FOREWORD

FOCUS CONTENTS

Vaccine renaissance

Vaccines are one of the most useful and cost-effective tools for reducing the morbidity and mortality that are associated with infectious diseases. Here, Jeffrey Almond discusses the selection of articles in this Focus issue, in the context of the challenges and opportunities facing vaccine developers today.

Jeffrey W. Almond
sanofi pasteur, 1541 Avenue Marcel Mérieux, 69280, Marcy L'Etoile, France.
e-mail: Jeffrey.Almond@sanofipasteur.com

The devotion of this Focus issue of *Nature Reviews Microbiology* to topics in vaccinology is timely. Vaccine research and development has been quietly undergoing a renaissance in recent years, fuelled by various factors. Commercially, over the past decade, although representing only ~2% of the overall pharmaceuticals business, the vaccines sector has outperformed most of the rest of the industry in terms of revenue growth. This growth includes the introduction of Prevnar, a 7-valent pneumococcal glycoconjugate, the first vaccine to achieve 'blockbuster' status in the pharmaceuticals marketplace. The recently licensed human papilloma virus (HPV) vaccines, Gardasil and Cervarix, as well as offering major contributions to human health[1], are likely to achieve even bigger sales. There will also be significant contributions to future growth from the tetravalent meningitis vaccine Menactra, the two new rotavirus vaccines Rotarix and Rotateq, and the herpes zoster vaccine Zostavax. Through the launch of such products, the vaccines sector is throwing off its dowdy image as a litigation-ridden, low-cost, low-profit industry, which is traditionally eschewed by most of the pharmaceutical industry. Indeed, recent corporate acquisitions testify to the renewed interest in vaccines from the traditional 'big pharma' players.

A recent revival

The recent rejuvenation in vaccine research makes sense in a world in which healthcare authorities are increasingly recognizing the benefit and cost-effectiveness of vaccines. Prevention rather than cure of disease must be the highest objective of any healthcare policy. It is recognized that relieving the infectious disease burden is one of the biggest factors in enabling developing countries to escape from poverty, and vaccines are the most cost-effective way of achieving this goal. Indeed, protection from vaccine-preventable diseases is increasingly being recognized as a right of all individuals, regardless of their economic status. The 1999 establishment of the Global Alliance for Vaccines and Immunization (now called the GAVI Alliance) has done much to boost the use of vaccines in many parts of the world. Since 2000, GAVI Alliance resources have enabled the poorest countries to introduce additional vaccines (for example for hepatitis B, *Haemophilus influenzae* B and yellow fever) and increase access to basic childhood vaccines (including vaccines for polio, diphtheria, tetanus, pertussis and measles) for an estimated 138 million children who would otherwise have been left unvaccinated.

Another significant new player is the Bill & Melinda Gates Foundation, which has declared war on poverty-related diseases and provided substantial funding for research and development. To this can be added significant increases in public investment, fuelled in part by concerns over bioterrorism and the possible emergence of a pandemic influenza virus. The US government in particular has increased funding for vaccine-related research through its Project BioShield and National Strategy for Pandemic Influenza, in which the supported activities range from securing the supply of existing vaccines and improving production technologies, to researching new vaccine approaches. This includes, for example, research into vaccines that might provide multi-seasonal and perhaps cross-subtype protection against influenza.

The revival of interest in vaccines has of course been underpinned by a rapidly expanding body of knowledge in the fields of microbial pathogenesis and immunology. For example, the search for protective antigens using 'reverse vaccinology'[2] — the rationale of mining bacterial genomes for potentially protective antigens through bioinformatics and then testing each candidate in a relevant animal model — has provided candidate antigens for vaccines against a range of pathogens, including *Neisseria meningitidis*, *Chlamydia pneumoniae*, group A and B streptococci, *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*[3,4]. Variations on this theme, for example using signature-tagged mutagenesis[5] to identify genes that are essential for pathogenesis *in vivo*[6] or epitope libraries to identify those antigens against which antibodies are elevated in human convalescent sera[7], have added focus to this search.

