Striking a balance: engineering effective live vector vaccines

Professor James E Galen, PhD, is a Professor of Medicine at the Center for Vaccine Development at the University of Maryland School of Medicine. He is a passionate genetic engineer whose work to date has focused on vaccine development. Most recently, he has turned his attention to live vector vaccines, which offer promise for tackling prevalent bacterial infections without the use of antibiotics.

Q&A

How does a live vector vaccine differ from other vaccines?
Live vector vaccines are made from bacteria that have either been engineered to be non-pathogenic (such as attenuated *S.* Typhi bacteria) or from non-virulent bacteria such as *Escherichia coli*. Once the vaccine strain is available, we can then take it to the next level. We further engineer the original vaccine to deliver *additional* vaccine antigens (such as smaller pieces from TcdA, TcdB, and Cdt) that the original vaccine strain would not normally produce. Essentially, we are replacing the needle often used for vaccinating people with proteins (think hepatitis B vaccine), with a living bacterial organism capable of delivering the desired vaccine antigens to the immune system.

How easy is it to find a balance between attenuation and immunogenicity?
Although I would *love* to convince you that I have finally figured out how to do this… I'm not sure yet. Only clinical trials with our best candidates will prove whether we got it right or not. The key concept to appreciate here is that finding the right balance on paper is easy; getting it to actually work in a human being is a much more daunting, but far more interesting, challenge.

What is the most exciting aspect of your research?
I have had the privilege of participating in the design and construction of a live vaccine against the human pathogen *Vibrio cholera*, testing it in animals, watching this vaccine become approved by the Food and Drug Administration (FDA) for clinical testing, then actually handing a cup of vaccine to a fellow human being, watching that person drink the vaccine, and finally finding out that the resulting immune response was fabulous. Through the heroic efforts of my mentor, Dr Myron Levine, this vaccine was actually licensed in the United States (read more here). This just doesn't happen that often in a scientist's life. If I could see such a thing happen with a live carrier vaccine against *C. difficile*, I would be "over the moon".

Has your experience as a technician as well as an academic helped you in your work?
My journey to full Professor has been unorthodox at best. But I have always been grateful to have worked both as a technician and PhD in both academia and industry. Academia taught me how to think critically and to read the literature *before* doing the experiment. But industry taught me something just as valuable: the value of time. Tomorrow is for the *next* experiment, not the one that should have been completed today.

Where do you see this line of research going next?
I would like to see an optimised live carrier vaccine progress through clinical trials and be proven to be safe and protective against disease. It does not actually have to be my vaccine that makes it through clinical trials and succeeds. I would be grateful to have contributed in some way to the proper engineering of the right live vaccine, and would be thrilled to see this important vaccine strategy be proven useful and valuable to the field of vaccinology. I would like to see carrier vaccines proven to be as useful as injectable vaccines, at least in cases where vaccination against mucosal diseases is required.

Antibiotic-resistant bacteria (ARB) threaten the treatment of bacterial diseases. The over-use of antibiotics significantly increases the incidence of ARB and of disease.

Clostridium difficile is a bacterium that particularly affects the elderly, especially after treatment with antibiotics for unrelated infections. Infection causes diarrhoea and can cause colitis. Recurrent strains are more infectious and once it has recurred, patients are more likely to suffer from the infection again (i.e. it is more likely to keep coming back). Antibiotic resistance causes *C. difficile* to be more prevalent and recur more frequently.

PREVIOUS WORK
Studies characterising the genome of *C. difficile* reveal that three toxins are frequently found, sometimes together, in hyper-virulent strains. These toxin proteins are referred to as 'virulence factors': molecules produced by an organism like a bacterium that affect its pathogenicity, or its ability to cause disease.

The main virulence factors in *C. difficile* are called TcdA and TcdB. They are usually found together in epidemic strains, which suggests that they cause disease when they are co-expressed. These enterotoxins, as they are called, cause cells to swell and burst (apoptosis), and disrupt the cell structures, both key features of conditions like colitis,

to which *C. difficile* is linked. An additional toxin, Cdt, also enhances the virulence of *C. difficile* and causes the bacteria to adhere better to the intestinal wall.

DEVELOPING A VACCINE

If these virulence factors that cause *C. difficile* to be more virulent can be targeted by immune responses, then the risk of infection and recurrence can be reduced. Animal models show that this has been effective.

