Unlocking the vault

Professors Kathleen Kelly and Leonard Rome are conducting research into mysterious nanostructures termed ‘vaults’. Here, they discuss their ambitious and innovative attempts to use vaults as targeted delivery molecules for immunogenic proteins.

What are your research backgrounds, and what expertise do you each bring to the current project?

KK: I received my PhD in Medical Microbiology and Immunology from The Ohio State University in Ohio, USA, where I studied how to trick the immune system into producing immune responses against orally ingested antigens. During my postdoctoral training at Washington University in St Louis, USA, I continued studying the development of the immune response in germinal centres. In the mid-1990s, I concentrated on researching an infection within the female genital tract: *Chlamydia trachomatis*. This pathogen induces a strong immune response in the genital tract, which destroys the organism but fades overtime, leaving individuals prone to repeat infections. I joined the Department of Pathology at the University of California, Los Angeles (UCLA), in 1999 and have since been working to find a way of hoaxing the immune system into producing a long-lasting immunity to *C. trachomatis* in an attempt to advance vaccine development.

LR: I have a PhD in Biological Chemistry and have been on the faculty of the Department of Biological Chemistry at UCLA since 1979. My research centres on the biology of nanoparticles, and I am currently collaborating on a number of interdisciplinary research projects aimed at engineering nanoparticles called ‘vaults’ as flexible nanoscale therapeutic delivery vehicles. *Chlamydia* and other sexually transmitted infections constitute a major burden to global healthcare systems; do you feel there is enough investment in developing treatments for these diseases?

KK: There is increased interest in the development of a *C. trachomatis* vaccine. A number of companies are working to this end but have not produced a vaccine to test in human trials. This field is in need of basic research support to unravel some of the critical developmental hurdles. The number of basic researchers with the goal of creating a vaccine has increased slightly in recent years but is unfortunately limited by the amount of funding available for this research.

LR & KK: We see two distinct advantages of using vaults as delivery particles for immunogenic proteins. Firstly, the vault vaccine does not require the addition of an adjuvant to stimulate a robust immune response. Exogenous vaults stimulate pattern recognition receptors – specifically, the Nod-like receptor family – giving them adjuvant properties. Secondly, the particle directs the immune response toward development of CD4 and CD8 T cell responses, a distinct advantage when mucosal immunity is desired.

Can you outline the particular advantages of using vaults as delivery particles for immunogenic proteins rather than alternative methods?

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Have you encountered any major challenges while undertaking work in the previously unexplored field of vault research? How have you overcome these challenges?

LR: One of the greatest challenges has been raising funds to support this research. It is very difficult to get funded in an area in which no other researchers are working, as there is no peer group at the National Institutes of Health (NIH) that can properly evaluate our research proposals. Every time we submit a grant, we...
Almost 30 years ago, researchers from the University of California, Los Angeles discovered tiny, novel structures in eukaryotes that are now showing potential as vaccine platforms for infectious diseases such as the sexually transmitted Chlamydia trachomatis. Have the uphill battle of educating the reviewers about vaults as it is unlikely that they have ever heard of them before.

What does the future hold for this research?

LR: About a year ago I partnered with a CEO, Michael Laznicka, to form a company to commercialise vault therapeutics. This company, Vault Nano Inc, has formed a partnership with UCLA to transfer vault technology from the laboratory into the clinic.

KK: I have been fortunate to obtain continuous NIH funding to work on development of a C. trachomatis vaccine. Indeed, the formation of Vault Nano Inc has allowed our group to continue investigating this nanoparticle as a unique and promising vehicle for development of a vaccine against C. trachomatis infection. We hope that our investigations will lead to real world clinical impacts in the future.

AS THE MOST common bacterial agent of sexually transmitted disease, Chlamydia trachomatis is responsible for over 1 million reported infections annually in the US alone. Spread through vaginal, anal or oral sex with someone who already has the disease, the consequences of infection are serious. Not only does it result in local inflammation, but it also can cause permanent damage to the reproductive system, making it difficult or impossible for women to become pregnant in the future. C. trachomatis can only be cured by antibiotic treatment administered very soon after the infection – however, since most infections are asymptomatic, treatment is often not started in time to prevent reproductive dysfunction.

