BOOSTING OUR BEST SHOT

Illustrations by Marina Corral

Vaccines work by training the immune system to target pathogens, but many types of shots need added substances called adjuvants to elicit a robust response. Despite the power of adjuvants, only one, called alum, is approved in the US. **Charlotte Schubert** looks at recent discoveries that could translate into a wider range of adjuvants and perhaps help provide future protection against diseases ranging from malaria to H1N1 'swine' flu.

Max Theiler never thought it would be easy to vanquish one of the biggest killers of his time. Yellow fever had already stumped a previous generation of microbe-hunters. And in the early 1900s it killed subjects who volunteered for experiments in which they received bites from mosquitoes, proving that the insects transmit the disease.

Theiler's work was painstaking. He spent more than a decade isolating the yellow fever virus, growing different strains in test tubes, mouse brains and chicken embryos, and testing various imperfect versions of the vaccine in animal experiments. Ultimately, his persistence paid off. In 1937, he finally hit upon a version of the yellow fever virus that did not itself cause disease but could be injected into people as a vaccine. The achievement won Theiler a Nobel Prize, and the vaccine is still one of the most effective ones known: one shot will protect against yellow fever for more than 30 years.

Despite his arduous path, Theiler was lucky. His vaccine is based on a live virus—one particularly good at bumping up the immune response. By comparison, most vaccines developed today rely on bits of microbes, such as short protein sequences—and they don't work quite so well on their own. To elicit an immune response, these vaccines typically need a jolt from an adjuvant, a substance named from the Latin 'adjuvans', which means 'to help'. But only one adjuvant, based on aluminum salts, is approved for use in the US. Dubbed alum, it spikes common vaccines such as shots for hepatitis B and tetanus.

New adjuvants, say researchers, have the potential not only to improve existing vaccines but also to quell diseases that, more than half a century after Theiler conquered yellow fever, still lack an effective jab. And adjuvants may help provide protection against the H1N1 pandemic 'swine flu' virus—as vaccine production faces limitations, adjuvants based on oil and water emulsions (such as one made by Novartis dubbed MF59) have the potential to increase vaccine potency and also to stretch the supply by reducing dosage. Whether the FDA is poised to give the green light to such adjuvanted vaccines, lined up for approval in Europe, remains unclear (see sidebar).

For many years, researchers such as Theiler moved their vaccine candidates forward with little mechanistic understanding of how they worked. That empirical approach is ending, says Bali Pulendran, an immunologist at the Emory Vaccine Center in Atlanta, and it's a boon to the development of new adjuvants.

"What we are now seeing in the adjuvant field is immunology and vaccinology coming together," says Pulendran. Research in his lab, for instance, is finally beginning to unravel how the yellow fever vaccine builds such a marvelous immunological defense without an adjuvant—work that could lead to new ways to augment other vaccines. Other researchers are applying findings from immunology to intelligent design of adjuvants. And just last December, malaria researchers made a splash with positive results in a trial of the RTS,S vaccine, which contains a carefully chosen adjuvant[1,2].

Scientists are getting steadily closer to their ultimate goal of making adjuvants into ideal helpers, intelligently tailored to the specific needs of each vaccine. "The dark ages are coming to the end," says Pulendran.

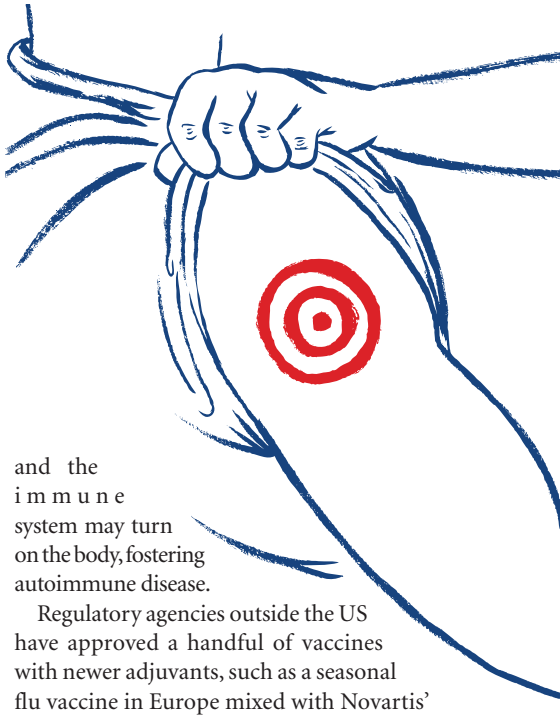
Safety first

Despite the excitement about the emerging science of how adjuvants work, researchers developing new vaccines face major regulatory hurdles. "The FDA [US Food and Drug Administration] is risk averse—particularly in their response to the safety of vaccines," says Ripley Ballou, who currently serves as deputy director for vaccines at the Bill and Melinda Gates Foundation and previously researched the RTS,S malaria vaccine.

