New Decade of Vaccines 1

The next decade of vaccines: societal and scientific challenges

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Vaccines against microbial diseases have improved the health of millions of people. In the next decade and beyond, many conceptual and technological scientific advances offer extraordinary opportunities to expand the portfolio of immunisations against viral and bacterial diseases and to pioneer the first vaccines against human parasitic and fungal diseases. Scientists in the public and private sectors are motivated as never before to bring about these innovations in immunisation. Many societal factors threaten to compromise realisation of the public health gains that immunisation can achieve in the next decade and beyond—understanding these factors is imperative. Vaccines are typically given to healthy individuals and safety issues loom high on the list of public concerns. The public needs to regain confidence in immunisation and trust the organisations responsible for the research, development, and implementation of vaccines. In the past, by use of a judicious amalgam of knowledge and empiricism, successful vaccines were largely developed by microbiologists who identified antigens that induced immune responses to conserved pathogen components. In the future, vaccines need to be developed against deadly diseases for which this strategy is often not feasible because of the extensive antigenic variability of relevant pathogens. High microbial diversity means that immunity after natural infection is often ineffective for prevention of disease on subsequent exposure, for example in HIV infection and malaria. Additionally, vaccines need to be generated to protect the people who are most vulnerable because of age or underlying diseases. Thus, in the future, a much deeper understanding of the immunological challenges—including the diversifying role of host genetics and environmental factors, leading perhaps to more personalised approaches—will be the touchstone for rational design and development of adjuvants that result in novel safe and effective vaccines.

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This is the first in a Series of five papers about the new decade of vaccines

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Introduction

This Series aims to review the potential contributions of immunisation to improvement of global health, with special attention to identification of the major scientific and societal challenges for development and implementation of safe and effective vaccines in the next decade and beyond. In the past century, the judicious use of vaccines against microbial diseases has improved the health of millions of people. Vaccines have eliminated or substantively diminished the toll of major scourges, including smallpox, poliomyelitis, measles, pertussis, tetanus, yellow fever, and diphtheria. In the past two decades, great progress has been made in prevention of meningitis, pneumonia, and hepatitis. Furthermore, an amalgam of new technologies encompassing genetics, structural biology, and biochemistry are enabling the classical disciplines of epidemiology, microbiology, and immunology to expand the portfolio of vaccines against respiratory infections and diarrhoeal diseases. Scientists in the public and private sectors are motivated as never before to bring about incisive advances through the development of new and improved vaccines in the next decade and beyond.

The spectacular past achievements and the exciting prospects for development and implementation of improved and new vaccines should be tempered by a realistic appraisal of the many challenges now and in the long term. Notably, in the past, many successful vaccines were based on induction of immunity to conserved target antigens of microbes. By contrast, many of the most

Key messages

- Vaccines against microbial diseases have already improved the health of millions of people but, in the next decade and beyond, there are extraordinary opportunities to expand the portfolio of immunisations against viral and bacterial diseases and to pioneer the first vaccines against human parasitic and fungal diseases
- In the past, successful vaccines were largely developed by identification of antigens that induced immune responses to conserved pathogen components, whereas, in the future, a major challenge is to develop vaccines against deadly diseases in which the target antigens have extensive antigenic variability
- Development of successful vaccines against pathogens with extensive antigenic diversity requires an in-depth knowledge of their molecular epidemiology, and evolutionary and population biology
- Although science is the key to development of improved and novel vaccines, it is not sufficient; the next decade demands that we also build public trust and confidence in immunisation through effective communication with policy makers and the public
- To increase vaccine safety, use of subunit antigens has been increasing, but these antigens are devoid of the natural adjuvant properties typical of whole-cell vaccines, and, therefore, are less immunogenic, and need novel strategies to increase immunogenicity
- Identification of the molecules and signalling mechanisms through which antigen presenting cells elicit specific and effective T cells is crucial for development of effective vaccines
- A major challenge is how to develop vaccines capable of conferring lifelong protection without the need for repeated booster immunisations
- Research is needed to address the particular challenges of immunisation of very young people, elderly people, and people with chronic diseases
- Increasing knowledge of the individual variations in immune responsiveness will provide a rational basis to question the appropriateness of mass immunisation and to replace mass immunisation with personalised strategies

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important diseases for which future vaccines are urgently needed are caused by viruses, bacteria, or parasites that are characterised by extreme variability of their surface structures.[1] Microbes displaying extensive antigenic diversity, such as HIV and *Plasmodium* spp, are particularly challenging; immunity after natural infection is often ineffective in prevention of disease on subsequent exposure. To induce comprehensive and durable protection to these microbes, understanding of immune responses will need to be much more detailed than at present, and vaccine formulations will need to include many different, judiciously selected antigens.

Antigenic diversity of pathogens has major implications for how science, through the public and private sectors, can identify the relevant vaccine antigens and formulate them into safe and effective vaccines. These issues are addressed, especially from an industry perspective, in the second[2] and third[3] papers in this Series. Estimates of annual total sales of vaccines projected for 2020 are in excess of US$30 billion compared with $6 billion in 2000; industry investment in research and development is estimated to be 15% of these figures, respectively.[4] The global agenda has been galvanised through, inter alia, the initiatives of the Bill & Melinda Gates Foundation, the GAVI Alliance, WHO, and public-private and product development partnerships. Vaccine safety issues heighten the scope of what is needed for a vaccine to be publicly acceptable. Because vaccines are largely given to healthy individuals to protect against a future threat of disease, safety is paramount, a concern that substantively affects an already complicated and costly research and development process. Achievement of an acceptable balance of benefit and risk is an enormous challenge; no vaccine can be exempt from all risk of adverse events. Furthermore, when safety issues are raised, gathering the appropriate data can take years and, during that time, anxieties cannot be disquieted. Nonetheless, the evidence unequivocally supports that immunisation is an absolutely fundamental component of global efforts aimed at efficient and cost-effective improvement of public health.