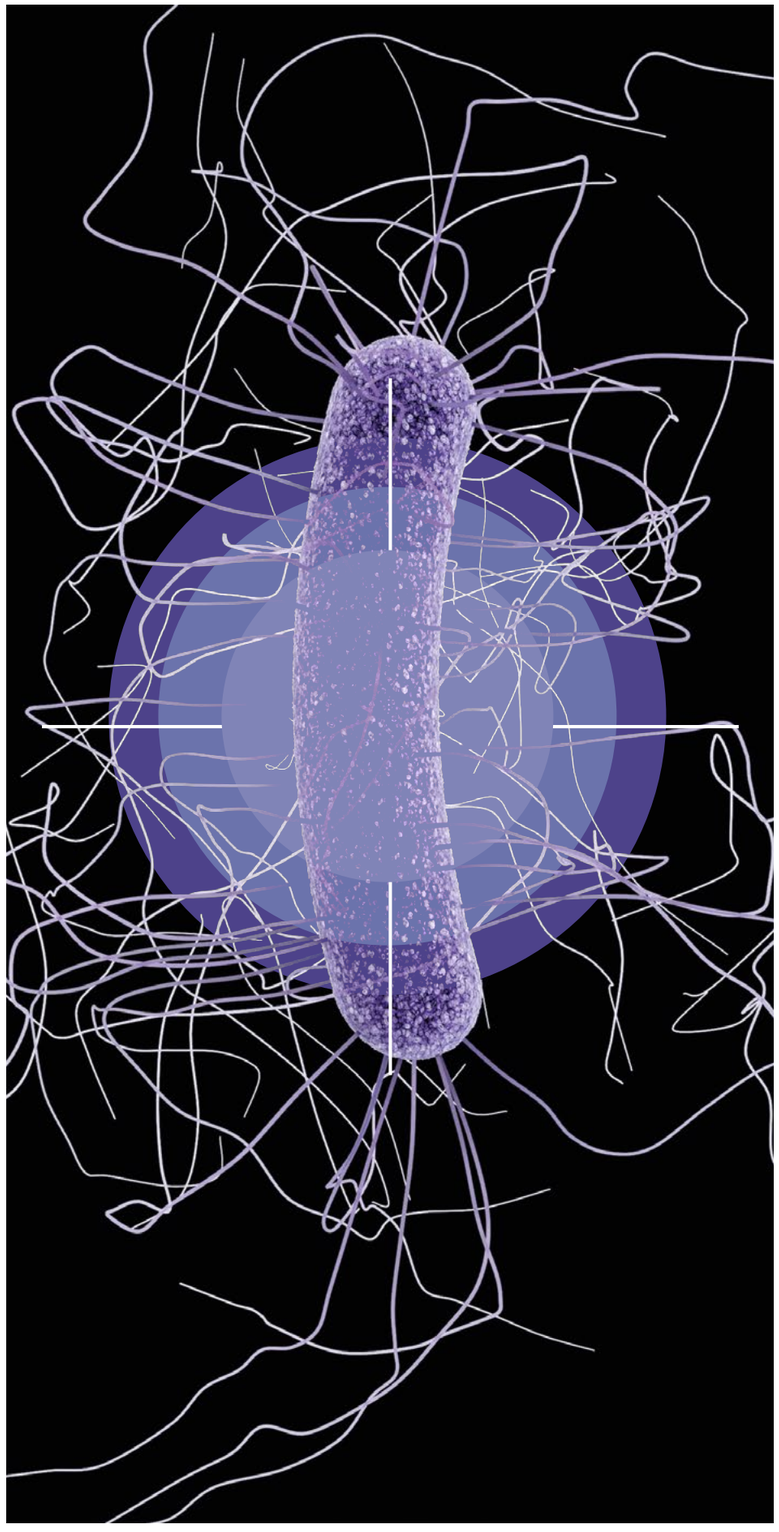
Treatment with a vaccine is preferable to antibiotic treatment because antibiotic resistance is the reason that *C. difficile* thrives and recurs. Professor Galen and his team are developing live vaccines that provide immunity to disease by stimulating an immune response using safe doses of antigens (smaller parts of the toxins that stimulate an immune response, e.g. TcdA, TcdB, and Cdt).

The vaccine will initially be given to people already infected with *C. difficile*. The team hypothesise that this will trigger a response to the bacteria because the immune system has already encountered it, and will be able to produce antibodies to fight off the infection (an anamnestic response).

PICKING YOUR TARGETS

Evidence from animal models suggests that targeting immunity to inactivate the toxins TcdA and TcdB, as well as also targeting the binding ability of Cdt to enhance the adherence of *C. difficile* to cells, would be successful in blocking recurrence. There is clear evidence that the antibody immunoglobulin G (IgG), capable of binding to and inactivating the toxins from *C. difficile*, is linked to resistance to disease.

