Immunomic Discovery of Adjuvants and Candidate Subunit Vaccines

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Preface

Within the wider context of immunovaccinology and vaccine discovery, this book will address and explore a range of new strategies and technologies, both informatics-based and experimental, which support and complement both traditional and emerging approaches to vaccine design and discovery. This book looks in turn at reverse vaccinology and the identification of putative candidate antigens, at the discovery of a wide range of different types of adjuvants, and finally at the development of sophisticated new delivery mechanisms, such as liposomes and other applications of nanotechnology. The expectation of this book is very straightforward: to foster and foment interest in those areas of vaccinology, which have thus far not received the level of interest that they perhaps deserve. We have tried to balance the optimism of which we are all guilty with some rationality. Not all of the approaches described will ultimately bear fruit, but each should nonetheless be investigated with the same diligence.

When writing a book, it is usual to acknowledge the contributions made by a whole tranche of people, and acknowledge this sooner rather than later.

First, we would like to thank all the authors for their contributions and for their patience and forbearance. Without their help none of what follows would have been possible.

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Lastly, we would like to extend our thanks to everyone at Springer for their efforts in bringing this work to fruition.

Birmingham, UK

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Chapter 1
Immunomic Discovery of Adjuvants, Delivery Systems, and Candidate Subunit Vaccines: A Brief Introduction

Darren R. Flower and Yvonne Perrie

Abstract Mass vaccination, when coupled to profound improvements in general sanitation, has given rise to the most remarkable transformation in public health in human history. Yet the development of vaccines remains largely trapped in the past, a hostage to the methodology of Pasteur. Infectious disease continues to threaten humanity, with new and renascent diseases emerging continually. The last two decades have seen a breath-taking revival in the commercial market for vaccines and the simultaneous emergence of a whole tranche of new technologies that promise to free vaccine development from the muddle of empirical thinking. In this short introduction, we set the scene for this renaissance, and explore how the combination of computational and experimental techniques promise so much for the future development of vaccines and the science of vaccinology.

The following statement long ago became a truism: that the development of mass vaccination coupled to profound improvements in general sanitation have engendered the most startlingly and amazing transformation in public health. If we travel backwards about hundred years, to the years directly preceding the First World War, human mortality was caused, in the main, by influenza, pneumonia, diarrhoea, and enteritis: totalling about 30 % of fatalities. At the same time, the great killers of today, cancer and heart disease, brought about no more than 12 % of deaths. Journey back another two hundred years—to the dying days of the seventeenth century—and average life expectancy seldom exceeded four decades. The main causes of death were again contagious disease: tuberculosis, smallpox, yellow fever, malaria, and dysentery, affecting infants, children, and adults, all alike. In the early years of the twenty-first century, things are radically different. Infectious disease is responsible for less than 2 % of deaths in the developed world, while chronic disease causes over 60 % of deaths.

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Vaccination and sanitation are indisputably the most efficient, and thus cost-effective, prophylactic treatments for infectious disease. Together they are the firm bedrock upon which the modern world resides. The truth of this is often ignored by those intoxicated by the many distractions of life in the early twenty-first century, from burgeoning social media to the recondite discoveries of the Large Hadron Collider. People say that the Internet or the iPad or even Facebook have transformed the world. If there is any truth in such assertions, then such transformations are at best shallow and superficial compared to the extraordinary reworking of lives that has transpired over hundreds rather than tens of years. The source of such change can be traced back, in part at least, to the discovery and exploitation of vaccines, vaccination, and vaccinology.

For around the first 100 years of the vaccine story, the story was solely that of smallpox. As recently as the late 1960s, some 10–12 million cases of smallpox were recorded in 31 countries, with annual deaths of 2 million. Yet today smallpox has, with the exception of a few well-guarded stockpiles, been completely eradicated: there have been no new cases for 30 years. The story of smallpox is thus the high point of the vaccination story; no other disease has been eradicated. Polio or poliomyelitis is the next nearest to full eradication, having long been targeted by a systematic, coordinated, worldwide eradication campaign. In 1991, one such programme run by the Pan American Health Organization effected a partial eradication of polio in the western hemisphere. Subsequently, the Global Polio Eradication Program has radically reduced polio throughout the rest of the world, so that today we can count cases worldwide in the tens or hundreds instead of the hundreds-of-thousands or millions.

