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Invited Review

Genome-based vaccine design: the promise for malaria and other infectious diseases

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ABSTRACT

Vaccines are one of the most effective interventions to improve public health, however, the generation of highly effective vaccines for many diseases has remained difficult. Three chronic diseases that characterise these difficulties include malaria, tuberculosis and HIV, and they alone account for half of the global infectious disease burden. The whole organism vaccine approach pioneered by Jenner in 1796 and refined by Pasteur in 1857 with the "isolate, inactivate and inject" paradigm has proved highly successful for many viral and bacterial pathogens causing acute disease but has failed with respect to malaria, tuberculosis and HIV as well as many other diseases. A significant advance of the past decade has been the elucidation of the genomes, proteomes and transcriptomes of many pathogens. This information provides the foundation for new 21st Century approaches to identify target antigens for the development of vaccines, drugs and diagnostic tests. Innovative genome-based vaccine strategies have shown potential for a number of challenging pathogens, including malaria. We advocate that genome-based rational vaccine design will overcome the problem of poorly immunogenic, poorly protective vaccines that has plagued vaccine developers for many years.

1. Vaccines – the global need

The term 'vaccine' is derived from *Variolae vaccinae*, literally smallpox of the cow, and was coined by Edward Jenner in 1798 in an article describing the protective effect of cowpox against smallpox (Jenner, 1798; Cohen et al., 1961; Baxby, 1999; Tuells, 2012). Since then, vaccines have been established as one of the most efficient and cost-effective interventions for the control and eradication of disease, and the prevention of morbidity and mortality worldwide. No other modality has had such a major effect on reducing mortality and improving public health, except for water sanitation (World Health Organization, 2006). Moreover, vaccinology is the only science that has eradicated an infectious disease (Andre, 2003), with the landmark achievement in 1977 of the eradication of smallpox (Fenner, 1982), a disease that plagued humankind and shaped our history since earliest civilisation (Fenner et al., 1988). It is anticipated that poliomyelitis will soon be eradicated, although some challenges remain (Pallansch and Sandhu, 2006).

Infectious diseases are responsible for one-third of all deaths worldwide, killing at least 15 million people each year (http://www.who.int/healthinfo/global_burden_disease/gbd/en/). They are clearly established as the leading cause of death of children globally and are responsible for 64–68% of deaths in children under 5 years of age, approximately 5 million children each year (Black et al., 2010; Liu et al., 2012). It is estimated that at least 3 million deaths per year are prevented by licensed vaccines currently in use (World Health Organization, 2007, 2009). Mass smallpox vaccination of children became compulsory in the United Kingdom (UK) in 1853 and vaccines are now available for most viral and bacterial diseases common in children including diphtheria (1923), whooping cough (1926), tetanus (1937), influenza (1942), pertussis (1949), polio (1958 and 1961), measles (1963), mumps (1967), rubella (1969), bacterial meningitis (1974), pneumonia (1983), varicella (1995) and rotavirus (1998) (http://www.who.int/immunization/policy/position_papers/en/). However, many of these existing vaccines are underutilised and the World Health Organization has estimated that 2.5 million children under the age of 5 years die from vaccine-preventable diseases each year, more than 6800 child deaths every day (World Health Organization, 2007). There are also many serious pathogens for which effective vaccines are not yet available including hepatitis C virus (HCV), human immunodeficiency virus (HIV), Dengue, respiratory syncytial virus (RSV) and cytomegalovirus (CMV); bacteria (e.g. *Mycobacterium tuberculosis* (TB), Group A streptococcus (GAS), Group B

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Streptococcus (GBS), *Staphylococcus aureus*, *Meningococcus* Group B (MenB), *Shigella*, pathogenic *Escherichia coli*); and parasites (e.g. *Plasmodium*, *Leishmania*, *Schistosoma*, *Trypanosoma*) and these are estimated to claim in excess of 3 million more lives each year (World Health Organization, 2005, 2007).

It is noteworthy that despite the demonstrated success of vaccines in preventing illness caused by viral and bacterial pathogens, there are not yet any licensed vaccines for parasitic infections of humans or for any chronic infections by complex pathogens (World Health Organization, 2006; Moorthy and Kieny, 2010). The pathogens causing these diseases have adapted to long-term coexistence with the human immune system and have evolved sophisticated immune evasion strategies. Many express hundreds or thousands of potential antigenic targets, often in distinct phases of their life cycles, so it is perhaps not surprising that vaccine efforts to develop vaccines based on only a limited number of antigens, often selected on an ad hoc basis and without knowledge of the antigenic repertoire of the organisms, have not been successful.

Moreover, all currently licensed vaccines for infectious diseases are prophylactic, preventing the effects of a future infection by the target pathogen, and there are no licensed therapeutic vaccines for any chronic or acute infectious disease. The only therapeutic vaccine approved by the United States Food and Drug Administration (US FDA) is for a certain type of metastatic prostate cancer (Provenge®, DendreonCorp, USA; approved in 2010). Prophylactic vaccines have been developed to prevent human papilloma virus (HPV) and hepatitis B virus (HBV) which cause chronic infections and in some cases cancer (Plotkin, 2008; Levine and Esparza, 2009). However, effective vaccines against the three pathogens responsible for more than half of the global burden of infectious diseases (malaria, HIV, TB) (World Health Organization, 2006) will need to be therapeutic, given the chronicity of these infections in endemic regions.

2. History of vaccines

The field of vaccinology originated on 14 May 1796 when Edward Jenner inoculated an 8 year old boy named James Phipps with vaccinia virus contained in pus from lesions on the hand of a milkmaid with cowpox and showed that Phipps did not become infected with smallpox when subsequently variolated (inoculated, or in today's parlance – challenged, with smallpox). Jenner's seminal study predated formal evidence for the germ theory of disease (microbial origin) obtained by Louis Pasteur in 1857 and Robert Koch in 1876 (D'Argenio and Wilson, 2010) which provided the foundation of empirical vaccine development. A century after Jenner's observation, proof-of-concept was established with the development by Louis Pasteur of an attenuated vaccine for chicken cholera in 1879, an anthrax vaccine in 1881, and a rabies vaccine in 1885 http://www.historyofvaccines.org).

These and subsequent "first generation" vaccines (e.g. Bacillus Calmette Guerin (BCG)) consisted of live-attenuated pathogens (typhoid, pertussis, measles, mumps, rubella) or inactivated killed pathogens (polio, rabies, cholera, hepatitis A, bubonic plague) and were developed according to the classical "isolation, inactivation and injection of disease-causing pathogen" approach to vaccine development established by Pasteur, and using Koch's postulates as a general guide.

