Hallenbeck and Weiner agree that the suppression is mediated by anti-inflammatory cytokines released by regulatory T cells – primarily transforming growth factor (TGF)-β and interleukin (IL)-10 – rather than by an antibody to ES. (Antibody to ES was not detected in the rats given the tolerizing treatment.)

The scientists took advantage of evolution by delivering the antigen in a nasal spray. Cells lining the gut and respiratory tracts are continuously being presented with proteins of various types and origins. 'If every time a protein appeared that wasn't 'self' we broke out every weapon in the arsenal to mount an attack against it, we'd be tearing ourselves apart,' Hallenbeck pointed out. Oral tolerance, in which antigens are ingested, works the same way.

Dosage is critical

Proper dosage is a key to developing tolerance. A single, large dose of the antigen wipes out the antigen-reactive T cells, which is the population of cells that mediates immune suppression. By contrast, a single administration of a low dose of the antigen was not enough to tolerize the animals: rats given a single dose of ES had 12-fold more infarcts than those given continuing booster shots. 'This is not like pharmacology, where dose effects are linear, and at some point you reach toxicity,' said Hallenbeck.

Other diseases are poised to reap the benefits of tolerance as well. 'It's a very broadly applicable concept,' Weiner observed. Studies are under way in animal models of atherosclerosis, Alzheimer's disease and various autoimmune diseases [5]. But Hallenbeck says that stroke is among those with greatest potential for success, because the targeted inflammation is at the molecular level, rather than in a hot, swollen arthritic joint, for example.

Weiner is optimistic about plans for the stroke vaccine to go to Phase I clinical trials, but notes that success in humans is never certain after an animal study. There have been mixed results in clinical trials attempting a similar strategy against other ailments, notably multiple sclerosis and rheumatoid arthritis. Although tolerance has yet to be shown as a miracle cure (or prevention), the failures seem to lie in the details, says Weiner. The good news is that mucosal delivery of tolerance-inducing antigens in humans 'was safe and didn't have side effects, so it's attractive in that regard.'

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News in brief

Viral targets and mechanisms

Adenovirus: a molecular Houdini

New light has been shed on the mechanism by which a virion exits its host tissue into the wider environment, allowing it to spread further. Researchers at the University of Iowa (http://www.uiowa.edu/) have revealed the molecular details of how adenovirus makes its way through the protective layers of epithelial cells lining the airway [1].

Adenovirus, which causes flu-like symptoms, infects epithelial cells lining the respiratory tract. These cells are held together by adhesion molecules, including one known as CAR (cozsackie and adenovirsu receptor). The researchers found that a protein called Fiber on the surface of adenovirus can bind to CAR and disrupt its adhesive properties, thus opening a gap in the endothelial lining. Fiber alone, without the rest of the virus, is enough to cause a breakdown of cell adhesion. In addition, blocking Fiber prevents the virus from escaping into the airway.

To improve its chances of escape, the replicating virus makes excess Fiber and defective viral particles that possess Fiber but are not infectious. Thus, when an infected cell dies and spills its contents, nearby CAR molecules are overwhelmed by a variety of viral protein forms. The virus also uses Fiber as part of its mechanism to gain entry into host cells. Robert Walters, the lead author of the study, explained the potential applications of these findings: 'In most viral infections, people shed the virus before they are noticeably sick. So being able to understand how the shedding occurs might allow us to prevent spread of infection among people in close quarters, such as in schools or military barracks.' The authors speculate that this mechanism could be used widely by other viruses and even bacteria.

1 Walters, R. *et al.* (2002) Adenovirus Fiber disrupts CAR-mediated intercellular adhesion allowing virus escape. *Cell* 110, 789–799

Viruses driven to extinction

Treating a common virus with a mutation-inducing cancer drug causes the virus to mutate so much that it can no longer reproduce and is driven to extinction. This intriguing find could lead to new methods to treat and eliminate viral infections.

  PII: S1359-6446(02)02513-8

To date, efforts to halt viruses from spreading have led to the development of drugs that prevent reproduction, but the effectiveness of these drugs has been limited. Viruses replicate and mutate so quickly that they become resistant to individual antiviral drugs.