However, the difficulties in establishment of robust and sustainable policies and financing of global immunisation programmes cannot be underestimated; these issues are reviewed in the fourth paper in this Series.[5] As we look to the next decade and beyond, the global landscape of vaccinology is undergoing substantial changes. A third of the world's population lack the basics of daily living and, by the middle of this century, the world population will exceed 9 billion.[6] The world is more closely networked than ever before and the burgeoning amount of air travel means that the spread of any pathogen across the globe can occur within hours.

The spread of information—and misinformation—has also changed through the internet and blogging. Advocates of immunisation should continue to interact broadly with society, but this engagement should be keenly appreciative of the contemporary and changing media environment. A high priority is placed on freedom of speech, irrespective of the accuracy of its content. Misinformation about immunisation, whether intentional or not, needs to be countered urgently to help recipients of vaccines seek reliable facts and trust health professionals. Although the benefits of vaccines are unequivocal and based on rigorous scientific evidence, how should advocates of immunisation respond to people who are sceptical or even aggressively opposed to immunisation? Antipathy, distrust, fear, and even outright rejection of scientific evidence are unwelcome to its proponents, but should not come as a surprise. Lessons from other contemporary challenges, such as those posed by climate change or genetically modified organisms, could help with building public trust in immunisation. In informing the public, advocates need to achieve a balance between the benefits of immunisation and acknowledgment of possible adverse outcomes.

Although evidence should inform public discussion and policy development, perception is to a great extent in the hands of consumers and the media. Championing the benefits of scientific evidence to a world made up of individuals of differing age, sex, religious affiliation, culture, education, socioeconomic status, and aspirations is a tall order. Nonetheless, professional education is paramount as advocates of immunisation strive to distinguish scientific evidence from misinformation and strengthen public confidence in vaccines, which is an aim of this Series. Important initiatives have already been taken—for example, the US Institute of Medicine has addressed key issues of the measles, mumps, and rubella (MMR) vaccine and autism, thiomersal and neurological developmental disorders, multiple immunisations, hepatitis B and neurological disorders, and anthrax safety, and has set up a national vaccine safety board.[7]

But the provision of clear, concise, and authoritative information is not enough. Horizons should be broadened with an eclectic range of expertise: for example, anthropologists can help to improve understanding of the cultural basis of behavioural responses, including religious extremism; validated questionnaires developed by social scientists can be used to probe issues of risk perception that are not tractable through quantitative approaches; and evolutionary biologists can explain the theories underpinning altruism and cooperation, which are major issues affecting herd immunity. In the next decade, information for the public needs to be systematic, through knowledge of our strengths and weaknesses, comprehensive, through inclusion of all scientific expertise, and credible, through understanding of how to generate trust and communicate effectively with policy makers and the public.

In the first paper of this Series, we discuss the challenges posed by antigenic variation of bacteria, viruses, and parasites, and the reciprocal challenge of

eliciting protective immune responses in genetically diverse individuals and differing geographical environments.

Pathogen diversity

The diversity of pathogens, whether based on genotype or phenotype, is astonishing and poses enormous challenges with respect to treatment and prevention of microbial diseases. Although the practice of variolation to prevent smallpox dates back many centuries, vaccines effectively came of age in the late 19th and early 20th centuries, in the golden era of bacteriology. The germ theory—the idea that particular species of microbes caused specific diseases—was axiomatic to the successful development of the earliest vaccines, and paved the way for use of whole organisms (eg, for pertussis and tuberculosis) or microbial toxins (eg, tetanus and diphtheria toxins) to induce protective immunity. For many decades, microbial diversity did not cause vaccine failure. Early vaccines stood the test of time because protective immunity was directed to invariant antigens. With the discovery and characterisation of viruses, such as influenza and poliovirus, the need to consider antigenic variation became clear. In the early 1950s, Sabin and colleagues[8] showed that immunisation with each of three distinct variants of poliovirus was needed to induce comprehensive protection. The antigenic shift and drift of influenza A viruses provided another example of the need to base effective vaccine strategies on a detailed knowledge of antigenic variation, specifically the neuraminidase and haemagglutinin antigens. Research on parasites, such as those causing malaria, trypanosomiasis, and leishmaniasis, also revealed extensive antigenic diversity.[1] At the time of writing, no vaccine has been licensed to prevent any human parasitic disease, despite the devastating morbidity and mortality wrought by these diseases.

For most of the diseases that can be prevented by existing vaccines, the target antigens are constant and do not vary over time or place, as exemplified by strain-specific vaccines against MMR, yellow fever, tetanus, diphtheria, and *Haemophilus influenzae* type b. Vaccines against poliovirus (live or killed) are only slightly more complicated than are strain-specific vaccines because three variants need to be included, but these multivalent formulations have remained highly effective worldwide, and efforts towards global elimination of poliomyelitis are ongoing. Occasional outbreaks of poliomyelitis have been caused by recombination between pathogenic vaccine-derived polioviruses and enteroviruses, which is an interesting model of viral evolution and emergence.[9] By contrast, vaccination against influenza A is complicated by antigenic variation in the target vaccine antigens.[10] Hence regular changes in the formulation of the vaccine are needed, but, at any one timepoint, few viral variants are in global circulation so a viable strategy is feasible. The major problem, as recently shown by the influenza A H1N1 pandemic, is that existing technology to make the vaccine is not fast enough to keep pace with the rapid spread of a pandemic strain.[3]

For many important pathogens, vaccine development needs to overcome the problem that many of the relevant candidate vaccine antigens are present in only a proportion of disease-causing strains of the species or, because of allelic variation, the chosen antigens induce protective immune responses that are not cross-protective against all strains. Therefore, vaccine development in the next decade and beyond needs to be based on a detailed understanding of the origins, maintenance, and dynamics of pathogen diversity and the relevant evolutionary and population biology underpinning this microbial variation (panel 1, panel 2, figure 1).