In the second half of the 20th Century, significant advances in many fields including cell culture (enabling the growth of viruses in vitro), polysaccharide chemistry, recombinant DNA technology and immunology, allowed the development of "second generation" vaccines which comprised purified pathogen components such as protein antigens or polysaccharides (e.g. polio, measles, mumps, rubella, chickenpox, tetanus, diphtheria, anthrax, influenza, hepatitis A, hepatitis B, rotavirus, influenza, pneumonia and human papillomavirus (Hilleman, 1999; Finco and Rappuoli, 2014; Rhee, 2014). Maurice Hilleman is widely recognised as a pioneer and the most prolific developer of vaccines in this period, developing more than 40 vaccines (Hilleman, 1999; http://www.historyofvaccines.org).

It was in this era that Jonas Salk, who developed the first (inactivated) vaccine against polio, defined vaccinology as "the application of the basic requirements for effective immunisation" which include (i) stimulation with a sufficient quantity of antigen, (ii) use of a suitably specific antigen, and (iii) the induction of an appropriate immune response for the prevention of the pathological consequences of infection; also noting that vaccinology "requires an understanding of the etiologic agents, the pathogenic mechanisms, and the epidemiology of the individual diseases" (Salk and Salk, 1977). The majority of currently-licensed vaccines consist of either killed (inactivated) or live attenuated pathogens, or pathogen-related biomolecules including toxoids or polysaccharides (Grimm and Ackerman, 2013; U.S. Food and Drug Administration, 2014). It is notable that most currently licensed vaccines target pathogens with a relatively low degree of antigen variability and work mainly by eliciting functional antibodies (De Gregorio and Rappuoli, 2012).

For many significant pathogens, the generation of broadly protective vaccines has remained elusive. Such pathogens often present technical obstacles to the vaccinologist that include their inability to be cultured in vitro (e.g. *Mycobacterium leper*, papilloma virus type), have antigenic hypervariability (e.g. serogroup B meningococcus, HIV, HCV), or whose life cycles have an intracellular phase that puts them out of the reach of antibodies and therefore require a cellular immune response - controlled predominantly by T cells (e.g. malaria, tuberculosis); furthermore, traditional approaches to vaccine design and development do not allow the rapid development of new vaccines for pandemic agents (Finco and Rappuoli, 2014). Most second generation vaccines do not target these types of pathogens or elicit the correct types of responses and we have reached a point where most of the low-hanging fruit has been taken. These challenges are significant and explain why we have reached the end of the age of second generation vaccine development.

Advances in genomics and other "omics" over the past two decades have given rise to a "third generation" of vaccines (e.g. Meningococcus group B, group A streptococcus, group B streptococcus, *S. aureus*, *E. coli*, *Clostridium difficile*) based on technologies such as reverse vaccinology pioneered by Rappuoli (2000), structural biology and synthetic vaccines (Delany et al., 2014; Finco and Rappuoli, 2014). This activity has resulted in vaccines that protect against an increased range of vaccine-preventable diseases, that are multivalent and target different serotypes, or highly purified vaccines with an improved safety profile, and replace the more reactogenic whole cell vaccines (Rappuoli et al., 2011). These advances, combined with knowledge gained from successes with vaccine development against acute diseases in the 20th Century, provide the foundation for the development of vaccines that have thus far proved elusive, including those that are therapeutic (for chronic diseases), require cellular immunity for protection, for pregnant women and elderly or immunocompromised people, or for new indications such as autoimmune disease and cancer (Poland and Barrett, 2009; Rappuoli et al., 2011). They also enable more efficient pathways for vaccine development and new technologies for assessment of vaccine safety, which are especially pertinent given increased public scrutiny of adverse events associated with vaccination and stringent regulatory requirements for vaccine approval (Rappuoli et al., 2011).

On May 8, 1980, the World Health Assembly certified the world free of naturally occurring smallpox, representing a landmark achievement in the history of vaccinology (Fenner, 1982). The

Global Alliance for Vaccines and Immunisation (GAVI) was launched at the World Economic Forum on 31 January, 2000 with the involvement of the major players in global immunisation, including representatives and leaders from key United Nations (UN) agencies, bilateral aid agencies, the pharmaceutical industry, major philanthropic foundations and government bodies, all partnering with the aim to deliver vaccines to millions of the world's poorest children and boost immunisation coverage (http://www.gavialliance.org/). A decade later, on 29 January 2010, Bill and Melinda Gates issued a challenge to participants at the World Economic Forum's annual meeting to make this the "Decade of Vaccines" with a commitment of $10 billion over the next 10 years to help research, develop and deliver vaccines for the world's poorest countries, with a specific goal to prevent the deaths of approximately 8 million children under the age of 5 years from 2010 to 2019 (http://www.gatesfoundation.org/Media-Center/Press-Releases/2010/01/Bill-and-Melinda-Gates-Pledge-$10-Billion-in-Call-for-Decade-of-Vaccines). In December 2010, stakeholders from the global health community, including the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), the US National Institute of Allergy and Infectious Diseases (NIAID) and the Bill & Melinda Gates Foundation, formally launched the Decade of Vaccines Collaboration to generate a Global Vaccine Action Plan (GVAP) 2011–2020 (World Health Organization, 2013). Following extensive consultation, the GVAP was endorsed by the 194 Member States of the World Health Organization Assembly on 11 May 2012 to achieve the Decade of Vaccines vision by delivering universal access to immunisation and preventing millions of deaths by 2020 and beyond. The GVAP outlines six strategic objectives to strengthen routine immunisation, and develop and introduce new and improved vaccines and vaccine technologies (Keith et al., 2013).

3. Empirical to rational vaccine development

Historically, vaccine development has been based on empirical trial-and-error approaches designed to mimic, by vaccination, the immunity induced by natural infection, which originated in the late 17th Century with Jenner. With this classical "isolate, inactivate and inject" approach, protection against a wide range of bacterial and viral pathogens (but not parasitic pathogens of humans) has been achieved, and whole-organism vaccines still represent a large proportion of the vaccines licensed today.

There are both advantages and disadvantages associated with whole-organism vaccines (Zepp, 2010; Rappuoli et al., 2011; Grimm and Ackerman, 2013). A major advantage is that they do not require prior knowledge of immune responses associated with protection, or of the pathogen genome or proteome. Moreover, they typically induce sustained protective immune responses without the requirement for adjuvant. Importantly, whole-organism vaccines provide an extensive repertoire of pathogen-derived B cell and T cell epitopes for recognition by the immune system of the vaccine in the context of multiple genetic backgrounds found in heterogeneous human populations, although only a fraction of these epitopes may be important targets of protective immunity. In contrast, highly purified antigens likely present only a limited number of epitopes and therefore may not be recognised by all individuals (immunological non-responsiveness).