Yet, vaccine-preventable disease still kills millions. Infectious and contagious disease cause approximately 25 % of world mortality, particularly in children under five. While in developed countries, mortality for diseases such as diphtheria, polio, or measles is less than 0.1 %, in other parts of the world deaths from such infectious diseases is significant. Pertussis, tetanus, influenza, Hib, hepatitis B are all responsible for deaths that number in the hundreds-of-thousands. Perhaps the most execrable situation is measles, which accounts for 70,000 (over 5) and 540,000 (under 5) deaths. However, the leading global causes of death worldwide remain tuberculosis; diarrhoeal illnesses, especially rotaviruses; HIV/AIDS; and malaria. In 2010, 8.8 million contracted TB and 1.4 million died. Disturbing though these numbers may seem, they nonetheless represent a significant reversal of a once ever-escalating trend. The number with latent TB peaked in 2005 at 9 million, while deaths from TB reached their peak at 1.8 million in 2003.

However, these bald numbers are likely to be significant underestimates. Let us also look at malaria. Murray et al. have recently provided evidence that deaths from malaria over the thirty-year span to 2010 are much higher than previously believed [1]. Their epidemiological figures show a peaked distribution over this period, increasing from around a million in 1980, peaking at approximately 1,820,000 in 2004, and then reducing to about 1,240,000 in 2010, with the greatest number dying in Africa. These figures are roughly twice the values published by the WHO. It

seems unlikely that the WHO's estimates for other major diseases are uniformly more accurate.

Why are these numbers so high? A principal reason is that there are no effective vaccines for either malaria or HIV, two of the WHO's big three diseases; nor is there expectation that such vaccines will appear in the near future, irrespective of the optimism of those working in the area. And as for tuberculosis—which is carried by around 2 billion people worldwide—the only licensed vaccine has limited efficacy. Many viral infections remain recalcitrant threats of the first order. About 350 million people are infected with hepatitis B, 170 million by hepatitis C, and 40 million by human immunodeficiency virus type 1 (HIV-1). This dire situation is further compounded by the threat from the 35 new or previously unknown infectious diseases identified in the past 30 years: HIV, West Nile fever, Ebola, dengue fever, SARS, and the potentially pandemic H5N1 influenza. Every year, between 5 and 15 % of the global population becomes infected with a new influenza strain, causing upwards of half-a-million deaths. It is widely thought that there will be a continual emergence of new infectious diseases during the present century: emerging zoonotic infections and antibiotic-resistant bacteria prominent amongst them.

In the face of declining birth rates, coupled to decades of ever-enhancing nutrition, advances in treatment regimens and medicines are leading the population of most developed and developing countries figuratively to age: that is for larger and larger proportions of the population to live to maturity and beyond. Growth in life expectancy is matched by growth in the diseases of old age. These include neurodegenerative diseases, principally Parkinson's or Alzheimer's disease; cardiovascular diseases; and stroke. Disease has altered significantly in the preceding century. It will continue to alter during the century to come. Some alterations we can predict; others will escape the forecaster's eye. Disease, particularly infectious disease, has been beaten, or, at least, severely restrained. Many factors have conspired to effect this—improved water quality, better precautionary hygiene, improved nutrition, decreased overcrowding—as well as many interventionary measures, principally antibiotic therapy and vaccines.

Hitherto, vaccines have been an uncompromising success, yet, as we see, so much more needs to be done if the full potential of vaccines is to be achieved. Although the licensing and use of vaccines varies between countries, 25–30 commonly licensed vaccines target a range of viral or bacterial infectious diseases, with approximately 14 paediatric diseases targeted during the first few years of life. Other than paediatric vaccination, most vaccines are used by travellers to tropical or subtropical regions; a significant minority fight infection in the developing world.

Vaccination also works to greatly reduce the morbidity of disease, often imbuing lifetime protection; this is particularly important for benign yet economically important infections, such as the so-called common cold. Diverse sporadic or epidemic infections of the human respiratory track—as caused by an excess of 200 distinct viruses, such as RSV or, more properly, respiratory syncytial virus, coronaviruses, influenza A and B, rhinoviruses, parainfluenza virus, and cytomegalovirus—remain a principal cause of hospitalisation and community morbidity with

an estimated 60 % of GP referrals associated with such infections, and cause the loss of enormous numbers of working days in developed countries.

Given the recalcitrance, and the immense investment in treatment and prophylaxis, it may be that many of the diseases alluded to above will never be eradicated, as smallpox was, and that vaccines alone will not be enough. We may instead need a complex network of prophylactic and therapeutic measures at least as complex as the diseases themselves in order to effectively reduce the prevalence of the disease and to treat those who become infected. Whatever other countermeasures we may have recourse to—artemisinin-based drugs, genetically manipulated vectors, or insecticide-treated bednets—the clinical and cost effectiveness of vaccines means they remain the must-have component in the ongoing search for better means of combating endemic infectious disease.

