both cellular and humoral type immune responses,' Jackson says.

Clinical trials

Human clinical trials will concentrate on female urogenital disease. The route of administration has not been decided, although the researchers have noted that the intranasal route produces a good immune response in the vaginal canal. 'In Phase I trials, as well as testing safety, we need to see if we get a basic immune response comparable to what we've seen in mice,' says Jackson. 'In later-stage trials, we would be looking for a good cellular [immune] response in the lower female reproductive tract. Without a strong cellular [immune] response, you won't get protection.' Antex is also in discussion with potential partners interested in trialing the vaccine against ocular trachoma.

References

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2 Maisonneuve, J-F. *et al.* (2001) Immunization with a high molecular weight protein (pmpG) from *Chlamydia trachomatis* confers heterotypic protection against infertility. *American Society for Microbiology 101st General Meeting*, 20–24 May 2001, Orlando, FL, USA (Abstract E-23, p. 197)

3 Jackson, W.J. *et al.* (2002) Mucosal immunization with recombinant pmpE from *Chlamydia trachomatis* serovar L2 confers protection against serovar F-induced infertility. *American Society for Microbiology 102nd General Meeting*, 19–23 May 2002, Salt Lake City, UT, USA (Abstract E-53, p. 182)

News in brief

Targets and mechanisms

'Fountain of youth' receptor identified

Scientists have uncovered evidence of a cellular G-protein-coupled receptor (GPCR) through which the hormone dehydroepiandrosterone (DHEA) acts [1]. DHEA – often referred to as the fountain of youth because of its protective properties – stimulates nitric oxide synthase, which helps to regulate blood pressure, inhibit blood clotting and prevents narrowing of arteries.

Until now, the hormone had no known cellular receptor or identifiable mechanism of action, and this has been a major obstacle to the understanding of how the hormone affects the body. However, researchers at the University of Iowa (UI) Health Center (http://www.uiowa.edu) decided to look in an unusual place for a steroid hormone receptor: 'All steroid hormones have receptors within cells. We found the DHEA receptor on the outside, not inside of cells. We also looked at cells which have not previously been examined. Other investigators have focused their research on immune blood cells or the liver, but we looked at the endothelium, or cells lining the blood vessels,' said the study's lead investigator Joseph Dillon, Assistant Professor of Internal Medicine at UI.

This research shows that there is a receptor for DHEA in the inner lining cells of blood vessels that responds to physiological levels of DHEA and this finding links the hormone to the production of nitric oxide, according to Dillon. This will provide a good starting point for studying the risks and benefits of DHEA as a potential therapy, and could help researchers design clinical trials of the hormone. 'The significance of our study is that it provides a scientific basis for further study of the DHEA action in humans,' said Dillon. The next step in his research will be to define the mechanism by which DHEA produces its effects.

1 Liu, D. and Dillon, J. (2002) Dehydroepiandrosterone activates endothelial cell nitric-oxide synthase by a specific plasma membrane receptor coupled to Gαi2,3. *J. Biol. Chem.* 277, 21379–21388

New cholesterol disorder predicted, then discovered

A team lead by John Kane, Professor of Medicine at the University of California San Francisco (http://www.ucsf.edu) has discovered a new cholesterol disorder that results from a single gene defect [2]. The disorder, which causes severely elevated blood cholesterol levels, is estimated to affect several hundred thousand people in the USA and Europe.

Unusually, the disease was hypothesized before it was discovered. The function of the gene was already known; it codes for the enzyme cholesterol 7-α hydroxylase (CYP7A1), which is essential for the breakdown of cholesterol in the liver. The researchers therefore predicted that mutation of CYP7A1 would block this mechanism and lead to an accumulation of cholesterol in the liver. This, in turn, should reduce the expression of the number of receptors in the liver that can bind to low-density lipoprotein (LDL) from the blood. Hence, it was hypothesized that a mutation in the gene for CYP7A1 should lead to an accumulation of LDL.