Panel 1: Evolution of pathogen diversity

Life forms date back at least 3·5 billion years. Microbial species, including those for which new or improved vaccines are urgently needed, evolve continuously: "....nothing in biology makes sense except in the light of evolution".[11] Today's microbes, including pathogens, evolved from ancestral forms that over vast periods of time were subject to sequential losses and gains of DNA through mutation and genetic exchange. All microbes, including pathogens, have been selected on the basis of their biological fitness, a composite property indicative of the replication and survival of a microbe, captured in the term basic reproductive rate (R_0). In any population of microbes, R_0 shows the number of surviving progeny produced, on average, by each individual microbe. To persist, R_0 of a microbial population must exceed one and the extent to which it does so is a quantitative measure of fitness. Importantly, many of the products of genes for adaptation are surface-expressed or secreted molecules that are vaccine candidates. The surface location or release of such molecules contribute to microbial fitness within the host, but microbes are also subject to selective pressures exerted by the host innate and adaptive immune mechanisms. Thus, host immune selection provides a major evolutionary drive for diversification of surface-located pathogen molecules. Unsurprisingly, the capacity of microbial surface structures to vary is subject to selection. The different strains that make up the population of a particular pathogenic species differ in genotype (gene content and organisation) and these variations have phenotypic consequences, including the antigenic variation that is such a challenge for vaccine development. Two major factors determine variation in microbial populations—mutation and genetic exchange (panel 2, figure 1).

Glycoconjugate vaccines

The different levels of vaccine complexity needed with respect to antigenic variation, and the implications of such complexity for vaccines, is exemplified by glycoconjugate vaccines, each of which is made from

Panel 2: Generation of diversity in asexual microbial populations by mutation and genetic exchange

Figure 1 shows how diversity evolves in asexual microbial populations (which contrasts with sexual reproduction of many parasites, yeast, and hosts, including man). Over time, many phenotypic variants arise, only one of which is depicted (variant A'), through accumulation of mutations in the ancestral organism (variant A). Most of the progeny of the ancestral organism will not be variants and genetic divergence of the population occurs slowly. The resulting population of variants is clonal, meaning that the diversity can be traced to a common ancestor. But many microbial populations are non-clonal, meaning that variations occur through lateral genetic transfer. Any life form can provide genetic novelty through lateral genetic transfer (variant B), although the most frequent source is within-species transfer (variant C). For example, lateral genetic transfer is needed for acquisition of novel surface antigens such as the shifts in haemagglutins or neuraminidases in influenza, and for switching of capsular antigens in the bacterium *Streptococcus pneumoniae* or in the variant adhesins (*var* genes) of the malarial parasite *Plasmodium falciparum*. Lateral genetic transfer is fundamental to understanding the diversity of microbial populations and, importantly, is a mechanism that greatly accelerates the spread of genetic diversity within populations of pathogens.

The amount of diversity is determined by two factors, the age of the species and interstrain genetic transfer. First, the more recent that speciation has occurred, the less time available for the species to accumulate genetic variation through mutations. Second, interstrain genetic transfer (recombination) profoundly affects the amount and rapidity of genetic divergence between strains. Recombination not only accelerates genetic divergence, since classical mutations introduce variation quite slowly, but it also impedes prediction of genetic associations. Loss or gain of DNA (or RNA) complicates the relatedness of progeny to their ancestors in a clonal population, resulting in a non-clonal population. This disruption of clonal population structure through genetic transfer leads to discordant associations of antigens, complicates predictions of antigen coverage for a multivalent vaccine, and requires detailed analysis of individual strains. To index the diversity in the strains in a population of pathogens, methods need to be able to distinguish variations between strains and establish the relatedness of one strain of a species to another. To this end, nucleic acid sequencing has been pivotal and can be used to characterise very large numbers of strains of any pathogenic species. The compilation of sequence data from many isolates provides a powerful profile of the evolved and evolving diversity of the natural population, and the implications of this diversity for potential vaccine antigens. For diseases in which future vaccines will need to cope with substantial antigenic variation, researchers need to rethink strategies for antigen discovery and development, and the associated regulatory framework and epidemiological surveillance.

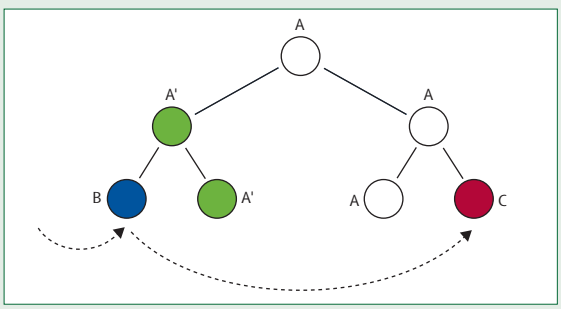

Figure 1: **Generation of diversity in microbial populations**
This figure shows the processes outlined in panel 2. An ancestral organism A (white) mutates and results in a phenotypic variant A' (green). The solid lines represent many generations. The interrupted lines represent lateral genetic transfer from any life form for variant B (blue) and within-species genetic transfer for variant C (red) of DNA (or RNA in the case of some viruses).

different but stable capsular polysaccharides. In the case of *H influenzae* type b, only strains expressing serotype b capsule, the target antigen for protective immunity, show associations with systemic disease that are of major public health importance.[12,13] Thus, glycoconjugate vaccines against *H influenzae* type b are fairly uncomplicated; the type b capsular antigen is stable over time and in different countries. A legitimate concern is that genetic exchange between different strains of the species might result in capsule switching, but, in the two decades since introduction of these glycoconjugate vaccines, there has been no indication that capsule switching is a substantial threat.[14]