The development by Wyeth of the vaccine Prevnar, as well as being a commercial success, was also a technological triumph. Conceptually, the approach was not

new; pneumococcal polysaccharide vaccines had been known for many years to be effective in certain age groups, and conjugation of bacterial polysaccharides to protein carriers incorporating epitopes for T helper (T_H) cells was known to improve immunogenicity in non-primed (young) individuals and stimulate stronger immunoglobulin G (IgG) responses and immunological memory. Nevertheless, this is an effective vaccine and after just a few years of use the benefits have exceeded expectations. Not only is the vaccine highly effective at protecting recipients, it also provides significant herd immunity[8], thereby significantly reducing the burden of pneumococcal disease in non-vaccinated individuals, even among the elderly. Indeed, it is estimated that the herd immunity effect has so far prevented more disease than direct vaccination has. True, there has been some evidence for the emergence of serotypes not present in the vaccine (such as serotype 19A), but so far this has been of minor significance compared with the protection provided by the vaccine. The race is now on for the development of 11-, 13- and even 15-valent glycoconjugate vaccines that could provide even broader protection, target emerging serotypes and be more relevant to the predominant circulating serotypes in developing regions, where the disease burden is highest. So far, the success of this glycoconjugate strategy has not diminished parallel efforts to find a protein-based pneumococcal vaccine that could potentially offer even broader protection and be produced in a more cost-effective manner. A protein-based vaccine could be a competitor to the glycoconjugate vaccines in the years ahead.

Improving on nature

One striking and probably crucial finding in the development of the new HPV vaccines Gardasil and Cervarix was the observation that these vaccines induce far higher levels of antibodies than are induced by natural HPV infection[9]. This might be related to the dose of antigen and adjuvant, and to the fact that in natural infection, HPV late proteins are expressed exclusively in terminally differentiated epithelial cells that are distal from sites of active immune surveillance. The possibility of 'improving on nature' — that is, to provide an immune response by vaccination that is quantitatively and perhaps qualitatively different from that induced by natural infection — is an exciting challenge in the industry and could be the key to developing vaccines against a range of target diseases, including malaria, tuberculosis, meningitis B and chlamydia, and possibly also to providing multi-seasonal and cross-subtype influenza vaccines. The ability to stimulate and/or manipulate the immune system to achieve this goal is becoming a reality, following the recent increase in our understanding of the control mechanisms in the immune system, and of the interplay between the innate and acquired immune responses.

In particular, the role of Toll-like receptors (TLRs) in recognizing pathogen-associated molecular patterns (PAMPs), and the ability to stimulate these receptors using a range of new agonists of varying specificities has revolutionized the adjuvants field, which has been shrouded in empiricism and developmental conservatism for too long. This topic is developed in the article by Guy, who argues for the 'perfect mix', a reference to the combination of antigen and immunostimulant, and the formulations that allow optimal immune responses. He points out that as health authorities push for well-defined, purified vaccines, and even vaccines with minimalist compositions, it is increasingly unlikely that any natural PAMPs will be present as part of the antigen preparation, unlike the situation with the traditional cruder vaccine preparations of years ago. It is therefore becoming necessary to add back immunostimulants in the form of TLR agonists that have been formulated to ensure the delivery of antigen and stimulant to the same endosomal cargo in antigen-presenting cells. New adjuvants also offer the prospect of dose-sparing, which might become crucial for vaccines for which there could be global shortages, such as a pandemic influenza vaccine, and dose schedule reduction. Dose schedule reduction would be attractive for the traveller market (where immune protection needs to be acquired quickly), for use in the developing world (where follow-up for second, third and fourth doses is often less than optimal) and for improving effectiveness in poor responders such as infants and the elderly. But if we get it right, why stop at prophylaxis? The principles of immuno-manipulation could be used to redirect the immune response in the therapy of chronic diseases and in immune disorders such as allergy and autoimmunity.