Beyond infectious disease lies what is possibly the most underexplored area within the ever burgeoning field of vaccinology and vaccination: vaccines against allergy and allergic disease; vaccines that target so-called lifestyle diseases, such as those deriving from addiction; and vaccines that target chronic diseases, the most important of which is cancer. Therapeutic vaccines against cancer are probably the best studied amongst the more novel, innovative, and underexplored areas at the forefront of vaccine discovery. The present overall whole-life risk from cancer, at least in developed countries, runs at or about 40 %. The figure is currently rising. There are approximately 3·2 million new cancer cases in Europe each year, resulting in around 1·7 million deaths. In the USA, over one and half million new cases are reported annually. Clearly, this is an important disease burden, and thus a key target for the pharmaceutical and biotechnology industries.

Lifestyle vaccines are another innovation, of which much is expected in certain quarters. They target all kinds of medical problems, ranging from drug addiction through dental caries, all the way to major genetic and multifactorial diseases, including obesity. Versatility and flexibility are major hallmarks of the vaccination concept. Vaccines take many forms and work in many ways. This facet has been exploited in the development of life-style vaccines. Let us look at serious addiction. During 2009, the United Nations Office on Drugs and Crime estimated 3.3–6.1 % of the global population abused so-called illicit substances—a serious problem indeed with an enormous implicit health, economic, and behavioural burden, with the worst excesses coming from cannabis abuse and the abuse of amphetamine, cocaine, and opiates. Anti-drug vaccines generate antibodies able to bind particular drugs; the drug–antibody complexes thus generated should have too high a molecular weight to penetrate the blood–brain barrier effectively, thus reducing the amount and rate of drug egress into the brain and so inhibiting psychoactive effects at the system level. Anti-addiction vaccines have been with us for some time, beginning over 40 years ago, when two proof-of-principle studies [2, 3] demonstrated in rats and in rhesus monkeys that morphine could be used as a hapten in order to create an antibody against morphine addiction. Today, addiction vaccines are being developed to target a range of major abused drugs, such as nicotine, cocaine, various amphetamines, and heroin.

Anti-allergy vaccination also offers great potential for successful commercial exploitation. The prophylaxis and treatment of allergy can now be addressed in many ways, including, notably, recombinant proteins and DNA vaccines. Vaccines against the common cold or anti-allergy vaccines are similar in mechanism to many lifestyle vaccines. These do not save lives directly but do help to greatly reduce the vast economic burden of disease morbidity.

An array of interconnecting factors that have made the pharmaceutical and biotechnology industries re-evaluate the potential of vaccines as a commercially viable product. Prior to 1980, there were relatively few vaccines, most targeting major pandemic diseases of the developed or developing worlds. Subsequently, partly as a result of enhanced technology as discussed at length in the current book, many vaccines have become available, most recently the cervical papillomavirus vaccine. Likewise, there are hundreds upon hundreds of vaccines in trials. The growth rate in the sales of vaccines reflects this feverish and febrile activity: $5 billion in 2000, $6 billion in 2003, $11 billion in 2006, rising to $19 billion in 2009 and $22 billion in 2010; projected sales for 2015 are about $35 billion. The rate of sales growth for vaccines is something like 16 %, compared to the sluggish drugs market, meandering its desultory way at 4 % per annum. There is profit in vaccines, clearly; what remains problematic for the profit-driven decision-making processes of big pharma is the haphazard and probabilistic nature of vaccine discovery. What the pharmaceutical industry needs is the capacity to apply the same systematic, automated, high-technology approaches used to identify new small-molecule drugs to the discovery and development of vaccines.

No right-minded scientist, looking back across the last 200 years, would wish to argue seriously with the contention that the design and development of vaccines is an innately labour-intensive process. The processes deployed to meet the objective of creating new and better vaccines are in desperate need of change. This change must be radical if we hope to simplify such processes. Simple processes are hopefully also fast and efficient processes. In the search for subunit vaccine antigens, one technical development—reverse vaccinology—has proved the most profound and hopeful.

Just over a decade ago, Rino Rappuoli used the expression "reverse vaccinology" to describe development of vaccines using a genomic-based approach, rather than the ponderous empirical methods favoured then, and still in use today. Reverse vaccinology seems about to deliver on its early potential: the European Medicines Agency is in the process of evaluating Novartis's Bexsero, the first commercial vaccine developed using the reverse vaccinology approach. The vaccine may become the first vaccine effectively to combat meningococcus B, a disease causing over 50 % of global meningococcal meningitis. A decision on Bexsero is expected shortly.

During the development of Bexsero, new protective protein antigens were identified using genomics: initially over 600 surface-exposed proteins were predicted from the *N. meningitidis* proteome as molecules liable to host immune surveillance, of which about 350 were then expressed in *E. coli*. This number was reduced by using these proteins to immunize 350 sets of mice, identifying 91 that

could induce antibodies in vivo, 29 of which killed *N. meningitidis* in vitro. By comparing the genomes of 31 clinical strains, a further subset of proteins offering broad protection could be identified.