By contrast, concerns are justified with respect to glycoconjugate vaccines against *Neisseria meningitidis*, which became available in 2009.[15] At least six of the 14 different *N meningitidis* capsular antigens cause potentially fatal diseases, and the epidemiology of infection (carriage and disease) is subject to variations over time and geography.[15] For example, in Africa, capsular serogroup A and, to a lesser extent, serogroup C were the most important causes of epidemics and outbreaks of invasive meningococcal disease until the 1980s.[16] However, subsequently, strains expressing W and X capsular polysaccharides[16,17] have become important causes of mortality and morbidity. Furthermore, prevention of diseases caused by capsular serogroup B strains—the major causes of invasive meningococcal disease in Europe, the Americas, and Australasia—is problematic because glycoconjugate vaccines against serogroup B, which are similar to those now licensed for other serogroups (A, C, W, and Y), are limited by poor immunogenicity and safety concerns.[18] These considerations have resulted in research to develop a vaccine that is not based on targeting the serogroup B capsular polysaccharide.[19]

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Prevention of *Streptococcus pneumoniae* infections is arguably the most complex future challenge to development of glycoconjugate vaccines because serious disease is associated with strains making any one of many antigenically different capsular polysaccharides. Thus, the inclusion of many different antigens (up to 13 capsular polysaccharides, each conjugated to a carrier protein) is needed to achieve broad coverage. The major variable for glycoconjugate vaccines against *S pneumoniae* is the changing prevalence of serotypes by geographical location.[20] Thus, the challenge for vaccine developers is to consider how many antigens (different capsular polysaccharides) should be included, and how these formulations can be modified to cope with geographical variations. Another important issue, well documented for *S pneumoniae* but also applicable to other glycoconjugate vaccines, is that immunisation induces herd immunity by reducing human carriage and spread,[21] but only to strains expressing capsules included in the vaccine.[22] Thus, vaccine selection pressure can result in altered carriage (serotype replacement) in which disease results from pathogenic serotypes that are not included in the vaccine.[22,23] This important factor merits close scrutiny through appropriate epidemiological monitoring, and has stimulated research to develop pneumococcal vaccines with formulations including one or more antigens to provide universal coverage.[24]

Antigenic variation of HIV-1 and *Plasmodium falciparum*

A dominant future challenge is to develop vaccines against major pathogens, such as HIV and *P falciparum*, which have substantial variability of target antigens compounded by unpredictable behaviour over time and geographical location.[25] The number and frequency distribution of antigenic variants should be considered in the initial choice of vaccine antigens, requiring a detailed knowledge of the population structure and molecular epidemiology of the pathogen. The evolutionary factors shaping population structure include the accumulation of mutations and the patterns of interstrain genetic exchange (panel 2), and the effect of natural selection. Furthermore, because of the importance of herd immunity and strain replacement, disease carriage and incidence should be known, and the relevant strains should be characterised by use of robust systems for vaccine typing.

Pandemic HIV-1 represents one of the greatest challenges in vaccine development because it has high antigenic variation and the epidemiology of strains changes over time and location.[26] The biggest obstacle to development of an effective HIV-1 vaccine is the failure to identify one or more viral antigens (immunogen) that stimulate broadly cross-reactive, protective antibodies.[27,28] In natural infection, many different human antibodies are produced to the two major glycoproteins exposed on the viral surface (gp120 and gp41), but very few antibodies are able to neutralise the virus effectively and identification of these antibodies and their cognate viral structures has proved difficult.[29] However, structural biology could pave the way towards rational vaccine design.[30,31] Wu and colleagues[31] screened individuals infected with HIV-1 for high potency neutralising antibodies, and then solved the crystal structure of the viral target glycoprotein (gp120 bound by these antibodies), which is a trimeric spike in its native state. These studies, reviewed by Walker and colleagues,[30] have negated a major concern, namely that achievement of adequate potency of protective immunity would inevitably be traded off against breadth of strain coverage.

The surface proteins of *P falciparum* also have extensive genetic diversity.[32] *P falciparum* causes the form of malaria that is associated with appalling mortality and morbidity, especially in Africa.[33] During its life cycle, the parasite expresses different antigens specific to each of its several stages. Furthermore, the longstanding co-evolutionary history with its human host has resulted in immune selective pressure manifested in extensive polymorphisms of its surface antigens.[32] Both mutation and genetic exchange (the parasite reproduces sexually) contribute to generation of the dynamic diversity that makes *P falciparum* a moving target. The problem is that vaccines directed against a subset of surface antigens, an exception could be the circumsporozoite antigen, might provide only a time-limited solution as novel variants rise in prevalence because of vaccine selection pressure. Consequently, vaccine development and the associated clinical trials have to take into account the distribution and dynamics of antigenic variations in endemic populations.[34] In the design of clinical trials of candidate vaccines, variant-specific efficacy needs to be a key endpoint and, therefore, molecular epidemiological studies should be done before development and testing of vaccines. Similar principles of study design for complex vaccines will apply to other pathogens, including viruses such as dengue virus, hepatitis C virus, and HIV, and the multivalent formulations envisioned for prevention of bacterial diseases such as *Staphylococcus aureus*, *Escherichia coli*, and *N meningitidis* serogroup B (panel 3[19,35]).

Human diversity: induction of potent antibody responses to weak immunogenic determinants

Vaccines that are effective after induction of only modest antibody responses have all been developed. To target diseases that have so far escaped vaccine prevention, future vaccines need to elicit appropriate effector and memory responses in diverse populations with maximum safety.

The quest for maximum vaccine safety is leading development away from whole-cell inactivated or live attenuated vaccines and towards subunit or non-replicating recombinant vaccines. These purified vaccines, which are essentially deprived of endogenous danger signals, are intrinsically less immunogenic than are

Panel 3: Implications of antigenic variation in assessment of vaccine effectiveness

Dengue

Dengue flavivirus is a mosquito-borne pathogen with four serotypes. The virus is reported in tropical and subtropical regions with an estimated 50–100 million infections per year and the number is increasing.[36] Sequence variation (30–35%) of the virus is such that vaccination against the variant antigen of one serotype does not provide protection from infection with the other serotypes. Many candidates for dengue vaccines are under development and some have already been investigated in human beings (NCT00875524), but the vaccines might need to elicit neutralising antibodies to each of the serotypes. Furthermore, the antibodies stimulated by the vaccine are likely to be neutralising (protective) against homologous serotypes, but not against heterologous serotypes. Indeed, the partial cross-reactivity induced by the vaccine might actually increase infection to heterologous strains through opsonisation that increases viral uptake, intracellular replication, and disease severity.[37,38] Vaccine development against dengue virus shows how antigenic variation complicates vaccine formulation and poses potential issues with vaccine safety.

