IMMUNE DISORDERS

Oral antibody against autoimmunity

Monoclonal antibodies (mAbs) have become a powerful tool in the treatment of a range of diseases. However, as their therapeutic potential is generally destroyed in the gut, mAbs are usually administered intravenously. Now, researchers in the laboratory of Howard Weiner have taken advantage of the unique properties of the mucosal immune system, and show that oral delivery of mAbs directed against CD3, a component of the T-cell antigen–receptor complex, might in fact constitute a novel and powerful therapeutic approach that could be widely applicable for the treatment of human autoimmune conditions.

Tweaking the immune system to revert autoimmune disease and to prevent graft rejection after transplantation remains one of the major challenges in immunology. Over the past decade, considerable attention has been focussed on naturally occurring T-regulatory cells (Tregs) for the modulation of undesired immune responses. As these cells have an important role in ensuring oral tolerance and are preferentially induced at mucosal surfaces, Ochi *et al.* were first to investigate whether Tregs could be activated by oral administration of immunomodulatory antibodies.

The antibodies used in this study were based on intravenous anti-CD3 mAbs approved for transplant rejection more than 20 years ago. Recently, it became apparent that these antibodies can do more than simply depress all antibody responses — they can also induce tolerance to specific antigens by activating Tregs.

As intravenous administration of anti-CD3 mAbs can have severe side effects due to the induction of cytokine release, the authors examined whether this problem could be circumvented by orally delivering anti-CD3 mAbs.

In two different types of experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis, the authors showed that a low dose of antibody could suppress and/or delay the onset of disease. Interestingly, unlike intravenous anti-CD3, oral CD3 could also prevent disease, and the fact that the same antibody was effective in both models points to a potential universal mechanism that is not antigen specific and which could be useful in a wide range of autoimmune conditions.

The authors found a fundamental immunological difference between intravenous and oral CD3-specific mAb: oral mAb did not modulate the T-cell receptor–CD3 complex on T cells, deplete T cells or induce cell division, even at high doses. Instead, the results suggest that the antibody exerts a weak signal to T cells in the gut that enhances the regulatory function of a unique subset of Tregs. These are $CD4^{+}CD25^{-}$ T cells that express surface latency-associated peptide (LAP+), and their function depends on the anti-inflammatory cytokine transforming growth factor-β. How exactly they are related to other Tregs remains to be determined, and the authors speculate that they take part in the natural recovery mechanism in EAE.

> “Oral delivery of mAbs directed against CD3 might constitute a novel and powerful therapeutic approach.”

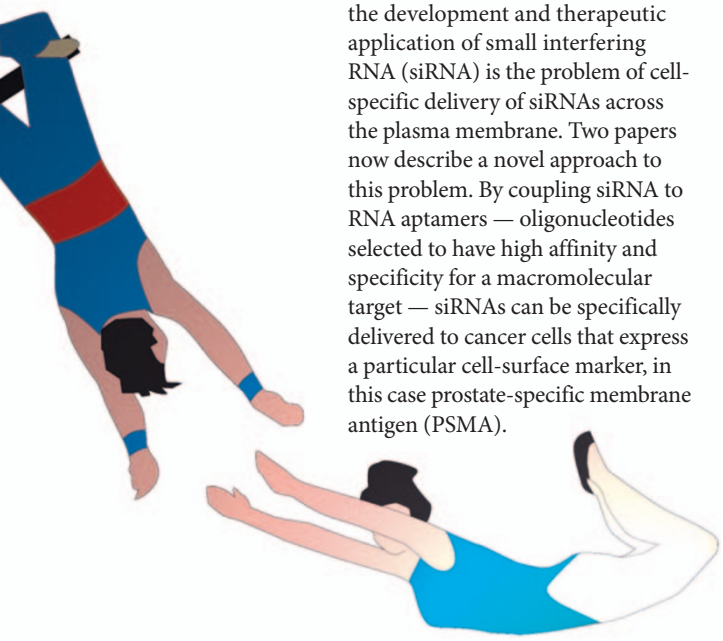
The authors report that ongoing studies also demonstrate the effectiveness of oral anti-CD3 in models of diabetes, arthritis and transplantation, and are also effective when administered nasally. The simplicity of the method and presumed good tolerance, even for chronic use, make this a particularly exciting approach for the management of inflammatory T-cell-mediated diseases.

