Overview of vaccines and immunisation

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The biotechnology revolution

During the past two decades, advances in biotechnology and increased knowledge of the inductive and effector components of immune responses have revolutionized the field of vaccine development. This has opened a vaccine ‘pipeline’ that has already resulted in the licensure of many new and improved vaccines including recombinant hepatitis B vaccine, acellular pertussis vaccines, and conjugate vaccines against Haemophilus influenzae type b, group C Neisseria meningitidis and Streptococcus pneumoniae. The application of recombinant DNA technology, polymerase chain reaction amplification, monoclonal antibodies, peptide synthesis, genomics (in particular, the sequencing of the entire genome of various pathogens\(^1\)), proteomics and high throughput informatics are examples of the powerful tools driving the development of desirable new and improved vaccines\(^2\). Combined with advances in adjuvant technology, specific methods of modulating immune responses (\textit{e.g.} by the administration of various cytokines), and novel ways of delivering antigens, many interesting new vaccine candidates and technologies are reaching the stage of clinical testing.

The vaccine development paradigm

Considerable attention has been focused in recent years on the versatile advances in modern biotechnology that are giving rise to the exciting new candidates that fill the upstream portion of the vaccine development pipeline. On the other hand, less notice has generally been paid to the series of sophisticated clinical vaccine studies that must be properly executed to advance a vaccine candidate, incrementally, towards ultimate licensure, based on proof of the vaccine’s safety, immunogenicity and efficacy in target populations.

\textbf{Phase I trials}

Phase I trials preliminarily examine the candidate’s safety and immunogenicity in small numbers of healthy adults. Such early dose/response
tests detect common adverse reactions and provide an initial glimpse of whether relevant immune responses are generated.

**Phase II trials**

Subsequent phase II trials, which assess the vaccine in increasingly larger numbers of subjects, are typically placebo-controlled to better measure the rate of adverse reactions versus background rates of complaints. The level of shedding of a live viral or bacterial vaccine or of a recombinant strain is often intensively examined in phase II trials, as is its propensity to be transmitted to household contacts and to survive in the environment. For vaccines that will ultimately be used in infants and children, phase I and II trials must be undertaken in progressively younger subjects. Particularly demanding is the design of phase II clinical trials to evaluate the reactogenicity and immunogenicity of the new multivalent combination vaccines in infants. The ultimate objective of combining vaccine antigens into a single inoculation is worthy, but experience has shown that interactions may occur that depress the immune response to some antigens or that enhance the overall reactogenicity. Thus, phase II clinical trials must rigorously demonstrate that acceptable immune responses to all antigens can indeed be stimulated without undue reactogenicity. Phase I and II trials of certain candidate vaccines require special considerations (e.g., vaccines against RSV and group A *Streptococcus pyogenes*) because of safety concerns.

**Experimental challenge studies**

In some instances, as with candidate vaccines to prevent influenza, *Shigella* dysentery, cholera or *Plasmodium falciparum* malaria, preliminary assessments of vaccine efficacy can be obtained through carefully performed experimental challenge studies with wild-type organisms in fully informed, consenting, adult community volunteers.

**Phase III trials**

Large-scale, randomized, controlled phase III field trials remain the gold standard for demonstrating the efficacy of a vaccine. Such trials tend to be expensive, require several years to complete and are subject to the vagaries of year-to-year variation in disease incidence. Moreover, in prelicensure efficacy trials, the protective activity of a vaccine is measured under idealized conditions where extra personnel participate in the vaccination
and only fully vaccinated subjects are included in calculations of efficacy; therefore, the practicality of programmatic use of the vaccine is not readily estimated. Epidemiological methods to estimate vaccine ‘efficacy’ (i.e. effectiveness) after licensure and large-scale use have also been developed.

Phase IV trials

Most phase IV assessments involve case/control studies which are relatively inexpensive and simple to perform, but have inherent limitations that can distort the estimation of ‘efficacy’\(^1\). Nevertheless, a few controlled phase IV post-licensure selective vaccination trials have been performed that directly measure effectiveness of vaccine used under real-life, programmatic conditions\(^2\).

Enhanced post-licensure epidemiological surveillance has proven its value by demonstrating herd immunity effects (as with \textit{H. influenzae} type b and meningococcal C conjugate vaccines), non-target consequences of vaccine use (e.g. the rare occurrence of vaccine-associated paralytic poliomyelitis in household contacts of infants who have received Sabin live oral polio vaccine) and rare vaccine-associated adverse events.