Reverse vaccinology has become perhaps the most famous well-developed approach amongst many advanced approaches now available within the discipline of vaccinology. Indeed, a whole range of other, high-technology methods and techniques have been and are being developed to complement and optimise reverse vaccinology. Capitalising on its success, these offer new hope in our constant struggle with infection; all we need is for this technology to be fostered, developed, and utilised.

This book is intended to fill a gap, if not a void, in current thinking within vaccine design and development by attempting to draw together several disparate strands; and, by doing so, also identify and illuminate some important areas replete with potential. Science, in much the same way that all human activities, from the most profound to the most trivial, follows fashion and progresses by tracking trends. Whether we think of publically funded science or the pharmaceutical industry, similar phenomena are observed. Science follows the money, and money follows consensus. The decision-making process underpinning the strategic direction that policy in both publically funded science and the pharmaceutical industry takes is only in part influenced by science. It is also regrettably in thrall to many, sometimes contradictory, voices: the fickleness of public opinion, vested interests of many hues and flavours, and the myopia of the profit margin, amongst many others. This is because the decision-makers in such organisations are seldom if ever scientists engaged in doing science directly; management and policy, at both the strategic and tactical levels, are often swayed by the prevalence of opinion.

In the pharmaceutical industry, for instance, this is manifest as the next big thing: combinatorial libraries, genomics, high-throughput screening, antisense, even molecular modelling; all were hailed as transformative saviours that would remove happenstance and unpredictability from drug discovery—yet as the current parlous state of the pharmaceutical industry readily attests, while all promised much, none really delivered. No single technique can achieve everything, which is why we should always develop a large range of alternatives, both informatics-based and experimental, all running in parallel.

Central to computational immunology is the capacity to make accurate predictions. Yet, obtaining routes to prediction that are accurate, robust, and dependable continually eludes us. Immunoinformatics deals with empirical, data-dependent methods. The success and utility of such methods depends very much on the data used to propagate and parameterise them; they cannot escape the severe limitations imposed by the data used to create them. The data from which we build models forms a complex phase space of structural and property variation, which can be extremely multidimensional, with a high degree of interdimensional correlation. When the data we work with is reliable and our knowledge of it is complete, then we can create useful models by applying standard methods from computer science to build accurate and predictive models relating observed biological activity to underlying measurable or predictable properties. Usually, such approaches are also

much superior when used to interpolate than they are when used to extrapolate. We need complete and thorough data sets effectively and efficiently able to explore the complex relationships between structure and function, necessitating continuous improvement in all aspects of data quality.

Within the wider context of vaccine design and discovery, we shall in this book describe and explore a range of key alternative strategies and technologies, both informatics-based and experimental, which are, by degrees, both supportive and complementary to reverse vaccinology and more traditional approaches to vaccine discovery. This book looks in turn at reverse vaccinology and the identification of putative candidate antigens, at the discovery of a wide range of different types of adjuvants, and finally at the development of sophisticated new delivery mechanisms, such as liposomes and other applications of nanotechnology.

In Chap. 2, Cafardi et al. review the present state of play with respect to reverse vaccinology, with particular emphasis on how completion of bacterial genomes impinges upon the vaccine discovery. They show how this approach allows the development vaccines that are difficult or near impossible to address with conventional approaches. They also highlight how advances in genome-based techniques and in so-called next-generation sequencing approaches and technologies will help to enhance reverse vaccinology, enabling timely identification of novel candidate antigens for new, emerging, or recrudescent infectious diseases.

In Chap. 3, Flower et al. review the discovery of candidate vaccine antigens in more detail. Placing their analysis in the context of emerging ideas about the possible nature of immunogenicity and how it may be propagated by elements of the immune response at the system level, the authors discuss the three main approaches to the identification of novel immunogenic antigens: sequence similarity-based approaches, whereby the antigen nature of a protein is inherited from similar sequences; methods based on identifying the subcellular location of microbial proteins, on the basis that proteins with only certain locations would be accessible to immune surveillance; and the use of empirical alignment-independent approaches to the prediction of antigens. Usefully, the chapter also includes discussion of expert systems for antigen discovery.

In Chap. 4, Vordermeier et al. review how genomics and the development of bioinformatics have radically transformed the cattle vaccinology of bovine tuberculosis. Within the context of a generalised infrastructure of bioinformatic analytical techniques, the authors describe in detail how the application of comparative *in silico* transcriptome and genome analysis is able to undertake prospective prioritisation of immunogenic antigens for experimental testing, leading to the identification of candidate subunit vaccines.