"The possibility of 'improving on nature' — that is, to provide an immune response by vaccination that is quantitatively and perhaps qualitatively different from that induced by natural infection — is an exciting challenge in the industry…"

The article by Kaufmann delves provocatively into which immune responses are required to combat different bacterial pathogens, giving examples for which simple stimulation of pre-existing antibodies is protective (Level 1), for which strong cellular responses also need to be induced (Level 2) and for which the holy grail of sterilizing immunity seems to be required for efficient protection (Level 3). Examples in all three categories expand on the idea of developing vaccines by improving on the responses provoked by natural infection. He also provides a laudably concise and up-to-date account of the complexity of T- and B-cell function and regulation, and discusses the need to balance T_H1 and T_H2 responses, depending on the pathogen. He also discusses recent work identifying the roles of T_H17 cells,

programmed death 1 (PD-1, also known as PDCD1) and regulatory T cells in modifying immune responses that could be relevant to the immunotherapy of chronic infections.

Safety, as well as efficacy, is a crucial requirement for any vaccine. Welsh and Fujinami remind us how T-cell responses, as well as being important for clearing an infection, can contribute to immunopathology and can occasionally be the basis of autoimmune disease. They suggest that the private nature of T-cell responses (different individuals, even identical twins, respond differently because their T-cell repertoires are generated from independent random DNA recombination events) can be key to whether a given infection is severe or mild, or chronic or resolving, and can also be significantly influenced by the individual's history of infections with crossreactive pathogens. They argue against strategies for vaccination that rely on a restricted number of immunodominant epitopes, noting that broad and diverse T-cell repertoires against a range of epitopes have been associated with better protection. Their ultimate goal is epitope engineering of important vaccine antigens, although the feasibility of this for large, out-bred populations of multiple races is likely, in practice, to be difficult.

Challenging targets

The different but overlapping challenges posed by different targets are discusssed in the articles on rotavirus, dengue and *Shigella*. As described by Angel *et al.* the rotavirus vaccine story is an intriguing one, which over the past few years has significantly shaped both the regulatory authorities and the industry in terms of what is required to demonstrate safety in clinical development. The first licensed rotavirus vaccine, Rotashield, was withdrawn from the market within a year of its launch, because of an association with a low level of intussusception in recipients[10]. Even though the attributable risk of intussusception was well below the risk of contracting severe, even life-threatening rotavirus diarrhoea, especially in developing countries, the vaccine was not used further. This might seem absurd, given that the number of lives that would certainly have been saved, especially in the developing world, would have surpassed by several orders of magnitude the number that would have been threatened by intussusception. Nevertheless, it is an illustration of the exacting standards required when developing a new vaccine in a risk-averse world[11].

Angel *et al.* describe the two new rotavirus vaccines that now have begun to replace Rotashield — GlaxoSmithKline's Rotarix[12] and Merck's RotaTeq[13]. The development of both vaccines involved large safety trials and different immunological strategies, both illustrating that even today a good measure of empiricism can be involved in vaccine development. It was clear early on in development that natural infection with rotavirus not only gives good protection against homologous strains but also protects against severe disease caused by heterologous serotypes. It was also known that animal strains and some human isolates are naturally attenuated or can be attenuated classically by passage in cell culture. However, then and now, we know little of the basis of host-range restriction of virulence and the mechanisms of attenuation; the correlates of protection are far from clear and the relevance of different virus serotypes to vaccine strategies remains controversial. Nevertheless, these vaccines seem to work, be they multivalent as in RotaTeq or a single human strain as in Rotarix. It remains to be confirmed, however, that one or both of these vaccines will provide protection against disease in all epidemiological settings and against all serotypes.

As discussed by Whitehead and colleagues, there is an urgent need for an effective dengue vaccine. Dengue infections have increased significantly over the past two decades and there are now tens of millions of cases per year, with >3 billion people living in areas of the world at risk. The paradigms of yellow fever, tick-borne encephalitis and Japanese encephalitis confirm that vaccines can be developed for both viscerotropic and neurotropic flavivirus species. However, for dengue there is the added challenge of multiple virus serotypes and the problem of dengue shock syndrome (DSS). The leading hypothesis to explain the occurrence of this severe form of dengue is enhancement of infectivity by mis-matched, partially or non-neutralizing antibodies. Thus, the waning of maternal antibodies in infants, or the presence of antibody against one or two of the four serotypes, but not the remainder, is considered to elevate the risk of DSS. Although the evidence supporting this hypothesis is still not comprehensive (indeed, the article by Welsh and Fujinami offers an alternative hypothesis), this notion creates pressure for the vaccine developer to ensure that vaccine candidates provide almost simultaneous induction of neutralizing antibodies to all four serotypes. Evidence suggesting that cellular immune responses might be important in providing some level of cross-protection argues in favour of a live-attenuated approach, but as with *Shigella* (discussed below), finding the optimal ratios of attenuated strains, which can have slightly differing fitness, and stimulating broad responses in infants with minimal time lapse between shots, remains a challenge. Several attractive candidates are in clinical development however and the prospects for success at this stage seem good.