Now, scientists at Cedars-Sinai Medical Center (http://www.cedarsmed.com) and the Universidad Autonoma de Madrid (http://www.uam.es) have capitalized on the tendency of viruses to make mistakes during replication [2]. Two types of mutation-causing cancer drugs were investigated in cells infected with lymphocytic choriomeningitis virus (LCMV), a virus that can infect both mice and humans. Infected hamster cells were grown in culture, then treated with either fluorouracil (FU) or 5-azacytidine (AZC). Replication ability and mutation rate were measured and compared at various doses of FU or AZC. The results show that, even at low doses, both FU and AZC increase the number of mutations in the viral genome. Moreover, high doses of FU were able to drive LCMV to extinction.

'Given that FU eliminated this particlular virus while AZC did not, this suggests that different mutagens will be more effective than others in the treatment of viruses,' commented Pedro Lowenstein, Director of the Board of Governor's Gene Therapeutics Research Institute at Cedars-Sinai Medical Center. 'This work also lays the groundwork for future studies to determine how mutagens actually slow down the replication process and, at the same time, may lead to new treatments that succeed where others have limited effectiveness.'

Although viral extinction was probably caused by an increase in the mutation rate induced by FU, the team were unable to track the mutations as the virus progressed closer to extinction.

2 Grande-Perez, A. *et al*. (2002) Molecular indetermination in the transition to error catastrophe: Systematic elimination of lymphocytic choriomeningitis virus through mutagenesis does not correlate linearly with large increases in mutant spectrum complexity. *Proc. Natl. Acad. Sci. U. S. A.* DOI 10.1073/pnas.182426999 (epub ahead of print; http://www.pnas.org)

α-Defensins named as anti-HIV agents

After more than 16 years of extensive research, scientists have now identified an activity that allows some HIV-1-infected individuals to remain free of AIDS [3]. This result represents a significant step forward for AIDS research.

HIV-infected individuals who remain healthy are termed 'long-term non-progressors' (LTNP). It has long been known that the $CD8^+$ cytotoxic T lymphocytes (CTLs) of certain LTNP individuals secrete an activity known as $CD8^+$ antiviral factor (CAF), which can suppress replication of HIV. Part of the anti-HIV activity of CAF has been attributed to β-chemokines, but its other components have remained elusive. Now, a collaboration between scientists at Ciphergen Biosytems (http://www.ciphergen.com/) and the Aaron Diamond AIDS Research Center at Rockefeller University (http://www.adarc.org/research/) has revealed that the rest of CAF comprises α-defensins-1, -2 and -3. Linqi Zhang *et al.* used Ciphergen's ProteinChip® system to compare supernatants from $CD8^+$ CTLs of LTNP individuals with HIV-infected patients with normally progressing immunodeficiency, as well as control subjects.

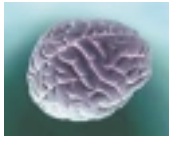
Analysis indicated that the $CD8^+$ CTLs from immunodeficient patients lacked α-defensins, but that these proteins were produced by the $CD8^+$ CTLs from the other two groups. The researchers went on to show that CAF deficient in both α-defensin activity and β-chemokine activity was virtually unable to prevent HIV replication or suppress HIV viral activity *in vitro* using purified or synthetic α-defensins.

Future work will focus on which subpopulation of $CD8^+$ CTLs produces these proteins and how the α-defensins inhibit HIV-1 replication. The possibility that α-defensins might act on viral transcription could constitute a new mechanism of action on which to base novel therapies.

3 Zhang, L. *et al.* Contribution of human α-defensin-1, -2, and –3 to the anti-HIV activity of CD8 antiviral factor. *Science Express* 10.1126/science.1076185 (in press)

Neurodegenerative diseases

Alzheimer's origins in white matter?



Scientists at the Sun Health Research Institute (http://www.shri.org/), led by Alex Roher, suggest that biochemical changes in the white matter of the brain could have a major role in disease onset [4]. The origin of Alzheimer's disease (AD) has long been baffling and has traditionally been associated with the grey matter of the brain. Despite white matter constituting ~50% of total brain tissue and being substantially altered during AD, the majority of research has focused on the grey matter. These latest findings question the accepted theory that changes in white matter are simply effects of the initial grey matter changes.

