that levels in the rabbits' drinking water was 'one-tenth that', raising questions about the safety of this limit.

This report does not indicate that copper pipes are unsafe, because metal pipes are inert, but acid in water can cause copper to leach out of the pipes. Research in this and other areas will continue while the precise causes of AD remain a hot debate.

2 Sparks, L. and Schreurs, B.G. (2003) Trace amounts of copper in water induce β-amyloid plaques and learning deficits in a rabbit model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.1832769100 (epub ahead of print; http://www.pnas.org/)

Single-handed control of vessel formation



Two molecular events that are key to blood vessel formation have been discovered by scientists at the University of North Carolina (http://www.unc.edu). One is the process by which endothelial cells are prevented from overrunning in the developing embryo [3], and the other is the factor that activates the first steps in vasculogenesis [4].

Endothelial cells line the blood vessels, and determine what a blood vessel does. Formation of new blood vessels involves endothelial cells differentiating from precursor stem cells. It is a single transcription factor, HoxB5, which alone starts this process. Flk1, the earliest molecular marker of endothelial precursors, and the first gene turned on in endothelial progenitor cells, was used to find the transcription factor that turns flk1 itself on. A screening procedure (yeast-one hybrid system) identified HoxB5 as the responsible transcription factor. To validate this finding, HoxB5 was further shown to increase expression of flk1 by binding the genetic regulatory element (cis-acting element in the first intron) in the *flk1* gene, especially when HoxB5 was overexpressed in stem cells. Vessel formation in stem cell cultures was also hugely increased.

The *BMPER* gene (BMP-binding endothelial precursor-derived regulator) was found to inhibit the whole process of endothelial cell differentiation from stem cells. BMPER is expressed only in endothelial cells and their precursors, the former secreting the protein as the cells differentiate.

These are important findings because the possibility for using HoxB5 to create renewable populations of endothelial cell precursors is heavily laden with therapeutic potential.

3 Moser, M. *et al.* (2003) BMPER, a novel endothelial cell precursor-derived protein, antagonizes bone morphogenetic protein signaling and endothelial cell differentiation. *Mol. Cell Biol.* 23, 5664–5679

4 Wu, Y. *et al.* (2003) HoxB5 is an upstream transcriptional switch for differentiation of the vascular endothelium from precursor cells. *Mol. Cell Biol.* 23, 5680–5691

  PII: S1359-6446(03)02821-6

Heart-to-heart

Heart transplant cells that were previously rejected in *in vivo* animal models can now survive after genetic manipulation, report scientists from Brigham and Women's Hospital (http://www.brighamandwomens.org) [5].

Adult bone marrow-derived mesenchymal stem cells can be used to repair cartilage and bone defects successfully but have shown limited reparative capacity in the pig heart due to poor cell viability associated with transplantation.

By overexpressing the prosurvival gene *Akt1* in these stem cells, the transplanted cells were prevented from dying in experiments with rats. In fact, there was a remarkable amount of reparative growth, which almost completely restored cardiac function. In rats that have been given artificial heart attacks, the stem cells again generated more heart-like cells.

This process of genetic engineering to increase the likelihood of transplant cell survival could be used in gene therapy for humans.

5 Mangi, A.A. *et al.* (2003) Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat. Med*. 10 August, doi:10.1038/nm912 (epub ahead of print; http://www.nature.com)

Improved diagnosis for dementia cause

Attributing the correct causes to dementia in the incredibly complex human brain is not always easy. Conditions such as Alzheimer's disease (AD) are often difficult to differentiate from vascular diseases of the brain, such as strokes.

By combining magnetic resonance imaging (MRI) and magnetic resonance spectroscopic imaging (MRSI), researchers have improved diagnostic accuracy [6]. This should not only assist diagnosis and therapy, but also improve the selection of patients for AD drug trials.