> "… even today a good measure of empiricism can be involved in vaccine development."

As well as being faced with the need to protect against multiple serotypes (16 in this case), the development of a comprehensive *Shigella* vaccine poses additional challenges, as discussed in the article by Levine and colleagues. For the live-attenuated approaches, as usual, the balance between attenuation and immunogenicity is crucial; under-attenuate and there will be unacceptable residual pathogenesis but over-attenuate and immunogenicity will be too low and, perhaps equally importantly, the dose size will be too

high to make industrialization feasible at a production cost that is commensurate with being able to deliver the vaccine to where it is needed most — the developing world. Again, if multiple live-attenuated strains are to be used, it is probable that competition will occur in establishing the subclinical infections of the gut at an intensity required to provoke an immune response. This problem portends that multiple doses will probably be required to stimulate immunity against all strains. In endemic regions, ensuring compliance with a multi-dose schedule is often difficult, and children can be left partially immunized. Additionally, for travellers (a customer base in the developed world often necessary to repay development costs), the use of multiple doses across an extended time window is less than optimal. Thus, the challenge is not only constructing strains of appropriate attenuation and immunogenicity, but also in designing the right cocktail of the multiple strains of slightly differing fitness, so as to provide immunity against all components simultaneously, that will work in infants through to adults, irrespective of the background gut flora that will be encountered in different regions of the world.

Levine *et al.* also air the possibility of a glycoconjugate approach based on lipopolysaccharide O-antigens linked to a carrier protein, and there is clinical efficacy data supporting such a concept. Again it is likely that two or three doses will be required and manufacturing costs — and hence the price — could be higher than with a live-attenuated approach. Finally, it should be remembered that *Shigella* is only one of the bacterial genera that cause diarrhoeal disease and are prevalent in the developing world. To make a real impact on health, and to be attractive to travellers, a more comprehensive diarrhoea vaccine would be the goal. Such a vaccine would ideally incorporate, in addition to multiple *Shigella* strains, the multiple types of enterotoxigenic *Escherichia coli*, *Vibrio cholerae* and *Salmonella enterica* serovars Typhi and Paratyphi.

Conclusions

These examples illustrate just some of the challenges and opportunities facing vaccine developers today. In constructing a research and development pipeline, each vaccine company develops its own priorities from the list of obvious targets. A principal driver is medical need, which often aligns well with market attractiveness. Contrary to popular belief, this can be true even when the disease is predominantly one affecting the developing world, where prices need to be low to ensure the widespread uptake of the vaccine. In these cases, the value of the market can often be increased by serving travellers from richer nations, with differential pricing. Obviously, vaccines of greatest medical need include vaccines for HIV/AIDS, malaria and tuberculosis (the so-called poverty-related diseases; short updates on vaccine development for these three diseases can be found in the News & Analysis section of this Focus issue), and difficult targets such as respiratory syncytial virus, meningitis B and *Chlamydia trachomatis*.

The vaccine industry, like any other industry, needs to make a profit. Investors therefore need to balance medical need and market attractiveness with technical feasibility before embarking on a project. Assessing technical feasibility is often a matter of judgment, but guidance can be taken by considering the pathogenesis of the organism in question, its route of transmission, the nature of naturally acquired resistance (if any) following infection and the examples offered by historical successes in both the human and veterinary vaccine sectors. For example, the successful development of inactivated polio vaccine after growth in cell culture and treatment with formalin was an excellent paradigm for the development of a vaccine against the related picornavirus, hepatitis A virus, in the 1990s. The same applies to the rotavirus vaccines, which were developed on the principles established for oral polio vaccine and the Jennerian principles of using related viruses of animals.

