PART 1

Introduction
CHAPTER 1
Concept and Scope of Modern Vaccines

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Introduction

Historically, vaccination has probably had the greatest impact on human health of any medical intervention technique. Immunization is the only cost effective solution that can arrest and even eradicate infectious diseases. The science of vaccinology can be traced to the ancient Chinese, who protected against smallpox by the process of variolation, in which small quantities of scabs from a lesion of an infected person were intranasally inoculated [1]. This process was revived in the early 18th century when Lady Mary Montagu, who had observed variolation being practiced in Turkey, advocated its use to prevent smallpox. Modern vaccinology started as a proper scientific endeavor by Edward Jenner’s findings that cowpox pustules would prevent smallpox infection [2]. His work was the first to be evaluated scientifically and established the scientific basis for using a related but less dangerous pathogen to engender immune responses that are cross-protective against the more virulent pathogen [3]. The seminal work and findings of Jenner lay unexploited for nearly a century until Louis Pasteur demonstrated that chickens could be protected from cholera by inoculation with attenuated bacteria [4]. Similar experiments also showed that sheep could be protected from anthrax [5]. This concept of weakening a pathogen to invoke the immune system to produce a response forms the basis of immunity elicited by the Bacille Calmette-Guérin (BCG) tuberculosis vaccine, first administered in 1921 [6] and still in wide use today.

Vaccines are defined as immunogenic preparations of a pathogen that evoke an immune response without causing disease. While attenuation and inactivation of pathogens are conventional approaches, and are still used, modern vaccines also exploit recent developments in immunology, genomics, bioinformatics, and structural and protein chemistry. At the heart of all vaccines is antigen – the ligand of the receptors of T and B lymphocytes. Lymphocytes are the effector cells of the adaptive immune system that mediate immunologic memory responses – the very hallmark of vaccination – which set vaccination apart from other forms of modern immune system manipulation, such as broad-spectrum immunopotentiators, cytokine therapy, or passive transfer of specific hyperimmune globulins derived from human plasma.

The scope of modern and future vaccines has widened considerably since the empirical approaches of the pre-genomic era. Vaccines can now be designed rationally, even customized to individual needs. Developments in many areas of vaccinology, from adjuvants, proteomics, expression library immunizations (ELI), and sub-unit vaccines, to innovative funding and philanthropy, continue to reach new milestones. However, there are challenges in the road ahead. The vaccines that have not yet been made either exceed the limits of
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current technology or there is a lack of incentive. Here we outline the limitations of current vaccine technology and, through the following chapters, identify technologies that may help the field of vaccinology to advance.

Triumphs and limitations of current vaccination

After access to affordable nutrition, clean drinking water, and sanitation, low cost vaccines are the single most cost effective healthcare measure that can be taken to protect human health. This is highlighted by the fact that mass immunization programs have directly resulted in the control of several infectious diseases. For example, rates of incidence of diphtheria, measles, mumps, pertussis, and a number of other common diseases have been reduced by over 99% in the United States (Table 1.1). In the case of smallpox, global eradication was achieved through a concerted effort led by the World Health Organization (WHO). For polio, a concerted eradication program has reduced the incidence year after year from approximately 35,000 cases annually to fewer than 4000 in 1996 (Figure 1.1). Similarly, as the number of immunizations against measles has risen over the past two decades, the number of reported measles infections has fallen (Figure 1.2).

In addition, by controlling infections, vaccines reduce expenditure on future treatment (Table 1.2). Such costs are highlighted by the Centers for Disease Control (CDC) [8], which estimates that for every dollar invested in immunization, between $2 and $29 are saved. In addition the entire cost of the global smallpox eradication program, approximately $32 million, is returned every 20 days in not having to vaccinate travelers. A specific case in point is made by the combined measles, mumps, and rubella (MMR) vaccine. Immunization with this combined MMR vaccine was estimated to provide $5.1 billion direct and indirect cost savings in the USA for 1992 alone [9].