Alexandra Flemming

ORIGINAL RESEARCH PAPER Ochi, H. *et al.* Oral CD3-specific antibody suppresses auto-immune encephalomyelitis by inducing CD4+CD25-LAP+ T cells. *Nature Med.* **12**, 627–635 (2006)

RNA INTERFERENCE

Targeted delivery by an RNA double act



One of the major hurdles facing the development and therapeutic application of small interfering RNA (siRNA) is the problem of cell-specific delivery of siRNAs across the plasma membrane. Two papers now describe a novel approach to this problem. By coupling siRNA to RNA aptamers — oligonucleotides selected to have high affinity and specificity for a macromolecular target — siRNAs can be specifically delivered to cancer cells that express a particular cell-surface marker, in this case prostate-specific membrane antigen (PSMA).

In their study published in *Nucleic Acids Research,* Chu and colleagues generated aptamer–siRNA conjugates in which the RNAs were first biotinylated and then non-covalently joined via the biotin-binding protein streptavidin. siRNAs were designed to target either lamin A/C or glyceraldehyde 3-phosphate dehydrogenase (GAPDH), whereas the aptamer targeted PMSA. Aptamer–siRNA conjugates were able to deliver functional siRNA molecules to cells expressing PSMA and rapidly inhibit expression of lamin A/C and GADPH. The presence of the streptavidin molecule had no effect on the function of the attached siRNAs. Importantly, because siRNAs can potentially activate nonspecific inflammatory responses that can lead to cellular toxicity, exposure to the aptamer–siRNA conjugates did not result in increase of interferon-β mRNA.

In the second study, reported in *Nature Biotechnology*, McNamara and colleagues used a sequence-extension method to generate aptamer–siRNA chimeras, in which the aptamer portion targeted PMSA and the siRNA portion targeted one of two survival genes overexpressed in most human tumours: polo-like kinase 1 (*PLK1*) and B-cell lymphoma 2 (*BCL2*). Using flow-cytometry and RT-PCR, it was shown that both chimeras specifically bound PSMA on prostate cancer cells and silenced either *PLK1* or *BCL2* gene expression. In addition, aptamer–siRNA chimeras reduced cellular proliferation and induced apoptosis in PSMA-expressing cells.

McNamara *et al.* then sought to determine whether the mechanism by which aptamer–siRNA chimeras silence gene expression was dependent on the activity of the endonuclease Dicer. Silencing of *PLK1* expression was inhibited by co-transfection of Dicer siRNA, suggesting that aptamer–siRNA chimera-mediated gene silencing is indeed dependent on Dicer and occurs via the RNA interference pathway. Again, treatment of PSMA-expressing cells with aptamer–siRNA chimeras did not induce production of interferon-β, suggesting that delivery of aptamer–siRNA chimeras did not trigger a type I interferon response.

Finally, the authors assessed the efficiency and specificity of the Plk1 aptamer–siRNA chimera for its capacity to limit tumour growth in a mouse

NEUROLOGICAL DISORDERS

Slicing into stroke therapeutics

> This study suggests that Na^+/K^+-ATPase might be a promising target for neuroprotection.

Neuroprotection remains a key goal for stroke therapy, yet the lack of clinically effective neuroprotective drugs suggests that innovative strategies for the selection of target pathways and lead compounds are needed. A new approach, published by Lo and colleagues in *PNAS*, could help to achieve this and has already revealed an unexpected new target for ischaemic stroke therapy.

Much of the damage caused by stroke results from interruption of the blood supply, and consequently glucose and oxygen delivery, in affected brain areas. In this study, potentially neuroprotective compounds were tested in rat brain slices subjected to oxygen–glucose deprivation (OGD). To monitor neuronal damage, the slices were transfected with the gene for yellow fluorescent protein, which labelled a subset of cortical neurons that acted as 'sentinels' in providing a measure of neuronal survival. This slice-based approach provides a cellular environment similar to that found *in vivo*, yet allows relatively large numbers of compounds to be tested in the context of drug discovery programmes.