Despite these advantages, whole-organism killed, inactivated or live attenuated vaccines are typically complex and their poorly characterised products have often raised safety concerns including reversion to virulence for live attenuated pathogens, induction of autoimmunity, or unacceptable reactogenicity associated with the induced inflammatory response (Rappuoli et al., 2011). Additionally, critical conformational epitopes may be disrupted by the inactivation process (Grimm and Ackerman, 2013). The most infamous example of whole-organism virulence may be the "Cutter Incident" in 1955 when thousands of American children were inadvertently immunised with live polio virus contained in what was supposed to be an inactivated polio vaccine. This tragically resulted in a number of deaths and many cases of paralysis (Brown, 1993), and damaged the public's perception of vaccine safety. Some of these problems have been overcome with improved technology and quality control, but even in recent years, unanticipated outcomes have resulted from the use of whole-organism vaccines. Recently, in Australia, an unexpectedly high number of adverse events in children was associated with receipt of the 2010 trivalent influenza vaccine (FluVax) manufactured by CSL Biotherapies (Australia), possibly due to suboptimal virus splitting (Maraskovsky et al., 2012). Even with the application of more advanced technologies to genetically attenuate the pathogen, the potential for the pathogen to circumvent the attenuating mutation has been noted (Mikolajczak et al., 2014). Moreover, increased public and regulatory scrutiny of vaccine safety has raised the bar for what constitutes acceptable risk for vaccines (Kennedy and Poland, 2011) and indeed many, if not most, of the first generation whole-organism vaccines that have proved successful and overwhelmingly safe (and saved millions of lives) would not be approved for use today.

Additionally, the whole-organism approach is almost exclusively restricted to pathogens that can be cultured in vitro, and although it has worked for a number of "simple" pathogens with relatively low antigen variability, it has not succeeded for many complex pathogens including those that cause chronic infections and are capable of evading or misdirecting the host immune response. Also, traditional vaccine design is based on the premise of mimicking the immunity induced by natural exposure, but for many pathogens this is suboptimal and robust sustained protection may require inducing immunity that is better than nature, with minimal adverse effects associated with stimulating the inflammatory response (Zepp, 2010). This is especially true for a chronic infection, where the pathogen is able to co-exist with the host for an indefinite period of time despite the presence of immune responses induced by the host and targeted against the pathogen. Finally, for effective long-term protection, it is important that the vaccine-induced immunity be sustained even in the absence of pathogen exposure to allow for rapid recall of the required immune memory.

To overcome these challenges, vaccine design has become more tailored, focusing on the antigen moieties targeted by protective immune responses (Zepp, 2010) with the conceptual framework changing from a reductionist view to a holistic view, with a broad perspective of the pathogen and its interaction with the host immune system (D'Argenio and Wilson, 2010). Thus, the modern era of vaccinology is multidisciplinary, exploiting enhanced knowledge or improved understanding and technological innovations in basic immunology, molecular biology, microbiology, host-pathogen biology, microbial pathogenesis, genetics, biotechnology, bioinformatics and computational science, as well as other fields (D'Argenio and Wilson, 2010; Kennedy and Poland, 2011). As noted by Rino Rappuoli, "Vaccines have progressed from the crude preparations used to prevent smallpox to one of the most technologically advanced and effective public health interventions devised by man" (Rappuoli et al., 2011).

4. Rational vaccine development in the modern era

The ideal vaccine would induce robust and sustained immune responses in all individuals in all populations, regardless of age or immune status, in the absence of any adverse effects, and would

be effective against all strains and species even for complex pathogens (modified from Zepp, 2010). Rational vaccine design offers the potential to achieve this goal, facilitated by advances in three core areas: (1) conceptual advances, with improved understanding of basic immunology, host-pathogen interactions and pathogenesis; (2) technological innovations in molecular biology, biotechnology, delivery systems and other enabling technologies; and (3) emerging knowledge in 'omics' and other data-intensive sciences, combined with bioinformatics and computational biology capability as well as cross-disciplinary approaches.

4.1. Conceptual advances

4.1.1. Learning from nature

Historically, vaccines have been designed to mimic the immunity induced by natural exposure to the target pathogen (Zepp, 2010). In a number of cases, the phenotype of the critical responding cells, mechanisms of immunity and immunological memory, and immune correlates of protection are unknown. As an example, the BCG vaccine for tuberculosis developed in 1927 has been administered approximately four billion times but its mechanism of protective immunity is still not known (Kaufmann et al., 2014). The premise of rational vaccine design is to induce the desired immune response against the key pathogen antigen(s) or epitopes which are targeted by protective immune responses. Dissecting the immune response to the target pathogen following natural infection in humans is a logical step to identify the immune response(s) that should be induced by vaccination and the pathogen antigens targeted by that response(s). A number of factors should be considered.

4.1.1.1. Immune activation – innate immunity, adaptive immunity, and the innate/adaptive interface. The requisite steps leading to in vivo immune activation have been reviewed in detail elsewhere (Moser and Leo, 2010; Zepp, 2010; Six et al., 2012). Although traditional vaccine design has focused on the adaptive immune response, it is now recognised that innate immune responses and the innate-adaptive interface are critical for induction of robust protective immune responses. The current state of knowledge indicates that the rate limiting step for vaccination is antigen processing and presentation by dendritic cells, which ultimately directs the differentiation of functionally distinct subsets of immune cells with different effector functions. Soluble proteins appear to be particularly poor in inducing dendritic cell maturation because, presumably, they lack the so-called "danger signal" but this deficiency can be overcome at least in part by adjuvant formulations (Moser and Leo, 2010). The choice of adjuvant has been demonstrated to significantly impact vaccine efficacy (e.g. RTS,S vaccine; (Stewart et al., 2006)). Nonetheless, until recently, Alum was the only adjuvant globally approved for human use (Eisenbarth et al., 2008), but MF59 and monophosphoryl lipid A-containing formulations have received regulatory approval in defined human vaccines (Rueckert and Guzman, 2012). Considerable efforts over the past few decades have been devoted to adjuvant discovery, and some promising adjuvants are now moving through the regulatory pipeline (Reed et al., 2013).

4.1.1.2. Activation of multiple arms of the immune system. Almost all licensed vaccines currently available, as well as those that were previously available but have been removed or replaced, rely on the induction of functional antibodies which neutralise or opsonise the target pathogen (Six et al., 2012; Delany et al., 2014). Some (e.g. tetanus) work by neutralisation of the toxin. Although some of these vaccines do induce cellular immune responses (e.g. pertussis, (Mills et al., 1998); hepatitis B (Ren et al., 2003)), the protection is thought to be antibody-mediated and, to the best of our knowledge, there are no licensed vaccines that are specifically designed to induce protective cellular immune responses. However, for many pathogens, including intracellular pathogens (Seder and Hill, 2000), cellular immune responses play a critical role in protection, either directly via $CD8^+$ and $CD4^+$ T cell cytotoxicity targeting pathogen-infected cells or indirectly via cytokines or other mediators produced by the T cells. Additionally, indirectly, helper T cells are required for optimal B cell activation and high affinity antibody responses, and influence the antibody isotype as well as the development of immune memory (Moser and Leo, 2010; Zepp, 2010; Delany et al., 2014). Indeed, the development of vaccines against those pathogens that have thus far proved elusive will likely require activation of all arms of the immune system with functional antibodies as well as efficient $CD4^+$ and $CD8^+$ T cell responses, with appropriate selection of pathogen antigen relevant for each immune response. Since conventional technologies have proved ineffective in generating robust cell-mediated immunity, innovative vaccine delivery systems will be crucial (see Section 4.2).

