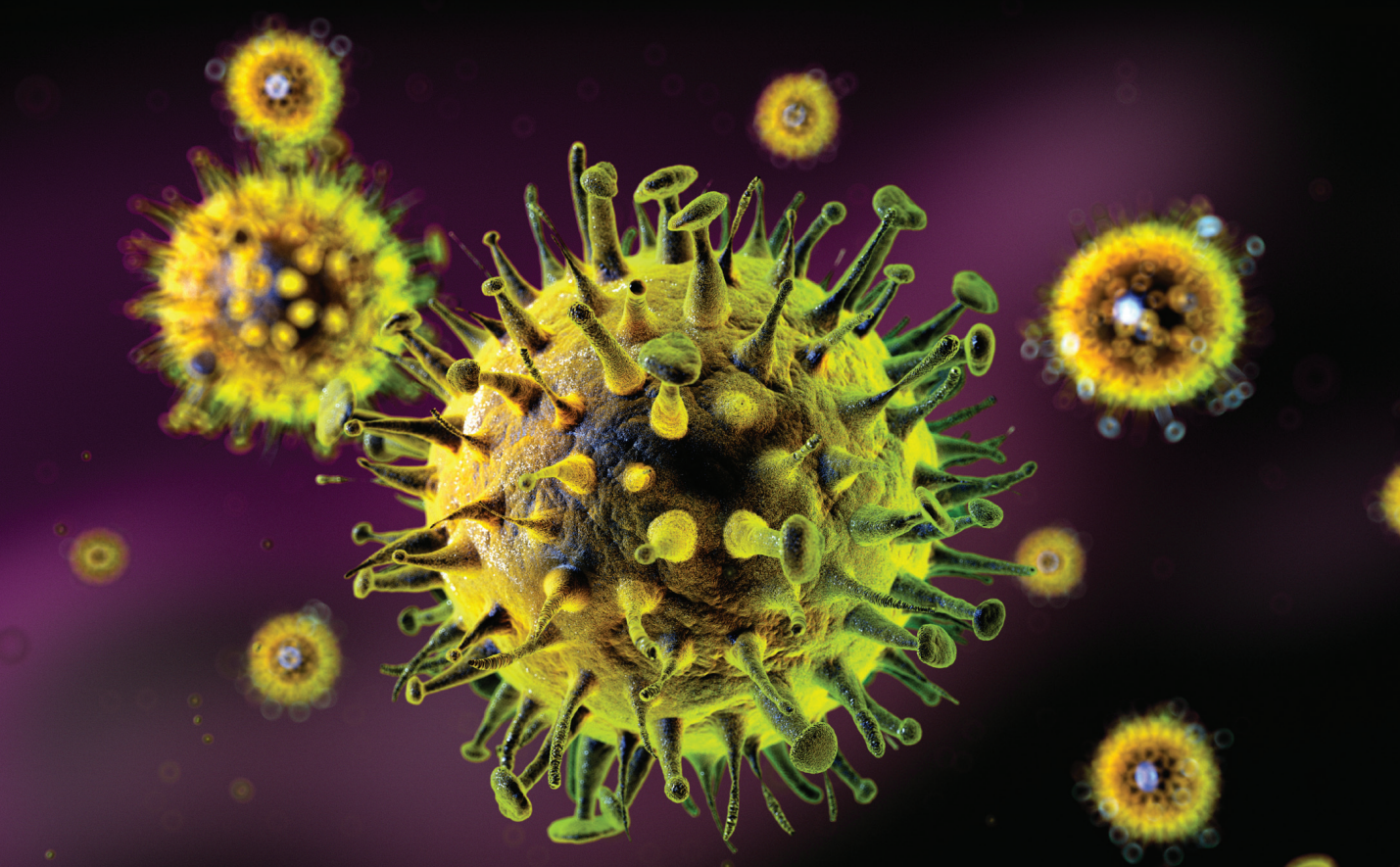
VACCINOLOGY

PRINCIPLES AND PRACTICE

EDITED BY

W. John W. Morrow | Nadeem A. Sheikh

Clint S. Schmidt | D. Huw Davies



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Vaccinology

Principles and Practice

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A John Wiley & Sons, Ltd., Publication

Blackwell Publishing was acquired by John Wiley & Sons in February 2007. Blackwell's publishing program has been merged with Wiley's global Scientific, Technical and Medical business to form Wiley-Blackwell.