Neisseria meningitidis

The poor immunogenicity and safety issues associated with capsular polysaccharide-based glycoconjugate vaccines against *N meningitidis* capsular serogroup B (Men B) disease (meningitis and septicaemia) has resulted in research to develop vaccines based on membrane proteins of this bacterium.[19] For example, one candidate formulation[35] consists of three antigens that are targets for bactericidal (protective) antibodies, which for simplicity are designated antigens A, B, and C. Antigen A shows allelic variation such that antibodies are protective against only a proportion of strains, antigen B is present in few strains, and antigen C is present in all strains but the amount expressed varies and, in some cases, is no longer quantitatively sufficient to sustain antibody-mediated protection.

In view of the rarity of Men B disease (<1 to ≤25 per 100 000 population),[16] a validated surrogate of protection, provided by the bactericidal assay, is needed to estimate efficacy in clinical trials. Clinical trials to test the vaccine and predict efficacy depend on identification of the proportion of individuals who have serum bactericidal activity to one or more of the three antigens after immunisation,[39] assuming that all individuals had no bactericidal antibodies and were therefore susceptible before immunisation. A detailed knowledge of the prevalence of Men B strains is also needed with respect to the three target antigens, which requires a typing scheme to assign any individual Men B strain to one of seven possible vaccine types (VTs): VT-1 (A, B, C); VT-2 (A, C); VT-3 (B, C); VT-4 (A, B); VT-5 (A); VT-6 (B); or VT-7 (C).

A major unknown is whether bactericidal activity to each of the antigens is sufficient to protect against disease. Bactericidal assays are done with strains that are grown in vitro. The antigens that are targets for protective immune responses are subject to changes in expression, for example through phase variation or post-translational modification, and the amount of each target antigen that is expressed in vitro might not correlate with that occurring in the host during infection. Careful consideration should be given to the choice of strains used in immune assays of host responses to estimate efficacy in clinical trials.[39] Another major unknown is the contribution of herd immunity to protection. Trials to assess the effect of a vaccine on carriage and transmission of disease before implementation can be difficult because of the large sample sizes needed. In such cases, with careful prospective planning, efficacy studies of direct (individual) and indirect (herd immunity) protection by the vaccine can be obtained after implementation by comparison of disease rates in individuals who are and are not immunised by use of case control or screening.[40,41] Because the natural population of meningococci evolves quite rapidly, detailed prospective studies of carriage and disease strains should be done to assess the adequacy of coverage and the need for changes in vaccine formulation.

naturally adjuvanted whole-cell or live vaccines.[42] However, many diseases need higher titres of neutralising antibodies to be induced than might be achieved with subunit vaccines, even in schedules with several doses.[43] Thus, specific immunisation strategies need to be developed to increase antibody responses to vaccines through improvement of B-cell targeting and activation (panel 4, figure 2). A role for novel adjuvants is best shown by the major effect of adjuvants on primary responses to influenza A H5N1.[44] Such adjuvants also address, at least in part, the issue of antigenic diversity by eliciting broader responses capable of cross-strain neutralisation.[45] As a rule, the breadth and neutralising potency increases with the antigen-specific antibody affinity.[46] However, polyreactive antibodies might engage in heteroligation, which implies that they could have an unsuspected role in viral neutralisation.[47]

Most vaccines have been developed for intramuscular or subcutaneous administration, but injection safety remains an issue worldwide and delivery without needles would improve the safety and logistics of immunisation.[48] Alternative routes of immunisation would also be immunologically relevant because pathogens naturally invade their hosts and disseminate through mucosal or skin surfaces. However, elicitation of protective immunity on mucosal surfaces has proven tricky: potent inflammation-inducing adjuvants are generally needed to overcome mucosal barriers while avoiding potentially harmful excess inflammatory reactions.[49,50] However, basic immunological knowledge is providing new insights into how the regulation of tissue-specific lymphocyte trafficking and differentiation could be used to induce protection directly at the mucosal surface.[51] The skin is an attractive route for

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vaccine administration, being densely populated by antigen presenting cells capable of efficiently migrating towards the draining lymph nodes.[52] The intradermal route could be revived by the development of easy-to-use delivery devices.[53] An interesting societal issue will be whether the induction of superficial skin or soft-tissue inflammatory reactions, effects obvious to immunised individuals, might limit vaccine acceptance.

Even the most potent vectors or adjuvants given by the best possible route will not transform weakly immunogenic polysaccharides or proteins into potent vaccine antigens. Thus, the antigen itself might have to be modified. The success of this approach was shown by glycoconjugate vaccines, which elicit B-cell responses of increased potency by provision of immunogenic epitopes to CD4 T cells.[42] An important future goal is to combine high-resolution structural analysis and immunobiological assays to identify the essential antigenic determinants of protection, and thus advance vaccinology from empiricism to the rational design of immunogens.[54]

Instruction of antigen presenting cells towards appropriate T-cell responses

Increasingly, novel immunological assays are directed towards the characterisation and identification of the functional (protective) roles of CD4 and CD8 T cells. Elicitation of appropriate T-cell responses has become a key milestone for novel vaccines. A complex combination of appropriate activation and regulatory signals properly spaced in time and location[55] are needed to direct T-cell differentiation along specific effector pathways and towards memory cells (panel 4, figure 2). These signals can be modulated by specific adjuvants and vectors, which might also affect the fine specificity and clonotypic diversity of antigen-specific CD4 T-cell responses[56] and thus modulate the induction of cytotoxic or regulatory CD8 T cells. Thus, most novel subunit vaccines will include immunomodulators to preferentially induce CD4 T-cell differentiation along specific pathways, whereas novel vectors should become available to elicit potent CD8 T-cell responses (panel 4, figure 2).