4.1.1.3. Quality of the immune response is not the same as quantity. Conventional assessment of vaccine-induced immune responses or correlates of immunity has typically focused only on the frequency and magnitude, or quantity, of the immune response; e.g. ELISA titres as a measure of the peak antibody response; cytotoxic capacity or frequency of antigen-specific T cells as a measure of the T cell response. However, the quality of the immune response is at least as important as the quantity, and quality can be a key determinant of protection (Zepp, 2010). For example, the standard ELISA assay for antibody responses quantifies antibodies that bind to the pathogen but this provides no indication of the capacity of those antibodies to be functional (Plotkin, 2008). In the case of antibody responses, quality can be assessed via affinity, avidity, diversity and biological function, as well as Ig subclass (Plotkin, 2008; Courtin et al., 2009; Feng et al., 2009). For T cell responses, quality can be assessed via phenotypic markers, differentiation state (central memory, effector memory, effector T cells), profile of secreted cytokines and polyfunctionality, TCR avidity and TCR repertoire diversity (Germain, 2010; Sallusto et al., 2010). For both antibodies and T cells, the generation and maintenance of immunological memory is critical to ensure rapid reactivation upon pathogen exposure. Recent advances in immune monitoring and immune profiling, and establishment of consortia such as the Human Immunology Project Consortium (http://www.immuneprofiling.org), provide the foundation for comprehensive evaluation of vaccine-associated immune parameters, and identification of novel immune mediators and pathways (Newell et al., 2013; Poland et al., 2013; Brusic et al., 2014).

4.1.1.4. Immune evasion, antigenic polymorphism and chronic diseases. With the conceptual and technological advances of the past decades, we have an improved understanding of antigenic polymorphism and immune evasion, and the associated challenges for vaccine development, especially for chronic diseases. It is now recognised that some pathogens are able to evade host immunity by mutating key antigens or epitopes with evolutionary pressure or during the course of disease. For example, the *Plasmodium* spp. parasite has co-evolved with the human host over millions of years with selection of polymorphisms within the human genome that represent some of the most common monogenic human disorders, such as α-thalassemia and hemoglobinopathies including sickle-cell trait (Mackinnon and Marsh, 2010; Taylor et al., 2013). Concomitantly, on the parasite side, polymorphisms within antigens or epitopes targeted by host immune responses have been demonstrated within relatively restricted time frames as a means

of circumventing the host immune response (Doolan et al., 1991; Barry et al., 2009; Takala and Plowe, 2009). In addition to polymorphism at the nucleotide level, allelic polymorphism whereby more than one allele exists for specific regions of a protein (e.g., *Plasmodium falciparum* merozoite surface protein 1 or apical membrane antigen 1; *Plasmodium vivax* Duffy Binding Protein), as well as antigenic variation of the asexual blood stages of *Plasmodium* where the parasite expresses at any given time only one member of a family of multi-copy variant surfaces antigens (e.g., PfEMP1), are also well established (Takala and Plowe, 2009; Kirkman and Deitsch, 2012). Examples of immune escape through antigenic polymorphism or allelic variation have been also demonstrated for other pathogens, with well known examples including influenza (Yewdell, 2013), HIV (Moore et al., 2002; Boutwell et al., 2010; Julien et al., 2012) and hepatitis C virus (Petrovic et al., 2012). Such variation poses significant challenges for vaccine development, since a vaccine that has high strain-specific efficacy against the antigen included in the vaccine would nonetheless be poorly effective against variant circulating strains in the field (Moorthy and Kieny, 2010). This has been demonstrated experimentally with a candidate three-component blood-stage vaccine against malaria known as Combination B (Genton et al., 2002) and subsequent field studies with recombinant merozoite surface protein 1 (MSP1) (Ogutu et al., 2009; Otsyula et al., 2013) and apical membrane antigen 1 (AMA1) vaccines (Ouattara et al., 2010, 2013; Thera et al., 2011). However, recent technological advances (see below) have allowed the development of multivalent vaccines against pathogens with multiple strains or serotype (e.g. the licensed 7-valent, 10-valent or 13-valent conjugate vaccines for *Pneumococcus* or the 4-valent vaccine for *Meningococcus* (De Gregorio and Rappuoli, 2012). Other approaches have demonstrated potential in preclinical evaluation, including chimeric vaccines (e.g. *P. falciparum*, Dutta et al., 2007; Faber et al., 2013) mosaic vaccines (e.g. HIV, Corey and McElrath, 2010), or structure-based vaccine design (see Section 4.3.3).

4.1.1.5. Not all antigens are equal. It is now recognised that not all antigens or pathogen-derived epitopes are equal in terms of their capacity to be recognised by the host immune response. This phenomenon, where pathogen-specific immune responses target only a small fraction of the full range of possible antigens or peptide epitopes, is known as immunodominance (Sercarz et al., 1993; Allen et al., 2001). The factors underlying the hierarchy of pathogen-specific epitope recognition are largely unknown, especially in the context of a complex pathogen, but could result from features intrinsic to the protein antigen such as protein abundance, kinetics of protein expression during infection, and rate of synthesis or decay. It could also result from properties intrinsic to the epitope(s), including efficiency of antigen processing and presentation, epitope abundance, major histocompatibility complex (MHC) binding affinity or precursor frequency (Yewdell and Bennink, 2001; Assarsson et al., 2007; Kotturi et al., 2008; Tan et al., 2011). The identification within this hierarchy of antigens (or epitopes) that are key targets of protective immune responses and that will stimulate effective immunity against the target pathogen is a key component of rational vaccine design (Rueckert and Guzman, 2012). There is currently no algorithm or universal approach to identify the important antigens and epitopes, and historically antigens for inclusion in subunit vaccines have been selected empirically, often based on antigen density and cell surface accessibility. In some cases, cell surface proteins have proved to be effective targets of neutralising antibodies; e.g. hepatitis B surface antigen (Schillie and Murphy, 2013). However, in many cases, antigens identified empirically may not be the optimal target of protective immune responses, may be poorly immunogenic, or may have associated safety concerns (Rappuoli et al., 2011). Advances in the genomic era, with the sequencing of pathogen genomes and systems immunology approaches to define the requisite immune response, offer the potential to overcome these limitations, particularly when the genome of the pathogen is large.