But, paradoxically, it is because of safety concerns that vaccines often require an adjuvant. For example, although laboratory experiments suggest that a vaccine built using a live, attenuated version of HIV might generate decent protection, such a live vaccine is unlikely to receive regulatory approval because of safety concerns. So vaccine developers generally err on the side of caution and create vaccines using building blocks such as lifeless protein fragments from microbes, which are also generally cheaper and easier to manufacture in a standardized way.

The catch is that most nonliving vaccines are too weak on their own and therefore need an adjuvant. The parts of the vaccine based on the offending microbe, the antigen, instruct the body to attack the microbe. But it's the adjuvant—mixed with antigen, welded to it or positioned together with the antigen in a tiny, spherical particle—that can amplify the immune response and make the vaccine more potent.

Herein lies the rub: adjuvants work by boosting the immune system: Boost it too much and the immune system may turn on the body, fostering autoimmune disease.



Regulatory agencies outside the US have approved a handful of vaccines with newer adjuvants, such as a seasonal flu vaccine in Europe mixed with Novartis' adjuvant MF59, an emulsion of oil in water. MF59 boosts protection against flu in older people, who have relatively frail immune systems, although it's still unclear exactly how it achieves this.

The FDA's view toward new adjuvants will be tested as early as this fall, when initial clinical data on adjuvanted pandemic H1N1 vaccine becomes available (sidebar). An advisory group for the agency is also scheduled to consider on 9 September whether to recommend approval of Cervarix, a cervical cancer vaccine available in Europe that is produced by GlaxoSmithKline (GSK). That vaccine contains an adjuvant developed by GSK dubbed AS04, containing alum plus monophosphoryl lipid A (MPL), which stimulates the immune system in a distinct way.

Last year, the FDA put on hold clinical trials of a version of the hepatitis B vaccine containing an experimental adjuvant, dubbed CpG oligonucleotides. One subject in a clinical trial of the vaccine, developed by Dynavax Technologies Corporation, a biotechnology company based in Berkeley, California, developed a severe autoimmune disease—Wegener's granulomatosis, in which blood vessels become inflamed.

More than 2,500 people have received Dynavax's experimental vaccine in clinical trials, and the illness may be due to statistical chance. Nonetheless, the FDA is reviewing the full analysis of the event before deciding whether trials can proceed. Notably, Dynavax's vaccine improves on an already available vaccine for hepatitis B, setting a high bar for FDA approval. "It enters in the inevitable risk-

Illuminating alum

Alum, the world's most widely used adjuvant, got its start in the 1920s when vaccinologists found that mixing it into their preparations gave a boost to the diphtheria vaccine. Researchers then proposed that it worked by glomming onto vaccine components, causing them to be released slowly into the bloodstream.

That theory held for 80 years. It is only now falling by the wayside, as immunologists get their hands on alum, a term for various immune-activating aluminum salts.

"Alum has been used in billions of doses since 1920," says Rino Rappuoli, head of research for Novartis Vaccines in Sienna, Italy, "and, up until about a year ago, we had no clue about the mechanism of action."

A 2008 study by Richard Flavell at Yale University in New Haven, Connecticut and his colleagues helped to crack open alum's secrets. Their research suggests that alum activates a complex of proteins in immune cells dubbed the 'inflammasome'. Mice lacking the inflammasome did not respond to alum, although other adjuvants still worked (*Nature* **453**, 1122–1126; 2008).

In response to alum, the inflammasome seems to cleave and activate certain immune-stimulating proteins, such as interleukin-1β and interleukin-18. These proteins are thought to subsequently bump up the body's production of antibodies.

