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News in brief

Neurodegenerative diseases

Turning back time in brain damage

Scientists at the Department of Neurology at the University of California, Los Angeles (UCLA; http://www.neurology.medsch.ucla.edu) have shown that a pattern of cellular activity in early hindbrain development can trigger repair to damaged adult brain [1]. These mechanisms were previously unknown and their discovery holds promise for the treatment of brain damage resulting from stroke and other disorders.

'Our research shows for the first time that this activity works to trigger repairs in adult brains,' said Marie-Francoise Chesselet, Professor of Neurology at the David Geffen School of Medicine at UCLA and co-author of the study.

The team used rat models to show how brain cells that were damaged with stroke-induced cortical lesions develop slow synchronous activity, which triggers new connections to develop between cells. They isolated signals that are specific to the development of new connections – known as axonal sprouting – and measured the frequency, power and synchronicity of brain activity, comparing it with a model that did not induce axonal sprouting. Synchronous neuronal activity occurred on day one following lesion, with a frequency of 0.2–2.0 Hz, followed by a later pattern on days 2–3 with a frequency of 0.1–0.4 Hz.

These findings suggest that this pattern of activity could signal a repair process in the human adult brain following injury. S. Thomas Carmichael, Assistant Professor of Neurology at UCLA said, 'On its own, a damaged brain has a limited ability to repair itself. Recovery is partial.' He added, 'A better understanding of how the brain recovers from injury will allow us to manipulate the repair process and to maximize recovery caused by stroke and other disorders.'

1 Carmichael, S.T. and Chesselet, M-F. (2002) Synchronous neuronal activity is a signal for axonal sprouting after cortical lesions in the adult. *J. Neurosci.* 22, 6062–6070

Neurons die a different death

Researchers at Johns Hopkins University School of Medicine (http://www.hopkinsmedicine.org) [2] have identified a novel form of programmed cell death in neurons that is different to those already known (apoptosis and necrosis). This finding defines, for the first time, a window of opportunity to prevent neuronal death, thus leading to potential new targets to treat Parkinson's disease (PD), stroke and brain injury.

Valina Dawson, Professor of Neuroscience at Johns Hopkins, said: 'All cell death is programmed in that it results from a particular series of events. But up to a certain point, the outcome is not inevitable and interference with the process can prevent or delay cell death. Knowing when that window of opportunity closes is critical.'

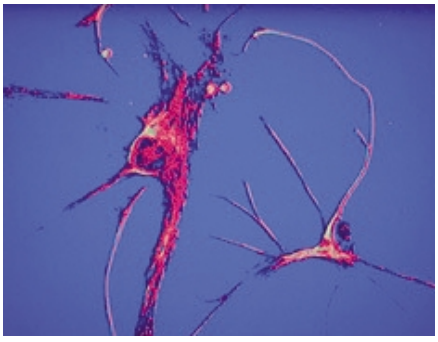
The study, conducted in mouse cells, builds on the knowledge that activation of poly (ADP-ribose) polymerase-1 (PARP-1) initiates neuronal death, and found an apoptosis-inducing factor (AIF), which is the final step in the event of cell death. This research showed that PARP-1 activation is necessary for AIF translocation from the mitochondria, where it is made, to the cell nucleus. Identifying the molecules that are involved in this process could aid in the interference and prevention of cell death. Dawson said, 'AIF entering the nucleus seems to be the point of no return – once it gets in, the cell is going to die no matter what you do.'

PARP-1 functions as the 'guardian of the genome' because it recognizes damaged DNA; in cells with too much damage, it triggers events that result in cell death. 'This study links PARP-1 activation with mitochondrial function for the first time,' said Dawson, and added that they had identified this link as being AIF.

'The classic definitions of necrosis and apoptosis are meaningless in the nervous system because the terms were defined in tissues outside of it. Cell death in the nervous system uses some of the pathways of necrosis and apoptosis but in a slightly different sequence.' The team showed that AIF transfer to the nucleus came before the release of the enzyme caspase, an initial step in apoptosis. Preventing PARP-1 activation and blocking AIF release prevented cell death in neurons but blocking caspase did not, showing this as a caspase-independent pathway of programmed cell death.

2 Yu, S.W. *et al.* (2002) Mediation of poly (ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. *Science* 297, 259–263

Inosine promotes nerve regrowth after stroke



Axon growth within the brain and spinal cord can be induced by inosine, a naturally occurring compound. Scientists from Children's Hospital, Boston (http://web1.tch.harvard.edu) in conjunction with Boston Life Sciences (http://www.bostonlifesciences.com) showed that the compound improves motor function after stroke in animal models [3].

In the USA, approximately 750,000 people suffer strokes every year and it is the third leading cause of death after heart disease and cancer. Stroke occurs when the brain is deprived of a blood supply from either the blockage of a blood vessel (ischemic stroke) or a ruptured vessel in the brain leading to blood leakage and damaged tissue (hemorrhagic stroke). Current therapies are limited to preventing the spread of damage and increasing blood flow; no treatments are available to regenerate the damaged nerves.