This guidance, however, is by no means foolproof. The glycoconjugate paradigm that has proved successful for Prevnar and Menactra has recently failed to provide convincing results when applied to *Staphylococcus aureus*. Some authors have suggested that the probability of success in developing new vaccines is critically linked to the incubation period of the disease[14], although there are undoubtedly many other factors. What is clear is that today, vaccine developers have at their disposal an impressively broad range of technical options and unprecedented insights into what it takes to develop successful new vaccines. It is a good time to renew investment in vaccines.

1. Jones, T. Vaccines against human papilloma viruses. *Curr. Opin. Investig. Drugs* **7**, 699–701 (2006).
2. Rappuoli, R. & Covacci, A. Reverse vaccinology and genomics. *Science* **302**, 602 (2003).
3. Pizza, M. *et al.* Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. *Science* **287**, 1816–1820 (2000).
4. Kelly, D. F. & Rappuoli, R. Reverse vaccinology and vaccines for serogroup B *Neisseria meningitidis*. *Adv. Exp. Med. Biol.* **568**, 217–223 (2005).
5. Hensel, M. *et al.* Simultaneous identification of bacterial virulence genes by negative selection. *Science* **269**, 400–403 (1995).
6. Shea, J. E., Santangelo, J. D. & Feldman, R. G. Signature-tagged mutagenesis in the identification of virulence genes in pathogens. *Curr. Opin. Microbiol.* **3**, 451–458 (2000).
7. Meinke, A., Henics, T., Hanner, M., Minh, D. B. & Nagy, E. Antigenome technology: a novel approach for the selection of bacterial vaccine candidate antigens. *Vaccine* **23**, 2035–2041 (2005).
8. Poehling, K. A. *et al.* Invasive pneumococcal disease among infants before and after introduction of pneumococcal conjugate vaccine. *JAMA* **295**, 1668–1674 (2006).
9. Garland, S. M. *et al.* An evaluation of non-inferiority in antibody response to human papillomavirus (HPV) 16 in subjects vaccinated with monovalent (HPV 16) and quadrivalent (HPV 6,11,16,18) L1 virus-like particle vaccines. *Clin. Vaccine Immunol.* 11 Apr 2007 (doi:10.1128/CVI.00478-06v1).
10. Rothman, K. J., Young, X. Y. & Arellano, F. Age dependence of the relation between reassortant rotavirus vaccine (RotaShield) and intussusception. *J. Infect. Dis.* **193**, 898 (2006).
11. Parashar, U. D. & Glass, R. I. Progress toward rotavirus vaccines. *Science* **312**, 851–852 (2006).
12. O'Ryan, M. Rotarix (RIX4414): an oral human rotavirus vaccine. *Expert Rev. Vaccines* **6**, 11–19 (2007).
13. Matson, D. O. The pentavalent rotavirus vaccine, RotaTeq. *Sem. Ped. Infect. Dis.* **17**, 195–199 (2006).
14. Kaslow, D. C. Biological feasibility of developing prophylactic vaccines for viral pathogens. *Hum. Vaccin.* **3**, 1–7 (2007).

Competing interests statement
The author declares competing financial interests: see web version for details.

FURTHER INFORMATION
GAVI Alliance: http://www.gavialliance.org
Bill & Melinda Gates Foundation: http://www.gatesfoundation.org/default.htm
Project BioShield: http://www.whitehouse.gov/infocus/bioshield/
National Strategy for Pandemic Influenza: http://www.whitehouse.gov/homeland/pandemic-influenza.html
Access to this interactive links box is free online.

Online Only

Links

Global Alliance for Vaccines and Immunization
http://www.gavialliance.org/

Bill & Melinda Gates Foundation
http://www.gatesfoundation.org/default.htm

Project BioShield
http://www.whitehouse.gov/infocus/bioshield/

National Strategy for Pandemic Influenza
http://www.whitehouse.gov/homeland/pandemic-influenza.html

Competing financial interests
Jeffrey W. Almond
Vaccine renaissance. *Nature Reviews Microbiology* **5**, 478–481 (2007); doi:10.1038/nrmicro1702.

Jeffrey W. Almond is Head of Research and External R&D at sanofi pasteur.