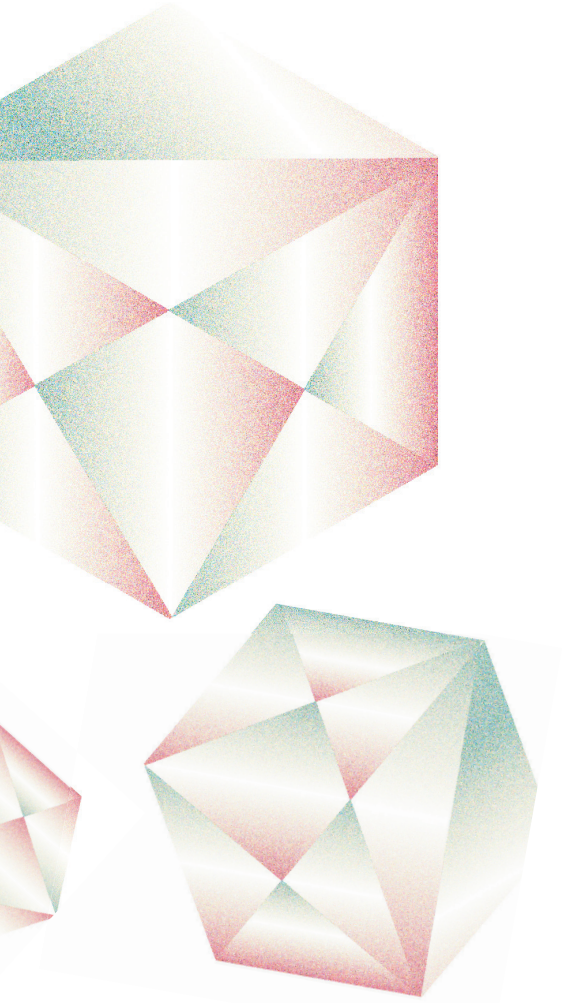
Next-generation vaccines: potent but safe

Dr Michael A Barry is using genes and viruses to protect against a range of difficult diseases. He reveals the reasons behind why he is attacking pathogens where they first occur and elaborates on his future plans

What inspired your work developing gene- and virus-based treatments for infectious and non-communicable diseases?

I am motivated to attack the pathogens that scare me the most, such as the methicillin-resistant *Staphylococcus aureus* (MRSA) superbug, Middle East respiratory syndrome, HIV and Ebola.



How has your background influenced your career in research?

I grew up in the Midwest of the US. My family was not wealthy, so I learnt early on in life to repair broken things. The other choice was to have nothing. You can also fix science and disease 'things', but you have to work hard to achieve that goal.

You have tested many vectors for vaccination. Can you detail the rationale behind virus selection for your gene-based viral vaccines?

I started with 'naked DNA' and have moved to more robust viral vectors. There is greater value in comparing different vectors in your own hands than having different labs 'champion' their favourite vector. For example, when we compared DNA and adenovirus vaccines against MRSA, the plasmids generated barely detectable responses, whereas adenoviruses provoked strong antibodies after single immunisation. Evolution has done the heavy lifting to engineer viruses like adenoviruses to infect and deliver genes at mucosa. We are simply stealing this ability to serve a better purpose.

Do you have particular concerns that you must address relating to the safety of gene therapies, DNA-based vaccines and drug-delivery systems?

The primary dangers arise from replicating vectors or the immune reaction to the vectors. For vaccines, you are harnessing what could be negative, the immune response, to target a pathogen. With regards to replication, the single-cycle vectors we invented resolve this problem directly by removing the threat of infection.

What is the importance of testing if vaccines are successful at producing an immune response at key mucosal sites of entry, rather than systemic responses?

Most infections occur at the mucosa and they generally begin with only one or a few infectious agents. Combating a few pathogens makes more strategic sense than trying to destroy billions of pathogens after they are let loose in the body.

In layman's terms, could you explain 'serotype switching' and its critical role in the development of vaccine vectors?

I like to describe this as a 'shell game'. First, you use one serotype of an adenovirus to deliver your vaccine. The immune system becomes resistant not only to the intended vaccine but also to that serotype of the vector. If you try to use the same serotype again, it will be neutralised by the immune system and have little to no effect. If you then change serotypes or 'shells', the immune system cannot see the new incoming vector and succeeds in delivering the vaccine.

