until we try it, we don't know,' whether a particular glycoprotein will be effective. Kobinger points out that effective viral vectors are not likely to be revealed through identification of the receptors they use. Most viruses, in fact, use a multitude of receptors to gain entry to different cell types, many of which are not identified, so it should come as no surprise that McCray saw entry independent of FRα.

McCray will attempt to identify the epithelial receptor, however, in hopes of optimizing gene delivery specifically to the lung. He suggests the potential for various viral packages specialized – by the addition of distinct glycoproteins – to deliver genes to hepatocytes, neurons and other cell types [5,6].

Perhaps a more important goal in the treatment of cystic fibrosis is to determine which epithelial cells the EBOΔO glycoprotein binds and enters. Some epithelial cells, McCray explains, have 'progenitor capacity'. This population will continue to divide repeatedly, passing on its genome. Because the retroviral vector has the ability to integrate its genome with that of the host, the therapeutic gene – in this case CFTR (cystic fibrosis transmembrane conductance regulator) – would be passed along to the progeny. 'The worst case scenario,' says McCray, would be one in which the virus tranduces only the terminally differentiated cells, which have a life span of only about three months. In this case, you could repeatedly deliver the gene therapy, but the immune system would inevitably come to recognize the viral intruder, rendering the delivery system inoperable.'

Ebola: good from bad

Both McCray and Kobinger know that the viruses they have manipulated evoke a sort of knee-jerk reaction. 'It sounds kind of crazy,' McCray realizes, 'to say that you are using Ebola for gene therapy.' But he urges us to view it another way. 'We take a part from a bad virus and use it for a good purpose.' Much about the filoviruses – including the details of pathogenesis and their origin in nature – remains a mystery. But as strange as it sounds, the Ebola glycoprotein could turn out to be a key element for successful gene therapy.

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New mode of intervention in sepsis treatment

Vida Foubister, freelance writer

A monoclonal antibody directed against a component of the inflammatory response pathway has been found to promote survival among monkeys with sepsis-induced hemodynamic failure. Innogenetics (http://www.innogenetics.com), a biotechnology company based in Belgium, released the results of a preclinical study on this molecule – INNO202 – in May 2003 and has begun looking for a partner to bring it into clinical trials.

INNO202 proved effective in a physiological model of sepsis and this has raised the expectations of scientists involved in the research for its use as a therapeutic. 'This is the first preclinical study in a primate model that has shown effectiveness when the treatment was initiated after the development of bacteremic shock,' said Lyle L. Moldawer, Professor of Surgery at the University of Florida College of Medicine (http://www.med.ufl.edu). 'None of the other drugs that have gone through clinical trials have been tested in this manner.'

A significant medical problem

Sepsis is the systemic inflammatory response to severe microbial infection that is common in patients following trauma and burn injuries. The body's immune system becomes overwhelmed by the infection and, in the case of severe sepsis, this can lead to failure of one or

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more vital organs such as the lungs, kidney, heart and/or liver. 'The mortality rates for severe sepsis are extremely high – somewhere between 20 and 40% in the industrial world,' Moldawer said. The most fatal condition, septic shock, is associated with unresponsive low blood pressure and has a mortality rate of about 50%.

More than two million people worldwide suffer from sepsis or severe sepsis each year. In addition, the incidence of this condition is rising due to the aging of the population and increasing numbers of immunocompromized and critically ill patients [1,2]. Currently, sepsis is a leading cause of death in noncoronary intensive care units and the eleventh most common cause of death in the USA.

Despite these figures, there is only one drug that has been approved in Europe and the USA for the treatment of severe sepsis. Xigris® (http://www.xigris.com), an activated protein C marketed by Eli Lilly and Company (http://www.lilly.com), was approved in the USA in 2001 and in Europe the following year. However, treatment with Xigris results in relatively modest improvements in patient outcomes – estimated in one study to be a 6.1% reduction in absolute mortality [3].

Not surprisingly, clinicians and researchers who work in the field believe there is a compelling need for the development of new therapeutics. 'There is much room to salvage patients who have not been salvaged and additional patients who can not take Xigris because they have some underlying bleeding problems or a propensity to bleed from major surgery, trauma or other reasons people get admitted to ICU,' said Steven Opal, Professor of Medicine in the Infectious Disease Division at Brown Medical School (http://bms.brown.edu/med).

Relevant primate model

Scientists at Innogenetics previously completed a prophylactic trial of INNO202 in Rhesus monkeys that had been given an intravenous injection of a nonlethal dose of *Escherichia coli*. The result? 'INNO202 appeared to protect the monkeys against the development of clinical septic shock,' said Katrien Lorré, Therapeutic Program Manager at Innogenetics.

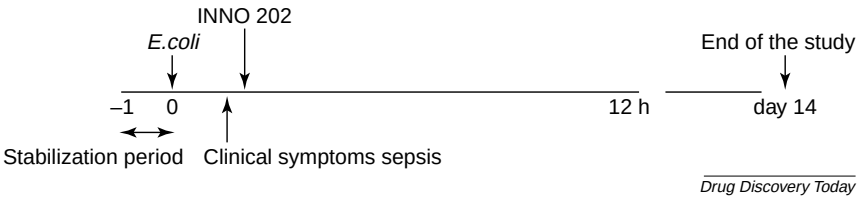

Figure 1. A primate model of sepsis. Cynomolgus monkeys were anaesthesized, allowed to equilibrate for at least 1 hour and then intravenously infused with a lethal dose of *Escherichia coli*. At the time septic-induced hemodynamic failure was first observed, INNO202 or a placebo was administered. The criteria for hemodynamic failure was met when the animals exhibited two or more of the following symptoms: a decrease of mean pressure of >30%; increase of heart rate of >30%; and/or urine flow less than 1 ml kg^{-1} h^{-1}. The monkeys were evaluated at fixed time points from 1 hour before the injection to 12 hours afterwards, and then daily for 14 days. Figure provided by Katrien Lorré, Therapeutic Program Manager at Innogenetics (http://www.innogenetics.com).