To prove this theory, the team searched a genetic database to see if those with a mutated CYP7A1 gene did indeed show signs of increased LDL levels. After screening 12,000 patients, 11 were found who carried the mutation. The family of one patient was studied in depth: of 37 people in this family, nine carried the same mutation. Three siblings who carried two mutant copies of the gene had cholesterol levels above 300 mg dl^{-1} – nearly double the family average, and levels that put them at high risk of coronary heart disease. Even family members with just one copy of the mutated gene had significantly elevated cholesterol levels – equivalent to a heart attack risk more than 50% higher than average.

'We went from a hypothesis to identifying the disorder in patients, rather than the more conventional route of

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seeing a disorder in patients and searching for the causes,' said Kane. 'By understanding the mechanism – how this gene affects cholesterol regulation – we can diagnose those at risk earlier and choose better treatments for them,' he added.

2 Pullinger, C.R. *et al.* (2002) Human cholesterol 7-α hydroxylase (CYP7A1) deficiency has a hypercholesterolemic phenotype. *J. Clin. Invest.* 110, 109–117

Lung cancer genes link

Researchers have identified two sets of genes that behave abnormally in malignant lung cells [3]. The team, at Ohio State University (http://www.osu.edu), found 14 genes that are overexpressed and 12 that are underexpressed in cancerous lung cells; approximately half of these genes have previously been linked to lung cancer. These findings could lead to new tests for the diagnosis of the disease.

Ming You, Professor of Molecular Virology, Immunology and Medical Genetics, who led the research, said: 'The overexpression and underexpression of certain genes in lung cells may directly contribute to the initiation or progression of lung cancer. Alternatively, the abnormal expression of these genes may be secondary effects of the tumour development process.'

You and colleagues analyzed cancerous lung tissue and compared it with normal lung tissue from the same patients. They found that the level of certain proteins in the diseased cells was higher than that in normal cells; this was traced to the overexpression of 14 genes, in the same way that underexpression of 12 genes was traced from the low level expression of 12 proteins.

The genes include ones that regulate intracellular communication, cell growth and apoptosis, and also include some known oncogenes – which promote tumour growth when overexpressed – and some tumour-supressor genes – which fail to stop tumour growth when underexpressed.

'In the past five years, lung cancer has killed more people in the US than breast cancer, prostate cancer and colon cancer combined,' said You, who continued, 'We estimate that about 20% of the genes identified in our study show signs of alteration at a very early stage of tumour development.'

The next step in the research is to find out which of these 26 genes start to behave abnormally at an early stage in lung cancer. You added, '… to find diagnostic markers for the disease, we need to identify genes that show aberrant behaviour before it is too late for treatment.'

3 McDoniels-Silvers, A.L. *et al.* (2002) Differential expression of critical cellular genes in human lung adenocarcinomas and squamous cell carcinomas in comparison to normal lung tissues. *Neoplasia* 4, 141–150

Ocular infection with HSV

Researchers have discovered that DNA from the herpes simplex virus (HSV) is essential for the formation of new blood vessels in the cornea, which can lead to the potentially blinding condition of stromal keratitis [4]. The cause of the formation of these new blood vessels as one of the major early events in stromal keratitis has long puzzled scientists because none of the proteins expressed by HSV have angiogenic activity.

A team from the Department of Microbiology at the University of Tennessee, Knoxville (http://www.utk.edu), used a murine micropocket assay and found that HSV DNA induces angiogenesis. This DNA contains an excess of motifs known as CpG motifs, which are potentially bioactive. Synthetic oligodeoxynucleotides (ODNs) that contain these motifs attract inflammatory cells and stimulate the release of vascular endothelial growth factor, which triggers new vessel formation. The team observed that the immune system stimulation could be involved in this angiogenesis process.

The study involved the use of beads coated with HSV DNA or synthetic ODNs that contained CpG motifs. Angiogenesis was induced in the corneas of mice, whereas control beads, either uncoated or coated with herring sperm DNA, did not have the same effect.