A long journey fraught with many potential pitfalls and considerable attrition awaits any vaccine candidate as it attempts to run the gauntlet from inventive concept to licensed product and public health tool. Few of the vaccines that enter phase I trials reach the point of a phase III efficacy trial, and only a handful of vaccine candidates ultimately become licensed by regulatory agencies. Moreover, the step-wise paradigm by which vaccine candidates are advanced from initial phase I dose response safety/-immunogenicity trials to phase II reactogenicity/immunogenicity trials in larger numbers of subjects, and finally to large-scale phase III efficacy trials is becoming increasingly complex and expensive. In particular, the cost of generating clinical trial data while strictly adhering to the rules and regulations of Good Clinical Practice and of performing quality assurance and monitoring to verify the validity of such data has greatly escalated during the past decade.

Some undercurrents influencing the flow of vaccine development

Even as the tools of modern biotechnology are expanding the horizons of what is achievable in the arena of vaccine development, a number of other counteracting forces are exerting pressures that retard vaccine development or channel it in certain directions. Some of these forces are discussed below.
Societal perceptions and expectations of vaccine safety

There is an increasing expectation on the part of the general public in industrialised countries that vaccines must be ‘completely’ safe. These expectations are in part being driven by vocal anti-vaccine groups that lobby legislators, influence mass media, and foster negative perceptions about vaccines among the public.

The ‘busy’ infant/toddler immunisation schedule

Several vaccines licensed since 1985, including acellular pertussis vaccines, *H. influenzae* type b and pneumococcal conjugates and meningococcal group C, are targeted for parenteral administration to infants and/or toddlers. Understandably, the licensure and recommended introduction of these vaccines into the infant immunisation schedule raised concern over the number of additional injections that had to be given. This led to great efforts being expended in the 1990s on the part of industry and the public sector to develop combination vaccines that deliver multiple antigens via a single parenteral inoculation. The advantages of combination vaccines are counterbalanced by the complexity and cost of clinical trials that must be performed to document their safety and the immunogenicity of their various component antigens.

Another technology-based approach to simplify the infant/toddler immunisation schedule is to develop more immunogenic vaccines that will require fewer doses to immunise successfully. Alternatively, it may be possible to achieve the comparable immunologic responses with fewer doses by utilizing alternative immunisation schedules (based on knowledge of the factors that influence immune responses).

The desirability of administering vaccines by non-parenteral routes

The Sabin oral polio vaccine set a precedent among vaccines for practicality and ease of administration to subjects of any age. There is great interest to identify ways to administer other vaccines by non-parenteral routes, *e.g.* orally, nasally or transcutaneously. Certain live vector vaccines, antigen delivery systems and powerful adjuvants offer promise as strategies to successfully administer vaccines via mucosal and transcutaneous surfaces.

There already exists considerable experience with several other oral and intranasal vaccines including: Ty21a live oral typhoid vaccine; a live oral cholera vaccine (CVD 103-HgR) and a non-living oral cholera vaccine (whole vibrio cells plus B subunit); and a live (cold adapted) and a non-
living (virosomes plus LT adjuvant) intranasal influenza vaccine. From this considerable experience, several observations have been made:

1. In most populations, oral or intranasal vaccines are preferred over parenteral vaccines, thereby increasing compliance.

2. Mucosal immunisation precludes problems of injection safety found in some non-industrialised countries where the sporadic use of non-sterile needles and syringes can result in the inadvertent spread of hepatitis B, hepatitis C and HIV.

3. Specialized microfold cells overlying mucosa-associated lymphoid tissues found both along the intestine and in the nose constitute competent portals of entry to inductive sites for immune responses.

4. Because they elicit IgA (usually in addition to systemic immune responses), mucosal vaccines are particularly attractive for pathogens that primarily cause mucosal infection of the gastrointestinal, respiratory or genito-urinary tracts or that invade via the mucosa lining those tracts.

5. Properly formulated, mucosally administered vaccines can be adapted to stimulate any relevant type of immune response, in addition to secretory IgA, including serum IgG neutralizing antibodies (against toxins and viruses) and a variety of cell-mediated responses including lymphocyte proliferation accompanied by release of cytokines, and classical MHC I-restricted CD8+ lymphocytes.

6. Some mucosal vaccines (e.g. Ty21a) have stimulated long-term protection enduring for up to 7 years.

7. Mucosal immunisation is not a panacea. Problems that require research include the observation that several oral vaccines are less immunogenic in subjects living in under-privileged conditions in non-industrialised countries and whether oral immunisation with certain vaccines (such as live rotavirus strains) increases the risk of intussusception during a short period of time immediately following vaccination.