Registered office: John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial offices: 9600 Garsington Road, Oxford, OX4 2DQ, UK
The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK
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Library of Congress Cataloging-in-Publication Data
Vaccinology : principles and practice / edited by W. John W. Morrow . . . [et al.].
p. ; cm.
Includes bibliographical references and index.
ISBN 978-1-4051-8574-5 (hardback : alk. paper)
I. Morrow, John, 1949–
[DNLM: 1. Vaccines. 2. Drug Design. 3. Vaccination–methods. QW 805]
615.3′72–dc23
2012002555

A catalogue record for this book is available from the British Library.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Set in 9/12pt Meridien by Aptara® Inc., New Delhi, India

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Preface

"Vaccinology" is a term that encompasses the whole process of producing vaccines – from basic research and preclinical demonstration of efficacy, to approval and clinical trial in humans. While there are many excellent books that detail the various steps, such as antigen discovery or delivery systems, there are fewer that also cover so called "downstream development," such as the design of clinical trials, or their regulation in the United States and the European Union. In this book we have aimed to fill this gap by providing the reader with a comprehensive and authoritative reference that describes the design and construction of vaccines from first principles to implementation. We hope it will appeal both to scientists engaged in vaccine research and development, and to clinicians, or indeed anyone, with an interest in the opportunities and challenges facing the development of new vaccines.

To tackle this vast subject we have organized the chapters into sections. We start with an examination of the concept and scope of modern vaccines. We follow this with the basic tenets of the immune system that govern our thinking about vaccines, with chapters on innate immunity, antigen processing and presentation, mucosal immunity, immunological memory in T and B cells, and the utility of mouse and nonhuman primate models for testing vaccine efficacy. In the following section we explore antigen discovery in the postgenomic era, during which there has been remarkable progress in proteomic mining for potential vaccine antigens, and powerful predictive algorithms and high-throughput assay and display technologies. Together these offer unprecedented opportunities for the rapid development of new vaccines. This is then followed by a selection of chapters on antigen engineering and delivery: attenuated microbe vaccines, virus-like particles, recombinant viruses (orthopox, avipox, lentivirus, and adenovirus) and bacteria, DNA vaccines, and artificial cells. In parallel we explore methods for antigen delivery, with chapters on transcutaneous vaccination, needle-free jet delivery, and oral vaccines. The need to potentiate otherwise inert proteins is the subject of the next section, with chapters on designing adjuvants, particulate delivery systems such as PLGs and microspheres, co-administration of co-stimulatory moieties, and the role of TLR signaling in adjuvanticity. We then transition from basic research to vaccine implementation. The first of these sections discusses regulatory considerations, with chapters on working with the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), developmental pipelines, the design of clinical trials, immune monitoring and biomarkers, and vaccine safety. This is followed by chapters on mass immunization strategies, and mathematical models and epidemiological monitoring.

This book would not be possible without the impressive array of experts who have contributed chapters. We wish to thank every one of you for making this possible and bearing with us on this ambitious project. Finally we wish to thank the production team at John Wiley, especially Julie Elliott, Maria Khan, and Michael Bevan. This has been a team effort, but ultimately any omissions or errors are the responsibility of the editors. We welcome comments and feedback for future editions of this book.

W. John W. Morrow
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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

Vaccinology: Principles and Practice, First Edition. Edited by W. John W. Morrow, Nadeem A. Sheikh, Clint S. Schmidt and D. Huw Davies.

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

Table 1.1 Incidence of disease and the year of peak rate in the USA prior to and after mass immunization programs were initiated.

Disease	Peak incidence (year)	Incidence		Percentage change 1997
		1996	1997	
Diphtheria	206 939 (1921)	2	5	−99.9976
Measles	894 134 (1941)	508	135	−99.9849
Mumps	152 209 (1968)	751	612	−99.9959
Pertussis	265 269 (1934)	7796	5519	−99.9791
Polio, paralytic (wild poliovirus)	21 269 (1952)	0	0	−100.000
Rubella (German measles)	57 686 (1969)	238	161	−99.9972
Rubella, congenital syndrome	20 000 (1964–5)	4	4	−99.9998

Adapted from [7] for 1996 statistics and from *Vaccine and Immunization News*, No. 6, March 1998.