This research confirms that DNA containing CpG motifs induces angiogenesis and suggests that HSV DNA could contribute to blindness caused by stromal keratitis. The authors suggest that these neutralizing ODNs could be a potential therapy for this condition, and an ODN that is rich in CpG motifs could artificially stimulate angiogenesis in ways that could be potentially beneficial, such as the treatment of vascular disease, baldness or for use in tissue grafts.

4 Zheng, M. *et al.* (2002) DNA containing CpG motifs induces angiogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 99, 8944–8949

PCOS linked to heart disease and stroke

Women with polycystic ovarian syndrome (PCOS) are more likely to suffer from coronary artery disease and stroke because they have less elastic carotid arteries [5]. PCOS is a condition that affects at least 20% of women of reproductive age and causes the ovaries to enlarge and develop many small cysts. In addition, sufferers often have elevated cholesterol levels, high blood pressure, obesity and insulin resistance. This recent study has shown that PCOS predicts vascular dysfunction independently of any other risk factor.

The study consisted of three groups of 20 women: one group with symptomatic PCOS, one group with asymptomatic polycystic ovaries (PCO) and a control group of healthy women. The women were examined using a duplex ultrasound equipped with an echo-locked arterial wall-tracking system that measures the stiffness of the common and internal carotid arteries. The results showed that the PCOS and PCO groups had a significantly lower compliance (the artery's ability to dilate when the heart needs to pump more blood through the body) in their common carotid artery and significantly higher arterial stiffness in comparison with the control group.

'The implication is that a common condition thought to be primarily a gynaecological problem may predispose women to heart disease,' says Paul Hardiman, lead author of the study and senior lecturer in obstetrics and gynaecology at Royal Free and University College Medical School (http://www.ucl.ac.uk/medicalschool). 'Hopefully this study will inform cardiologists about an important risk factor for cardiovascular disease in women,' he said.

5 Lakhani, K. *et al.* (2002) Impaired carotid viscoelastic properties in women with polycystic ovaries. *Circulation* 10.1161/01.CIR.0000020681.19400.8A

Gene expression

Gene expression more diverse than previously thought

The analysis of mRNA transcript 3′ ends has shown that they are spread out over much greater distances (>1000 nucleotides) than previously thought [10]. The data, which has implications for the design of cDNA microarray probes, indicates that as many as half of all human genes encode multiple mRNA transcripts. The mRNA samples used for DNA chips are usually from a region near the poly(A) tail, which acts as a cut-off point to distinguish one mRNA transcript from another. However, by using only this portion, it is thought that the array will miss other possible transcripts and will provide only part of the full picture of gene expression.

This latest study, performed in collaboration with the National Cancer Institute (http://www.nci.nih.gov/), examined in detail a portion of human chromosome 21 and found that long-range variations in the mRNA ends affect up to 20,000 of the possible 35,000 genes that are thought to make up the human genome.

'These findings have profound implications for understanding how genes function,' said Victor Jongeneel, director of the Office of Information Technology at the Ludwig Institute (http://www.licr.org/03_bra/lausanne.htm). 'We need better DNA chips to match the increasing complexity of our genes,' he said.

10 Iseli, C. *et al*. (2002) Long-range heterogeneity at the 3′ ends of human mRNAs. *Genome Res.* 12, 1068–1074

First genetic profile of the aging eye

The first-ever gene profile of the aging human retina has shown that significant changes, particularly in stress response and energy metabolism, occur in gene expression during ageing [11]. This research could provide greater insight into disorders such as age-related macular degeneration (AMD).

'We still don't know what causes AMD, we do know that the strongest factors are age and family history,' said Anand Swaroop, lead author of the paper and director of the research team at University of Michigan Kellogg Eye Center (http://www.kellogg.umich.edu/). 'We are likely to find that AMD is caused by a complex interaction between genetic and environmental risk factors,' he said.

Approximately 50% of the genes expressed in the retina were identified by microarray analysis using commercially available DNA slides. The expression profiles of young and old individuals showed that ageing altered the expression of 24 genes.