Economics and vaccine development

There is increasing recognition of the fundamental role that economic factors play in driving the development of specific vaccines and in the setting of vaccine development priorities within industry. Major vaccine manufacturers in the US and Europe have played a pivotal role in the vaccine innovations that have resulted in most new vaccines becoming available as licensed products during the past two decades. The investment that ‘big pharma’ makes for this purpose is enormous, estimated at ~$300 million to bring a new vaccine to licensure. New or expanded production facilities may cost an additional $100–200 million. To remain viable and competitive, industry must recoup these
enormous investments. Moreover, this return on investment provides resources to support development of the next generation of innovative vaccines.

Since most vaccine revenues for the major multinational manufacturers come from the sale of vaccines in industrialised countries, it is useful to consider that vaccines can be categorised into one of four groups in relation to whether or not there exist credible markets for the vaccine in industrialised countries: (i) global market vaccines; (ii) industrialised country market vaccines; (iii) impeded vaccines; and (iv) developing market vaccines.

**Global market vaccines**

Whereas the diseases against which ‘global market vaccines’ are directed exhibit a substantial burden among populations in both industrialised and non-industrialised areas of the world, their development is overwhelmingly driven by the anticipated industrialised country market. Nevertheless, the public health need for these vaccines in the non-industrialised world is generally more compelling because of the greater frequency of severe clinical syndromes and fatalities. Examples of licensed ‘global market vaccines’ include the *H. influenzae* type b conjugates and hepatitis B vaccines. Important global market vaccines that are not yet licensed but are in advanced development include 9-valent and 11-valent *S. pneumoniae* conjugate vaccines and several new candidate rotavirus vaccines.

**Industrialised market vaccines**

The power of the market place to influence vaccine development decisions is exemplified by ‘industrialised market vaccines’ intended to prevent diseases that are considered relevant targets only in the context of industrialised country settings. Vaccines against Lyme disease targeted at populations in the Northeastern and Northern midwest US are examples.

During the past two decades, two categories of vaccines have languished in development, albeit for quite different reasons.

**Impeded vaccines**

Vaccines in this category would almost certainly have substantial markets in industrialised countries if they were shown to be safe and effective, but certain scientific, ethical or public perception obstacles raise the risk that they might not reach product licensure and commercialization. As a consequence, such vaccines are generally lower priority for investment by the vaccine industry. The legacy from experiences with earlier generations of RSV and M protein-based *S. pyogenes* vaccines (that were incriminated as having caused severe adverse events or as having predisposed to the development of immunopathology when vaccinees were exposed to the wild-type pathogen in the course of clinical trials) has stifled the pace of development of more modern vaccine candidates.
Developing market vaccines
Vaccines that fall into this category face ethical as well practical dilemmas. ‘Developing market vaccines’ mainly aim to prevent diseases for which the burden is prominent in non-industrialised country populations but little, if any risk, is posed for individuals in industrialised countries (unless they travel to non-industrialised areas). Some examples include vaccines against malaria, tuberculosis, *Shigella* and enterotoxigenic *Escherichia coli* diarrhoea, cholera, typhoid fever, group A meningococcal infections, dengue fever, hepatitis E, leishmaniasis and schistosomiasis. The fact that industrialised country markets are either lacking or relatively small (usually limited to travellers) provides little incentive for industry to invest in the development of these vaccines. The term ‘developing market vaccines’ not only reflects that these are particularly targeted for use in non-industrialised countries but also conveys the notion that ‘non-traditional’ markets for these vaccines will have to be stimulated in the less developed world in order to increase the attractiveness of investment.

Fostering the development of ‘impeded’ vaccines
Two examples of ‘impeded vaccines’ are RSV and group A *S. pyogenes*. Because of the perceived risk to return on investment, within industry, these vaccines have not received the priority one might otherwise expect based on their public health need and expected market in industrialised countries.