A collection of 1,200 compounds, selected for their drug-like properties, was examined using this method. This approach allowed a broad range of target pathways to be investigated, including many that have not been associated with neuroprotection, increasing the chances of identifying a new target. Importantly, the drugs were tested for neuroprotective effects when added at delayed time points after OGD onset, reflecting the probable time-frame for their use in stroke patients. The most effective compound was the cardiac glycoside neriifolin, which provided neuroprotection when tested more than 6 hours after OGD. The neuroprotective action of neriifolin was subsequently confirmed in a second brain-slice model and in two *in vivo* models of ischaemia. Significant but less potent neuroprotection by other cardiac glycosides was also demonstrated.

Cardiac glycosides, which are used clinically to treat cardiac dysfunctions, block Na^+/K^+-ATPase, an enzymatic pump that maintains ion concentration gradients across cell membranes. The demonstration of neuroprotection by cardiac glycosides was unexpected because many of these compounds can be toxic in other situations. However, the authors propose that the drugs might reduce cellular metabolic demand, or counteract a drop in calcium levels caused by ischaemia.

This study suggests that Na^+/K^+-ATPase might be a promising target for neuroprotection, although further work will be needed to determine whether the delayed efficacy of neriifolin can be replicated *in vivo*. Furthermore, the high biological content of this new

xenograft model of prostate cancer. PSMA-expressing tumours injected with Plk1 aptamer–siRNA showed a greater than twofold reduction in tumour volume, compared with nearly a fourfold volume increase in controls. Notably, no morbidity or mortality was observed following 20 days treatment with the aptamer–siRNA, suggesting it was not toxic to the animals.

In summary, aptamer–siRNAs can specifically delivery siRNAs, and might provide benefits with regard to specificity and lack of side effects compared with other siRNA-targeting strategies that have been developed so far, such as the use of lipids, peptides and antibodies. Issues such as pharmacokinetics and biodistribution of aptamer–siRNA conjugates and chimeras need to be addressed before they can be used therapeutically, but in principle this approach could have potential for treating prostate cancer and other diseases.

Charlotte Harrison

ORIGINAL RESEARCH PAPERS Chu, T. C. *et al.* Aptamer-mediated siRNA delivery. *Nucleic Acids Res.* **34**, e73 (2006) | McNamara, J. O. *et al.* Cell type-specific delivery of siRNAs with aptamer–siRNA chimeras. *Nature Biotechnol.* 26 Jun 2006 (doi: doi:10.1038/nbt1223)

screening strategy might contribute to improvements in the prediction of the clinical efficacy of compounds in preclinical phases of drug testing and could lead to the identification of further promising targets.

Katherine Whalley

ORIGINAL RESEARCH PAPER Wang, J. K. T. *et al.* Cardiac glycosides provide neuroprotection against ischemic stroke: Discovery by a brain slice-based compound screening platform. *Proc. Natl Acad. Sci. USA* **103**, 10461–10466 (2006)

CHEMICAL BIOLOGY

Modulating molecular gateways

Imagine if you no longer had to worry about whether your new drug was able to cross biological barriers, because you were able to transiently modify such barriers to allow compounds to pass through. New research recently published in *Nature Chemical Biology* could make this fantasy a reality.

Scientists working within the National Institutes of Health Molecular Libraries Screening Centers Network Initiative have identified an antagonist of a receptor that modulates barrier function and can be used together with a known agonist to finely regulate barrier activity. Being able to manipulate barriers in the brain, immune system, lung and skin could have wide-ranging applications for treating oedema, organ rejection and autoimmune diseases such as multiple sclerosis, and could also be used to deliver drugs to impermeable tissues.