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The researchers found that inosine stimulates nerve cells from undamaged regions of the brain to grow connections into the damaged areas. This resulted in a significant improvement in a range of mobility types in rats. Anatomical evidence correlated with the behavioral enhancements: after stroke, inosine stimulated growth of new brain connections three- to four-times more effectively than in mice that were not treated with the compound.

Larry Benowitz, head of the laboratory at Children's Hospital and Associate Professor at Harvard Medical School commented on the study: 'Inosine induces a great deal of rewiring in the brain after stroke. This rewiring is apparently sufficient to promote substantial functional recovery. In terms of clinical implications, inosine, which appears to have no apparent side effects in animals thus far, has potential as a novel nerve regeneration approach to treatment of stroke and other types of brain injury.' Mark Lanser from Boston Life Sciences, which is developing inosine for the treatment of stroke, spoke of the potential of the therapeutic, 'In a separate study (not included in this publication), we have found that inosine is effective even when administered as much as 24 h after stroke. This is an additional important potential advantage over other current therapies.'

3 Chen, P. *et al.* (2002) Inosine induces axonal rewiring and improves behavioral outcome after stroke. *Proc. Natl. Acad. Sci. U. S. A.* 99, 9031–9036

Infectious diseases

A deadly combination

Common bacteria can turn deadly if they have the right set of genes, scientists at the National Institute of Allergy and Infectious Diseases (NIAID; http://www.niaid.nih.gov) have recently discovered [4]. This research highlights an important mechanism for bacterial evolution and identifies several potential targets for new vaccines and drugs against infections.

The study showed that bacteriophages are responsible for the emergence of virulent new bacterial strains; they do this by infecting bacteria, capturing their genes and transferring them to other bacteria. The team focussed on group A *Streptococcus* (GAS) bacteria, which commonly cause throat and wound infections, as well as toxic shock syndrome (TSS), 'flesh-eating' disease (necrotizing fasciitis) and scarlet fever.

James Musser, from the Laboratory of Human Bacterial Pathogenesis at NIAID, seeks to understand why some strains of GAS cause deadly infections, whereas others lead to milder infections, by comparing the genomes of different bacterial strains. 'We are trying to move past the technical phase of genome research and start asking what we have learned from gene sequences to develop new ways to prevent and treat infections and so understand how new, virulent strains emerge,' he said.

In this study, the sequence of the serotype M3 strain of GAS, isolated from a patient with TSS, was determined. Of its 1.9 Mb, 1.7 Mb was found to be related to serotype M1 and M18 strains, with phage-like elements contributing to the majority of variation. Among the variable genes were several bacterial toxins. These molecules could prove useful targets for new drugs or vaccines. 'What we have discovered is that bacterial viruses have important crucial new toxin genes to create new virulence strains,' said Musser.

This research has opened up a new avenue to a previously neglected area of research. Musser added, 'Scientists have known about bacteriophages for a long time but they have not been extensively studied for their indirect contributions to infectious diseases. Now we have shown their importance in bacterial evolution, there is much we need to know about them.'

4 Beres, S.B. *et al.* (2002) Genome sequence of a serotype M3 strain of group A *Streptococcus*: phage-encoded toxins, the high-virulence phenotype, and clone emergence. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.152298499 (epub ahead of print; http://www.pnas.org)

New RNA inhibition technique could stop infection in its tracks

RNA interference (RNAi) technology has been successfully used for the first time to silence gene expression in mice [5]. Researchers at Stanford University (http://www.stanford.edu) were able to suppress transgene expression by injecting naked, synthetic small interfering RNAs (siRNAs) and small hairpin RNAs (shRNAs).

To observe the RNAi process, Anton McCaffrey, first author on the paper, injected mice with a luciferase gene and, in half of the mice, he co-injected siRNA that inhibits luciferase gene expression. The results were convincing: in mice receiving both DNA and RNA, whole body scans revealed that the luciferase gene had been inhibited by 80–90% compared with control mice. Subsequent experiments showed that a gene construct consisting of the luciferase gene and a portion of the hepatitis C virus was also suppressed to a similar extent as the luciferase transgene alone, suggesting that it could be possible to silence viral genes and halt virus replication.

However, Mark Kay, senior author on the paper, cautioned that, 'Although these results look promising, they rely on injected RNA, [which] doesn't last long in cells.' An easier option would be to inject DNA, which is more durable, to produce the RNA. However, current methods for injecting DNA produce only single-stranded RNA, which is no use for RNAi.

To overcome this, McCaffrey and Kay injected mice with a plasmid that encodes a cognate shRNA, which doubles back on itself to form a single, double-stranded RNA molecule. Inhibition of luciferase using this method was found to be as successful as using siRNAs, and had the advantage that after the RNA breaks down, the plasmid DNA remains in the cell and continues to produce more shRNA. Kay said, 'The ultimate goal is to use this to treat a disease…we can do this by placing these molecules into standard gene therapy vectors.'