4.1.2. Improving on nature

As stated above, traditional vaccines aim to mimic the immunity induced naturally following infection with the pathogen. However, this approach has not been successful for many diseases, including those caused by pathogens where immunity is typically slow to induce and non-sterilising (e.g. malaria, RSV, *Pseudomonas aeruginosa*) or where infections are chronic or have a latent phase (e.g. HIV, hepatitis C virus, *S. aureus*) (Delany et al., 2014). Thus, modern vaccinology must improve on nature. There are a number of examples which demonstrate that this is possible, specifically: (i) higher titres of neutralising antibodies can be induced in adults by vaccination with a human papilloma virus virus-like particle (VLP) vaccine adjuvanted with AS04 than are induced naturally (Schwarz and Leo, 2008; Zepp, 2010). (ii) Human papilloma virus VLP vaccines can induce a systemic protective immune response, even though the virus enters only via the mucosal system and remains localised to the basal cell layer of the squamous epithelium (Schwarz and Leo, 2008; Zepp, 2010). (iii) Higher titres of neutralising antibodies can be induced in children by vaccination with *Haemophilus influenzae* type b, pneumococcal or meningococcal polysaccharide-protein conjugate vaccines that convert the induced antibody responses from T cell-independent to T cell-dependent responses than are induced naturally (D'Argenio and Wilson, 2010). (iv) A candidate vaccine against *Chlamydia trachomatis* infection comprising the major outer membrane protein (nMOMP) solubilised with amphipols can induce more robust chlamydia-specific humoral and cell-mediated immune responses and protective immunity in mice, by stabilising the protein and increasing epitope accessibility (Tifrea et al., 2014). Additionally, recent advances in approaches such as structural vaccinology are specifically tailored to design vaccines that do better than nature (see Section 4.3.3).

4.2. Technological innovations

Rational vaccine design in the modern era is facilitated by technological innovations in many areas including: (i) technical advances in recombinant DNA technology and biotechnology; (ii) manufacturing improvements including fermentation, production, purification and consistency in manufacturing scale-up; and (iii) enabling technologies which include novel vaccine platforms (adjuvants, viral vectors, DNA vaccines, RNA vaccines, virus-like particles (e.g. human papilloma virus vaccine, liposomes, nanoparticles, mosaic vaccines)) as well as delivery systems (skin patches, electroporation). These have been reviewed in detail elsewhere (Hilleman, 1999; Zepp, 2010; Grimm and Ackerman, 2013).

4.3. Emerging knowledge from genome-scale datasets

Perhaps the most important influences on rational vaccine design have been the tremendous advances made in the genomic era, which began with the sequencing of the complete genome of *H. influenzae* in 1995 (Fleischmann et al., 1995). Since then, the genomes, proteomes and transcriptomes of many pathogens have been elucidated (http://www.genomesonline.org/). This information provides the foundation for novel 21st Century approaches to identify target antigens for the development of vaccines, drugs and diagnostic tests; for the development of innovative vaccine delivery systems; and for the development and application of methods to identify immune correlates of protection. These data-rich sets of information have given rise to a large number of new scientific fields

known as "omics" (Baker, 2013; http://www.genomicglossaries.com/content/omes.asp), as well as revolutionary conceptual and technological advances which are largely cross-disciplinary. Those most relevant to vaccine design include reverse vaccinology, immunomics, structural vaccinology and systems immunology. These, and others related to the field, have been reviewed elsewhere by us (Doolan, 2011; Schussek et al., 2014) and others (Dormitzer et al., 2008, 2012; Pulendran et al., 2010; De Gregorio and Rappuoli, 2012; Grimm and Ackerman, 2013; Thomas and Luxon, 2013; Delany et al., 2014; Finco and Rappuoli, 2014), so will be only briefly summarised here to provide context.

4.3.1. Reverse vaccinology

Reverse vaccinology, pioneered by Rappuoli and colleagues in 1999 (Rappuoli, 2000), is considered a milestone in vaccinology and biotechnology because it exploits the power of in silico analysis to identify, from the complete genome of the target pathogen, a subset of genes encoding proteins with characteristics associated with vaccine-induced immunity (e.g. location on cell surface) which are then systematically evaluated for capacity to induce the desired immune response/immunogenicity (Rappuoli, 2000). Proof-of-concept for reverse vaccinology was established with *Neisseria meningitidis* (Pizza et al., 2000), identifying in just 18 months more surface exposed antigens than had been discovered in 40 years of conventional vaccinology; a vaccine based on three of these novel antigens and outer membrane vesicles is now licensed in 30 countries (Gorringe and Pajon, 2012).

4.3.2. Immunomics

Immunomics is the study of the subset of pathogen-derived proteins or their epitopes that are recognised by the host immune system. In contrast to reverse vaccinology, immunomics-based identification of vaccine candidates relies not only on in silico prediction algorithms, but also takes advantage of biological samples from humans or animals with immunity to the disease of interest, to define the set of antigens or epitopes that interface with the host immune system (Doolan, 2011; Sette et al., 2005). Thus, immunomics specifically addresses the interface between the host immune system and the pathogen proteome (Klysik, 2001). No vaccines derived from immunomics have yet reached the stage of clinical testing but a number of promising candidate antigens have been identified by us in the malaria model using antibody-based (Doolan et al., 2008; Trieu et al., 2011) or T cell-based (Doolan et al., 2003; Doolan, 2011) approaches.

4.3.3. Structure-based vaccine design or structural vaccinology

Most recently, advances in three-dimensional structural biology enabling determination of protein tertiary structure and potential conformational B cell epitopes has provided the foundation for structural vaccinology or structure-based vaccine design (Dormitzer et al., 2008, 2012; Thomas and Luxon, 2013; Delany et al., 2014). The underlying rationale is that protective epitopes (rather than complete proteins) should be sufficient to induce immune responses and provide protection against pathogens, and constructs can be engineered such that they are more stable, expose hidden cryptic epitopes, or enable broadly cross-protective responses against pathogen variants. Proof-of-concept has been demonstrated in preclinical studies for a number of pathogens, including the highly variant *N. meningitidis* serogroup B surface-exposed factor H-binding protein, the group B *Streptococcus* pilus protein, the F glycoprotein of RSV and influenza HA (Dormitzer et al., 2012).

4.3.4. Systems immunology

Systems immunology (systems vaccinology) falls under the broad umbrella of systems biology and aims to study the immune system in an integrated perspective rather than studying isolated components, in order to identify immune correlates of protection or signatures of immunogenicity (Querec et al., 2009; Pulendran et al., 2010; Kaufmann et al., 2014). This approach, which successfully predicted the immunogenicity of the highly efficacious yellow fever vaccine in humans (Querec et al., 2009), incorporates a holistic view of the overall host-pathogen interaction as opposed to the simple conventional readouts of vaccine-induced immune responses such as ELISA or ELIspot assays (Six et al., 2012). Such studies would be expected to provide a comprehensive understanding of the host-pathogen interaction and its regulation; to identify novel immune mediators and pathways, and correlates of vaccine efficacy, facilitating vaccine evaluation in the clinic.

