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Emory University's Bali Pulendran and Dmitri Kazmin use systems biology to look for the 'molecular signatures' of the antibody response.

DRUG DEVELOPMENT

Searching for patterns

Tapping into big data could inform better vaccine design.

BY TOM PAULSON

Immunization is often hailed as one of medicine's greatest triumphs. Yet its success has been largely accomplished without fully understanding exactly how it works. Take, for example, one of humanity's best vaccines — YF-17D, which protects against yellow fever. A single immunization with this vaccine provides decades of almost complete protection, but until recently its mechanism was shrouded in mystery. It worked, but “nobody really knew why,” says Bali Pulendran, an immunologist at Emory University in Atlanta, Georgia.

That mystery can be traced back to the origins of the yellow fever vaccine. YF-17D was developed in the early twentieth century by Max Theiler, who in 1951 won the Nobel Prize in Physiology or Medicine for his work. Theiler relied on methods that barely departed from those used by Edward Jenner in his discovery of the smallpox vaccine in the eighteenth century. “Vaccine development has been a trial and error process,” says Pulendran. “We really

don't understand the basic mechanism of how vaccines produce immunity.”

The traditional approach to developing vaccines entails identifying the causative agent, killing or crippling it (for example by heating or irradiating it) and then injecting the inactivated virus or bacterium into animals or people, to produce an immune response — a sequence described as ‘isolate, inactivate and inject’. There are some more sophisticated variations on this theme, such as producing only the immunogenic proteins using recombinant genetics rather than inactivating a whole virus. But the empirical approach to vaccine development can hit a wall when confronting the trickiest pathogens.

“The search for vaccines against several incurable diseases, including AIDS, malaria, tuberculosis (TB) and dengue fever, has largely failed,” says Rafick-Pierre Sékaly, chief scientific officer at the Vaccine & Gene Therapy Institute of Florida in Port St Lucie, and his colleague Lydia Trautmann. This failure demands a new, more comprehensive and rational strategy.

Scourges such as HIV, TB and malaria are caused by microbes that have evolved to evade and undermine the immune response (see ‘Beating the big three, page S4’). “I don't think we will be able to develop vaccines against these three diseases without understanding a lot more about how the immune system works,” says Alan Aderem, an immunologist and president of Seattle Biomed in Washington, a non-profit organization that focuses on infectious disease research. Aderem is pursuing a less haphazard approach — called systems biology — that he and many other researchers are convinced will dramatically improve vaccine discovery and development¹.

TAMING THE DATA TORRENT

Systems biology is often described simply as the marriage of high-throughput computation and biological research, with the goal of sifting through massive datasets to look for emergent, often unanticipated, properties of genes, proteins or other factors as they interact in a cell

or organism. The aim is to measure a dynamic network of multiple biological interactions simultaneously without having to know in advance which single variable or process to focus on. Systems biology shows promise as a powerful strategy for dealing with the ever-increasing torrent of biological information. Proponents of systems biology hope that it will help researchers to identify hidden and complex interactions that can reveal new insights into what drives the immune response and find new targets for candidate vaccines.

In 2005, Pulendran and his colleagues chalked up the first big vaccinology win for systems biology when they used the approach to solve the mystery of the yellow fever vaccine. First, they vaccinated individuals with YF-17D. Next, they collected blood samples over time and analysed them to compare gene activity to immune response. What emerged was a network consisting of some 100 genes; this allowed the researchers to identify five key proteins (toll-like receptors) that trigger innate immune activity, which usually provides a rapid, non-specific response to invading pathogens². “That was the first demonstration of a vaccine mediating its effects by triggering such receptors in the innate immune system,” Pulendran says. Follow-up studies allowed the researchers to predict the magnitude of the antibody and T-cell responses to the vaccine, and hence its efficacy³.

More recently, Pulendran’s analysis of the biological response to the yellow fever vaccine has revealed a key insight into the vaccine’s mechanism. It turns out that the *GCN2* gene, which encodes a protein that is expressed in response to amino-acid deprivation, also promotes the T-cell response — involving ‘CD8’ or ‘killer’ T cells — that makes the yellow fever vaccine so effective⁴. “The *GCN2* link to immunity was not appreciated,” he says. “This is a good example of how the systems approach can give critical new insights you could not have anticipated.”