5 McCaffrey, A.P. *et al.* (2002) Gene expression: RNA interference in adult mice. *Nature* 418 10.1038/418038a (epub ahead of print; http://www.nature.com)

Asthma targets and mechanisms

Virus in infancy linked to asthma

Scientists have proposed that a viral infection in the first years of life could leave a lasting mark on the immune system that causes asthma later on [6]. Michael J. Holtzman and colleagues at the

Washington University School of Medicine (http://www.washington.edu/medical/som/index.html) have shown that the increase in allergic response that occurs during an asthma attack is also accompanied by an antiviral response.

Using a mouse model, the team found that a single paramyxovirus infection (the most common cause of respiratory infections in babies) caused acute bronchiolitis, but also triggered a chronic response with airway hyper-reactivity and goblet cell hyperplasia that persisted for a year after the original infection. 'Since both of these changes also is a long-term symptom of asthma, these findings provide a link between the response to viral infection and the development of asthma,' Holtzman said. By contrast, stimulation of the immune system with allergen alone only caused acute airway inflammation and hyper-reactivity.

The role of the inflammatory mediator ICAM-1 was investigated by using *ICAM-1*-null knockout mice. As expected, mice lacking ICAM-1 that were infected with paramyxovirus were initially healthier than their wildtype counterparts. However, the long-term inflammatory effects of the virus were still observed, suggesting that the two phenotypes (acute versus chronic inflammation) can be distinguished by their dependence on the *ICAM-1* gene. Holtzman added, 'These results in mice provide a further basis for determining exactly how similar events may develop in children and adults with asthma.'

6 Walter, M.J. *et al.* (2002) Viral induction of a chronic asthma phenotype and genetic segregation from the acute response. *J. Clin. Invest.* 110, 165–175

Gene implicated in asthma pathogenesis

Genetic linkage studies have led to the discovery of a gene on chromosome 20p13 that is thought to be implicated in asthma [7], a disease that affects 14 million people and 8 million people in the USA and UK, respectively. Researchers at Genome Therapeutics (http://www.cric.com), the University of Southampton in the UK (http://www.soton.ac.uk), and Schering-Plough (http://www.schering-plough.com) performed a genome-wide scan on an outbred population consisting of 460 Caucasian families, and identified a locus that was linked to asthma and bronchial hyper-responsiveness.

A survey of 135 polymorphisms detected in 23 genes identified the *ADAM33* gene as being significantly associated with asthma. ADAM proteins are membrane-anchored metalloproteases with diverse functions, such as the shedding of inflammatory cytokines and cytokine receptors. 'The airways in asthma patients undergo a number of changes such as thickening of the airway walls and subsequent narrowing of the airway passage,' said Stephen Holgate, a lead collaborator on the project, 'Our studies suggest ADAM33 plays a role in this remodeling and may underlie abnormalities in asthmatic airway function.' The discovery of this genetic predisposition to asthma could aid the diagnosis of susceptible individuals, who could then be treated early to try to prevent any permanent damage to the respiratory tract.

7 Van Eerdewegh, P. *et al*. (2002) Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 10.1038/nature00878 (epub ahead of print; http://www.nature.com)

Miscellaneous

Bleach and blood vessels

An enzyme that produces chlorine bleach to kill bacteria and other pathogens can also turn off a blood vessel dilation signal during inflammation, it was discovered by scientists at the University of California Davis School of Medicine and Medical Center (UCDMC; http://www.ucdmc.ucdavis.edu) [8]. This research identifies a previously unrecognized function for this immune system protein and could lead to a new molecular target for the development of drugs to treat inflammatory vascular diseases.

The enzyme, myeloperoxidase (MPO), is found in white blood cells and releases locally produced hypochlorous acid as a bactericidal agent. Jason P. Eiserich, lead author of the paper and Assistant Professor of Medicine and Human Physiology at UCDMC, said: 'MPO… is present in very high concentrations in white blood cells and provides an important line of defence against invading micro-organisms. Since neutrophils are also known to contribute to impaired vascular function during acute inflammatory responses, we reasoned that MPO may be a central player.' He added, 'Our studies show that MPO does affect the vasculature, but by a pathway independent of its well-characterized capacity to produce chlorine bleach.'

Normally, nitric oxide (NO) produced by endothelial cells lining the wall of blood vessels acts as a vasodilator. This study found that, in rodent models, following induction of acute inflammation, MPO is released from white blood cells and is deposited in the blood vessel wall where it 'mops up' NO, thus blocking the blood vessel dilation signal; these changes were not seen in MPO-deficient rodents.

'Identifying a protein that modulates NO-dependent blood vessel dilation has important implications for the potential treatment of inflammatory vascular diseases,' said Eiserich. He added that drugs aimed at mimicking this activity could be used to treat systemic hypotension during septic shock and drugs that block MPO activity could treat chronic vascular disorders, such as atherosclerosis, which are characterized by a lack of NO and accumulation of MPO in blood vessel walls. This research could also help identify whether individuals that lack MPO display abnormal vascular responses during inflammation.

8 Eiserich, J.P. *et al.* (2002) Myeloperoxidase, a leukocyte-derived vascular NO oxidase. *Science* 296, 2391–2394

News in Brief was written by *Matt Brown*, *Daphne Chung*, *Joanne Clough and Joanna Owens*