The group's analysis of AD white matter showed increased quantities of β-amyloid (Aβ) peptides and total fatty acid content, and significant decreases in myelin proteins and cholesterol levels. 'These profound white matter alterations undoubtedly contribute to the origin and development of AD, and might possibly be the initiating step,' says Roher.

Two interesting relationships also discovered were that women tend to have more alterations in white matter than men, consistent with the predominance of AD in women. An increased level of apolipoprotein E, associated with a higher risk of AD, also correlated with white matter biochemical alterations.

'Only if the full extent of AD pathology is known will present attempts to intervene in the progression of the disease have any reasonable chance of success,' says Roher. The success of future therapeutic interventions will require a complete appreciation of the full scope and extent of AD pathology.

4 Roher, A.E. *et al.* (2002) Increased Aβ peptides and reduced cholesterol and myelin proteins characterize white matter degeneration in Alzheimer's disease. *Biochemistry* 41, 11080–11090

New genetic model of oxidative stress-mediated neurodegeneration

New research has identified a gene that protects neurons against oxidative stress, leading to the prevention of neurodegeneration [5].

Oxidative stress is caused when the concentration of oxygen free radicals, produced by many normal metabolic processes, exceeds the antioxidant capacity of a cell. This leads to cell damage, and has been implicated in a variety of conditions affecting humans, including aging, cancer, heart disease and neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease).

Using harlequin mice that have a mutation (*Hq*) in the gene encoding apoptosis-inducing factor (*Aif*), researchers at The Jackson Laboratory (http://www.jax.org/) showed that, in these mice, neurons suffer oxidative stress, because of the reduced AIF levels. Furthermore, under oxidative stress, these neurons duplicate their DNA in a process known as re-entering the cell cycle, but they are unable to divide successfully and die in the attempt.

To date, ALS is the only neurodegenerative disease that is known to be caused by oxidative stress, but this mechanism has been suggested as a possible cause of other late-onset neurodegenerative conditions; there is some evidence that neurons in patients with Alzheimer's disease show cell-cycle re-entry before they die.

This work by Ackerman *et al.* is the first genetic model of neurodegeneration mediated by oxidative stress and cell cycel re-entry, and provides an excellent opportunity for studying the effects of these factors on several neurodegenerative diseases.

5 Klein, J.A. *et al.* (2002) The harlequin mouse mutation down-regulates apoptosis-inducing factor. *Nature* 419, 367–374

Polyglutamine protein aggregates and neurodegeneration

Scientists at Northwestern University (http://www.northwestern.edu/) have deciphered a key step towards our understanding of loss of nerve function in a variety of diseases, including Parkinson's, Alzheimer's, cystic fibrosis and Creutzfeldt–Jakob disease [6].

In all neurodegenerative diseases in humans, clumped damaged and misfolded proteins form toxic species that then aggregate, leading to the loss of cell function and disease. The cellular toxicity associated with these aggregates have been suggested to result from sequestration of essential cellular proteins. Molecular chaperones (special protective proteins) can suppress these effects but, until now, it was not known how they interacted with the aggregated proteins.

By observing interactions between the toxic protein aggregate and Hsp70, which is a molecular chaperone, researchers led by Richard I. Morimoto, have to come to understand more about how the chaperone works to prevent protein aggregation. They observed that Hsp70 continuously binds to and then releases the aggregate, preventing healthy proteins that are essential to cell function from binding to the aggregate. The chaperone appears to release any healthy proteins from the aggregate, which could suppress its growth, prolong cell life and therefore delay the onset of neurodegeneration.

Although it is unknown exactly how the chaperone can act in this way, the researchers suggest that this is the next step leading to the prevention of neurodegenerative diseases and to the

MIscellaneous

Burning chili pepper link to inflammation

New research shows that the pain associated with arthritis shares a common component with the burning sensation produced by chili peppers [10]. This could help in the development of strategies to block the pain hypersensitivity associated with inflammation.