Vaccines against chronic diseases
The next great frontier for vaccine development will be vaccines against chronic diseases such as peptic ulcer disease, cancer (*e.g.* gastric carcinoma, cervical carcinoma), atherosclerotic heart disease, type I and II diabetes, and Alzheimer’s disease, to mention a few. In some instances the feasibility for vaccination is based on the discovery that infection with a specific pathogen is (or is likely) responsible for the chronic disease. Whereas the association between hepatitis B virus and hepatocellular carcinoma has long been known, some pathogens that have more recently been associated with chronic diseases include human papilloma virus with cervical cancer, *Helicobacter pylori* with peptic ulcer disease and gastric carcinoma, an association between *Chlamydia* and atherosclerotic heart disease (and perhaps with cervical cancer). In other instances, vaccine development is based on immunisation with chemical moieties that play a role in the pathogenesis of the chronic disease. Thus, immunisation against certain lipids may be an approach
to prevent atherosclerotic heart disease and vaccination with β-amyloid protein may thwart the progression of Alzheimer’s dementia.

Target populations for vaccination other than infants and toddlers

In both industrialised and non-industrialised countries, most immunisations are administered to infants. Moreover, most revenues for the multinational vaccine industry come from the sale of vaccines used in infant immunisation in North America and Europe. However, for purposes of epidemiological control of disease, some existing vaccines and many new vaccines under development in the future will be utilized in target populations of older children, adolescents, young adults and the elderly. Already, in many industrialised countries, senior citizens are the target for receipt of annual influenza and periodic pneumococcal (polysaccharide) vaccinations. Vaccines against certain other diseases are likely also to be given to these groups. Adolescents and young adults were important targets for mass vaccination with group C meningococcal conjugate vaccine in the UK’s national programme to control invasive group C disease. The degree of compliance of the population, public perceptions, epidemiological impact and cost effectiveness in the UK of the meningococcal C vaccination campaign are being scrutinized by health authorities in other countries as a possible model to be adapted to other societies. Future vaccines against certain sexually transmitted diseases such as Chlamydia, Herpes simplex virus and Neisseria gonorrhoea are also likely to be targeted to high risk teenage and young adult populations.

The Global Alliance for Vaccines and Immunization (GAVI)

Some momentous achievements of vaccines globally are the result of various international agencies and interested parties working together in coalitions and alliances with common goals. The Smallpox Eradication Program, the Polio Eradication Initiative and the Expanded Program on Immunization, (EPI, which is concerned with delivering a series of basic vaccines to infants throughout the non-industrialised world), represent examples. In the waning years of the 20th century, three glaring gaps became apparent with respect to vaccines at the international level: (i) EPI coverage had stagnated globally from the peak coverage reached circa 1990 and had begun to fall in certain areas; (ii) ‘new’ vaccines that were routinely being given to infants in industrialised countries (such as H. influenzae type b conjugate and hepatitis B) were not being expeditiously introduced for routine use in non-industrialised countries; and (iii) inadequate resources were being channelled to develop vaccines of particular importance for populations in non-industrialised countries.
It is against this background that a new coalition, the Global Alliance for Vaccines and Immunization (GAVI), came to exist in January 2000, as various traditional partners (such as WHO, UNICEF, the World Bank, the Rockefeller Foundation, national governments and bilateral agencies) teamed with new partners, including industry and the Bill and Melinda Gates Foundation, to forge a novel alliance to address the perceived gaps and weaknesses. It was hoped that GAVI would invigorate the commitment of all partners to provide safe and effective vaccines for immunisation of all the world’s children. GAVI has promulgated five specific objectives. They include, to: (i) improve access to immunisation services; (ii) expand the use of existing cost-effective vaccines; (iii) accelerate the development and introduction of new vaccines; (iv) accelerate research and development efforts for vaccines and related products specifically needed by non-industrialised countries, particularly vaccines against HIV/AIDS, malaria and TB; and (v) make immunisation coverage an integral part of international development efforts.

One extraordinary resource available to GAVI is its Global Fund for Children’s vaccines, which was initially capitalized by the Bill and Melinda Gates Foundation with a contribution of $750 million. The Fund now exceeds $1 billion, owing to additional contributions from several national governments. The Global Fund, which is primarily targeted to the world’s least affluent countries, provides flexible financial support to strengthen the infrastructure needed to increase immunisation coverage and (for countries that already have a DTP3 coverage >70%) offers hepatitis B vaccine and, where appropriate, *H. influenzae* type b conjugate and yellow fever vaccines.

**Globalization**

Many infectious diseases have global reservoirs that do not respect national borders. During the 1990s, the public in industrialised countries came to accept the notion that protection from infectious diseases often requires supporting surveillance and control activities (including vaccine development) on an international level, particularly in non-industrialised countries. There was also an increasing recognition that many infectious diseases that pose notable risks for travellers (including deployed military personnel), such as malaria, typhoid fever, shigellosis, enterotoxigenic *E. coli* diarrhoea, tuberculosis and dengue fever, constitute major endemic disease problems facing populations in non-industrialised countries.