The level of complexity of T-cell responses implies many challenges, including how to select the vaccine candidates most likely to survive the stringent selection process leading from preclinical trials to clinical trials, licensing, and implementation. Indeed, although the limitations of most animal models are well established, human trials cannot realistically be done for all potential vaccine candidates. Although improved cellular immunological techniques could possibly improve the selection process, they will not circumvent the major limiting factor that blood samples are accessible but not representative of the processes in tissues, nodes, or bone marrow compartments.

Lifelong vaccine-induced protection without repeat boosting

Prophylactic vaccines can only contribute to individual and public health if they induce sustained protective responses. Ideally, vaccine responses should be elicited by a single immunisation and last a lifetime. This objective can indeed be achieved, as shown by smallpox vaccines.[57] However, a lifelong persistence of antibody responses is the exception rather than the rule: in the absence of antigen exposure, vaccine-induced antibodies progressively decline to eventually reach undetectable concentrations.[42] Research has not yet identified precisely why certain antibodies persist for several decades whereas others wane after a few years.

However, evidence now suggests that the type and duration of immune memory might be largely determined by the magnitude and complexity of the innate immune signals provided at induction, opening new perspectives.[58] For example, triggering of both toll-like receptors 4 and 7 induces early programming towards B-cell memory, in both mice and macaques.[59] We now understand that the reactivation of resting memory B cells into immunoglobulin-secreting plasma cells requires both antigen exposure and time. Thus, protection against diseases with a rapid pace of pathogenesis or absence of pathogenic invasion beyond mucosal surfaces requires long-term maintenance of needed of serum antibodies.[60] A new challenge is to identify determinants of the induction, size, and persistence of sufficient pools of antibody-secreting plasma cells, memory B cells, or T cells (panel 4, figure 2), and to define the extent to which these determinants could be modulated by immunisation strategies.

Herd immunity is another important mechanism that might contribute to the maintenance of vaccine-induced protection despite primary or secondary vaccine failures. The success of this approach was shown by the introduction of glycoconjugate vaccines into carrier age-groups (infants, young children, or adolescents).[61,62] It also applies to sexually transmitted diseases such as hepatitis B virus[63] or human papillomavirus infections. According to mathematical modelling and a few studies,[64,65] the best societal use of influenza vaccines could be to target pre-school or school children as the main transmitters, in addition to or instead of elderly people or other high-risk groups in whom vaccine efficacy is often poor. Shifting of vaccine targets from the high-risk groups to the high-transmitter groups, or even vaccination of target groups with little direct benefit, would represent a substantial societal challenge in view of the tension between individual rights and public health.[66]

Limitations of young or advanced age and chronic disease

A major scientific and societal challenge is to develop effective vaccines for individuals in whom the functions

Panel 4: Immunological challenges for new vaccines

At the site of injection

To initiate a successful immune response, vaccines need to include sufficient danger recognition patterns to activate local cells that participate in innate responses, such as stromal cells, tissue-resident monocytes, muscle cells, or mucosal cells, to produce chemokines and inflammatory signals (figure 2, label 1). These early signals trigger circulating monocytes, leucocytes, or dendritic cells (label 2) to exit from blood vessels, migrate to the injection site, and provide further inflammatory signals. The combined direct and indirect activation of locally resident or attracted APCs (label 3) should elicit increased uptake of antigen or adjuvant molecules, antigen processing and display by surface MHC molecules (T-cell activation signal 1), expression of activation markers (signal 2), and cytokine production (signal 3). This activation is needed for antigen-decorated, adjuvant-activated APCs to migrate towards the lymph nodes draining the site of injection (label 4). The main immunological challenge for novel vaccines is thus to trigger sufficient local activation signals to elicit the best possible vaccine responses, while avoiding both excess inflammatory reactions and the non-specific activation of bystander immune cells.

In the draining lymph nodes

Soluble antigen or adjuvant molecules reach the capsular sinus of the draining lymph nodes within minutes (free-fluid diffusion), followed a few hours later by locally activated antigen-bearing APCs (label 4). For potent, sustained vaccine responses, both T and B cells need to be induced. Antigen-expressing, activated APCs migrate into the T-cell area (label 5). APCs need to display antigenic peptides on MHC class II molecules to induce the differentiation of antigen-specific CD4 T cells into Th1, Th2, or Th17 effector cells, Tfh cells, or Treg cells, whereas APCs with antigens displayed on MHC class I lead to optimum activation of CD8 T cells (label 6). A sufficient pool of Tcm cells, which constitute a reservoir of antigen-primed memory cells in the skin and nodes, and of ready-to-function Tem cells in peripheral tissues is needed to induce sustained vaccine responses (label 7). Activation of distinct pathways is needed for optimum induction of antibody-producing cells. Vaccine antigens reach the B-cell follicles (label 8) by free-fluid diffusion or channelling by subcapsular sinus macrophages. B cells binding to the antigen surface (signal 1) and sufficient coactivation signals from activated APCs and CD4 helper cells are needed to activate antigen-specific B cells to migrate towards B-cell follicles and induce the germinal center reaction. Through a selection process of proliferation, hypermutation, differentiation, and affinity maturation, which is affected by the nature and intensity of B-cell activation, B cells differentiate into antibody-secreting plasma cells or memory B cells (label 9). Large pools of long-lived antibody-secreting plasma cells surviving in specific bone marrow niches, and a sufficient reservoir of antigen-specific memory B cells ready to differentiate into antibody-producing cells within a few days, are needed to generate sustained vaccine responses.