In their most recent study, the researchers went one step further. They used a humanized form of the monoclonal antibody and treated animals after they developed the clinical symptoms of septic shock. A total of 14 Cynomolgus monkeys were given a lethal dose of *E. coli* to induce hemodynamic failure, defined as two or more of the following symptoms: >30% decrease in mean arterial blood pressure; >30% increase in heart rate; and/or ≤1 ml kg^{-1} body weight h^{-1} urine output (Fig. 1). They animals were then treated with INNO202 or a placebo in a blinded experiment. All six control animals developed symptoms of severe septic shock and died early; by contrast, six of the eight animals given the candidate drug survived for seven days or more.

This approach more closely approximates the clinical onset of the disease than other sepsis models in which treatment is administered prior to or at a fixed time after bacterial challenge. However, 'it is still a contrived, artificial environment,' said Opal. 'No animal model can duplicate the complexity of what we see in clinical medicine and the final analysis is to actually do a clinical study.'

Future potential

Innogenetics is currently seeking a partner to take INNO202 into a clinical trial. At this point, the company is not disclosing the target of its anti-inflammatory molecule. 'Monoclonal antibodies, in principle, are protein-based therapeutics that are directed against a specific mediator,' Moldawer explained. 'Generally they are either directed against a protein ligand or its receptor.' Although monoclonal antibodies to tumor necrosis factor alpha – one component of the inflammatory response pathway – only show modest, if any, benefit in the treatment of sepsis [4], they remain optimistic that INNO202 will prove effective.

Opal also believes that an anti-cytokine approach is conceivable. However, he emphasized the need to combine general sepsis studies with technologies such as genomics and proteomics to more intelligently identify which patients to treat with a given drug. 'We know more than we used to about how to target a drug to patients who would likely benefit from it,' he explained.

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Solving insoluble drug delivery

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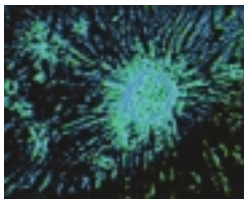

An innovative way of getting macromolecular drugs past the body's natural defences offers a new and promising way of delivering poorly soluble drugs directly to the sites they are required, say pharmaceutical scientists.

Getting through the barriers

With its hostile epithelial barrier and efficient immune system, the human body is well adapted to destroy or clear foreign objects that it encounters, even when those objects are life-saving drugs. Packaging drugs in structures such as liposomes has proved successful for getting water-soluble pharmaceuticals past the immune system. But such structures are not designed to transport poorly soluble drugs – a characteristic of many anti-cancer agents. The delivery of such pharmaceuticals 'continues to represent a challenge', says Vladimir Torchilin, Professor of Pharmaceutical Sciences at Northeastern University, Boston (http://www.neu.edu).

However, Torchilin continues, micelles, which in water assemble with a hydrophobic core, are able to carry sparingly soluble anti-cancer drugs, such as taxol, into the heart of a tumour before the body can recognize and clear it (see review [1]).

Once loaded with a cancer-killing drug, Torchilin says that tumour-specific antibodies can be attached to take the micelles straight to the target tissue. 'Drug-loaded cancer-specific micelles recognize a variety of cancer cells *in vitro* and provoke an increased killing of cancer cells *in vitro* and *in vivo*,' he told BioMedNet News (http://www.bmn.com).

PEGylation

This method has certain advantages over established ways of masking macromolecular drugs, such as pegylation, where the active macromolecule is coated in polyethylene glycol (PEG), thereby masking it from the immune system (see review [2]).

'With the PEG, you have to have a covalent linkage between the protein and the polyethylene glycol,' said Ijeoma Uchegbu, Professor of Pharmaceutical Sciences at the University of Strathclyde, Scotland (http://www.strath.ac.uk). 'Some people don't want to alter their drugs in this way because you could alter the activity and you could be putting the PEG on the active site,' she said.

Liposomes get around this problem, she says, but because of their aqueous inner compartment, they are better suited for transporting aqueous drugs. Although they can carry poorly soluble drugs like taxol, they can only hold them in the membrane and not in the spacious core, she says, which limits the amount of drug that can be transported.

The micelles could be the solution, says Torchilin. 'Our findings may lead to the development of a new, more efficient, and safer delivery system for taxol and other poorly soluble anticancer drugs,' he and his colleagues note in the abstract to a paper they will present at the *30th Annual Meeting of the Controlled Release Society* next month in Glasgow, Scotland (http://www.controlledrelease.org/).

Promise for drug delivery

Uchegbu agrees that this delivery system 'seems to improve cell kill over that seen with the plain drug.' However, she is not convinced that Torchilin's structures should really be called micelles. 'I would love to know how they were classified as such,' she said, 'as it is possible that they could be liposomes or even just plain small solid nanoparticles.' Nevertheless, whatever they actually are, Uchegbu says that Torchilin's packaging structures look like they have promise for the delivery of drugs with limited solubility like taxol and etoposide.

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