In non-industrialised countries, where about two-thirds of the world’s children live, the tasks of maintaining a cold chain and of delivering vaccines that require multiple-dose immunisation schedules to widely dispersed populations through understaffed health care infrastructures
represent enormous undertakings. Thus, from the global perspective, vaccines are sorely needed that: (i) can successfully immunise and protect after just a single dose; (ii) can be administered in combination with other vaccines; (iii) can be administered in the neonatal period or in very early infancy; and (iv) do not require special storage conditions (i.e. no cold chain). Moreover, for reasons previously mentioned, ideally these vaccines should be administered by non-parenteral routes.

Another by-product of globalization is the threat of bioterrorism. Groups representing diverse ideologies and agendas may contemplate the release of pathogenic agents within the US, the UK and other industrialised countries with the aim of causing widespread transmission, outbreaks of disease and panic and of influencing policy. Whereas many pathogenic viruses and bacteria or bacterial products are theoretically amenable to serve as bioterror weapons, particular attention has been given to *Bacillus anthracis*, *Yersinia pestis*, smallpox virus and botulinum toxin.

**Generic ‘platform’ technologies in vaccine development**

Some generic vaccine technologies and vaccination strategies hold special promise because of their flexibility, practicality and potential simplicity of production. Five that hold great promise are:

1. DNA vaccines as a means of priming the immune system of neonatal and very young infants.\(^8^\)

2. Bacterial live vector vaccines, in particular attenuated *S. Typhi*, *Shigella*, and *Mycobacterium* (BCG and new strains). These can either express foreign antigens of unrelated pathogens or can deliver DNA vaccines via mucosal administration.\(^3^1^\)

3. Viral live vectors such as adenovirus, which can carry the genes of unrelated pathogens.\(^3^2^\)

4. The concept of DNA prime/live vector boost.\(^3^3^\)

5. ‘Reverse vaccinology’, *i.e.* the identification and preparation of vaccine antigens using a genomic-based strategy.\(^3^4^\) This approach can function even with pathogens that cannot be readily cultivated *in vitro*.

A fundamental approach in vaccinology that has proven useful for poorly immunogenic antigens and that is flexible for many purposes is the ‘prime/boost’ strategy. When animals are primed with parenteral DNA vaccine and then boosted parenterally with a live vector expressing the relevant antigen, the immune responses and level of protection elicited are significantly superior to what may be achieved with other regimens. The efficacy of the DNA prime/live vector boost regimen markedly exceeds that achieved when either DNA or live vector is used for both
prime and boost or when protein is used to boost following priming with DNA. In animal models, the efficacy of DNA prime/live vector boost has been particularly impressive with antigens that are notoriously poor immunogens, such as Plasmodium and HIV.

The DNA prime/live antigen boost phenomenon, which has been confirmed in several laboratories, is now considered to be a critical tool of vaccinology and proof-of-principle clinical trials with HIV and malaria vaccines have been (or are being) initiated in Europe and the US. Heretofore, either a vaccinia virus or an attenuated (replication deficient) adenovirus live vector has usually been used for the boost and has always been given parenterally.

**Bioethical issues in vaccine development**

During the past few years, ethical issues related to vaccine development have been the subject of intense public scrutiny and debate. One revelation has been that some investigators involved in clinical trials, who are otherwise fine scientists or skilful clinicians, are unfamiliar with the rules and regulations of Good Clinical Practice, do not practice proper informed consent procedures, and do not know when and how they must communicate critical information (e.g. serious adverse events) to Institutional Review Boards (IRBs) and to regulatory agencies. Because of inadequate oversight of clinical trials, IRBs at a number of universities and investigators in the US have been temporarily barred from operation while their procedures were corrected. For clinical trials in non-industrialised countries, debate has raged over what should be the appropriate standard of care offered to the control group. Finally, debate has focused over the potential conflicts of interest for investigators who test vaccines and drugs that are proprietary to companies in which they hold equity.

**Concluding comments**

Vaccine development strategies driven by modern biotechnology, the need to combine vaccines into practical combinations, the complexity of testing new vaccines, economic issues of vaccine development and implementation and public perceptions of disease risk and vaccine safety are all in a state of evolution. An optimistic glimpse of the future envisions the administration of more vaccine antigens in new combinations by non-parenteral routes to target groups of all ages who, consequent to health education and responsible mass media messages, recognize the public health need for vaccines and their relative safety.
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