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New vaccines: damming for multi-strain organisms?

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A team from the University of California, Santa Barbara (UCSB; CA, USA) has developed a method to stimulate cross-protective host immune responses to multi-strain pathogens [1,2]. This could avoid the lack of efficacy often seen when vaccinating against continually mutating organisms.

Obstacles to effective vaccination

One of the main obstacles of vaccine development is that there are often many different pathogenic isolates of a given species contributing to disease. Vaccination against one strain might not elicit protection against another, or even against a variant of the parent strain. This is why protective immunity against some microbes – such as influenza – requires annual vaccination with different strains. It also explains why vaccine efficacy can depend on the specific pathogenic isolates endemic to a given geographical region, and why mutant variants can cause disease in populations that are immune to infection with the parent strain.

One way that traditional vaccine manufacturers have tried to overcome this problem is to take several different bacterial capsules (the loose layer outside of the cell wall) from different strains and combine them in one formulation. However, this approach has limitations because of the vast number of strains in the environment and their ability to mutate.

'Different strains have different proteins and carbohydrates on their surface and different cellular components that require different arms of the immune system to stop them. Some of these problems could be circumvented by the use of vaccine strains that ectopically express multiple antigens that are shared among different pathogenic strains,' suggests Michael J. Mahan, Associate Professor in the Department of Molecular, Cellular and Developmental Biology at UCSB, and leader of the project.

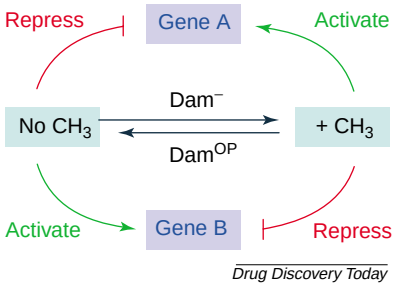

Figure 1. Altered levels of Dam lead to ectopic gene expression, attenuated virulence, and the elicitation of protective immune responses.

Vaccinating against multiple disease strains

Much of Mahan's work regarding the construction of live bacterial vaccines has been performed with *Salmonella* species because they establish an infection by direct interaction with the gut-associated lymphoid tissue, resulting in a strong mucosal immune response. *Salmonella* also represents a formidable vaccination challenge, because it has >2500 different pathogenic strains that cause a range of diseases including food and blood poisoning, as well as typhoid fever in humans.

'Our original interest was in understanding how bacteria cause disease. The idea was that bacteria are like a "Trojan horse" hiding their weapons until they are inside the animal,' says Mahan. By studying *Salmonella*, they knew that many genetically determined virulence functions of this pathogen are preferentially expressed during infection. The coordinated expression of virulence genes when cells are moved from synthetic media to host tissues suggested that the cells harboured regulatory factors that controlled this unique ability. The team reasoned that mutations in this regulatory mechanism would impair the capacity of *Salmonella* to cause disease, and thus devised a method to screen for mutations that permitted genes to be expressed when cells are growing *in vitro*.

The screening method yielded cells with mutations that resulted in the loss of activity of the enzyme, DNA adenine methylase (Dam). Dam is known to act as a regulator of gene expression and to affect a wide range of crucial cellular functions, including DNA replication, DNA repair, transposition and segregation of chromosomal DNA [3]. The team went on to suggest that downregulation of Dam activity disabled the ability of a pathogen to cause disease via aberrant virulence gene expression and contributes to the heightened immunity in vaccinated hosts through the ectopic production of an expanded repertoire of potential antigens.

Looking at *Salmonella typhimurium* strains that either lack or overproduce Dam (Dam$^-$ and Dam^{OP}, respectively), Mahan's group showed that both strains produced severe virulence defects when tested for their ability to cause typhoid fever in mice (Fig. 1). The mice survived and showed no signs of disease when inoculated with 10,000-fold more Dam$^-$ or Dam^{OP} cells than are needed for the

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wildtype (Dam+) strain to kill 50% of the animals [1,4].

The next step was to test whether Dam mutants could serve as a live vaccine to protect mice against infections from wildtype *Salmonella.* The group showed that inoculating mice with Dam mutants protected against subsequent exposure to virulent wildtype strains. They also recently showed that *Salmonella* Dam– and Dam^{OP} live, attenuated vaccines elicited cross-protective immunity to three different heterologous *Salmonella* serotypes in mice and chickens [1,2].

Mahan and colleagues also explored the role of Dam in the pathogenesis of two other enteric bacteria, *Vibrio cholerae* and *Yersinia pseudotuberculosis*, the causative agents of human cholera and gastroenteritis, respectively [5]. They found that Dam overproduction attenuated the virulence of both bacteria. In the case of *Y. pseudotuberculosis* it led to a fully protective immune response in vaccinated hosts. They concluded that, because mutations in Dam can attenuate the virulence of several diverse pathogens, the role of DNA methylation in virulence might emerge as a common theme in bacterial pathogenesis and could be used to elicit a class-protective immune response to more than one bacterial strain.

Future studies

Mahan is concerned that many regulatory hurdles will need to be overcome before a license using this technology is granted for a live vaccine for human use. For this reason much of the research effort is now being directed to see if Dam technology can be used to immunize animals with killed bacteria. Mahan believes that the first use of the Dam technology in clinical trials is likely to be against tumours in cancer patients where radiotherapy and chemotherapy have failed. 'The idea would be to get *Salmonella* to express the antigens that are being made in higher quantities by cancer cells in the hope of eliciting a heightened immune response,' explains Mahan.

'The discovery by Mahan's group that dysregulation of Dam renders a variety of bacterial pathogens avirulent, yet capable of stimulating cross-protective immunity, is a significant step towards rapidly generating efficacious vaccines for several infectious diseases,' comments Brad T. Cookson, Associate Professor in the Departments of Laboratory Medicine and Microbiology at the University of Washington (Seattle, WA, USA). 'Further, by understanding how Dam mutants stimulate cross-protection, the potential of efficiently eliciting immune responses to heterologous antigens expressed by viable bacterial vectors would have important applications in medicine outside of infectious diseases.

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Electronic DPI for insulin

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Researchers at MicroDose Technologies (Monmouth Junction, NJ, USA) have developed what they believe is the first completely electronic dry powder inhaler (DPI). The device has produced encouraging results in Phase I studies of pulmonary insulin delivery.

Millions of people with diabetes must inject themselves with insulin to control blood glucose levels. Many find this painful and inconvenient, and there have been numerous attempts to develop a non-invasive delivery system.

Insulin is a peptide and, therefore, cannot be administered orally because it would be degraded by digestive enzymes. Approaches to produce an oral formulation that bypasses this problem are being investigated, as are transdermal, buccal and nasal delivery. However, there is general agreement that pulmonary insulin delivery is viable [1]. Several inhaled insulin products are in clinical trials and the first are expected to reach the market within two years.

Pulmonary delivery

To be absorbed effectively, the insulin dose must be carried deep into the lungs to the alveoli. The problem is that particles deposited further up in the airway will not reach the bloodstream. Particle size is crucial for deep lung penetration and, therefore, efficacy. The insulin that is fused with the MicroDose inhaler is formulated by Elan Drug Delivery (Nottingham, UK), and is being developed through a joint venture by the companies called QDose. 'Pulmonary

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