5. Malaria vaccine development

5.1. Challenges and feasibility

The *Plasmodium* parasite has a large, complex genome encoding thousands of potential antigenic targets expressed at different stages of a complex life cycle. It has developed a range of effective strategies for evading the human immune response, including allelic and antigenic variation, a predominantly intracellular existence, and the ability to down-regulate the host's immune response. Current vaccine approaches have failed to yield an effective *P. falciparum* vaccine in spite of 50 years of dedicated effort. Nevertheless, two experimental human vaccine models suggest that a malaria vaccine is indeed a feasible goal.

Sterile infection-blocking immunity can be induced in mice, monkeys and humans by immunisation with *Plasmodium* sporozoites attenuated by radiation such that they can invade the host hepatocyte but do not fully develop (Nussenzweig and Nussenzweig, 1989; Hoffman et al., 2002). T cell responses (in particular $CD8^+$ T cells) directed against parasite antigens expressed in the infected hepatocyte are considered the primary immune effectors, although antibodies also play a role in inhibiting sporozoite invasion of the hepatocyte (Good and Doolan, 2010). Secondly, individuals surviving past early childhood in areas of high malaria transmission develop substantial clinical immunity and rarely die from malaria, although they are frequently infected (Baird, 1998). Antibodies directed against antigens exposed on the surfaces of merozoites or infected erythrocytes, or released from apical organelles at the moment of invasion, are thought to be the critical effectors since passive transfer of purified immunoglobulin from individuals with lifelong exposure to endemic malaria results in a marked decrease in blood-stage parasitemia and resolution of symptoms in the recipients (Cohen et al., 1961; Langhorne et al., 2008). The human models represented by irradiated sporozoite immunisation or naturally acquired immunity are powerful models for the development of a malaria vaccine to completely prevent infection or death and severe disease, respectively.

More recently, a practical demonstration of the feasibility of a malaria vaccine has been shown by the impact of the RTS,S vaccine. Although phase 3 results with RTS,S/ASO1 suggest that efficacy is very low in the target age group and is not sustained (Duncan and Hill, 2011; Olotu et al., 2013), the vaccine may nonetheless receive regulatory approval for implementation through the routine Expanded Program for Immunisation (Bouchie, 2013) since modelling suggests that it may have an impact on the number of "deaths averted" (Smith et al., 2012; Nunes et al., 2013). These data are especially encouraging since the vaccine is based on only a single target antigen (*P. falciparum* circumsporozoite protein) amongst the more than 5,000 expressed by the parasite. However these poor efficacy results highlight the need to include more antigens and induce a broad multi-

pronged immune response involving $CD4^+$ and $CD8^+$ T cell responses as well as antibody responses.

5.2. Progress, or lack thereof

Malaria vaccine development has focused on subunit vaccines against a very limited number of target antigens (World Health Organization, 2006; Schwartz et al., 2012). Nearly all *Plasmodium* antigens present in the genome have the potential to be targets for effective vaccines, drugs or diagnostic tests and remain so until each is systematically assessed. What distinguishes one protein from another for candidacy as a target for a new drug or as an immunogen for a new vaccine is often determined empirically. Selection of vaccine targets has been based on a variety of criteria which, while not irrational, are not systematic. For example, although some asexual blood-stage antigens were identified by probing a cDNA expression library with antibodies present in inhibitory sera from malaria-exposed individuals (Kemp et al., 1983), other antigens have come to attention due to historical reasons related to the ease with which murine or rabbit antisera were generated against them. Their selection as candidate vaccine targets may be validated by immunoepidemiological evidence of associations between immune responses and clinical immunity, neutralisation or adoptive transfer experiments in animal systems, or induction of protective immunity in animal models with homologous antigens from murine or simian malaria species.

For most of the past half century, malaria researchers attempting to develop a pre-erythrocytic stage malaria vaccine have focused almost exclusively on a single antigen, the circumsporozoite protein (CSP). Several lines of evidence supported the initial focus on the CSP as a pre-erythrocytic stage vaccine candidate (reviewed in Doolan and Martinez-Alier, 2006). In 1997, 16 years after the CSP was identified and 15 years after GlaxoSmithKline (UK) and the Walter Reed Army Institute of Research (WRAIR, US) entered a collaborative agreement to produce a malaria vaccine, it was demonstrated that the RTS,S vaccine could protect ~40% of malaria-naive volunteers against sporozoite challenge (Stoute et al., 1997; Cohen et al., 2010). A review of the history of the RTS,S vaccine is sobering since in the development effort nearly a dozen other constructs based on the CSP were tested preclinically and as many as six different vaccines were tested in the clinic in Phase 1, Phase 1/2a challenge studies and up to Phase 2b studies in malaria endemic regions; some were efficacious but the efficacy was marginal and significant research and development (including almost 40 different clinical trials) was required before the final vaccine candidate was identified (Cohen et al., 2010). After 30 years of dedicated commitment from the pharmaceutical partner, GSK, phase 3 data are finally available which show that the vaccine is far less efficacious in the target population than anticipated and that the effect on clinical malaria is short-lived (Olotu et al., 2013). Consistent with this, a number of studies have established that although the CSP is the dominant sporozoite surface protein, it is not a major component of the protective immune responses induced by immunisation with radiation attenuated *Plasmodium* sporozoites (Doolan et al., 2003; Gruner et al., 2007; Kumar et al., 2006; Trieu et al., 2011).

In the case of erythrocytic stage vaccines, there has been a similar, if slightly less restrictive, focus on a handful of candidate antigen targets, particularly MSP1 (Fowkes et al., 2010) and AMA1 (Remarque et al., 2008), with a marked lack of success in the clinic (Ogutu et al., 2009; Sagara et al., 2009).

There is reason to believe that such a narrow focus is inadequate and misplaced. A possible explanation for the unsatisfactory efficacy of CSP-based vaccines, for example, comes from analysis of immune responses in animals and human volunteers protected by immunisation with irradiated sporozoites. While such volunteers do generate antibody and T cell responses to CSP, they are often very weak responses, substantially weaker than those induced by immunisation with CSP itself (Doolan and Hoffman, 1997, 2000). This discrepancy suggests that immune responses to CSP are not a major component of the robust protection induced by immunisation with radiation-attenuated sporozoites, and is similar to the low-level responses elicited in the volunteers to other candidate pre-erythrocytic stage vaccine antigens, including SSP2/TRAP and LSA1 (Doolan et al., 1997; Doolan and Hoffman, 2000). These findings suggest two possibilities: either the protective immunity induced by radiation attenuated sporozoites depends on a strong response to a limited number of not-yet-identified antigens, or it depends on the summation of a large number of weak responses to many antigens. In either case, a focus on the large number of other potential pre-erythrocytic stage vaccine candidates present in the genome is warranted.