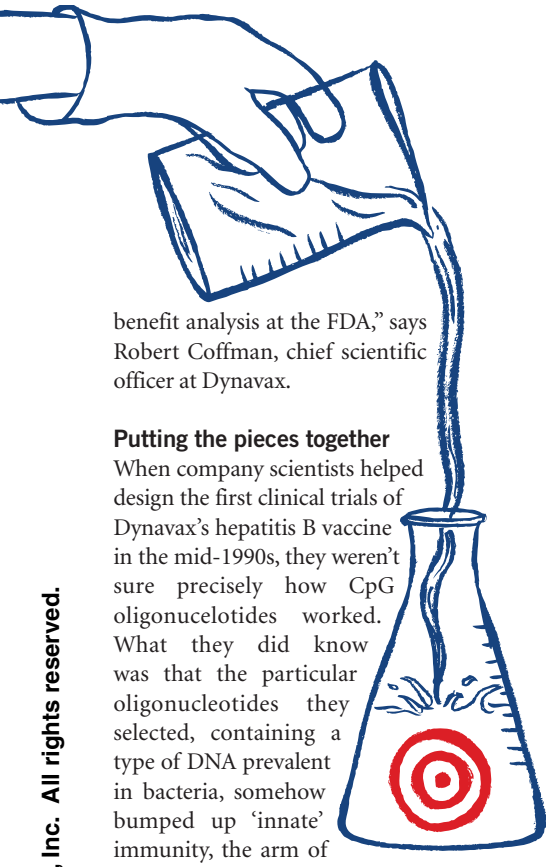
The new findings from Flavell and his colleagues, along with other studies implicating the inflammasome, potentially explain why alum is good at prompting an antibody response. A separate study in 2008 suggested that alum may initiate this response partly through the induction of cellular damage. Cells damaged by alum were shown to release uric acid, a known trigger of the inflammasome (*J. Exp. Med.* **4**, 869–882; 2008).

But subsequent research has muddied the waters. For instance, other immunologists, such as Philippa Marrack at the University of Colorado Health Sciences Center in Denver, have found evidence that alum works just fine as an adjuvant in the absence of inflammasome activity (*Eur. J. Immunol.* **38**, 2085–2089, 2008; *Nat. Rev. Immunol.* **4**, 287–293; 2009).

"In trying to understand the molecular mechanism of adjuvants, we will find a lot of redundant mechanisms, so I'm not surprised there is a controversy," says Rappuoli.

Marrack and other immunologists are using standardized reagents to help work out the discrepancies, which may have arisen because of differences in alum formulation and the analysis of the immune response. Ultimately, researchers may be able to design more effective alum-based adjuvants, she says.

Charlotte Schubert, Washington DC



benefit analysis at the FDA," says Robert Coffman, chief scientific officer at Dynavax.

Putting the pieces together

When company scientists helped design the first clinical trials of Dynavax's hepatitis B vaccine in the mid-1990s, they weren't sure precisely how CpG oligonucelotides worked. What they did know was that the particular oligonucleotides they selected, containing a type of DNA prevalent in bacteria, somehow bumped up 'innate' immunity, the arm of the immune response that acts a first responder to microbes.

The extra boost from the adjuvant resulted in a more rapid and potent antibody response than the currently approved hepatitis B vaccine. Their trials also showed that recipients require only two shots, in contrast with the three for the current vaccine[3].

Now, says Coffman, "we have backfilled our understanding." That understanding has emerged largely from years of basic research by immunologists revealing how the body responds to microbes.

CpG oligonucleotides bind and activate Toll-like receptor 9 (TLR9), a member of a family of receptors that recognize the components of microbes. These receptors stud the membranes of immune cells such as dendritic cells, which alert the body to foreign pathogens. Dendritic cells activate the immune response and help tailor it to the type of microbe—whether virus, bacterium or parasite.

People have at least 11 Toll-like receptors, each one recognizing distinct substances. For instance, MPL, part of the adjuvant in Cervarix, activates TLR4.

In fact, says Pulendran, TLRs are also what give the yellow fever vaccine much of its power. In 2006, Pulendran and his colleagues showed that the yellow fever vaccine activates four different Toll-like receptors, which, in turn, activate a range of dendritic cell subtypes, each with its own distinct booster effect on the immune system[4].