In Chap. 5, He explores the use of epitope-focused immunoinformatic analysis in the prediction of optimal vaccine candidates when undertaking a genome-wide reverse vaccinology exercise. Specifically, He describes the web-server Vaxign, concentrating on a case study: vaccine design against the virulent bacterium *Francisella tularensis*, where 12 candidates were chosen using a combination of pertinent selection criteria.

In Chap. 6, Dhillon et al. offer us a wide-ranging review of methods and strategies for two important areas of immunoinformatic analysis within the domain of vaccine discovery: predicting the immunogenic subcellular location of microbial proteins and identifying proteins encoded by so-called genomic islands. While in Chap. 7, Ansari et al. describe a variety of database systems that facilitate immunoinformatics and antigen selection.

Chapters 8 and 9 look at adjuvants and their discovery. In Chap. 8, Edwards describes the basis of adjuvant action, and the role played by macromolecular adjuvants. In Chap. 9, Flower explores and examines different varieties of molecular adjuvant and their discovery, concentrating on small molecule adjuvants, and their systematic identification using virtual screening technology. This topic is put into context by a thorough review of extant adjuvants, molecular mechanisms of adjuvant action, as well as macromolecular adjuvants and how various adjuvants engage pattern recognition receptors of the innate immune system.

In addition to the characteristics of the antigen and the adjuvant independently, how the antigen and adjuvant are presented to the immune system has a major impact on the biological output of the vaccine. Indeed, the co-delivery and continued association of antigen and adjuvant may be a prerequisite in effective immunisation. This is not a new idea; the ability of alum to promote an antigen depot effect at the site of action has been ascribed as one of its main mechanisms of action for several years. For example, since 1977 the WHO has recommended that over 80 % of diphtheria toxoid needs to be adsorbed to alum for its effective use [4]. Whilst this does not ensure that the antigen remains adsorbed to alum after injection and exposure to interstitial fluid, it is thought to at least initially promote co-location of the antigen with the alum adjuvant. However, alum is not the only adjuvant able to promote the co-delivery of antigens and adjuvants in one system; a range of particulate delivery systems can offer this. Examples of such delivery systems include lipid-based systems (e.g., liposomes, niosomes, ISCOMs) and microparticles. Each of these systems offer a suite of advantages and disadvantages and when considering the choice of delivery system attributes including antigen-loading capacity, antigen retention and protection both on storage and within the biological milieu, and the ability to incorporate adjuvants within the delivery system all require optimisation and this is without consideration of the ability of the delivery system to act as an adjuvant in its own right. In this book we aim to address these issues by considering several of the most commonly employed particulate vaccine delivery systems.

Out of these particulate delivery systems liposomes are one of the most established systems; liposomes were first reported as an effective immunological adjuvant for diphtheria toxoids by Allison and Gregoriadis in 1974 [5]. Since then, a large array of understanding on their design has been gathered and strong links between their formulation and function identified. Of these parameters, the composition of the liposomes will play a pivotal role. For example, the surface charge of liposomes used for vaccine delivery can influence the interactions between liposomes and protein antigens, and affect how liposomes interact with cells. This manipulation of surface charge can range from the inclusion of anionic lipids such

as phosphatidylserine, which may facilitate the targeting of antigen presenting cells through interaction with phosphatidylserine receptors. Alternatively, the use of cationic lipids can improve the loading of anionic antigens to the liposomes and upon injection promote a depot effect at the site of injection, promoting the co-delivery of antigen and adjuvant to dendritic cells (e.g., [6, 7]). However, this depot effect is more than electrostatically driven, with the choice of cationic lipids used in the formulation having an impact as explored in Chap. 10.

In addition to liposomes, there are a range of alternative surfactant-based delivery systems, such as niosomes. These are similar in many ways to liposomes; however, non-ionic surfactants form the main component of their bilayers. The most common composition of niosomes investigated for vaccine delivery is 1-monopalmitoyl glycerol, cholesterol, and dicetyl phosphate. One might argue this is not a niosome formulation due to the inclusion of anionic dicetyl phosphate. However, the addition of charged surfactants has proven to enhance the stability of these vesicles and their inclusion in these vesicles is common practice. Generally, niosomes exhibit many similarities to liposomes; however, potential advantages cited include their lack of predisposition to oxidative degradation, as well potential lower cost of components and reduced variability compared to natural phospholipids. Whilst with current manufacturing methods, the cost and reproducibility of phospholipids is less of an issue, niosomes still offer a useful alternative to liposome formulations. In particular, niosomes appear an attractive option for oral delivery of vaccines due to their ability to withstand the harsh gastrointestinal environment as outlined in Chap. 11.