11 Yoshida, S. *et al.* (2002) Microarray analysis of gene expression in the aging human retina. *Invest. Ophthalmol. Vis. Sci.* 43 (in press)

Genes do what the neighbourhood does

Approximately 20% of *Drosophila* genes lie in groups of 10–30 genes that are expressed similarly across a wide range of experimental conditions, even if they are not functionally related [12]. Paul Spellman and Gerald Rubin of the Howard Hughes Medical Institute (http://www.hhmi.org) and the University of California, Berkeley (http://www.berkeley.edu) suggest that there is a previously unsuspected mechanism of regulating gene expression that might work on blocks of genes depending on their position along a chromosome.

Rubin and Spellman analyzed gene expression data from 88 independent microarray experiments using *Drosophila melanogaster* DNA but instead of searching for groups of genes with similar expression profiles, they arranged the expression data in the order that the genes would appear on the chromosome. They found >200 groups of 10–30 adjacent and similarly expressed genes that accounted for 20% of the fruitfly genome.

Spellman and Rubin now speculate that the expression of these gene groups might be a result of the structure or activity of chromatin, and that allowing an important gene to be expressed could lead to the, almost accidental, expression of neighbouring genes.

12 Spellman, P.T. and Rubin, G.M. (2002) Evidence for large domains of similarly expressed genes in the *Drosophila* genome. *J. Biol.* 1 (available online at: http://jbiol.com/content/1/1/5)

In silico Hox modelling

Researchers have used computer modelling to simulate the genetic interactions involved in the development of the vertebrate hindbrain [6]. The team, at the Dept of Applied and Computational Mathematics, California Institute of Technology (http://www.caltech.edu), tracked the behaviour of each component of the Hox regulatory mechanisms. They produced computerized movies of the system and were able to reproduce key features of the wildtype gene expression patterns.

Development of the early hindbrain involves subdivision into segmental compartments, or rhombomeres, which each express a distinct set of transcription factors, receptors and ligands that define the function of each rhombomere in the brain. Kastner and colleagues focussed on rhombomeres 4 and 5, which are known to express the transcription factors *Hoxb1* and *Krox20*, respectively.

A stochastic simulation algorithm (SSA) was used to model the Hox network *in silico* using starting values representing the conditions in early hindbrain development. These results closely match those obtained in live embryos. It also predicted the misfiring of certain cells that deviate from their normal fate, which is possible to rectify if it occurs early enough in development. This model has been highly successful in predicting events and in highlighting novel occurrences that require further investigation *in vivo*.

6 Kastner, J. *et al.* (2002) Modeling a *hox* gene network *in silico* using a stochastic simulation algorithm. *Dev. Biol.* 246, 122–131

Combination therapy induces cellular immunity to HIV

An HIV vaccine coupled with immunostimulatory DNA has been able to shift the immune response from antibody- to cell-mediated immunity in mice infected with the parasite *Schistosoma*, according to the results of a recent preclinical study [7]. The results further support the combination vaccine approach of using REMUNE® in addition to an adjuvant.

In the study, mice infected with *Schistosoma* were immunized with inactivated glycoprotein-120-depleted

HIV-1 antigen (REMUNE®) and oligodeoxynucleotides (ODNs) containing cytosine-phosphate-guanosine (CpG) immunostimulatory sequences. The mice showed significantly lower levels of Th2-related cytokines and increased levels of Th1-related cytokines such as interferon-γ. Many scientists believe that cell-mediated, rather than antibody-mediated, immune responses are crucial to the development of both preventative and therapeutic vaccines for HIV.

'In light of the devastation AIDS is causing across the developing world, there is a crucial need for additional testing and study, including clinical human trials, in order to determine whether this approach can be useful in countries facing the HIV epidemic,' said Dennis Carlo, president and CEO for the Immune Response Corporation (http://www.imnr.com), which conducted the study in collaboration with the senior author of the paper, Ruth Ben-Ari from the Institute of Clinical Immunology and AIDS Center in Israel.

Ben-Ari commented, 'The relevance and implication of these findings to the situation in the developing world are obvious and very encouraging, especially in view of the difficulty in eradication of parasitic infections. We suggest that the addition of CpG immunostimulatory sequences to HIV antigens in incomplete Freund's adjuvant may optimize HIV-specific immune responses, and therefore should be included in future trials of REMUNE® and other candidate HIV-1 vaccines.'