Hugh Rosen and Chi-Huey Wong's groups at the Scripps Research Institute set out to use a pair of chemical 'probes' to study the sphingosine 1-phosphate (S1P)–S1P-receptor-1 ($S1P_1$) pathway, which is involved in the modulation and maintenance of biological barrier activity. Of the five S1P receptors, $S1P_1$ is known to be expressed predominantly on endothelial, cardiac and blood cells. They synthesized an antagonist of $S1P_1$ signalling that had improved potency and *in vivo* activity over previously described antagonists by replacing the phosphate ester with phosphonate to generate *R*- and *S*-enantiomers of 3-amino-4-(3-hexylphenylamino)-4-oxobutylphosphonic acid. The *R*-enantiomer was shown to be a full competitive antagonist of $S1P_1$, whereas the *S*-enantiomer was not, and so the *R*-enantiomer and the $S1P_1$-selective agonist SEW2871 were used as a pair of probes with which to study vascular and lymphoid responses regulated by SIP_1 activity.

The SIP_1 antagonist reversed the endothelial barrier protection conferred by endogenous and exogenous $S1P_1$ agonists against vascular endothelial growth factor (VEGF)-induced leakage of mouse vascular capillaries. However, when administered to mouse lung vasculature, the antagonist significantly enhanced pulmonary leakage in a dose-dependent manner in the absence of exogenous ligand. This suggests that there is variation in the level of basal receptor activity at barriers in different tissues, and that this could result in a tissue-specific effect of the exogenous antagonist.

The authors then studied the effect of the antagonist on lymphocyte egress — the process by which lymphocytes move from lymphoid tissue into the bloodstream. This was done by plotting the previously measured degree of vascular protection from VEGF-induced leakage against the induction of lymphopaenia, which revealed a positive correlation. SEW2871 induced lymphocyte sequestration from peripheral blood, whereas this was reversed in the presence of the $S1P_1$ antagonist. Moreover, the $S1P_1$ antagonist also inhibited the loss of expression of the lymphocyte activation marker CD69 on lymphocytes caused by $S1P_1$ agonists. Two-photon imaging of the effects of the antagonist in lymph nodes revealed that the actions of the exogenous agonist and antagonist pair were confined to the medullary region and had no effect on lymphocytes in other regions, showing that S1P-induced lymphocyte arrest is limited to medullary egress *in vivo*.

These findings provide valuable insights into the complexity of S1P–$S1P_1$ signalling but also cast a shadow on the usefulness of gene-deletion mutants studied alone for fully understanding the effects of modulating a single receptor. A comparison of these results with previous gene-deletion studies highlighted several areas of uncertainty regarding $S1P_1$ function. This suggests that a combined approach using knockouts and chemical probes could be more illuminating when studying receptor function *in vivo*.

Joanna Owens

ORIGINAL RESEARCH PAPER Sanna, M. G. *et al.* Enhancement of capillary leakage and restoration of lymphocyte egress by a chiral $S1P_1$ antagonist *in vivo*. *Nature Chem. Biol.* 9 July 2006 (doi: 10.1038/nchembio804)

IN BRIEF

CANCER

Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells.

Saito, Y. *et al. Cancer Cell* **9**, 435–443 (2006)

Following the recent discovery that micro RNAs (miRNAs) have a role in cancer, Saito *et al.* show that several miRNAs are subject to epigenetic silencing in tumours and that their expression can be induced by chromatin-modifying drugs. The oncogene *BCL6* was found to be a direct target of miRNA-127, and was downregulated by activation of miRNA-127 induced by a DNA-demethylating agent and a histone deacetylase inhibitor. Regulation of miRNA expression by epigenetic treatment might be a novel strategy in anticancer drug development.

HUNTINGTON'S DISEASE

Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant Huntingtin.

Graham, R. K. *et al. Cell* **125**, 1179–1191 (2006)

Huntington's disease is a devastating condition for which there is currently no treatment or cure. Hayden and co-workers have discovered the first molecular target for drug development in Huntington's disease. They show that the major pathology is caused by a specific toxic fragment of the molecule huntingtin, which is generated after specific cleavage of the huntingtin protein by caspase-6. Mice lacking this cleavage site are protected from the disease.

IMAGING

Photodynamic therapy agent with a built-in apoptosis sensor for evaluating its own therapeutic outcome *in situ*.