Despite the impact of vaccines on childhood infectious diseases such as measles, diphtheria, polio, and meningitis, there are many infectious diseases that continue to thwart vaccination programs, particularly in resource-poor countries, such as malaria, salmonellosis, and tuberculosis. There are several reasons why we lack vaccines to these diseases:

- Genetic instability. A major roadblock to vaccination against many pathogens is unpredictable antigenic variation. Antigenic variation per se is not an insurmountable challenge: current vaccine technology already protects us from pathogens with relatively small numbers of serotypes, for example polio (three serotypes) and rotavirus (four serotypes), and in principle scaling to dozens or even hundreds of strains of a pathogen, such as Streptococcus pneumoniae (ninety known serotypes), is possible. It is well known that an individual can become immune

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<tbody>
<tr>
<td>Diphtheria</td>
<td>206 939 (1921)</td>
<td>2</td>
<td>5</td>
<td>-99.9976</td>
</tr>
<tr>
<td>Measles</td>
<td>894 134 (1941)</td>
<td>508</td>
<td>135</td>
<td>-99.9849</td>
</tr>
<tr>
<td>Mumps</td>
<td>152 209 (1968)</td>
<td>751</td>
<td>612</td>
<td>-99.9999</td>
</tr>
<tr>
<td>Pertussis</td>
<td>265 269 (1934)</td>
<td>7796</td>
<td>5519</td>
<td>-99.9791</td>
</tr>
<tr>
<td>Polio, paralytic (wild poliovirus)</td>
<td>21 269 (1952)</td>
<td>0</td>
<td>0</td>
<td>-100.000</td>
</tr>
<tr>
<td>Rubella (German measles)</td>
<td>57 686 (1969)</td>
<td>238</td>
<td>161</td>
<td>-99.9972</td>
</tr>
<tr>
<td>Rubella, congenital syndrome</td>
<td>20 000 (1964-5)</td>
<td>4</td>
<td>4</td>
<td>-99.9998</td>
</tr>
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to a formidable number of strains of a particular pathogen, as evidenced by the acquisition of immunity to malaria or the common cold over the course of two or three decades of natural exposure. This could be replicated in a compressed time frame with the appropriate vaccine. The real challenge is to provide pre-existing immunity to pathogens or strains of pathogens that do not yet exist. While the pre-existing repertoire of the adaptive immune system clearly has the capacity to respond to and retain immunologic memory of any antigen, current vaccine production methods require these antigens.
Table 1.2 Cost effectiveness of childhood vaccines in the United States and the estimated returned savings, both direct and indirect, from vaccination.

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Direct medical savings</th>
<th>Direct and indirect savings</th>
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<tr>
<td>DTP</td>
<td>$6.0</td>
<td>$29.1</td>
</tr>
<tr>
<td>MMR</td>
<td>$15.3</td>
<td>$21.3</td>
</tr>
<tr>
<td>OPV</td>
<td>$3.4</td>
<td>$6.1</td>
</tr>
<tr>
<td>Integrated (DTP, MMR, OPV)</td>
<td>$7.4</td>
<td>$25.5</td>
</tr>
<tr>
<td>Haemophilus influenza type b</td>
<td>$1.4</td>
<td>$2.2</td>
</tr>
<tr>
<td>Hepatitis B (perinatal/infant)</td>
<td>$0.5</td>
<td>$2.0</td>
</tr>
<tr>
<td>Varicella</td>
<td>$0.9</td>
<td>$5.4</td>
</tr>
</tbody>
</table>

DTP, diphtheria, tetanus, pertussis; MMR, measles, mumps, rubella; OPV, oral polio vaccine.
Data from National Immunization Program, Centers for Disease Control and Prevention.

to be known beforehand. HIV is the worst-case scenario of such a pathogen. Although we know antibodies to gp120 can confer protection, the ability of the virus to generate a seemingly infinite number of antigenically distinct molecules as it replicates has thwarted most attempts to develop a vaccine. This intractable problem remains a major hurdle to development of vaccines against organisms with unpredictable antigenic variation.

- **Complexity.** This generally goes hand-in-hand with the size of the genome of a pathogen and the number of distinct stages in its life cycle. Most successful vaccines are against viruses, much fewer are against bacteria, and few are in development against parasites or fungi. In order to achieve immunity by vaccination, a vaccine has to be able to emulate the immunogenic components of natural infection without causing the disease. Attenuated organisms lack the pathogenicity of the parent pathogen and retain the ability to engender protective immunity. Often only a single immunization is required, and immunity is frequently life long. Using these criteria, the best vaccines are arguably therefore live, attenuated organisms. However, the trend today is toward the development of killed or subunit vaccines because they pose no risk of reversion. Killed organisms may retain some of the inherent immunogenic properties of the live organism (e.g., components in the cell wall) although such vaccines may contain live organisms if not prepared correctly. These risks mean killed organisms are also gradually being replaced in favor of safer subunit or recombinant protein-based vaccines or nucleic acid vaccination. Unfortunately, recombinant protein vaccines face the greatest challenges, particularly those aimed against bacteria or parasites. Unless pathogenicity is mediated by a single component (such as an exotoxin, which can be protected against using a simple toxoid vaccine) protective immunity appears to be mediated by responses to multiple antigens. To date, single recombinant protein vaccines have performed poorly in providing protection against bacteria.