Have you faced difficulties in selecting appropriate protein antigens for vaccine design and effectively constructing a viral vaccine containing the corresponding transgene?

Selecting protein antigens is relatively straightforward for viruses, but more complex for bigger bacteria. If the pathogen is extracellular, you target exposed proteins to generate antibodies. If the pathogen is intracellular, you can target many proteins for antibodies or T-cell responses.

Is there a specific impact you are aiming to have with your single-cycle adenovirus vectors?

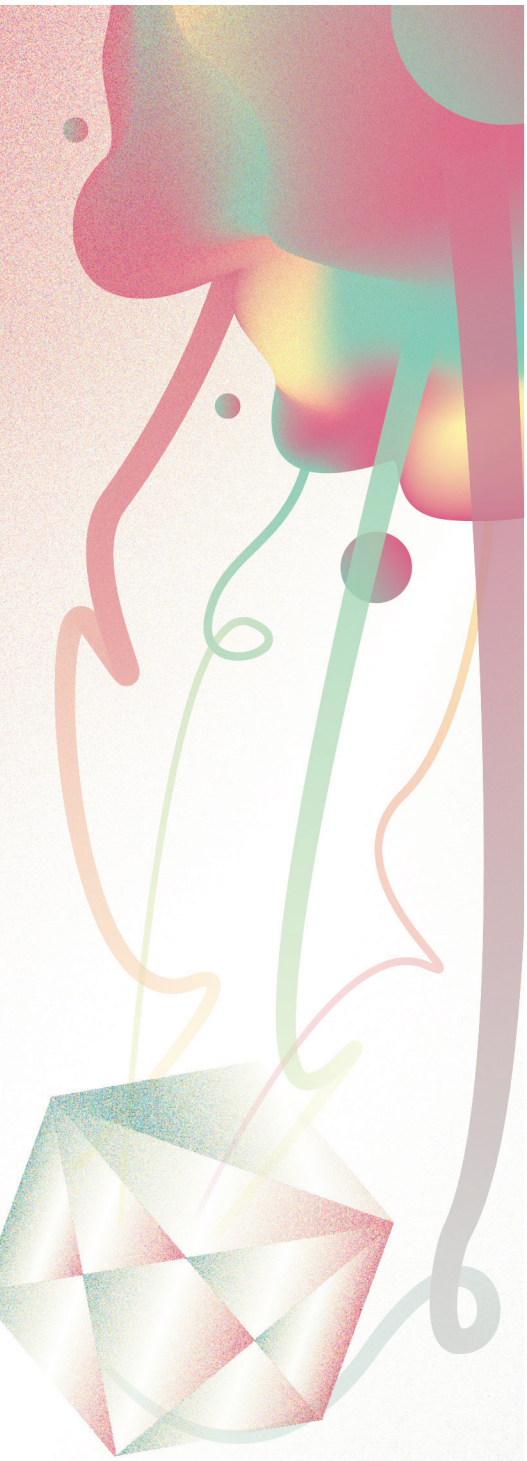
This technology is more robust than anything I have worked on in the last 22 years. Given this, we are applying this platform against every threat we can think of, including cancer. We are also harnessing it to develop vaccines that patients could take as a sublingual vaccine under the tongue, or simply swallow. This would be great for global vaccination and it would avoid issues with having to refrigerate vaccines.

How do you see the field of vaccine development changing in the next five to 10 years?

We use viral vectors simply because they are currently most potent, not because they are ideal. Evolution engineered them for gene delivery. I think the field will move more towards non-viral systems to increase safety. The trick will be in engineering them to be as robust as viruses. In this case, humans have to do the heavy lifting rather than relying on evolution for next-generation vectors.