With both liposomes and niosomes, immunostimulatory agents can be easily incorporated within the system and in some cases this can result in restructuring of the particulate delivery system as is the case with ISCOMs (Chap. 12). ISCOMs are prepared from a mixture of phospholipid, cholesterol, and a saponin (often Quil A). Whilst a phospholipid/cholesterol mixture would normally form liposomes, the addition of appropriate concentrations of saponin to the mixture can result in restructuring of the system to form spherical, open, cage-like structures around 40 nm in size, as nicely shown in Chap. 12. Given that their structures are open, ISCOMs cannot incorporate hydrophilic antigens, and antigens need to display a degree of lipophilicity for inclusion into ISCOMs. If required, antigens can be modified through a range of methods to incorporate lipophilic regions within their structure, thereby promoting their incorporation into the structure. Alternatively, similar to cationic liposomes, cationic ISCOMs (where cationic components are used to build their structure) can electrostatically bind a range of anionic antigens and enhance their delivery. In Chap. 12 the formulation, preparation, and application of ISCOMs as vaccine adjuvants is considered.

However, lipid-based systems are only one group of particulate delivery systems, and polymeric systems have also been extensively studied. In particular the use of biodegradable polymers to formulate nano- and microparticulate delivery systems for vaccines has been widely investigated. Much like the lipid-based systems, there is a wide selection of options to consider in the formulation of polymeric nanoparticles and microspheres for vaccine delivery, with polyester

polymers offering advantages due to their clinical approved use in a range of medical products. As with the other systems considered, immunostimulatory agents can be incorporated within these polymer constructs and thus these systems can act as delivery systems and adjuvants for antigens. Within Chap. 13, the design of polymeric microspheres as vaccine adjuvants is considered from the choice of base polymer, through to optimisation of process parameters, and finally to considerations of their stability as a product.

Whilst much of current research into the development of vaccines has focused on the design of vaccines for administration of a particulate suspension, dry powder vaccines may hold considerable advantages. In Chap. 14, the authors consider the design of dry powder vaccines. Such vaccines may offer low-cost, temperature-stable products suitable for pulmonary delivery, with the added advantage that the pulmonary route avoids the use of needles (and their associated risks). Furthermore, it can allow for the effective delivery of antigens to target cells of the immune system without the harsh conditions faced by orally delivered vaccines. The development of spray-drying methods outlined in Chap. 14 supports the ability of such powder vaccines to be delivered using conventional dry powder inhalers already clinically licensed for pulmonary delivery.

Therefore, by considering these vaccine delivery platforms in conjunction with the appropriate choices for antigen and adjuvant it is hoped that the threefold multi-component nature of a vaccine can be considered more completely than before. When viewed conceptually, vaccines comprise an important triad. The first part of the vaccine, and in a sense the most important, is the biological component. This is the whole protein, or whole organism, or epitope-based part which confers the ability to be recognised by the immune system. It is this part which differentiates one vaccine from another, an anti-flu vaccine from an anti-TB vaccine. The second part of the vaccine is the adjuvant, which is one of many alternatives, that confers an immunogenicity to many vaccines that they would otherwise not possess. It often does this in a generic fashion, such as via agonising the innate immune system, so that the same adjuvant is quite capable of functioning in many different vaccine formulations. The final and third part of the vaccine is the delivery vehicle, as opposed to the delivery mechanism, such as oral vaccines versus injectable. The vehicle can be things as different as a viral vector or a liposome. An attenuated or heat-treated whole-organism vaccine can be thought of as combining all three parts of this triad in one supra-molecular moiety. Of course, this is a gross simplification, and many other things go into deployable vaccine formulations, such as preservatives, contaminants, and other chemical or biological components that so exercise the anti-vaccine lobby. Hopefully, this book will encourage us to think of vaccines in these terms, and provides the background necessary to engage with each of the three components of the vaccine triad.

With this in mind, the anticipation inherent with this work is indeed simple and straightforward: to foster and foment interest in those areas of vaccinology that this far have not received the level of intense work that they richly deserve. To help achieve this, we have sought to balance optimistic positivity and cold, hard

rationality. Not all of the approaches described will ultimately bear fruit, but each should, nonetheless, be examined with equal diligence, sedulousness, and assiduity.