Pulendran’s analysis of the yellow fever vaccine was widely regarded as proof of concept for the systems biology approach. The National Institutes of Health (NIH), for example, cited the paper in 2009 in support of its US\$100 million Human Immunology Project Consortium, which focuses on applying systems biology to a broad spectrum of questions in human immunology. But Pulendran’s analysis was still a largely descriptive study of a vaccine that had already been proven to be effective. In that sense, this work offered only indirect evidence that systems biology could predict whether a new vaccine would offer protection. What are most needed to advance vaccine development are predictive findings.

Aderem, who worked with Pulendran on some of the YF-17D studies, says the findings support his belief that systems biology offers the best hope for deciphering the black box of immunity. “One of the clinical signs of

madness is doing the same thing over and over and expecting a different result,” he says. And when it comes to vaccine development, “we need a new approach.”

PICKING OUT PATTERNS

Aderem, Pulendran and others say we’re on the verge of entering a new phase of the study of immunological genomics — a more rational, systematic method of studying immunity and developing vaccines. Yet only a dozen or so labs worldwide are applying the systems approach to vaccine development.

Until recently, the field was stymied by lack of funding as well as by scepticism from scientists who regarded it as unproven and as a solution in search of a problem. One leading critic of systems biology — Nobel laureate and biologist Sydney Brenner — contends that it suffers from what might be called the ‘Big Data’ delusion. Brenner has repeatedly criticized the anti-reductionist approach of systems biologists because he believes it furthers the practice of collecting biological data without committing to a clear hypothesis or theory of action: “We are drowning in a sea of data and thirsting for knowledge,” he argues. “Most biology today is low input, high throughput, no output.”

The point, which others besides Brenner have also made, is that too many biologists today are merely sifting through reams of data hoping for magical conclusions. “There seem to be a lot of people around who think that if you measure absolutely everything, somehow

the truth will jump out and punch you on the nose,” says Nobel laureate and biochemist Tim Hunt. “Experience suggests that it’s more of a recipe for confusion.”

But the yellow fever vaccine analysis

showed that systems biology can offer new ways to manage and probe massive datasets, and was therefore a watershed moment for the field. The NIH initiative, Aderem believes, is a sign that scepticism is on the decline. Critics of systems biology, he says, tend to not give enough weight to the potential power of this approach to identify patterns and networks at work within the cell without having to commit to a specific theory of action or starting hypothesis. Systems biologists, according to Aderem, are not testing the mechanism of a specific antigen or protein; rather, they are looking across the hundreds or even thousands of interactions of different kinds that comprise the immune response to identify a pattern of gene or protein behaviours that hint at basic mechanisms. “It is an unbiased approach to trying to understand a biological system, starting with no preconceptions,” says Daniel Zak, a principal scientist at Seattle Biomed who is working with Aderem as well as researchers at

the University of Cape Town, South Africa, to try to figure out how *Mycobacterium tuberculosis* infection moves from a non-disease state known as latency to full-blown disease.

Zak’s study began in 2010 in collaboration with a team led by Willem Hanekom, director of the South African Tuberculosis Vaccine Initiative. The researchers recruited 6,000 adolescents, half of whom were infected with latent TB. Blood was collected from these adolescents every six months. After two years, 50 of the 6,000 adolescents had developed the disease. The Seattle–Cape Town team is using systems analysis techniques to identify and track changes in gene expression and protein activity in the cells of 150 adolescents — some who developed the disease and some who did not. The scientists have been sifting through the blood samples, looking for any odd patterns. The first clue may come from a novel chain of events carried out by white blood cells. Or it might be a unique pattern of protein–protein interactions, or gene activity. In this case, the first signature of TB disease found by Zak’s team turned out to be a particular pattern of gene activity.

“We are trying to identify which genes are turned on in the blood of people who progressed to active disease,” Zak says. “We are seeing predictive changes.” That is, the researchers have found that this unique pattern of gene activity indicates that a person infected with latent TB is progressing to active disease. These biomarkers, he says, could lead to the development of better drugs or a better TB vaccine candidate by identifying multiple key targets within these networks.

“The problem with drugs is they usually have a single target,” Aderem says. “If we can hit an entire network, or critical nodes in a network, it’s much harder for the bug to get around the treatment.”

Pulendran’s team has used systems biology to assess the seasonal influenza vaccine for any signatures that might predict an antibody response⁵. The team compared two flu vaccines — a trivalent inactivated vaccine and a live attenuated vaccine — and found signatures that predicted the human antibody response to the vaccine. Ultimately, Pulendran says, systems biology could help researchers to identify a universal biomarker that predicts the antibody response and reduces much of the guesswork that currently plagues the seasonal preparation of the influenza vaccine. “That would be tremendous advance.” ■

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