Viral vehicles

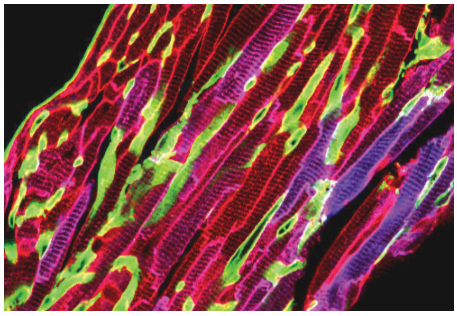
The **Laboratory of Vector and Vaccine Engineering** at America's Mayo Clinic is harnessing self-amplifying vectors to combat diseases

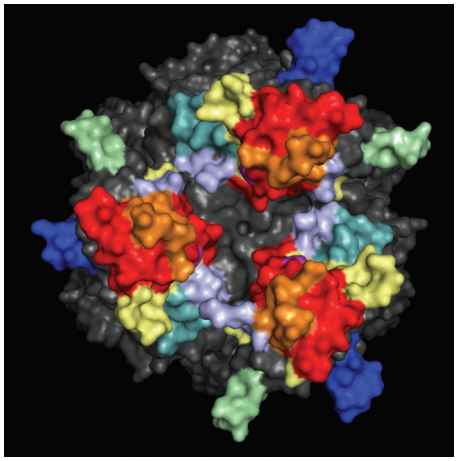


PATHOGENS GENERALLY ENTER

the body at mucosal surfaces and then spread systemically. An infection from an invading pathogen will either 'educate' the immune system or kill its host. In response, scientists have created vaccines as a pre-emptive measure with the aim of educating the immune system without creating the risk of disease or death. This education can occur either at mucosal surfaces or within the body. "Generating immune responses at mucosa is important to provide 'barrier' protection at the first site of pathogen entry," states Dr Michael Barry, who is leading a group at the Mayo Clinic dedicated to using genes and viruses to treat very difficult diseases.

Barry's group believes generating immune responses at mucosa makes great strategic sense, since a vaccine has a better chance of stopping an infection when fewer pathogens are present. "Controlling pathogens when they are outnumbered may be more achievable than trying to control the flood of infectious agents after they have spread throughout the body," he explains. It is also important to generate immune responses in the blood and the body in case the pathogen escapes the mucosal barrier. "While we know that most infections start at mucosal surfaces, most vaccines are delivered in the muscle, a site that may not educate the immune system well for mucosal protection," he continues. Barry's group is therefore interested in developing vaccines that can be delivered at mucosal surfaces or that amplify mucosal barrier protection.





Top: Blue fluorescent protein gene delivery into skeletal muscle. **Bottom**: Adenovirus hexon protein trimers.

SINGLE-CYCLE ADENOVIRUS VACCINES

Some of the most robust vaccines have been made by killing or attenuating pathogens. While they can be quite potent, there is always a finite risk that these vaccines will actually cause the disease they aim to prevent. Vaccines advanced substantially when recombinant DNA technology met gene delivery. In a gene-based vaccine, genes from pathogens are used as vaccines instead of the pathogen itself.

As scientists have sought to conquer some of the most dangerous pathogens using this technique, more robust viral vectors have been utilised to increase vaccine potency. There are two main forms of viral vectors: replication competent (RC) and replication defective (RD).

An RC vector is efficient. It can infect a cell and copy it and its genome, as well as a vaccine gene, thousands of times to generate very strong immune responses. However, it does not stop replicating in that one cell – it continues to reproduce, making thousands of new viruses that endanger the body; it even has the potential of infecting the medical personnel who inject the vaccine. On the other side of the spectrum are RD vectors, which were genetically engineered so they cannot spread beyond the first cell that is infected. While safer, RD vectors generate relatively weak immune responses.

Recognising that RC and RD vectors exist at two ends of a spectrum, Barry's group split this difference to create a vaccine that amplifies genes without risking infection. The solution came in the form of an engineered single-cycle adenovirus (SC Ad). This vector is based on potent adenoviruses that cause a variety of mild diseases. To inhibit infectious virus production while maintaining the ability to replicate vaccine genes, the scientists deleted a pivotal gene that encodes the viral IIIa protein.

After creating SC Ad, the group compared it to both RD and RC vectors. They first examined viruses expressing the green fluorescent protein-luciferase (GFPLuc) protein as a test gene. "GFPLuc serves as a model vaccine, since it generates T-cell and antibody responses like a pathogen protein, but it also allows us to

track genes and vectors in the body, which a pathogen protein cannot do," Barry explains.