References

1. Murray CJ, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Haring D, Fullman N, Naghavi M, Lozano R, Lopez AD (2012) Global malaria mortality between 1980 and 2010: a systematic analysis. Lancet 379:413–431
2. Berkowitz B, Spector S (1972) Evidence for active immunity to morphine in mice. Science 178:1290–1292
3. Bonese KF, Wainer BH, Fitch FW, Rothberg RM, Schuster CR (1974) Changes in heroin self-administration by a rhesus monkey after morphine immunisation. Nature 252:708–710
4. World Health Organization. Manual for the production and control of vaccines—tetanus toxoid. blg/undp/77.2 Rev.1, 1977 [ref type: serial (book, monograph)].
5. Allison AG, Gregoriadis G (1974) Liposomes as immunological adjuvants. Nature 252:252
6. Henriksen-Lacey M, Bramwell VW, Christensen D, Agger E-M, Andersen P, Perrie Y (2010) Liposomes based on dimethyldioctadecylammonium promote a depot effect and enhance immunogenicity of soluble antigen. J Control Release 142(2):180–186
7. Kamath AT, Rochat A-F, Christensen D, Agger EM, Andersen P, Lambert P-H, Siegrist C-A (2009) A liposome-based mycobacterial vaccine induces potent adult and neonatal multifunctional T cells through the exquisite targeting of dendritic cells. PLoS One 4:e5771

Chapter 2
Bacterial Genomes and Vaccine Design

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Abstract Since its introduction, vaccinology has been very effective in controlling and eliminating life-threatening infectious diseases. However, in several cases, the conventional approach to identifying protective antigens, based on biochemical, immunological, and microbiological methods, has failed to deliver successful vaccine candidates against major human pathogens. The availability of complete bacterial genome sequences has allowed scientists to change the paradigm and approach vaccine development starting from genomic information, a process named reverse vaccinology. This can be considered as one of the most powerful examples of how genomic information can be used to develop vaccines that were difficult or impossible to tackle with conventional approaches. The ever-growing genomic data, the new genome-based approaches and high-throughput sequencing technologies will help to complement reverse vaccinology to enable timely development of new vaccine antigens against emerging infectious diseases.

2.1 Introduction

Vaccines are currently available for infectious diseases caused by various viruses and bacteria and the prevention of disease and death by vaccination has profoundly improved the public health of many populations globally. Louis Pasteur, who developed the first vaccine against rabies, established in 1881 the basic paradigm for vaccine development, which included the isolation, inactivation, and injection of the causative microorganism. These basic principles have guided vaccine development during the twentieth century. All existing vaccines are based on killed or live-attenuated microorganisms or subunits purified from the microorganism such as toxins detoxified by chemical treatment, purified antigens or polysaccharide

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conjugated to proteins. At the end of the twentieth century, most of the vaccines that could be developed by these traditional technologies had been developed and they allowed the control and, in some cases, the eradication of many important infectious diseases [78]. Although very successful, in several instances these approaches were not able to deliver vaccines against certain pathogens and on other occasions the vaccines obtained with these classical approaches were no longer adequate due to safety concerns and low efficacy. Killed and attenuated vaccines, based on the whole organisms, may contain several factors that may have reactogenic activity and may induce undesirable inflammatory response. The attenuated vaccines could also revert to the virulent status and chemicals used for inactivating pathogens could be present as traces in the final composition. In addition, classical biochemical and microbiological methods used to identify protective subunits were hampered by the limited number of candidate antigens that could be identified as well as the time required for their identification. Remarkable progresses were recently made by the introduction of new technologies such as recombinant DNA and chemical conjugation of proteins to polysaccharides, as well as advances in the identification of novel adjuvants.

The genome era, initiated with the completion of the first bacterial genome, that of *Haemophilus influenzae* in 1995 [1], catalyzed a new revolution in vaccine development. Advances in sequencing technology and bioinformatics have resulted in an exponential growth of genome sequence information. The study of genomes by both computational and experimental approaches has significantly advanced our understanding of the physiology and pathogenicity of many microbes and has provided insights into the mechanisms of genome evolution as well as microbial population structures [2, 3].

Genomes and genome-based technologies have also the potential to help in the development of therapeutics and vaccines. The availability of whole-genome sequences has entirely changed the approach to vaccine development. The genome represents a list of virtually all the protein antigens that the pathogen can express at any time. It becomes possible to choose potentially surface-exposed proteins in a reverse manner, starting from the genome rather than from the microorganism with an approach called reverse vaccinology [4]. In this review we will describe how genomic information has been successful in the identification of novel protein antigens against various human pathogens. We will also focus on recent reports that have contributed to the discovery of novel vaccine candidates providing the proof of concept of genome-based approaches such as pan-genome investigation, subtractive reverse vaccinology, and DNA microarray analysis. A future view of how high-throughput sequencing methods might positively influence vaccine design will also be discussed.