A team led by Clifford Woolf, Director of the Neural Plasticity Research Group at Massachusetts General Hospital (http://www.mgh.harvard.edu/).found an increased production of the heat-gated ion channel TRPV1 (the chili-pepper receptor) following peripheral inflammation. This increase is mediated by activation of the signalling molecule p38 mitogen-activated protein kinase (MAPK), located within sensory s, which also induces nerve growth factor (NGF)-induced TRPV1 increases in the periphery. TRPV1 responds to capsaicin, the chemical responsible for the 'heat' in peppers, as well as to heat and to low pH, a condition that occurs in inflammation. 'With these findings, we're starting to understand why patients with arthritis or other inflammatory conditions are likely to have increased pain and sensitivity to heat,' says

Surprisingly, p38 activation causes a 20-fold increase in TRPV1 protein in the skin but does not increase the activity of the gene encoding TRPV1. 'This means that the chili-pepper receptor is not being regulated by the gene being switched on but by more protein being produced, an unexpected form of regulation,' says lead author Ru-Rong Ji, also of Massachusetts General Hospital. These findings could increase clinical options for pain management, and there is the potential to develop p38 inhibitors to block the TPRV1 increase, thus blocking pain in many inflammatory conditions.

10 Ji, R.-R. *et al.* (2002) p38 MAPK activation by NGF in primary sensory s after inflammation increases TRPV1 levels and maintains heat hyperalgesia. *Neuron* 37, 57–68

Athletes prone to motor disease

Patients with motor disease, such as amyotrophic lateral sclerosis (ALS), are more likely to have been athletes and of slim build, according to a new study in *Neurology* [11]. Nikolaos Scarmeas and colleagues from Columbia University (http://www.columbia.edu) compared the body mass index (BMI), sex, age of disease onset, slimness and athletics background of 279 patients with motor disease and revealed that the odds of having motor disease was 2.21-fold higher in subjects who reported that they had always been slim compared with those who had not. Furthermore, motor disease was 1.7-fold higher in patients who reported they had been varsity athletes.

It is not known why top athletes have a higher chance of developing motor disease than non-athletes and why only a few slim athletes develop ALS. However, some researchers have hypothesized that vigorous exercise might increase exposure, transport and absorption of environmental toxins to the brain, as well as increasing susceptibility to the disease through added physical stress.

11 Scarmeas, N. *et al.* (2002) Premorbid weight, body mass, and varsity athletics in ALS. *Neurology* 59, 773–775

development of effective drug treatments. 'How do we use this new information…to protect individuals from the molecular damage of disease?' said Morimoto, 'That is our next challenge'.

6 Kim, S. *et al.* (2002) Polyglutamine protein aggregates are dynamic. *Nat. Cell Biol.* 4, 826–831

Cancer targets and mechanisms

Revealed: the function of BRCA2

The function of the protein BRCA2 has been elucidated by scientists at the Memorial Sloan-Kettering Cancer Center (MSKCC; http://www.mskcc.org) [7]. Mutations in the *BRCA2* gene have been linked to breast and ovarian cancers.

BRCA2 was already known to be a tumour suppressor but its mechanism of action was unclear. Now, structural biologists at MSKCC have elucidated the 3D crystal structure of the protein to 3.1 Å, and shown that this 90 kDa protein aids the repair of genetic damage. The senior author of the study, Nikola P. Pavletich, head of MSKCC's Laboratory of Structural Biology of Oncogenes and Tumor Suppressors, said: 'If BRCA2 is altered or missing, it leads to a dangerous accumulation of genetic errors. By studying the normal function of BRCA2, we can understand how changes in the protein contribute to the development of cancer.'

The team showed that BRCA2 helps repair double-stranded breaks in DNA; these can be particularly lethal because they result in the complete loss of genetic information. They elucidated the crystal structure of the protein, which was revealed to be similar to other DNA binding proteins. They then showed that BRCA2 binds to DNA in regions found around DNA strands, and enables recovery of lost information via homologous recombination, where the missing DNA is copied from another part of the cell.

Pavletich said, 'We are now a step closer to understanding this particular type of inherited breast and ovarian cancers.'