However, simply eliciting an antibody response does not always work. Moreover, research suggests that targeting enterotoxins alone is not always effective. Therefore, to increase the chances of success, the vaccine must target Cdt as well. Galen has therefore proposed a three-prong approach which will hopefully target the different toxins present in *C. difficile* in different ways: firstly, the toxins are prevented from binding by triggering a serum antibody response (serum is the protein-rich, liquid component of blood) and therefore generating immunity; secondly, immunity is induced in the intestinal mucus, which reduces the ability of Cdt to bind to intestinal cells; thirdly, mucosal immunity is targeted to reduce colonisation, recurrence and transmission.



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FINDING A CARRIER

To be able to vaccinate against *C. difficile*, Galen's team has had to find an appropriate carrier, or vector. Galen and others have already used carriers derived from *Salmonella enterica* serovar Typhi effectively in animal models, for instance against plague. There are substantial benefits to using *S.* Typhi as a carrier: it can be delivered orally, targets specific immunity cells where vaccines are more likely to be effective, and can stimulate broad immune responses. However, to make *S.* Typhi safe for use as a carrier vaccine in humans, virulence factors from *S.* Typhi must be genetically removed to avoid producing disease, while still preserving the ability to deliver antigens from *C. difficile*. The resulting safe carrier is then engineered to express smaller, non-toxic pieces of the two enterotoxins TcdA and TcdB that cause *C. difficile* to engender an immune response.

GETTING THE BALANCE RIGHT

However, finding the balance between attenuation (reducing the virulence of a pathogen like *S.* Typhi, while still keeping it 'live') and immunogenicity (the ability to provoke an immune system response) is essential. If the carrier vaccine is not properly weakened, it can cause adverse clinical effects. However, if the vaccine is too weak, and doesn't provoke an immune response (its reactogenicity, or ability to cause expected adverse effects, is minimal), immunity is not developed. Essentially, you don't want the attenuation to be too effective; otherwise it defeats the purpose of vaccinating in the first place.

IT'S ALL IN THE METHODS

Developing a live vector vaccine is complex, and the methods employed to incorporate antigens are important. In a recent review paper [Galen & Curtiss] Professor Galen put it this way: "The manner in which these antigens are delivered to the immune system can have a profound effect on the resulting immune responses and ultimate success of a carrier vaccine".

There are two main ways of genetically engineering a suitable carrier vaccine candidate. Firstly, scientists can use plasmids, circular types of bacterial DNA that differ from linear chromosomal DNA. Plasmids can be used to introduce new genetic material (such as genes capable of making antigens from *C. difficile*) and alter the expression of the carrier. However, this can cause metabolic stress that triggers selective plasmid loss, minimising its effectiveness. Additionally, plasmid methods can often lead to over-attenuation, making the vaccine less powerful.

The second method irreversibly inserts antigen-encoding genes directly into a single location within the chromosome of the candidate live vector without any further need for plasmids. The problem with the chromosomal approach is that the amount of antigen made is often much lower than levels made using plasmids. However, one study [Wang et al] found that inserting genes in two locations on the chromosome, rather than just one, yielded results that were far better. They also found that foreign antigen synthesis could be "tuned" to the physiology of the carrier vaccine, a competitive advantage.

LOOKING AHEAD

So far, no human trials have been carried out with vaccines that get the balance right between attenuation and immunogenicity. The logical next step is therefore to do this with optimal live vaccines. Building on the success of previous work, an effective vaccine may soon be available for prevention of many bacterial diseases for which treatment with antibiotics is becoming less effective.

The technology developed in recent years by Galen and his team may even have future applications in oncology: engineered reagents may be able to promote tumour reduction in certain types of cancer.

Detail

RESEARCH OBJECTIVES

Professor Galen's work focuses on the development of live vector vaccines and their importance in the fight against antibiotic resistance. His current project aims to create a live vector vaccine for use against the increasingly resistant bacteria, *Clostridium difficile*.

COLLABORATORS

- Dr Jan ter Meulen (Merck)
- Dr Jimmy Ballard (University of Oklahoma)
- Dr Hanping Feng (University of Maryland Dental School)

BIO

Professor James E Galen is a genetic engineer with over 20 years' experience in both industry and academia. Starting out as a technician, he became Research Assistant to Dr Jim Kaper before earning his PhD from the University of Maryland Baltimore. After two years as a Research Scientist at MedImmune he moved to a faculty position at the Center for Vaccine Development. Here, he continued to focus on live vector vaccine development, contributing to significant advances in both vaccine development and innovative methods for testing the immunogenicity of candidate vaccine strains. Now Head of the Salmonella Live Vector Vaccine Unit, Prof Galen enjoys the teaching and mentoring element of his role as well as his research efforts.

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