- **Correlates of protection.** A related roadblock is a lack of knowledge of the antigen(s) required to engender protection. We measure immune responses to a pathogen using *in vitro* tools, such as ELISAs, neutralization assays, and γ-interferon release assays. These can be very sophisticated and map the antigens recognized, and even the epitopes within, in fine detail. The assumption is made that these detectable responses overlap, at least in part, with the antibodies and T cells that mediate protection. However, many may be immunologically irrelevant. Simply because we can detect a response to a particular antigen does not always connote a primary role in protection. Conversely, an antigen that is critical for engendering a protective response may not be detected by our *in vitro* assays. Thus the antigen(s) used for a vaccine do not necessarily
have to be particularly immunogenic in natural infection. The overlap between the measurable “reactome” and the “protectome” is a largely unexplored area.

- **Adjuvants.** Expectations of modern vaccines can sometimes be unrealistic. Current vaccines are prophylactic and are administered to healthy individuals. Therefore any safety issues have to be weighed carefully against the benefit. While subunit vaccines are the safest of all our options, the gain in safety is a tradeoff in efficacy. Most subunit vaccines and recombinant protein vaccines lack the inherent proinflammatory properties of attenuated organisms, which have to be replaced by the inclusion of an adjuvant. For reasons that are still not fully understood, immunity generated by proteins formulated in adjuvants decays more rapidly than that generated from live organisms, thereby requiring booster immunizations. Swelling, aching, and fever – the very proinflammatory properties required of a good adjuvant – are considered unacceptable side effects. (The stress suffered by a parent when their child has a fever will serve as a reminder of this.) These are trivial compared to the disease itself, yet relatively few adjuvants are approved for human use, and those that have been approved are all mild. Ultimately it may be impossible to engender complete immunity by vaccination without causing “disease” of some sort, and the best vaccines are likely to be a compromise.

**Modern approaches that impact vaccine design**

**Genomics**

In the pre-genomic era, vaccines were made from animal pathogens, or human pathogens either attenuated by abnormal growth conditions or killed by chemical inactivation. Many successful vaccines were developed using this empirical approach. This gave way to extracts of pathogens – or subunit vaccines – where components of the pathogen were used in place of the whole organism. In the post-genomic era, the production of subunit vaccines has become more rational and the preparations of antigens more precisely controlled. Despite their drawbacks, recombinant protein vaccines have had, and will continue to have, a major impact on diseases caused by simple pathogens, especially viruses, where a single antigen is often enough to provide immunity (e.g., human papilloma virus, HPV). Even for more complex pathogens such as bacteria and parasites, there is still the expectation that recombinant protein vaccines can provide protection, particularly if adjuvanted cocktails of protective antigens are used. Continued progress in this area has been hampered by the identification of candidate antigens. The problem has been the sheer size of the genome and the number of potential antigens available, and until recently the discovery of potential subunit vaccine antigens have been piecemeal and non-systematic.

Modern high throughput approaches to proteome-wide expression and screening technologies promise to revolutionize the discovery of new vaccine antigens for old diseases. A recent antibody profiling study of acquired immunity to malaria in the Gambia, for example, identified antibodies to several antigens present in children with acquired immunity that are absent from children who were still undergoing seasonal bouts of malaria [10]. These antigens would be considered prime targets for vaccine development. Importantly, the same study revealed the antigens currently being evaluated in clinical trials were not among these discriminatory antigens. The conclusion from studies like this is that non-biased screening approaches may lead to the discovery of different antigen sets than conventional “intuitive” approaches. It remains to be determined whether these new antigens lead to better vaccines, and Part 3 of this book focuses on these new technologies.

**Improved delivery systems and adjuvants**

Recombinant proteins are, for the most part, poorly immunogenic and require delivery in an immunogenic package. The most successful delivery systems for recombinant proteins are often based on macromolecular assembles of one sort or another, and can take the form of immune stimulating
complexes (ISCOMs), liposomes, or virus-like particles. Suspensions of antigen bound to inorganic particles such as alum are also immunogenic. It seems dendritic cells are particularly efficient at ingesting and responding to insoluble, particulate antigens, but less so to soluble proteins.