The best way to combat C. trachomatis is to prevent the infection from happening in the first place. Yet developing a vaccine that is capable of providing a long-lasting immune response has proved extremely challenging: “Vaccine trials in the 1960s used whole organisms and caused hypersensitivity or excessive inflammation in a number of individuals,” discloses Professor Kathleen Kelly, based in the Department of Pathology and Laboratory Medicine at the University of California, Los Angeles (UCLA). “This prompted the search for a ‘set’ of desirable antigens for vaccine development. Additionally, there are a large number of strains that can infect humans – and immunity against one strain does not protect against all other strains.” In response to these challenges, Kelly is collaborating with cell biologist Professor Leonard Rome, also at UCLA. Together, drawing on a chance discovery that happened nearly three decades ago, the scientists are making important progress in vaccine development for C. trachomatis.

A LANDMARK DISCOVERY

Rome and Kelly’s current research stems back to 1986, when Rome and his then postdoctoral assistant, Dr Nancy Kedersha, were investigating new ways of separating coated vesicles from rat liver cell lysates. In their project, they were using transmission electron microscopy to analyse the vesicles – and it was from one of the resulting images that they stumbled across something entirely new and unexpected: a tiny dark structure shaped like a barrel in the cytoplasm of a liver cell. At the time, the researchers had no idea what it was so Rome gave Kedersha the go-ahead to examine this mysterious nanoparticle.

Kedersha proceeded to isolate the coated vesicles and then stained and imaged them using electron microscopy. After treating the particles with proteases and enzymes to analyse their constituent molecules, she found evidence of three major proteins and an RNA component. Kedersha and Rome labelled the structures ‘vaults’ due to their resemblance to vaulted cathedral ceilings.

Following the initial discovery of vaults, Kedersha subsequently found that these tiny structures are widespread across cells in a huge variety of different animal species, including several human cell lines. Yet while their...
**Vaccine developments**

In addition to treating *C. trachomatis*, the vault vaccine platform could be used in other infectious diseases where mucosal immunity is needed. Rome and his colleagues used the chemokine CCL21-vault in a model of lung cancer, demonstrating that the particle has the threefold ability to release CCL21 in the tumour, attract antigen-presenting cells and activate a systemic cellular immune response against the tumour. With evidence strongly suggesting that the CCL21-vault is an effective cancer therapeutic, the researchers are hoping to move this vault into a phase 1 clinical trial by the end of next year. Moreover, they have recently established a partnership with University of California, Los Angeles’ Dr Otto Yang in order to develop vault vaccines for HIV.

**A KEY COLLABORATION**

It was Rome’s research into vaults that eventually led him into collaboration with Kelly. After attending one of Rome’s research seminars, an exciting idea occurred to Kelly: could vaults be used as a vaccine platform? Afterwards, she shared this theory with Rome – and he agreed that their relatively large, hollow morphology could make them an excellent tool for delivering biomaterials: “I thought that it would be a good idea to package an antigen into the particle and so we began working together to investigate the ability of vaults packaged with an immunogenic *C. trachomatis* protein to stimulate an immune response in mice,” he reveals.

In a research team composed of nanotechnologists and immunologists, Rome and Kelly pioneered a unique method for delivering immunity in *C. trachomatis*. They found that vault nanoparticles containing immunogenic proteins could act as ‘smart adjuvants’ for triggering protective immunity at mucosal surfaces while avoiding over-immunisation. For instance, after immunising female mice with recombinant vaults – with a component of *C. trachomatis* enclosed – and exposing them to a challenge infection, the researchers demonstrated that their reproductive tracts were protected from severe bacterial infection. Published in 2009, this initial study laid the groundwork for the development of a vaccine against *C. trachomatis*.

**A VAULTED FUTURE**

Rome, Kelly and their colleagues have successfully demonstrated that their engineered vaults induce protection in mouse models of *C. trachomatis* without causing hypersensitivity, marking a critical hurdle in the development of a vaccine against the infection. Looking ahead, they are eager to continue their investigations in this area, pushing the boundaries of vault technology and pursuing its translation from the laboratory to the clinic. The hope is that the wider scientific community will recognise the potential of the vault vaccine platform for a variety of infectious diseases and, consequently, that greater investment will be made in this exciting area.

**LEONARD ROME** is Distinguished Professor of Biological Chemistry in the David Geffen School of Medicine at UCLA. He was the Senior Associate Dean for Research in the School of Medicine from 1997 to 2012. He has been the Associate Director of the California NanoSystems Institute since 2004, and was Interim Director from 2007-09.

**KATHLEEN KELLY** is Professor in the Department of Pathology and Laboratory Medicine, co-Chair for Medical School Education in the David Geffen School of Medicine at UCLA and Associate Director of Clinical Chemistry for the Ronald Reagan UCLA Medical Center. Kelly is a recipient of the Young Scientist Award and past Chair of the Immunology Division for the American Society of Microbiology.