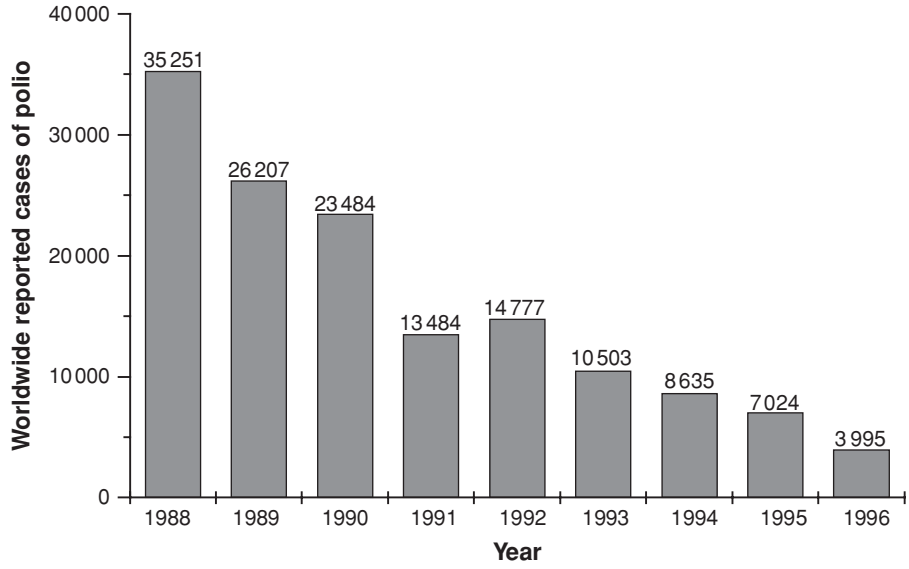

Figure 1.1 Impact of polio eradication program upon cases of polio infection worldwide. Each year there has been a gradual decrease in the numbers of polio cases reported. Adapted from *Vaccine & Immunization News* No. 5, 1997 (WHO Publications).

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

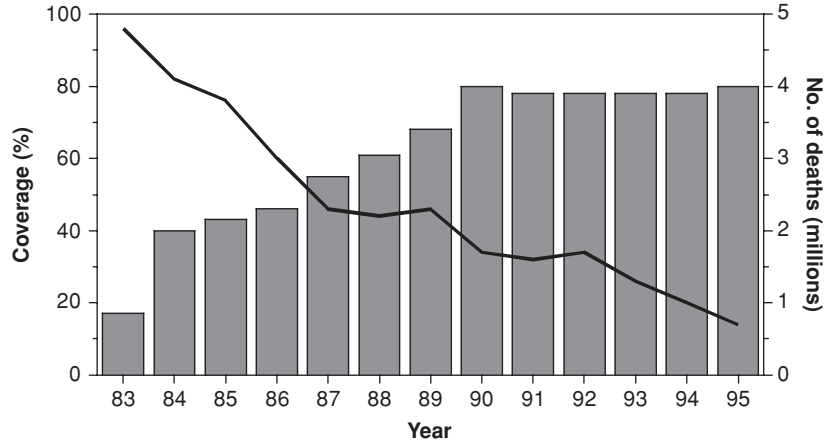

Figure 1.2 Impact of measles eradication program upon cases of measles infection worldwide. Shaded bars, coverage; line, number of deaths. Each year there has been a gradual decrease in the numbers of deaths due to measles. Adapted from *Vaccine & Immunization News* No. 4, 1997 (WHO Publications).

Table 1.2 Cost effectiveness of childhood vaccines in the United States and the estimated returned savings, both direct and indirect, from vaccination.

Vaccine	Savings per "vaccine dollar" invested	
	Direct medical savings	Direct and indirect savings
DTP	$6.0	$29.1
MMR	$15.3	$21.3
OPV	$3.4	$6.1
Integrated (DTP, MMR, OPV)	$7.4	$25.5
Haemophilus influenza type b	$1.4	$2.2
Hepatitis B (perinatal/infant)	$0.5	$2.0
Varicella	$0.9	$5.4

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

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.

