# Ebola glycoprotein: the key to successful gene therapy?

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Of the many potential clinical successes of genetic research, perhaps among the most promising is gene therapy for chronic diseases like cystic fibrosis. An absent gene could one day be delivered via a viral vector suspended in an inhalant; however, human pulmonary epithelial cells present a challenge: 'The lung is an immunologically smart organ. To think you could incorporate a new gene – introduced by a virus – and think the lung would not detect it is naïve,' says Paul McCray of the University of Iowa (http://www.uiowa.edu). But, he points out, there are pathogens that have devised ways to enter the cells. 'So it is not an insurmountable problem; we were just using the wrong tools.'

## An alarming origin

Recent work from several laboratories has shown that the right tool might be a protein of seemingly alarming origins: the Ebola virus. Now, McCray and his colleagues have examined a possible role for the folate receptor-alpha (FRα) as a means of virus entry to host epithelia [1].

Gary Kobinger of the University of Pennsylvania (http://www.upenn.edu), who is not involved with the current study, states the difficulty plainly: 'How do you get the gene into the cells?' Researchers have been frustrated in their efforts, apparently because many of the cellular receptors for the often-used retroviral vectors reside on the basolateral surface of epithelial cells. However, in an earlier work, Kobinger was able to show that with the right viral vector – an HIV envelope 'pseudotyped' with a glycoprotein from Ebola – the cells could be infected apically *in vivo* [2].

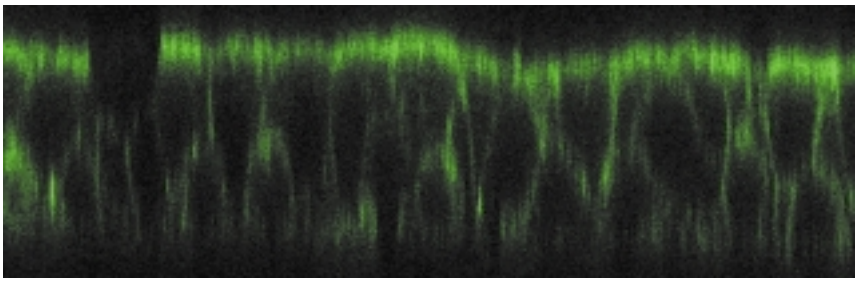

**Figure 1.** Vertical sections of primary cultured human airway epithelial cells, as seen with confocal microscopy. Cells were stained with an antibody to folate receptor-alpha (FRα), which is expressed abundantly on the apical surface of the cells. Image courtesy of Patrick Sinn, University of Iowa (http://uiowa.edu).

## Recombinant viruses

Together, Ebola (EBO) and Marburg (MRB) make up the *Filoviridae* family of viruses. Like retroviruses, the filoviruses express a glycoprotein that mediates receptor binding and fusion with host cells. This filoviral protein is added to the HIV virion to create the recombinant vector. McCray says it is 'basically like putting a new coat on the virus to allow it to enter these polarized cells from the apical surface'.

'The beauty of recombinant lentivirus system is that you can change those glycoproteins,' notes McCray. In the current work, he and his colleagues inserted the Ebola gene into a non-human lentivirus: the feline immunodeficiency virus (FIV). The researchers used a previously described mutant called EBOΔO, from which a 180-amino acid region of the heavily modified glycoprotein has been deleted [3]. The removal of the large *O*-glycosylated domain resulted in a dramatic 74-fold increase in titer compared to wild type – an effectual concentration. Despite similar manipulations of the Marburg virus-pseudotyped FIV, it never reached an efficient titer.

## The right receptor

Other recent work has shown that the EBO-modified virion enters cultured cells by binding to FRα *in vitro* [4]. Could this be the receptor that mediates viral entry to epithelia? To find out, McCray examined primary cultured human epithelia cells. The polarized cells appear to express FRα predominantly on the apical surface (Fig. 1). However, when the receptor was either blocked or cleaved, transduction by EBOΔO–FIV virus was not disrupted. 'We went in thinking it would be the folate receptor, and we came out realizing it wasn't,' says McCray.

So, how important is it to identify the true receptor? McCray notes that the use of various pseudotyped viruses is a 'trial and error' process: 'The fact is,

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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.

### References


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# New mode of intervention in sepsis treatment

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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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