"What we find is that there is this remarkable synergy in the immune response," says Pulendran. Activating one Toll-like receptor tweaks the immune response, but activating certain pairs of Toll-like receptors can more than double the effect.

Pulendran has applied this principle most recently to create experimental vaccines against the H1N1 'swine flu' virus and the H5N1 'bird flu' virus.

His lab created an adjuvant containing two TLR activators: MPL and a synthetic TLR7 ligand. They also paid attention to what vaccinologists call the 'delivery vehicle'. They encapsulated the vaccine in a nanoparticle, using polylactic glycolic acid, which aids in the immune response.

By combining two TLR activators, the researchers could radically decrease the dose of H5N1 antigen required in mice. In as yet unpublished work, 0.1 microgram of antigen, when combined with their experimental adjuvant, had the same effect as 10 micrograms of antigen combined with alum. The experimental adjuvant "beat alum, hands down," says Pulendran. He adds that in preliminary experiments it also elicited a more robust, longer-lasting response than alum.

Other researchers have observed a similar synergistic effect with multiple TLR activators, including Steve Reed, head of research and development at the Infectious Disease Research Institute, a Seattle-based nonprofit that fosters vaccine development for diseases such as tuberculosis, HIV/AIDs and malaria.

"We are pioneering the next generation of TLR ligands," says Reed, "it's a very aggressive

Swine flu agitates the adjuvant debate

US regulatory agencies will face a big decision, as early as this fall, as to whether to approve the use of adjuvants for pandemic H1N1 flu vaccine on an emergency basis.

Adjuvants used in some seasonal flu vaccines in Europe have the potential to boost the effectiveness of pandemic H1N1 vaccine and also substantially reduce dosage—thereby stretching supplies. If clinical trials underway bear out such advantages, the US will have to balance its concerns for safety against the benefits, particularly given the short supply of vaccine worldwide.

"If they decide to not use adjuvants, there is no doubt it will have a big impact on the supply of H1N1 vaccines globally," says Ripley Ballou, deputy director for vaccines at the Bill and Melinda Gates Foundation, based in Seattle, Washington. The only adjuvant currently used in the US is alum, whereas the adjuvants typically paired with flu vaccines are oil and water emulsions—such as Novartis' MF59, which is used in seasonal flu vaccines for the elderly in Europe.

There is clearly pressure to stretch the supply when it comes to pandemic H1N1 influenza vaccines. Ballou says his 'back of the envelope' calculations suggest the US is snapping up 40% to 50% of the world's supply, an estimate other public health experts say is reasonable. That is based on a worst-case scenario, in which two shots are required of a standard 15-microgram dose without adjuvant.

Given the potential of adjuvants to shrink the dosage, The World Health Organization has recommended their use in pandemic H1N1 flu vaccine.

The European Medicines Agency has a mechanism to quickly approve adjuvanted vaccines with novel antigens, such as H1N1, on the basis of presubmitted efficacy and safety data of vaccines with other antigens. Novartis and GlaxoSmithKline, for instance, are lined up to submit applications for pandemic H1N1 vaccine under this accelerated process.

The tone at the US Food and Drug Administration (FDA) seems to be more cautious. One reason the US treads more carefully than Europe is that the climate in the US tends to be "more litigious, with a very active antivaccine lobby," says Amesh Adalja, a fellow at the Center for Biosecurity in Pittsburgh, Pennsylvania and a physician at the University of Pittsburgh Medical Center.

The FDA has the authority to approve 'emergency use authorization' with input from other agencies such as the US National Institutes of Health (NIH). But in a July meeting of the committee that advises the FDA on vaccines, Norman Baylor, director of the agency's Office of Vaccines Research and Review said than an unadjuvanted H1N1 shot is "the most expeditious pathway for providing a safe and effective vaccine to the public."

program." Researchers there have combined oil-in-water emulsions with various TLR activators, such as one that activates TLR4 and another that activates TLR7 and TLR8. In unpublished experiments, Reed says, they have found that the combinations of TLR activators give extra zip to experimental vaccines for tuberculosis and malaria (tested in primates) and leishmaniasis (tested in dogs).

Vaccine researchers praise the Seattle institute for a program it operates in collaboration with the World Health Organization that makes standardized adjuvants, ready for clinical use, available to outside researchers and vaccine manufactures in developing countries. More than 40 researchers have used the service, which bypasses the difficulties some face in getting adjuvant from companies, says Reed.