Other steps can be taken to improve the immunogenicity of existing vaccines. For example, peptide vaccines suffer from short half life in vivo, which can be improved by chemical modification to improve stability. Nucleic acid vaccines, although showing great promise in animal models, currently have had less developmental success in humans. The reasons are still unclear but their efficacy can be improved by using live vectors to boost them. The immunogenicity of recombinant vectors such as vaccinia or adenovirus is blunted by pre-existing immunity. This can be overcome by using animal viruses as vectors, such as fowlpox, where pre-existing immunity does not exist. These examples and other antigen engineering technologies are examined in Part 4.

Therapeutic vaccination

Currently none of the licensed traditional vaccines for use in humans are therapeutic, but instead are prophylactic and depend on antibodies to block initial infection. A vaccine administered after infection in order to treat (not prevent) disease is a realistic goal of modern vaccination. Once a pathogen has established an infection, the type of immune response required to eliminate the infection depends largely on whether the pathogen remains extracellular or gains entry into cells, where it becomes inaccessible to antibody. The optimism for therapeutic vaccines comes from great strides in the 1980s and 1990s in understanding T-cell recognition and antigen processing/presentation, and the realization that vaccines specifically targeting cell-mediated immunity could engender protection against pathogens that reside within cells. Both CD8 and CD4 T cells can mediate killing of cells harboring intracellular pathogens, particularly viruses (CD8) and bacteria that reside in endosomal compartments (CD4). Many model systems in animals have shown proof-of-principle of therapeutic vaccination. The bottleneck to translating this to vaccine development is, as with antibody vaccines, the size of the pathogen genome. Uniquely with T cells, the problem is amplified if synthetic peptides are desired for vaccination. Again, high throughput proteomic screening platforms and ever improving predictive algorithms promise to define the antigens needed, while carefully selected delivery vehicles or adjuvants will ensure the correct T cell subset(s) is stimulated.

Although the field of therapeutic vaccination is still developing for infectious disease, some promising inroads have been made in the cancer immunotherapy field. These technologies are based on the ex vivo activation [11] and amplification of the specific cellular immune response, followed by re-infusion of immune cells to the patient, as opposed to the in vivo activation hopefully achieved by traditional vaccines. The oncology targets for this approach are many. However, the field is gaining momentum with the FDA approval of Dendreon Corporation’s Provenge™ (sipuleucel-T) for asymptomatic, or minimally symptomatic metastatic, androgen-independent prostate adenocarcinoma [12].

A return to attenuated organisms?

With the notable exception of toxoids, the disappointing previous performances of single recombinant protein subunit vaccines against complex pathogens (bacteria, fungi, and parasites) compel us to continue the development of live attenuated vaccines alongside subunit vaccine development, to ensure the highest probability of discovering a successful vaccine against any particular pathogen. Live attenuated vaccines have many advantages over killed or subunit vaccines, although the safety requirements are more stringent owing to the risk of reversion to a pathogenic phenotype. Attenuated live bacterial vaccines currently licensed for human use include Mycobacterium bovis strain Bacille Calmette-Guérin (developed in the 1920s), Salmonella typhi Ty21a (1970s), and Vibrio cholerae CVD 103-HgR (1980s) [13]. The latter was derived by site-directed mutagenesis of the cholera toxin A gene (ctxA), and in some respects it represents
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the flipside of the traditional cholera toxoid vaccine. Although “low hanging fruit” for an attenuated vaccine, it points toward the rational way in which such vaccines may be made in the future. For most bacteria, multiple virulence factors are linked to pathogenesis. Traditional approaches to attenuation, such as forced adaptation to unusual culture conditions or radiation/chemical mutagenesis, are too “hit and miss” for modern rational approaches. With increasingly rapid annotation of sequenced pathogens comes the potential for systematic identification of virulence factors and their targeting for mutagenesis or deletion. Technologies for screening large numbers of mutants for attenuation and immunogenicity need to be developed, and will likely involve in vitro models.