Researchers at San Francisco VA Medical Center (http://www.sf.med.va.gov/) compared elderly patients diagnosed with AD, patients who had suffered small strokes and been diagnosed with subcortical ischaemic vascular dementia (SIVD) and a control group of normal elderly subjects. MRI was used to form a 3D image of the brain of each subject. This enabled differences in structure to be determined, contributing to a 79% accuracy of diagnosis.

The researchers then used MRSI to analyze the amount of *N*-acetylaspartate (NAA) produced by active neurons in various areas of the brain. The amount of NAA detected is directly proportional to the density of active neurons in the area under study.

The team found patterns of NAA levels that could be used to distinguish subjects with AD from those with SIVD. In conjunction with the MRI results, this pushed the correct diagnosis rate up to 89%. The results also indicated that SIVD might be due to neuronal dysfunction rather than loss, offering hope of therapeutic intervention. Norbert Schuff, lead author, cautioned that the subjects of the study were carefully chosen, and that the techniques now need to be validated in a clinical setting.

6 Schuff, N. *et al.* (2003) Different patterns of *N*-acetylaspartate loss in subcortical ischemic vascular dementia and AD. *Neurology* 61, 358–364

Tailored vaccine for autoimmune disease

A new treatment for autoimmune disease turns immunotherapy on its head. After diagnosing the specific causes of disease, a tolerizing vaccine increases the body's tolerance to the causes, rather than priming the immune system to attack [7]. The methodology could spawn therapies for diseases such as multiple sclerosis and type-1 diabetes.

The technique, developed by researchers at Stanford University Medical Center (http://www.med.stanford.edu/), comprises identification of the malfunctioning immune-system components, followed by customized therapy. It was demonstrated in a mouse model of multiple sclerosis, in which the insulating myelin sheath of nerve cells is degraded.

Serum antibodies were introduced to microarrays containing various myelin proteins. Antibodies responsible for the autoimmune response attacked specific proteins on the microarray. Each mouse had a unique reactivity profile, and those with the highest number of reactive proteins went on to develop the most severe disease.

Next, the researchers sought tailored therapies for particular disease profiles. They used a technique known as tolerization, whereby the immune system is taught to tolerate rogue proteins after injection. Lawrence Steinman, lead researcher, clarified the idea: 'This is the opposite of what we try to do with traditional vaccines against bacteria and viruses, where we want to stimulate the immune system to attack the microbe,' he said.

Microarray profiles indicated which proteins to target in each mouse. DNA coding for the specific proteins was inserted into a plasmid vector. Injection of the DNA vaccine led to production of the desired repertoire of proteins and tolerization began, greatly improving the condition of the mice. As well as helping to tailor such therapies, the ability to profile which antibodies have gone awry also has diagnostic potential for predicting the future severity of autoimmune disease in a particular patient.

7 Robinson, W.H. *et al.* (2003) Protein microarrays guide tolerizing DNA vaccine treatment of autoimmune encephalomyelitis. *Nat. Biotechnol.* 10 August; doi: 10.1038/nbt859 (epub ahead of print; http://www.nature.com)

Cancer Targets and Mechanisms

Histone errors linked to cancer

The cell has numerous mechanisms for detecting and fixing damaged DNA. If these mechanisms are disrupted, cancer often ensues. A new study highlights such a consequence when histone protein becomes non-functional [8].

Histone is a non-specific repressor of transcription and a constituent of chromatin. New research indicates that when the *H2AX* histone gene is knocked out, errors in the genetic code persist, leading to lymphomas and solid tumours.

The new findings could have important implications for cancer research, as many cancers are caused by faults in the region of chromosome 11 where *H2AX* is located.

A multi-institutional team, headed by Craig Bassing at the Howard Hughes

Medical Institute (http://www.hhmi.org/) looked at the effects of knocking out *H2AX* in mice. The target was chosen because previous studies had shown that *H2AX* is activated when DNA breaks occur. The team created a double knockout that lacked both *H2AX* and *p53*, a tumour suppressor gene. These mice showed vastly increased tumour rates, well above those found with either mutation alone.