To date, few proteins in the *Plasmodium* spp. proteome have been eliminated from consideration as potential vaccine candidates, and many are being advanced as vaccine components in clinical trials without rigorous evaluation of efficacy. Moreover, subunit vaccines based on a single or few antigens may elicit a narrow breadth of response, providing neither optimal protection nor protection on genetically diverse backgrounds. This protein-by-protein approach has resulted in a long and largely unsuccessful history of malaria vaccine development, with many candidate vaccine immunogens being in the development pipeline for decades and many failures (World Health Organization, 2007; Schwartz et al., 2012). It is likely that failure to develop a malaria vaccine despite decades of effort can be attributed to this limited and arbitrary list of antigens, and that only by overcoming this restriction will it be possible to identify vaccine targets suitable for inducing protective humoral and cellular immune responses protecting against chronic infections such as malaria. The requirement is both to select optimally protective antigens and to ascertain whether a few or many such antigens underlie the protective immunity induced by immunisation with irradiated sporozoites (pre-erythrocytic stage immunity) or by repeated exposure to infection (blood-stage immunity). In our opinion, genome-based rational vaccine design is the most logical, and only, way forward.

5.3. Whole parasite vaccine trend

The long and challenging history of malaria vaccine development has caused a resurgence of interest in the whole-organism approach established by Jenner in the 18th Century. This interest builds on experimental evidence that whole parasite approaches utilising either radiation-attenuated sporozoites or parasitised red blood cells can protect in animal models of malaria, reviewed extensively elsewhere (Butler et al., 2012; Epstein and Richie, 2013; Good, 2013; Good et al., 2013; Loukas and Good, 2013; Vaughan and Kappe, 2013).

Briefly, the core approaches are: (i) metabolically active radiation attenuated sporozoites being championed by Sanaria (Hoffman et al., 2010; Seder et al., 2013); (ii) immunisation with infectious *P. falciparum* sporozoites under chloroquine chemoprophylaxis (CPS) (Roestenberg et al., 2009, 2011; Bijker et al., 2013); (iii) genetically attenuated liver stage and/or blood stage parasites (Mueller et al., 2005; Spring et al., 2013; Mikolajczak et al., 2014); and (iv) chemically attenuated blood stage parasites (Good et al., 2013).

Although some promising results have been obtained in preclinical models, it remains to be seen whether the many technical, logistical and regulatory hurdles can be overcome (Menard, 2005; Ballou and Cahill, 2007; Anders, 2011). In our opinion, the difficulties associated with progressing whole-organism malaria vaccines to the clinic are likely to be insurmountable and compel us towards

a modern genome-based rational vaccine design, rather than a return to the approach established over 200 years ago.

5.4. Genome-based rational vaccine design for malaria

Malaria represents an excellent model for genome-based vaccine design against complex pathogens because: (i) it is a significant public health problem in the developing world; (ii) there are two human models of protective immunity that establish the feasibility of developing a malaria vaccine (immunisation by radiation-attenuated *Plasmodium* sporozoites, relying on the generation of protective cellular responses against pre-erythrocytic stage antigens; and naturally acquired immunity, relying on the generation of protective antibody responses against blood stage antigens); (iii) candidate antigens can be assessed either in vitro (using an appropriate immune readout), in animal model challenge systems (using the corresponding *Plasmodium* spp.), or in humans by challenging with *P. falciparum* in a well-validated human challenge model; (iv) both cellular and humoral immune responses to several antigens simultaneously will be likely required, in accordance with the two models of protection in humans; (v) sub-optimal protection has already been achieved using current molecular vaccine technologies, suggesting their feasibility and potential for improvement; (vi) vaccines so generated can be tested for safety and protection against sporozoite challenge in the USA or Europe, and transitioned as appropriate to field testing; and (vii) malaria, similar to many other important human pathogens in the developing world, is a parasitic disease and thus has developed mechanisms to avoid or modulate the host immune system, meaning that the technological solutions developed for malaria vaccine development should be translatable to other chronic infectious agents.

5.4.1. Plasmodium 'omics'

The complete genomic sequences of the human parasites *P. falciparum* (3D7 clone), *P. vivax* (Sal I strain), *Plasmodium yoelii* (17XNL strain) and *Plasmodium knowlesi* (H strain) have been elucidated (Gardner et al., 2002; Carlton et al., 2002; Pain et al., 2008). Genome sequence data from nine *Plasmodium* strains are in various stages of completion and are available to the research community at PlasmoDB (http://www.plasmodb.org/) (Carlton et al., 2005, 2008; Aurrecoechea et al., 2009) together with partial genome sequence for an additional 16 geographically diverse *Plasmodium* parasites (15 *P. falciparum*, one *Plasmodium reichenowi*). With the advent of next generation sequencing technology, the number of completed and ongoing sequencing projects is rapidly increasing. The sequencing of at least 105 geographically diverse *Plasmodium* strains/isolates including 75 *P. falciparum* isolates, nine *P. vivax* isolates, *Plasmodium malaria* and *Plasmodium ovale*, representative simian malaria parasites (*P. reichenowi*, *Plasmodium cynomolgi, Plasmodium inui, Plasmodium coatneyi, Plasmodium fragile*), rodent parasites (*Plasmodium chabaudi, P. yoelii,* and *Plasmodium berghei*), an avian parasite (*Plasmodium relictum*), and a reptile parasite (*Plasmodium mexicanum*) is in progress (Cai et al., 2012).