The Mayo Clinic group first investigated viral genome replication in human cells. As expected, the RD vector DNA did not change over time. One copy of RD DNA remained one copy. In contrast, SC and RC vectors replicated their viral DNA and the GFPLuc gene up to 3,000-fold within one day. Importantly, only the RC Ad vector produced infectious progeny viruses. SC Ad amplified without the risk of infection.

FROM *IN VITRO* TO *IN VIVO*

To test if they could deliver genes and generate needed blood and mucosal immune responses, the researchers immunised hamsters with a single intranasal dose of the vectors. When luciferase was measured over time, RC and SC viral vectors generated levels that were a hundred times higher than those produced by traditional RD Ad vectors.

When antibodies were measured in the blood, they showed that SC Ad rapidly generated significantly higher levels that remained elevated for six months after a single vaccination. Importantly, only the SC Ad vector produced antibodies at mucosal barriers. These antibodies climbed in mucosa over months and remained elevated for six months after single immunisation. In contrast, the RD and RC vectors produced barely detectable antibodies at mucosa.

In macaques, SC Ad and RD Ad vectors were dripped under the tongue as a simple oral vaccine. In this case, SC Ad again induced higher and more persistent antibody and T-cell responses than conventional RD vectors after single immunisation.

AMPLIFYING HOPE FOR VACCINES

Given SC Ad potency, the researchers have replaced GFPLuc with genes from HIV, Ebola, methicillin-resistant *Staphylococcus aureus* (MRSA) and influenza, to name a few. For influenza, their benchmark for a protective vaccine was to produce antibody titers in the blood above 40. The SC Ad vector hit this benchmark after single immunisation using 100 times less vector than a standard RD Ad vaccine. This potency may translate into the ability to use 1/100 the amount of SC Ad than RD Ad vaccine in humans to achieve protection and reduce dose-related side effects. It may also allow manufacturers to generate 100 times the number of doses per batch of vaccine than an equivalent RD Ad vaccine, thus reducing production costs.

SIMPLE ORAL VACCINES

SC Ad vectors were engineered specifically for mucosal delivery. While one can apply these to mucosal sites like the nose, Barry's true Holy Grail for the technology is to develop it as an oral vaccine. "It was with this goal in mind that we engineered SC Ad vectors to take limiting gene delivery events and amplify their impact using replication," he continues.

The researchers' work in macaques of involving dripping the SC Ad vaccine under the tongue suggests that this goal may be achievable. They are currently combining this robust vector platform with their previous bioengineered oral vaccine technologies to develop simple oral vaccines that can target different sites along the digestive tract. This would also stabilise the vaccine for global use without need of refrigeration.

The team's work has shown that SC Ad vectors have huge potential as simple vaccines for worldwide use against some of the direst pathogens. "We have good data for HIV and influenza so far, and the others are following," he comments. His group's most recent results, published in January this year, show the potential of the recently developed SC Ad6 vector as a vaccine platform. Looking ahead, in addition to creating an oral vaccine for HIV that can repel the virus at its site of entry, Barry is also working on creating vaccines for drug-resistant pathogens like MRSA, Middle East respiratory syndrome and Ebola.

LIFE BEYOND BUGS?

Adenoviruses (Ads) can be engineered for gene therapy or as 'oncolytic viruses' to kill cancers. Like SC Ads, these oncolytic Ads are self-amplifying drugs, because each cancer cell that is killed produces thousands of new viruses. Barry's lab has found that different Ads have different appetites to kill different cancers. Their group is engineering these Ads as systemic therapies to hunt down and kill metastatic cancers, while sparing normal tissues.

The gene therapy and oncolytic scientists in the lab rub elbows with the vaccine researchers. This provides interesting opportunities for the three therapeutic approaches to cross-fertilise. Indeed, this proximity approach actually led to the invention of the SC technology by wedding replication-competent oncolytic-like Ads to replication-defective Ad vaccines. Given these bridging opportunities, Barry's lab is now turning SC Ad back to uses for gene therapy and cancer. In particular, SC Ads look promising as cancer vaccines, immune adjuvants and amplifying other therapeutic proteins.

AMPLIFYING VACCINES AND THERAPIES

OBJECTIVE

To develop safe, effective mucosal vaccines, gene therapies and oncolytic virotherapies.

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