Advanced genetic screening method may speed vaccine development

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Kathryn Sykes is a researcher at the Biodesign Institute's Center for Innovations in Medicine, Arizona State University. Credit: The Biodesign Institute at Arizona State University

Infectious diseases -- both old and new -- continue to exact a devastating toll, causing some 13 million fatalities per year around the world.

Vaccines remain the best line of defense against deadly pathogens and now Kathryn Sykes and Stephen Johnston, researchers at Arizona State University's Biodesign Institute, along with co-author Michael McGuire from the University of Texas Southwestern Medical Center are using clever functional screening methods to attempt to speed new vaccines into production that are both safer and more potent.

In a recent study appearing in the journal *Proteome Science*, the group

used high-throughput methods to identify a modulator of immune activity that exists naturally in an unusual pathogen belonging to the *Poxviridae* family of viruses.

Parapoxvirus infection causes immune cell accumulation at the site of infection; direct screening in the host for this biological activity enabled the isolation of an immunomodulator—labeled B2. Indeed, B2 by itself causes immune cell accumulation at the site of skin injection. When added to a traditional influenza vaccine, B2 improves the vaccine's protective capacity. Furthermore, the immunomodulator also demonstrated the ability to shrink the size of cancerous tumors, even in the absence of any accompanying specific antigen.

In the past, the process of vaccine discovery involved the random selection of naturally attenuated strains of viruses and bacteria, which were found to provide protection in humans. Examples of this approach include the use of vaccinia to protect against smallpox and attenuated mycobacterium bovis (BCG) to protect against tuberculosis.

In recent years, many vaccines have been developed using only selected portions of a given pathogen to confer immunity. These so-called subunit vaccines have several advantages over whole pathogen vaccines. Genetic components that allow a given pathogen to elude immune detection for example may be screened out, as well as any factors causing unwanted vaccine side effects. Through careful screening, just those elements responsible for eliciting protective immune responses in the host can be extracted from the pathogen and reassembled into an effective, safer subunit vaccine.

In practice, the process of narrowing the field of promising subunit candidates from the whole genome of a pathogen has often been time consuming, laborious and perplexing. In the current study, their earlier-developed strategy, known as expression library immunization, is

extended to develop a scheme to find the protein-encoding segments—known as open reading frames (ORFs)—from a pathogenic genome that have any biological function of interest.

This simple, yet powerful technique uses the host's immune system itself to rapidly reduce any pathogenic genome (viral, fungal, bacterial or parasitic) to a handful of antigens capable of conferring protection in the host.

The advantage of this in vivo technique is that it offers a means of rapidly screening entire genomes, with the results of the search displaying desired immunogenic traits. The mode of entry of vaccines designed in this way closely resembles the natural infection process of host cells—an improvement over live attenuated vaccines.

This promising approach has been used effectively to engineer a vaccine against hepatitis and may provide a new avenue for the development of protective agents against pathogens that have thus far eluded traditional vaccine efforts, including HIV and ebola.

"We had developed a method for screening for protective subunits against a specific disease," Sykes says. "However this type of safer vaccine design is notoriously less potent than the whole pathogen designs. What we needed was a method to find generally useful vaccine components that would serve to enhance and control immunity."

The group chose the pathogen parapoxvirus ovis (known as the Orf virus) for the current set of experiments, in which expression library immunization techniques were used to screen for an immunogenic factor buried in the pathogen's genome.

Parapoxvirus ovis causes a highly infectious disease known as Orf, which is prevalent in sheep and goats and may be transmitted

cutaneously to humans handling these animals, causing pustular lesions and scabs.

Once the group had sequenced the full genome of parapoxvirus, PCR was used to amplify all the viral open reading frames, which code for all of the viruse's proteins. Each ORF, comprising a library of genomic components, was compiled into a unique high throughput expression construct, and these were randomly distributed into sub-library pools. These pools were directly delivered into sets of mice for in vivo expression. Functional testing for the activity desired identified B2 as the immune cell accumulator.

In further experiments, the team co-delivered B2L as an additive or adjuvant for an influenza gene vaccine, to see if it could improve survival rates in mice challenged with the influenza virus. The co-immunized mice indeed displayed full protection against influenza compared with 50 percent protection of the control group, immunized with influenza vaccine alone.

In addition to infectious agents like Orf, non-infectious diseases including cancer may be amenable to vaccine defense. Thus far however, the discovery of tumor-specific antigens has been frustrating. One approach may lie in using non-specific immunogenic factors like B2.

In the current study, two forms of cancer were investigated in a mouse model, following the administering of B2 alone, in the absence of a disease antigen. The experiments evaluated B2's ability to enhance survival and shrink tumor size. In the case of an aggressive melanoma, tumor size was significantly reduced and survival rate improved. Administration of B2 to an infection induced by a breast cancer cell line also showed a modest but measureable reduction in tumor size.

With the growing popularity of sub-unit vaccines, the need arises for

more effective adjuvants, which may be used to compensate for the reduced immunogenicity of such vaccines compared with their whole-pathogen counterparts. Techniques similar to those applied here to isolate and evaluate B2 could potentially permit the screening of virtually any genome for any gene-encoded activity testable in an organism.

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