But getting new adjuvants such as Reed's and Pulendran's, with two TLR activators, into vaccines for everyday use is a long shot, cautions Dynavax's Coffman. "There is always the potential for complications," he says. "You might synergize with regards to efficacy but you might also synergize with regards to side effects. If you can get the type of response you want with a single agent, you might be better off."

Moving ahead with malaria

Safety has not been an issue to date with RTS,S, an experimental vaccine against malaria that is formulated with new-generation adjuvants, such as oil-and-water emulsions. The vaccine has been tested in more than 4,000 infants and children; recently, a study of 340 infants showed a safety profile comparable to the standard hepatitis B shot[1].

Developing the right adjuvant also was central to getting a vaccine that, after more than two decades of development, has begun to look promising. In his first years working on malaria vaccines, says Ballou, he and his colleagues could barely get an immune response. Alum just didn't cut it.

Alum is good at fostering an antibody response, but antibodies, found Ballou, were not sufficient to thwart the malaria parasite. Antibodies are decent at eliminating microbes in the bloodstream, but the malaria parasite worms its way to the liver, where it buries itself inside cells.

To get at the buried parasite, Ballou and his colleagues at the Walter Reed Army Institute of Research reasoned that they had to bump up the activity of T cells, which can kill parasites inside cells. The breakthrough came in 1995, when the researchers began testing experimental adjuvants produced by GSK, which had begun to take these substances seriously.

"GSK had made a conscious decision that one of the new technologies that would open the potential for new vaccines was the development of new adjuvant platforms," Ballou says. "This was the very beginning." After screening nine GSK adjuvants in animals, he and his colleagues tested three on human volunteers. They injected volunteers with the adjuvant mixed with RTS,S, which is based on a malaria peptide, and then exposed them to mosquitoes infected with the parasite. (That's an approach Ballou had subjected himself to in a previous study, where he unfortunately ended up getting infected and having to down a dose of antimalaria pills).

In 1995, the researchers found that one adjuvant, dubbed AS02, worked particularly well—it protected six out of seven volunteers bitten soon after their shot. And it produced not only a strong antibody response but also a robust T cell response[5].

That adjuvant consisted of an oil-in-water emulsion, which bumped up the antibody response, along with two substances—MPL and another immune-stimulating agent, QS21—that not only further enhanced the antibody response but also promoted the robust activity of T cells. That original adjuvant has since been updated; the emulsion was replaced with lipid vesicles called liposomes, creating a particle that gives the adjuvant an extra boost. This adjuvant, dubbed AS01, was used in phase 2 trials of the RTS,S vaccine recently conducted in Africa.

Using this updated vaccine, Ballou and his colleagues last fall reported an approximately 50% reduction in incidence of malaria in children who received the shot compared to those who did not[2]. This formulation of the vaccine is now in phase 3 trials.

Still, the US government is clearly keeping the option of adjuvants open: in May and July, the country's Department of Health and Human Services arranged to buy nearly $500 million worth of MF59 from Novartis and more than $200 million of a similar adjuvant, AS03, from GlaxoSmithKline. (If approved, the adjuvants would be mixed with the flu antigen at the site of vaccine administration.)

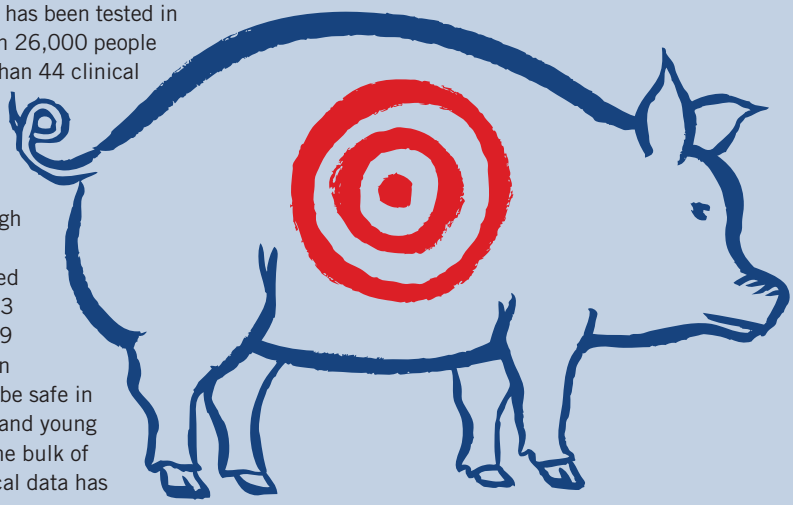
Dozens of studies with H5N1 vaccine for avian influenza have also shown a strong 'dose sparing' effect. Experiments with AS03 suggest that it could reduce the dose thought to be needed for protection in people from 90 micrograms to as little as 3.8 micrograms (*Curr. Opin. Mol. Ther.* **3**, 337–345; 2009). Adjuvanted flu vaccines can also provide stronger, longer-lasting protection.