7 Ayash-Rashkobsky, M *et al.* (2002) Generation of Th1 immune responses to inactivated, gp120-depleted HIV-1 in mice with a dominant Th2 biased immune profile via immunostimulatory oligonucleotides: relevance to AIDS vaccines in developing countries. *Vaccine* 20, 2684–2692

Miscellaneous

Explained: why fish is good for you

Scientists from the University of Texas Medical Branch (UTMB) at Galveston have uncovered the mechanism by which omega-3 fatty acids offer protection against colon cancer [8]. The discovery also has implications for the prevention and treatment of other types of cancer.

It has been accepted for many years that omega-3 fatty acids, commonly found in fish oil, can help prevent colon cancer, but little was known about why they are effective. A team from UTMB, led by Alan P. Fields, has found that omega-3 fatty acids block the action of protein kinase C β II (PKCβII), an enzyme associated with increased vulnerability to colon cancer. They created genetically modified mice whose colon cells over-produced PKCβII, which made the mice far more likely to develop colon cancer when exposed to a known carcinogen. The mice also showed evidence of the rapid cell growth that precedes cancer, even when not exposed to carcinogen. However, transgenic mice fed a diet rich in omega-3 fatty acids had a lower incidence of colon cancer and showed no signs of runaway growth of colon cells.

'We found that the hyperproliferation, which we normally see in these transgenic animals, is completely blocked by omega-3 fatty acids,' said Fields. 'Furthermore, now when we expose these animals to a carcinogen, they have the same cancer risk as non-transgenic mice.'

Examining the mechanism further, they found that cells with a high level of PKCβII produce much less transforming growth factor-β receptor type II (TGFβRII), a molecule that is crucial for regulating cell division. This finding widens the potential of omega-3 fatty acids as natural anti-cancer agents, because low levels of TGFβRII have been observed in many other cancer cell types, including breast cancer. These results suggest that PKCβII might play a significant role in more than just colon cancer and that inhibiting its action through diet or drugs could help prevent or treat many diseases.

8 Murray, N.R. *et al.* (2002) Protein kinase C βII and TGF βRII in ω-3 fatty acid-mediated inhibition of colon carcinogenesis. *J. Cell Biol.* 157, 915–920

From fat to nerves in one easy move

Scientists have transformed adult stem cells, taken from fat cells, into what appear to be nerve cells [9]. The researchers, from Duke University Medical Center (http://www.mc.duke.edu) and Artecel (http://www.artecel.com), are optimistic that these new cells could have the potential to treat CNS disorders.

'These experiments are proof-of-principle that it is possible to change one lineage of adult stem cells into another using fat,' said Henry Rice from Duke, paediatric surgeon and lead author of the paper. 'If future studies in animal models are successful, we'll have gone a long way toward demonstrating the power of these cells to treat human diseases.'

The researchers treated adipose cells from humans (taken from liposuction procedures) and mice with chemicals and growth factors and grew them in the laboratory. Rice said 'Within hours the treated cells in both models began to look like neuronal cells and began to produce measurable amounts of proteins normally expressed by nerve cells.'

Jeffrey Gimble, chief scientific officer at Artecel, said: 'This is a promising first step in the use of an abundant source of adult stem cells in the setting of CNS repair. While it is known that you can create neuronal cells from adult stem cells taken from bone marrow, we feel that our approach with fat offers a limitless supply of readily obtainable adult stem cells.'

However, there are hurdles to be overcome before these cells can be used in the clinical setting. The lifespan of these cells needs to be increased from several days, and it is not known whether these transformed cells function in the same way as nerve cells, even though they resemble native nerve cells.

The researchers believe that the first animal models will focus on acute injuries, such as stroke and spinal cord injuries.

9 Safford, K.M. *et al.* (2002) Neurogenic differentiation of murine and human adipose-derived stromal cells. *Biochem. Biophys. Res. Commun.* 294, 371–379

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