2.2 Reverse Vaccinology: A Novel Genomic Approach to Antigen Identification

The recent genome revolution has extended the confines in vaccine research. Genome mining has revolutionized the approach to vaccine development and provided a new innovation to antigen selection and design. The approach starting from the genomic information leading to the identification of potential vaccine candidates is termed reverse vaccinology [4]. The availability of complete bacterial genome sequences offers a comprehensive catalogue of genes encoding all the potential proteins of a pathogen, with the potential to rationally select vaccine candidates rather than empirically test them one at a time. Furthermore, the prediction of antigens is independent of the need to culture the pathogen in vitro. On the basis of the concept that surface-exposed proteins are susceptible to antibody recognition and are therefore the most suitable vaccine antigens, a complete genome sequence can be screened using bioinformatics algorithms to select open reading frames (ORFs) encoding putative surface-exposed or secreted proteins. Putative surface proteins can be readily identified based on the combination of several features including the presence of signal peptide sequences, membrane spanning regions, lipoprotein signature, and motifs such as sortase attachment sites (LPTXG sites). Moreover, proteins with homology to known virulence factors or protective antigens from other pathogens can be selected based on homology. Several computational methods are available to search for surface-associated or secreted proteins: PSORT is used for the prediction of protein sorting signals and localization sites in amino acid sequences; SignalP predicts the presence and location of signal peptide cleavage sites in amino acid sequences from different organisms, Gram-positive and Gram-negative prokaryotes; TMpred program makes a prediction of membrane spanning regions and their orientation. Although much progress can be made in silico, the experimental approach is necessary to establish unambiguously the localization of the protein in living bacteria. Furthermore, screening for sequence homologies to human proteins for their exclusion in the selection process can help to avoid problems of autoimmunity.

After candidate surface antigens are identified in silico, they are produced as recombinant proteins and their immunogenicity is assayed to measure their potential as vaccine candidates. The feasibility of the reverse vaccinology approach relies on the availability of a high-throughput system for protective immunity screening and also on good correlate of protection. In the paragraphs below, we will describe how genomic information has been successful in the identification of novel potential vaccine candidates against various human pathogens, such as *Neisseria meningitidis* serogroup B, *Streptococcus agalactiae*, and pathogenic *Escherichia coli*.

2.2.1 The First Vaccine Obtained Through Reverse Vaccinology: The Serogroup B Meningococcus Vaccine

The concept of reverse vaccinology was applied for the first time to serogroup B *N. meningitidis* (MenB). *N. meningitidis* is the major cause of meningitis and sepsis, two devastating diseases that can kill children and young adults within hours, despite the availability of effective antibiotics. It is a Gram-negative bacterium that colonizes asymptomatically the upper nasopharynx tract of about 5–15 % of the human population. However, in a significant number of cases, the bacterium can traverse the epithelium and reach the bloodstream causing septicemia. From the blood meningococcus is able to cross the blood–brain barrier and infect the meninges, causing meningitis [5, 6].

N. meningitidis can be classified in 13 serogroups on the basis of the chemical composition of the capsule polysaccharide. However, more than 95 % of total cases of invasive disease are caused by five major serogroups: A, B, C, Y, and W135. Vaccines against serogroups A, C, Y, and W135 were developed in the 1960s by using the purified capsular polysaccharide as antigen. Second-generation, conjugated vaccines have now been introduced. The chemical composition of the polysaccharide of serogroup B, which resembles a molecule present in human tissues, makes a polysaccharide-based vaccine poorly immunogenic and a possible cause of autoimmunity.

In the last 40 years a lot of efforts have been directed to the identification of meningococcus B antigens as the basis of new vaccines. However, the high variability of these proteins among the different MenB strains represents a serious obstacle to the production of a globally effective anti-MenB vaccine [5]. As a consequence there are no effective vaccines available for the prevention of MenB disease, which is responsible for one third of meningococcal disease in the United States, and up to 80 % of cases in Europe.

In 1998, the research team at Novartis Vaccines embarked on a large-scale genome project. To develop a universal vaccine against serogroup B, the genome of a MenB isolate (MC58 strain) has been sequenced and used to discover novel antigens [7, 8].

The identification of new previously unidentified antigens was a process that took the research team 18 months to achieve. The sequence of the virulent strain was determined by the shotgun strategy and in order to identify novel vaccine antigens a strategy has been aimed to select, among the more than 2,000 predicted proteins, those that were predicted to be surface-exposed or secreted and their potential to induce protection against disease was tested. *N. meningitidis* is essentially an extracellular pathogen and the major protective response relies on circulating antibodies: complement-mediated bactericidal activity is, in fact, the accepted correlate for in vivo protection and as such is the surrogate endpoint in clinical trials of potential meningococcal vaccines. On the basis of this evidence, the group worked on the assumption that protective antigens are more likely to be found among surface-exposed or secreted proteins. Hence the initial selection of