7 Yang, H. *et al.* (2002) BRCA2 function in DNA binding and recombination from a BRCA2-DSS1-ssDNA structure. *Science* 297, 1837–1848

Another piece in the puzzle

Researchers have determined that the gene *BRCA2* has a more extensive role in ovarian cancer than was previously thought [8]. This reinforces previous research with *BRCA1*.

The research, by a team at University of Iowa Health Care (UI; http://www.uihealthcare.com/) could lead to the development of therapies to restore *BRCA2* and/or *BRCA1* function in women with ovarian, fallopian, or primary peritoneal cancer.

The UI team looked for *BRCA2* dysfunction in tumours of the same 92 women who had been the focus of the previous study into *BRCA1*, at the Holden Comprehensive Cancer Center. Eighty-two percent of tumours had dysfunctional *BRCA1* or *BRCA2*.

According to Richard Buller, UI Professor of Obstetrics and Gynaecology and the principal investigator of the study, this high incidence rate is a dramatic contrast to the *BRCA* gene dysfunction rates that were previously associated with ovarian cancers. 'As recently as 2–3 years ago, people thought that only 5–10% of ovarian cancer cases had disrupted *BRCA1* or *BRCA2* function, and most of those dysfunctional genes were thought to be inherited,' he said. Buller continued, 'However, together, the two studies show frequent BRCA2 and BRCA1 dysfunction in sporadic, or non-hereditary, ovarian cancers…With that level of dysfunction, therapies targeted toward the return of *BRCA1* and *BRCA2* function are very important for virtually every woman with ovarian or related cancer.'

In sporadic, as opposed to hereditary, ovarian cancer, the *BRCA* genes eventually malfunction, and thus are unable to carry out the repair process of tumour suppression. The study also revealed an association between defective *BRCA2* and primary peritoneal cancer, which occurs in the lining of the abdomen in women who do not have ovaries; this is the first time that these have been linked.

The study is also the first to use a genetic screening test known as protein truncation assay, which, although not a new methodology, hs not been applied to this field of research before, acording to Buller. However, even with these advances in the study, the true incidence of hereditary *BRCA1* and *BRCA2*-related cancer is not completely known.

8 Hilton, J.L. *et al.* (2002) Inactivation of BRCA1 and BRCA2 in ovarian cancer. *J. Natl. Cancer Inst.* 94, 1396–1406

Getting up to monkey tricks in cancer research

Scientists at the University of North Carolina at Chapel Hill (http://www.unc.edu), led by Blossom Damania, have discovered similar gene activity profiles between a herpes virus that infects rhesus macaque monkeys – rhesus monkey rhadinovirus (RRV) – and a human herpes virus linked to Kaposi's sarcoma – Kaposi's sarcoma-associated herpes virus (KSHV) [9]. Kaposi's sarcoma is endemic among Mediterranean and sub-Sahara African populations, but in the past 20 years this cancer has been mostly associated with people infected with AIDS.

Unlike the human virus, the simian virus can be grown easily in large quantities, facilitating the production of recombinant viruses for use in a rhesus macaque model. 'By developing this model, we can determine the genes that are important for virus survival, growth and replication, and genes that enable the virus to induce malignancies in its host' said Damania, adding that, 'Once you've established the genes that are required to do all of these things, you can start thinking about developing drug therapies against these genes to prevent virus spread and to prevent the virus from inducing cancer in its host'.

The study details the transcription profile seen during RRV lytic replication and identifies three new spliced products of RRV genes that are structural homologs of KSHV genes. The authors suggest that a conservation of gene function exists between the key transcription factors of KSHV and RRV. This new research paves the way for future studies using rhesus viruses as an *in vitro* and *in vivo* model for KSHV and could eventually form the basis of targeted drug therapies against specific KSHV genes.

9 DeWire, S.M. *et al.* (2002) Kinetics of expression of rhesus monkey rhadinovirus (RRV) and identification and characterization of a polycistronic transcript encoding the RRV Orf50/Rta, RRV R8, and R8.1 genes. *J. Virol.* 76, 9819–9831

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