APC=antigen presenting cell. Th=T helper cell. Tfh=follicular T cell. Treg=regulatory T cell. Tcm=central memory T cell. Tem=effector memory T cell.

of the immune system are immature (infants), affected by chronic disorders (diabetes, cancer, or chronic disease of any organ), or declining because of ageing. Effective vaccines in early life need to overcome both immune immaturity, which limits antibody responses and preferentially polarises T cells away from inflammatory responses, and the inhibitory effects of maternal antibodies.[43,67] Furthermore, vaccines should not trigger excess reactogenicity, a challenging issue in early life.[68] The likelihood that high titres of functional antibodies can be elicited within weeks after birth is a remote objective.[43] The best approach is thus to promote early life protection through a combination of maternal immunisation, neonatal immunisation, and development of safe adjuvanted infant vaccines, which is a challenging strategy in view of the risk-adverse context of pregnancy and early life.

Another challenge is to develop vaccines capable of improving vaccine-induced protection in immunocompromised patients, a growing population because of the increased use of immunosuppressive treatments and the spread of HIV infection. Remarkably little is known about how specific anti-inflammatory and immunomodulatory treatments affect immune responses—for example, the inhibitory effect of prophylactic paracetamol on infant vaccine responses came as a surprise.[69] These effects limit the rational design of the best possible immunisation approaches for immunocompromised patients and raise several questions. Could adjuvanted vaccines including more potent immunomodulators prove more effective in certain immunocompromised patients? Could such vaccines prove safe in immunocompromised patients but be too reactogenic in healthy individuals? How could such vaccines be developed in today's stringent regulatory environment without the financial incentive of a large market size?

The elderly population is rapidly growing and needs progress in vaccinology. The estimated benefits of pneumococcal or influenza immunisation in elderly people have been largely overestimated.[70–73] Several mechanisms combine to result in age-associated immune frailty:[43,74] impaired responses to toll-like receptor-mediated signalling;[75] and progressive waning of cell-mediated immunity because of reduced thymic output and progressive expansion of dysfunctional terminally differentiated T cells driven by chronic viral infection.[76] Potent adjuvants could potentially improve immunogenicity through increasing responses to toll-like-receptor-mediated signalling. Virosomes, MF59, AS03, and other adjuvants indeed improve immunogenicity to influenza vaccination in elderly people, but do not restore immunogenicity to that of younger adults, suggesting the existence of other crucial factors.[77] Whether vaccine prevention can be improved by novel vaccines or would need strategies to slow the immune senescence process is a challenging issue.

Immunological safety of vaccines

The potent adjuvants that vaccinologists strive to develop and clinicians desire to have will not be used unless they prove safe.[2] From an immunological perspective, some key features of adjuvant safety are beginning to unravel. At the site of injection, inflammatory reactions are indicative of the onset of innate immunity, as shown by the number and type of recruited cells, the activation of these cells, and the associated cytokine release (panel 4, figure 2).[78] Consequently, adjuvants eliciting less intense reactions across a long period might show a better reactogenicity profile than do those with a hit-and-run

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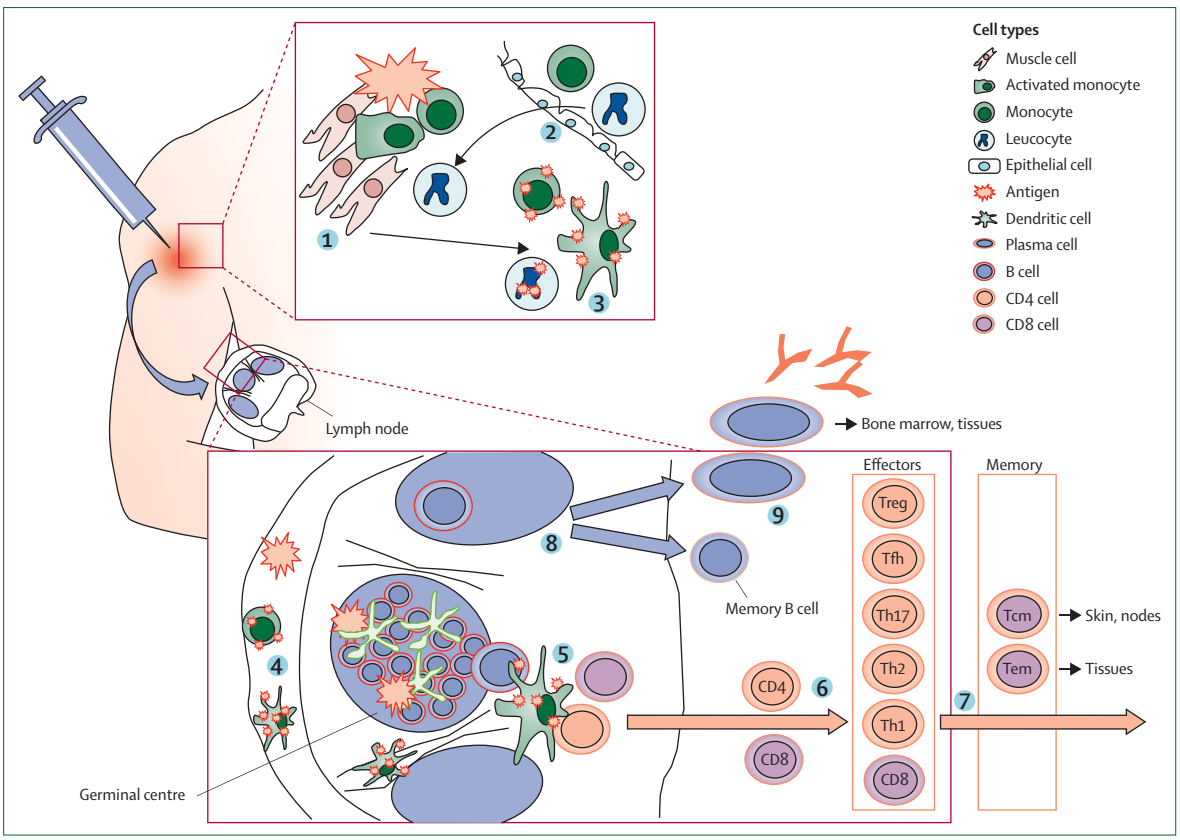

Figure 2: **Immunological challenges for new vaccines**
This figure shows the processes outlined in panel 4. Treg=regulatory T cell. Tfh=follicular T cell. Th=T helper cell. Tcm=central memory T cell. Tem=effector memory T cell.