Furthermore, double mutants lacking only one of the two copies of the *H2AX* gene showed a very different spread of tumours to those lacking both copies. Mice with only one *H2AX* gene and functioning *p53* also showed genetic instability, and carried only half as much H2AX protein as a normal mouse.

This 'haploinsufficiency' is unusual in a system of tumour suppression and would not have been readily detected in most screens for such factors. Frederick Alt, one of the paper's authors, offered a possible explanation, explaining that the protein is not an enzyme but a structural protein. Only having half the normal amount could cause problems in monitoring DNA breaks and recruiting repair enzymes.

8 Bassing, C.H. *et al.* (2003) Histone H2AX: a dosage-dependent suppressor of oncogenic translocations and tumours. *Cell* 114, 359–370

Aspirin: old drug, new use?

Aspirin, trusty staple of many home medicine cabinets, could have an exciting new application. Research suggests that the anti-inflammatory effects of the drug might be transferable to treating certain forms of cancer [9].

The possibility arose after researchers from Cancer Research UK (http://www.cancerresearchuk.org/), working with colleagues at the Alexander Fleming Biomedical Sciences Research Center (http://www.fleming.gr/), found molecular evidence that runaway inflammation processes are the cause of turban tumour syndrome.

This condition is a rare and occasionally life-threatening form of skin cancer in which disfiguring growths occur on the scalp and other haired skin areas. The disease is caused by inheriting a faulty version of the *CYLD* gene, but the function of the gene product was previously unknown.

Using a human cell line, the researchers studied this gene to elucidate why the mutant version leads to tumours. They found that *CYLD* codes for a protein that negatively regulates NFκB by deubiquitination. If NFκB is left unregulated, the inflammatory response is activated and proceeds unchecked, leading to tumour growth. The same mechanism might cause other types of cancer including certain breast cancers.

If the theory is correct, a simple therapy could be at hand, in the shape of anti-inflammatory drugs, such as aspirin. Alan Ashworth, lead scientist, suggested a possible scenario. 'We think anti-inflammatory drugs could be rubbed into tumours in gel form in order to shrink them,' he speculated. He further suggested that aspirin could be given to young patients at an early stage in the disease, as a preventative measure. The team is keen to move on from these cell-line studies to testing their theory in the clinic.

9 Trompouki, E. *et al.* (2003) CYLD is a deubiquitinating enzyme that negatively regulates NF-κB activation by TNFR family members. *Nature* 424, 793–796

Gene Therapy

Gene therapy hits a nerve

About 30,000 people in the US suffer from amyotrophic lateral sclerosis (ALS) and about 5000 new cases are diagnosed each year. In this disease, nerves that control muscles gradually die, leading to paralysis and death. The cause of ALS is unknown, and there is no effective therapy.

In a recent study, researchers from the Salk Institute (http://www.salk.edu/) and Johns Hopkins University (http://www.jhu.edu/) report a novel form of gene therapy that delays symptoms and almost doubles life expectancy in mice with the symptoms of ALS [10]. In mice destined to develop the condition, injection of the gene for insulin-like growth factor-1 (IGF-1) into muscles protected nerve cells, extended survival and improved strength.

Jeffrey Rothstein, an author of the study, said: 'Even in mice, progression of the disease is so rapid that we only test possible treatments before the mice get sick. It is amazing that this gene therapy can slow progression even after symptoms develop'.

Gene therapies use viruses to deliver genetic instructions to cells and usually have to deliver them to the exact site they are needed. This new study took advantage of the ability of adeno-associated viruses to migrate from muscle to the nerves that control them. Using this virus as a gene delivery vehicle, the gene for IGF-1 was transported to nerve cells where the protein was expressed. Producing IGF-1 in nerve cells rather than in muscle improved life expectancy and delayed symptoms. The authors hope to start clinical trials in the near future.

10 Kaspar, B.K. *et al.* (2003) Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. *Science* 301, 839–842

News in brief was written by *Matt Brown, Georgina Smyth, Clare Rathbone and Catherine Wild*