In addition to this genomic data, high throughput transcriptomics and proteomics technologies, including microarray DNA chip, mass-spectrometry, yeast two-hybrid (Y2H) screening and most recently RNA-seq, NSR-seq and CHIP-seq, have been used to characterise and profile the expression, regulation and interaction of *Plasmodium* genes at the level of the transcriptome, proteome, metabolome and interactome (Winzeler, 2006; Cai et al., 2012). Large-scale proteomic datasets are also available for several life cycle stages (*P. falciparum, P. berghei* and *P. yoelii*) (Florens et al., 2002, 2004; Hall et al., 2005; Khan et al., 2005; Tarun et al., 2008). Transcriptomic data are now available from multiple life-cycle stages or gene knock-out mutants of *P. falciparum* and *P. berghei* (Hall et al., 2005) as well as multiple stages of *P. yoelii* (mosquito, erythrocytic and liver stages) (Tarun et al., 2008; Mikolajczak et al., 2008; Zhou et al., 2008; Lasonder et al., 2008), *P. vivax* isolates (Bozdech et al., 2008) and other *P. falciparum* strains (Rovira-Graells et al., 2012). Other datasets include expressed sequence tag (EST) data from over 130 libraries (*P. falciparum, P. vivax, P. berghei* and *P. yoelii*) (Watanabe et al., 2007; Tarun et al., 2008; Florent et al., 2009) and serial analysis of gene expression (SAGE) data (*P. falciparum* only) (Patankar et al., 2001; Gunasekera et al., 2003, 2004). Most recently, transcriptome analysis by next generation sequencing (RNA-seq) has provided information about the abundance of all transcripts in the transcriptome by considering the real number and the level of different transcript isoforms (Otto et al., 2010; Lopez-Berragan et al., 2011; Lindner et al., 2013). Moreover the combination of transcriptomics analysis with ChIP-seq data has provided new insights to unravel the regulation of gene expression (Lopez-Barragan et al., 2011; Hoeijmakers et al., 2012). Other studies have provided transcriptomics data on clinical isolates using NSR-seq (Vignali et al., 2011). Expression profiling data throughout the intraerythrocytic cycle (oligonucleotide-based microarrays in both glass slide and Affymetrix formats) as well as single nucleotide polymorphism (SNP) analysis for 20 *P. falciparum* strains and 100 *P. falciparum* isolates (Jeffares et al., 2007; Mu et al., 2007; Volkman et al., 2007) are also available. Additional functional data sets include evidence of protein–protein interactions (Y2H and predicted interactome) (LaCount et al., 2005; Date and Stoeckert, 2006); Genome Ontology (GO) (Ashburner et al., 2000) and InterPro domain (Mulder et al., 2005) annotations for *P. falciparum*, *P. vivax*, *P. berghei*, *P. yoelii*, *P. knowlesi* and *P. chabaudi*; Enzyme Commission (EC) number (Ashburner et al., 2000) annotations for *P. falciparum*, *P. yoelii* and *P. knowlesi* (Ginsburg, 2006); and metabolic pathway assignments for *P. falciparum* (Ginsburg, 2006). Predictions of protein subcellular localisation (Bendtsen et al., 2004) and transmembrane domains (Krogh et al., 2001) for *P. falciparum, P. vivax, P. berghei*, *P. yoelii*, *P. knowlesi* and *P. chabaudi* are available, as well as parasite-specific predictions (*P. falciparum* only) for apicoplast localisation (Foth et al., 2003) and for export to the host cell (Hiller et al., 2004; Marti et al., 2004, 2005).

These analyses of genomics, proteomics, transcriptomics, interactomics and, most recently, next generation sequencing, allow for investigation at the molecular level of drug resistance, immune evasion and pathogenesis, and provide the foundation for antigen discovery and prioritisation on a genome-wide or proteome-wide scale. Web-based databases such as PlasmoDB (http://plasmodb.org/) and GeneDB (http://www.genedb.org/) providing public access to the data and advanced statistical and bioinformatics algorithms and analytical tools such as BioGRID (http://thebiogrid.org/), Pathway Commons (http://www.pathwaycommons.org/) and STRING (http://string-db.org/), allow the researcher to visualise, analyse, integrate and interrogate the data. Overall, these datasets provide a comprehensive foundation for genome-based vaccine design.

5.4.2. Applied 'omics'

With this wealth of genomic, proteomic, transcriptomic and related data, the tools to exploit these data, and biological samples from human volunteers, we have an unprecedented opportunity to develop and implement a rational approach to target antigen selection and vaccine design. In our view, the "post-genomics" era of vaccine research should focus on leveraging these genome-scale datasets for objective evidence-based assessment of candidate targets for rational vaccine development. Rational and systematic genome-based strategies overcome the deficiencies of the current ad hoc approach to target selection. Importantly, they take advantage of the two human models of immunity which depend on

exposure to whole *Plasmodium* organisms expressing hundreds to thousands of potential immune targets, while aiming to "improve on nature".

Already, exploitation of genomic data with systematic approaches to rational drug design and target selection (chemogenomics) has led to the identification of new classes of antimicrobial drugs (Bhattacharjee et al., 2013). Progress towards genome-based vaccine design is well under way and showing great potential. In our laboratory, we are using the power of immunomics to address two situations: one (predominantly pre-erythrocytic, based on the irradiated sporozoite model) in which T cell targets are selected, and one (predominantly erythrocytic, based on naturally acquired immunity) in which antibody targets are selected. We are using biological specimens (T cells and plasma/sera) from individuals immunised with irradiated sporozoites or naturally exposed to malaria, or immunoglobulin from clinically immune individuals, for in vitro immune screening to assess the capacity of the antigen to be recognised by recall of *Plasmodium*-specific immune responses in protective human models using clinically relevant selection criteria. We have generated unique 'omic'-scale datasets of proteome-wide T cell responses and antibody responses to *Plasmodium* by systematically screening the *Plasmodium* proteome using protein microarrays and epitope prediction algorithms, with specimens from humans, non-human primates or rodents naturally or experimentally exposed to malaria. (Doolan et al., 2008; Crompton et al., 2010; Barry et al., 2011). Analysis of these comprehensive datasets has revealed that the target antigens of antibody or T cell responses are not randomly distributed throughout the proteome, and that only approximately 30% of the proteome is recognised. We have also established that a large number of newly identified antigens are more immunogenic than antigens identified by traditional methods. Furthermore, antigens that are highly reactive for T cells are not serodominant for antibody responses (Doolan, D.L., unpublished data), suggesting that different approaches are required to identify the most effective targets of T cell and antibody responses. These data establish proof-of-concept for both T cell- and antibody-based approaches to identify antigens and epitopes which represent promising candidates for next generation malaria vaccine development. They also support the premise that omics-scale approaches offer promising solutions to the challenge of vaccine development for complex pathogens.

6. Conclusion

There is currently no accepted rational approach to vaccine design and target selection for malaria or any other complex pathogen. We advocate a rational approach to malaria vaccine design capable of exploiting the wealth of genomic, proteomic and bioinformatic information that has been developed in the genomic and post-genomic eras. We believe that rational genome-based vaccine design, enabling the selection of the best possible targets by prioritising antigens according to clinically relevant criteria (frequency and magnitude of clinically relevant immune response and/or biological function), will overcome the problem of poorly immunogenic, poorly protective vaccines that has plagued malaria vaccine developers for the past 25 years. Clearly, additional work will be required to bring rationally-designed vaccines to the clinic, including pre-clinical safety testing, optimisation of immunogenicity, phased human clinical trials, and large scale production and distribution. However, appropriate approaches and standard protocols for these steps are well-established and these remaining steps are not part of the critical roadblock to malaria vaccine development. There remain economic roadblocks to production and licensing of an effective vaccine even after efficacy and safety are demonstrated. We believe, however, that once an effective malaria vaccine has been developed, private and governmental sources will be persuaded to provide adequate resources to support production and distribution and, once distributed, the vaccine is likely to meet with wide acceptance in Africa. Extension of this approach to other pathogens will require a substantial research investment, however such effort will undoubtedly be worthwhile.

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