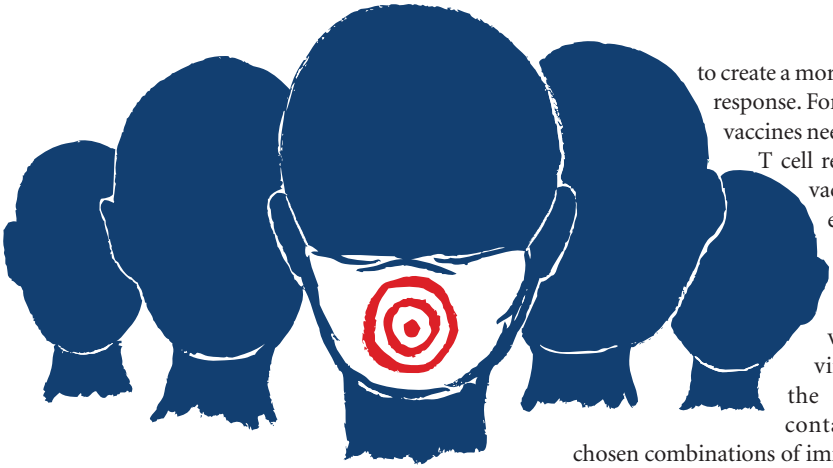
The decision of whether to use adjuvants will depend partly on results of such trials. The NIH, for instance, is initiating two clinical trials of H1N1 vaccine using AS03; Novartis, meanwhile, began trials in July with MF59 and expects early results in late September or early October, says Rino Rappuoli, head of research for Novartis Vaccines in Sienna, Italy. But the deliberation of long-term safety will have to incorporate data from other vaccines, such as seasonal flu jabs containing MF59 and AS03.

Both of these adjuvants "have an excellent safety profile in literally millions of doses," says Steve Reed, head of research and development at the Infectious Disease Research Institute in Seattle. MF59, for instance, has been tested in more than 26,000 people in more than 44 clinical trials, says Reed.

Although vaccines adjuvanted with AS03 and MF59 have been found to be safe in children and young adults, the bulk of the clinical data has been acquired in older adults. Given that, the US will consider using adjuvanted pandemic flu vaccine in children in "only extreme circumstances," in a scenario in which the unadjuvanted vaccine does not work, says Anthony Fauci, director of the US National Institute of Allergy and Infectious Diseases.

Charlotte Schubert, Washington, DC





"This is a great example of the approach for a new generation of vaccines," says Reed. "RTS,S would not have worked with alum or emulsions alone."

Back to the basics

Scientists are only just beginning to understand how commonly used adjuvants such as alum work (sidebar). Meanwhile, some researchers have gone back to the basics. They emphasize that potent, traditional vaccines such as yellow fever still have a lot to teach us. Ultimately, understanding how simple vaccines can work without adjuvants might shed light on the type of immune response that adjuvants need to create.

Last winter, Pulendran and other researchers applied large-scale gene analysis technology to come up with a gene 'signature' showing which genes are activated in people with an effective immune response to the yellow fever vaccine[6,7]. The studies are a "landmark in this field," says Coffman.

Genes for several other immune cell receptors, in addition to Toll-like receptors, popped up in the analysis and were found to mediate the response to the vaccine. Pulendran's group also found that the vaccine turned on the 'stress response', which readies the body for trouble such as oxygen deprivation or viral infection.

"It was surprising," says Pulendran. "I spent hours on PubMed reading up on this stress response protein I had never before heard of." Perhaps, he says, clues to the next generation of adjuvants could emerge from such data. Other clues could also arise from even more basic research into how the immune system deals with microbes.