Allied to this are vaccines based on animal pathogens – the “Jennerian” approach. The smallpox vaccine is often described as the prototype of all vaccines, and the only vaccine to have approached the eradication of a human disease. The principle of the original smallpox vaccine (which was cowpox) is somewhat different to the attenuated and killed vaccines that have followed. Cowpox is not an attenuated version of the human pathogen, but a closely related, less pathogenic, species of orthopoxvirus. The origins of vaccinia are not clear but modern phylogenetic analyses indicate it is a “domesticated” version of cowpox. Although vaccines based on animal pathogens are less pathogenic, attenuated strains are preferable. Replication-competent smallpox vaccines are being replaced by attenuated vaccinia strains such as MVA. The attenuated Mycobacterium bovis strain BCG, first produced as a vaccine against M. tuberculosis in the 1920s, also works on this Jennerian principle. More recent examples include the human-animal “reassortant” rotavirus vaccines that have been developed using animal rotaviruses engineered to contain antigens from the human rotavirus [14].

**Improve existing vaccines and vaccine uptake**

Most attenuated live organisms have limited efficacy, in part because the attenuation is so severe. Attempts to improve existing vaccines, such as with more potent adjuvants or adjuvant combinations, or improved manufacturing methods, is therefore another approach upon which modern technologies can be brought to bear. Basic research in immunologic processes will undoubtedly continue to reveal novel approaches to improving the immunogenicity of existing vaccines. The discovery of the role of Toll-like receptors [15,16] and the application of contemporary immunologic techniques [17] have helped our understanding of the basis of adjuvanticity. Likewise, our understanding of antigen processing pathways and different regulatory and effector T cell subsets has revealed the importance of antigen delivery in the type of immune response elicited. In the future, immunomodulators that switch off suppressive pathways and promote proinflammatory pathways, or ligands that target antigens to specific cells and tissues of the immune system, may be routinely engineered into vaccines. It is likely that our understanding of other critical processes, such as immunologic memory and immunodominance, will also become clearer in the near future and influence our design of vaccines and the adjuvants used.

It is worth remembering we do not need to discover new vaccines to make an impact on global health. The WHO estimates that 2.7 million children die annually from diseases that could be prevented with existing vaccines, almost half of which are caused by rotavirus and Streptococcus pneumoniae [18]. The majority of these are in resource-poor countries. The WHO’s Expanded Programme on Immunization (EPI), first introduced in 1974, aims to bring vaccination to children throughout the world. The scheme was recently expanded to cover the world’s poorest nations through the Global Alliance for Vaccines and Immunisation (GAVI) (www.gavialliance.org). Complacency and misinformation are problems in developing countries, and threaten to undermine vaccine-induced protection. Simply because a disease is no longer as common as it once was creates the illusion it is eradicated, allowing re-emergence if vaccination is not maintained. Clearly, mandatory childhood vaccination is important but remains essentially optional in most countries. Regardless of whether or
not one believes there is a role for the MMR vaccine in the development of autism, the reduction in uptake of the MMR vaccine in response to the recent hysteria had a direct effect on the rise in cases of childhood measles [19].

**Hurdles and challenges for the future**

**Non-infectious diseases as targets for modern vaccines**

The identification of autoantigens associated specifically with cancer and autoimmune disease has opened up new opportunities for vaccination. These are predominantly “therapeutic” T cell-based vaccines administered to individuals who already have disease. This considerably extends the concept of a “vaccine” beyond the traditional immunogenic preparation of a pathogenic microorganism, and indeed the recently approved HPV vaccines are a significant advance in the prophylactic vaccination against a virus-associated cancer [20].

**Transition from research to trial**

The pages of vaccine journals (and indeed this very book) are full of novel and ingenious vaccines, delivery systems, adjuvants, vectors, and scientific methods. Yet only the simplest and safest vaccines are ever considered for clinical trial. The realities of obtaining necessary approvals, producing a vaccine to current good manufacturing practice (cGMP) standards, and finding funding are far removed from most academic laboratories where basic vaccine research is conducted. Even if a candidate is evaluated in Phase I or II clinical studies, the investment required to enter Phase III trial is beyond the scope of most government funding agencies and requires the involvement of industry. For example, it is estimated that the research and development costs of bringing Gardisil™, an HPV vaccine comprising four recombinant proteins, to market was in excess of $1 billion. There have been several attempts to overcome the economic barrier against the development of less lucrative vaccines and diagnostics, such as with tax incentives and guaranteed government purchases. Additionally, non-profit organizations, such as the Wellcome Trust, and more recently the Bill & Melinda Gates Foundation, have become pivotal drivers for vaccine development. Thus, with the cooperation between scientific, industrial, non-profit, and political entities, the field of vaccinology will continue to advance, meeting the world’s unmet medical needs.

**References**

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