Stefflova, K. *et al. J. Med. Chem.* **49**, 3850–3856 (2006)

Functional photoacoustic microscopy for high-resolution and noninvasive *in vivo* imaging.

Zhang, H. F. *et al. Nature Biotechnol.* **24**, 848–851 (2006)

Two papers report advances in imaging technology that can be used to evaluate the effectiveness of drug treatment. The first is an elegant study in which the authors designed a probe that acts as a drug for photodynamic therapy of cancer but is also able to simultaneously detect apoptosis, and is capable thereby of measuring its own success at killing cancer cells. The probe consists of a photosensitizer that acts as the therapeutic modality and as a fluorescent label, and a fluorescence quencher that is bound to opposing sides of a caspase-3-cleavable peptide linker. Once this probe is activated by light in a cell, and if the damage is sufficient to initiate apoptosis (but insufficient to cause cell death), the subsequent activation of caspase-3 will cleave the quencher from the fluorescent label and the resulting fluorescence can be measured. The second paper describes an advance in the resolution of *in vivo* optical imaging for visualizing angiogenesis, melanoma and haemoglobin parameters in animals and humans. Existing methods to determine physiological status do not measure optical absorption of tissue directly and are unable to measure deeper than 1 mm below the tissue surface because of optical scattering. This paper describes a new technique called functional photoacoustic microscopy (fPAM) that detects absorbed photons ultrasonically through a so-called photoacoustic effect and enables spatial resolution beyond the 1-mm depth limit.

ANTI-OBESITY DRUGS

Fighting fat

Rising levels of obesity and associated health problems in many countries worldwide have led to a burgeoning interest in drugs that could promote weight loss. Writing in the *British Journal of Pharmacology*, Pauwels and colleagues now provide evidence that a novel 5-hydroxytryptamine (5-HT)$_6$ receptor partial agonist — known as E-6837 — could represent a novel approach for the management of obesity.

Several previous investigations have indicated that the 5-HT$_6$ receptor, which is distributed almost exclusively within the central nervous system, could have potential as an anti-obesity target. However, there have until now been no studies published that have assessed the chronic effects of 5-HT$_6$ receptor ligands on body weight in an animal model considered to closely mimic human obesity. So Pauwels *et al.* chose to evaluate the effects of chronic treatment with E-6837 in mature rats that had been given access to a highly palatable high-calorie diet, which develop obesity with insulin resistance and other metabolic disturbances.

During the 4-week treatment period, diet-induced obese (DIO) rats treated with E-6837 showed sustained weight loss and decreased food intake. Importantly, the weight loss was mediated exclusively by a ~30% decrease in fat mass, with no losses in protein or water, and the decrease in adiposity resulted in a significant improvement in glycaemic control. The rats were also studied for a 6-week period after treatment, and their weights were still significantly lower than those of vehicle-treated control rats at the end of this period, indicating that weight is not rapidly regained following treatment cessation.

The authors also assessed the effects of sibutramine, one of the few anti-obesity drugs approved so far, in DIO rats as a positive control. The onset of the weight-loss effect of E-6837 was slower than that of sibutramine, but the maximal effect was greater. Furthermore, although some weight regain occurred after treatment cessation with both E-6837 and sibutramine, the body weights after E-6837 remained lower.

At present, the mechanisms by which modulation of the 5-HT$_6$ receptor by E-6837 affects food intake are not clear. Nevertheless, the results of Pauwels *et al.* suggest that such ligands have promise as a CNS-mediated strategy for combating obesity, with the potential to result in greater sustained weight loss than sibutramine.

Peter Kirkpatrick

ORIGINAL RESEARCH PAPER Fisas, A. *et al.* Chronic 5-HT(6) receptor modulation by E-6837 induces hypophagia and sustained weight loss in diet-induced obese rats. *Br. J. Pharmacol.* 19 June 2006 (doi:10.1038/sj.bjp.0706807)
FURTHER READING Holenz, J. *et al.* Medicinal chemistry driven approaches toward novel and selective serotonin 5-HT$_6$ receptor ligands. *J. Med. Chem.* **48**, 1781–1795 (2005)