profile (ie, eliciting a highly intense reaction in a short period). An unmet challenge will be to learn how to translate preclinical observations about the type and magnitude of innate responses to the expected reactogenicity pattern in individuals differing in their antigen experience, health problems, and genetic background. In the draining lymph nodes, bystander activation of potentially autoreactive B or T cells should be avoided because of possible autoimmune manifestations.[79] This aim is likely to be best achieved with formulations deprived of polyclonal activation capacities, and by restriction of antigen presenting cell activation to the monocyte-derived dendritic cells generated at the site of injection rather than those residing in the lymph nodes. Avoidance of the systemic dissemination of immunomodulatory signals also seems crucial. Novel imaging techniques that allow in-vivo assessment of the fate of vaccine components should prove useful to characterise and select formulations with the most promising immunological safety patterns.

From mass immunisation to personalised responses: environmental and genetic factors

The one-size-fits-all approach is not sustainable in vaccine prevention of diseases. In the past three decades, vaccines have been shown to elicit variable responses in distinct geographical environments. For example, the same conjugate vaccine against *H influenzae* type b (PRP-D) proved effective in Finland but not in Alaska, and Chilean infants raised three-times higher antibody responses to immunisation than did Belgian children.[80] Subsequently, rotavirus vaccines that were proven effective in Europe, Latin America, and the USA were much less immunogenic in low-income countries.[81] Disentangling the relative contribution of varying epidemiology patterns, environmental factors, and host genetic factors contributing to the success of a vaccine, or to adverse vaccine outcomes, is a major challenge.

The potency of the immune system resides in its highly polymorphic nature, enabling sufficient immunological diversity to overcome many diverse pathogens. The severe or fatal outcomes of infection in otherwise healthy immunocompetent hosts result from genetic predispositions mediated by mutations in specific immune genes.[82] This genetic basis implies that researchers could predict the infections that individuals are genetically destined to be at risk from, or at least those infections that are severe in rare cases, and thus only these individuals would be selectively protected. For example, identification of the small proportion of

individuals who fail to rapidly clear viruses such as hepatitis B virus or human papillomavirus—and are thus at high risk of chronic infection or cancer[83]—would allow immunisation strategies to focus on high-risk individuals. In theory, genetic screening for the likelihood of severe complications from most or all vaccine-preventable pathogens could thus generate personalised recommendations for immunisation.

The diversity of the immune system similarly affects vaccine responses. Millions of single nucleotide polymorphisms, and even more genetic variants, have been identified. The successful development of pharmacogenomics has prompted probing of how host genetic markers might result in variations of vaccine-induced responses.[84] This research is expected to identify gene polymorphisms that predict the likelihood that a vaccine will be successful or have adverse outcomes of either vaccine failure or adverse events.[84] Epigenetic studies might help to better understand how environmental factors can affect innate and adaptive immune responses. This work is still in its infancy but holds promise, especially when combined with novel system vaccinology approaches.[85,86]

Paradoxically, however, the global successes of mass immunisation strategies contrast with the largely recognised failure of strategies targeting individuals at increased risk of complications, whether from underlying disease or treatment.[87,88] Such a move from community-based towards personalised genetic-driven immunisation strategies would thus raise major new challenges: high-risk individuals who should be immunised against specific pathogens, or should not be immunised because of increased risk of adverse event, would need to be identified; relative risks would need to be communicated to high-risk individuals; personalised recommendations would need to be generated for these high-risk individuals; and immunisation services capable of reaching all high-risk individuals, who would no longer be indirectly protected by herd immunity, would need to be implemented. Furthermore, individuals defined as low risk by genetic analysis might become high risk when they become immunocompromised by disorders or treatments. Replacement of the existing immunisation menu with à la carte vaccinations would probably be extremely costly, and thus would initially be restricted to a few novel expensive vaccines with potentially higher efficacy but also higher risk of adverse events than with existing vaccines. Interestingly, developments in personalised immunisation might be driven by the increasing consumer demand for personalised medical approaches that maximise the likelihood of benefits but mitigate the risks of severe adverse events.

Conclusions

Compared with their human hosts, microbial populations are greater in number, have higher rates of replication, generate more mutations, and have promiscuous mechanisms of genetic exchange. These factors underpin the diversity of microbial genotypes and phenotypes, and are major factors in the adaptive potential that determine their commensal and virulence behaviour. In the past, the major contributions to vaccine research were made by microbiologists who combined detailed knowledge of microbial diseases with no small measure of empiricism to choose components that could induce protective immunity. However, many of the most challenging pathogens are characterised by several mechanisms of antigenic variation, allowing evasion of host immune responses. For these pathogens, host-microbial interactions are highly complex: natural infection often does not confer adequate immunity to protect against future exposure. In the future, immunology will be a cornerstone of our efforts to understand in detail what is needed to formulate and present microbial components so as to engage innate and adaptive host immune responses efficiently and safely. To that end, reductionist approaches, especially those using in-vitro systems, have increased our understanding of the role of dendritic cells in innate immunity, and of T and B cells in adaptive immunity. But reductionist science can fall short of capturing the highly relevant complexity that characterises the combinatorial interplay between host genetic polymorphisms and environmental factors; we are, for example, only just beginning to unravel the crucial dialogue between commensal microbes (host microbiota) and immune responsiveness.[89]

Contributors

ERM and C-AS equally contributed to conception, writing, and revision of the report.

Conflicts of interest

ERM has received honoraria for work as a board member for Novartis and GlycoVaxyn, and as a consultant for Novartis. C-AS's institution has received grants from GlaxoSmithKline, Sanofi Pasteur, Nasvax, and DBV Technologies; and travel and accommodation expenses from GlaxoSmithKline and Sanofi Pasteur. C-AS and C-AS's institution have received royalties for the patent for Viavac; and C-AS has patents, which have not generated royalties, with Sanofi Pasteur and DBV Technologies.

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