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PART 2
Principles of Vaccine Design

CHAPTER 2
Strategies to Stimulate Innate Immunity for Designing Effective Vaccine Adjuvants

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Principles of vaccine design: stimulation of innate immunity

Stimulation of innate immunity is an important requirement for the induction of effective immune responses following vaccination. The majority of today's vaccines contain adjuvants that were added for the purpose of enhancing the magnitude, type, onset, and duration of the acquired immune response. The recognition of the role and importance of adjuvants in the stimulation of innate immunity and the relationship between innate and acquired immunity are more recent.

Innate and acquired immunity are intimately linked through antigen-presenting cells (APCs), in particular macrophages and dendritic cells (DCs). In an immature stage, these cells specialize in uptake of antigens and are equipped with a variety of pattern recognition receptors (PRRs), which facilitate the recognition of highly conserved pathogen-associated molecular patterns (PAMPs) such as bacterial and viral DNA, lipopolysaccharide (LPS), and flagellin (Table 2.1)[1]. Signaling through PRRs results in activation of multiple signaling pathways and the subsequent increase in expression of a plethora of effector molecules, including major histocompatibility complex (MHC), co-stimulatory molecules, and proinflammatory chemokines and cytokines (see Chapter 26 for more detail). Once activated, DCs begin to mature and home to the draining lymph node, where they present the antigen to naïve lymphocytes as part of the specific or acquired immune response (Figure 2.1). This maturation process is characterized by a loss of endocytic and phagocytic capacities and an increase in the surface expression of co-stimulatory molecules such as CD80, CD86, and CD40 [2,3]. With maturation, DCs also change expression of chemokine receptors (CCR) from those that are expressed in the peripheral tissues (CCR1, CCR2, CCR5, and CCR6) toward expression of CCR7, which recognizes CCL19 and CCL21. These two chemokines

Vaccinology: Principles and Practice, First Edition. Edited by W. John W. Morrow, Nadeem A. Sheikh, Clint S. Schmidt and D. Huw Davies.

Table 2.1 PRRs and their activating PAMPs.

PRR	Class of PRR	Agonists	Signaling event(s)
TLR1/TLR2	TLR	Triacyl lipopeptides lipoarabinomannan from mycobacterium, yeast/zymosan, glycosylphosphatidyl inositol-linked proteins	Promotes proinflammatory cytokine expression
TLR2	TLR	Zymosan, lipoteichoic acid, peptidoglycan(?)	Expressed most abundantly in peripheral blood leukocytes
			Promotes proinflammatory cytokine expression via NF-κB and MAPK signaling cascades
			May promote apoptosis in response to lipoproteins
TLR3	TLR	dsRNA	Expressed in placenta and pancreas, and dendritic cells
			Recognizes dsRNA associated with viral infection, and induces the activation of NF-κB and the production of type I interferons
			Promotes proinflammatory cytokine expression via NF-κB and MAPK signaling cascades
TLR4	TLR	LPS, taxol	Most abundantly expressed in placenta, and in myelomonocytic cells and B cells
			Induced by LPS found in most Gram-negative bacteria
			Promotes proinflammatory cytokine expression via NF-κB and MAPK signaling cascades
TLR5	TLR	Flagellin	Expressed in myelomonocytic cells
			Recognizes bacterial flagellin
			Promotes proinflammatory cytokine expression via NF-κB and MAPK signaling cascades
TLR6/TLR2	TLR	Di-acyl lipopeptides, lipoteichoic acid, yeast/zymosan, glycosylphosphatidyl inositol-linked proteins	Cooperatively with TLR2
			Promotes proinflammatory cytokine expression via NF-κB and MAPK signaling cascades
TLR7	TLR	Imidazoquinoline, loxoribine, ssRNA	Expressed in lung, placenta, and spleen
			Promotes proinflammatory cytokine expression via NF-κB and MAPK signaling cascades
TLR8	TLR	ssRNA	Expressed in lung and peripheral blood leukocytes
			Promotes proinflammatory cytokine expression via NF-κB and MAPK signaling cascades
TLR9	TLR	Non-methylated CpG-containing DNA	Expressed in spleen, lymph node, bone marrow, and peripheral blood leukocytes
			Mediates cellular response to unmethylated CpG dinucleotides in bacterial DNA
			Promotes proinflammatory cytokine expression via NF-κB and MAPK signaling cascades