Pulendran is currently applying for funds to the NIH to be part of a project to generate databases on how various vaccines and adjuvants affect the human immune system, using in part large-scale genomics approaches, now being ramped up in many vaccine labs.

In the future, Pulendran and other researchers envision, vaccinologists may be able to exactly tailor the adjuvant to each vaccine to create a more specific immune response. For example, malaria vaccines need to elicit a strong T cell response, and HIV vaccines may need to elicit an immune response in the mucosa of the rectum and the vagina, where the virus first contacts the body. Vaccines containing smartly chosen combinations of immune-stimulating agents could guide the immune response in the right direction.

Researchers caution that there is a long way to go before vaccinology is a precise science. "I think we are not nearly as smart as we like to think at predicting what type of adjuvant will work best for a type of vaccine," says Coffman.

"There is still a lot of trial and error," says Ballou. But he adds that things are getting better. "We are still relatively crude at this, but we are much better at it than we were, say, ten years ago," says Ballou.

Immunologists such as Pulendran are optimistic that some as yet undiscovered molecule could help researchers come closer to the vision expressed by the chairman of the Nobel Committee, Hilding Bergstrand, when he introduced Max Theiler before his Nobel lecture in 1951:

"Dr. Theiler's discovery gives new hope that in this manner we shall succeed in mastering other virus diseases, many of which have a devastating, effect and against which we are still entirely powerless."

Charlotte Schubert is senior editor for News and Views and is the Washington, DC correspondent for Nature Medicine.

1. Abdulla, S. *et al. New Engl. J. Med.* **359**, 2533–2544 (2008).
2. Bejon, P. *et al. New Engl. J. Med.* **359**, 2521–2532 (2008).
3. Halperin, S.A. *et al. Vaccine* **24**, 20–26 (2006).
4. Querec, T. *et al. J. Exp. Med.* **203**, 413–424 (2006).
5. Stoute, J.A. *et al. New Engl. J. Med.* **336**, 86-91 (1997).
6. Querec, T. *et al. Nat. Immunol.* **10**, 116–125 (2009).
7. Gaucher, D. *et al. J. Exp. Med.* **205**, 3119–3131 (2008).

Key TLR-independent adjuvants in development for prophylatic vaccines

Name	Company	Indication
Alum	various	various
AS03	GSK	Pandemic influenza
MF59	Novartis	Influenza
Provax	Biogen Idec	N/A
Montanide	Seppic SA, Bioven, Cancervax	Malaria, cancer
TiterMax	CytRx	N/A
Advax	Vaxine Pty	various, including Hepatitis B, influenza, rabies, and cancer immunotherapy
QS21	Antigenics	various, including melanoma, malaria, HIV
Quil A	Statens Serum Institute	various
Iscom	various, e.g. CSL, Isconova	various, including influenza
Liposomes	various, e.g. Crucell, Nasvax	various

Key TLR-dependent adjuvants in development for prophylactic vaccines

Ampligen	Hemispherx	Pandemic influenza
AS01	GlaxoSmithKline	Malaria, tuberculosis
AS02	GlaxoSmithKline	Malaria (Mosquirix), tuberculosis, HBV, HIV
AS04	GlaxoSmithKline	various, including Fendrix (HBV) and Cervarix (HPV)
MPL RC-529	Dynavax	Supervax (HBV)
E6020	Eisai/Sanofi Pasteur	N/A
TLR-technology	VaxInnate	Influenza
PF-3512676 (CpG 7909)	Coley/Pfizer, partnered with Novartis for some indications	various, including HBV and influenza (Novartis)
ISS	Dynavax	HBV (Heplisav), influenza
IC31	Intercell	various, including influenza, tuberculosis, malaria, meningitis, and cancer indications

APC, antigen-presenting cells; EBV, Epstein-Barr virus; HBV, hepatitis B virus; HPV, human papillomavirus; HSV, herpes simplex virus; MPL, monophosphoryl lipid A; N/A, not available; NSCLC, non small cell lung cancer; O/W, oil-in-water; RSV, respiratory syncytial virus; TLR, toll-like receptor

Source: *Vaccine Adjuvants-Uncertainties Rule*, Datamonitor, MedTRACK (2008), Thomson Pharma (2008)