Immunomics: a 21st century approach to vaccine development for complex pathogens

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SUMMARY

Immunomics is a relatively new field of research which integrates the disciplines of immunology, genomics, proteomics, transcriptomics and bioinformatics to characterize the host-pathogen interface. Herein, we discuss how rapid advances in molecular immunology, sophisticated tools and molecular databases are facilitating in-depth exploration of the immunome. In our opinion, an immunomics-based approach presides over traditional antigen and vaccine discovery methods that have proved ineffective for highly complex pathogens such as the causative agents of malaria, tuberculosis and schistosomiasis that have evolved genetic and immunological host-parasite adaptations over time. By using an integrative multidisciplinary approach, immunomics offers enormous potential to advance 21st century antigen discovery and rational vaccine design against complex pathogens such as the *Plasmodium* parasite.

Key words: Immunomics, immunome, antigen discovery, rational vaccine design, vaccine development, parasites, malaria, complex pathogens.

INTRODUCTION

The shift from an empirical to a rational method for vaccine development associated with an enhanced understanding of host–pathogen interactions is synergistic with advances in genomics and bioinformatics. Since the first complete sequencing of a DNA genome over 20 years ago, that of the phage Φ-X174, with only 5368 base pairs (Sanger *et al*. 1977), the number of sequenced genomes, proteomes and transcriptomes of different pathogens has risen exponentially. There are now over 6500 complete genomes published from over 59 000 organisms, representing bacteria, viruses, parasites and eukaryotes, as available on the GOLD database (https://gold.jgi-psf.org/index). This includes the genomes, proteomes and/or transcriptomes of human, nonhuman primate, and rodent species of *Plasmodium*. This wealth of information is a direct consequence of technological advances focused at the molecular level and allows for multidisciplinary approaches to identify target antigens for the development of vaccines, drugs and diagnostic tests, and for the development and application of methods to identify immune correlates of protection (Doolan *et al*. 2014). Complementary methods, such as next-generation sequencing of lymphocyte epertoires (Mehr, 2014), complete T- and B-cell phenotype analysis (Zarnitsyna *et al*. 2013), highly-sensitive gene expression measurement using Fluidigm (Spurgeon *et al*. 2008) or Nanostring (Geiss *et al*. 2008), high-throughput profiling technologies using CyTOF mass cytometer (Kidd *et al*. 2014; Hansmann *et al*. 2015), and biophotonic imaging for visualizing the infectious disease process (Andreu *et al*. 2011), among other advances, have the potential to enhance understanding of the interaction between host and pathogens at the molecular level. The availability and refinement of large-scale bioinformatic databases containing information on both host and pathogen can further advance the acquisition, analysis and application of research data to yield more clinically relevant outcomes, ideally leading to the development of vaccines that provide sterile life-long protective immunity without the need for boosting, or sensitive and specific biomarkers of pathogens exposure or protective immunity. Such applications are highly desirable in the malaria community.

The term 'immunomics' was coined in 2001 by Klysik (Klysik, 2001), who suggested that ongoing advances in technology should serve to address the correlations between genes and the functional properties of their protein products. Today, the term immunomics refers to an integration of molecular immunology, genomics, proteomics, transcriptomics and bioinformatics, effectively providing a much-needed link between these fields (Doolan, 2011) and enabling an effective correlation between immunology research and clinical application. Immunomics is the study of the immunome, which can be defined as the set of antigens or

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epitopes that interface with the host immune system (Sette *et al*. 2005). However, it is necessary to consider that the definition of immunomics might be subject to different interpretations, as usually happens with the fast-changing fields of study like the ones comprised in the general domain of –omics, and is likely to expand and embrace concepts that might still be under investigation. Thus, the evolution of methods and techniques in the fields that are currently under the concept of immunomics will likely influence the definition and context of this term. In this paper, we are using the Sette *et al*. definition for immunomics.

Immunomics is distinct from reverse vaccinology, systems immunology and vaccinomics. Reverse vaccinology aims to identify the complete repertoire of antigens that an organism is capable of secreting or expressing on its surface (Rinaudo *et al*. 2009). Systems immunology is a sub-discipline of systems biology (Schubert, 2011), and deals with molecular mechanisms of how the components of the immune system work together as a whole (Narang *et al*. 2012). Vaccinomics, on the other hand, integrates immunogenetics and immunogenomics with systems biology and immune responses (Poland *et al*. 2011), Aimed at creating vaccines that are personalized/individualized.

Immunomics is as dependent on the host as it is on the pathogen, since the immune system and the infecting pathogens have been co-evolving for thousands of years. Furthermore individual characteristics of the parasite (e.g. species, strain, virulence, etc) interact with individual characteristics of the host immune system (e.g. age, immune status, epigenetic traits) (Tournier and Quesnel-Hellmann, 2006; Stilling *et al*. 2014). Each of the components of the immune response is extremely complex on its own, and the interactions between them create an even more complex network of reactions. This complexity creates a challenge for in-depth, comprehensive analyses and increases the cost of experimental verification. An immunomics-based approach offers a solution to this challenge since high-throughput screening is performed (at least in part) *in silico*, prior to *in vitro* and *in vivo* experimental verification. High-throughput screening is used intensely (and successfully) for lead and drug discovery (Balls *et al*. 2012; Annang *et al*. 2015). Translation of this technique to immunomics in order to address the challenge created by the complexity of the immune response to a pathogen includes the use of powerful computational analysis of next-generation, high-density peptide microarrays for rapid discovery and mapping of antigenic determinants (Hecker *et al*. 2012; Carmona *et al*. 2015). This allows for a more thorough, unbiased and rational approach. In this fashion, data-driven studies of the immunome facilitate identification and characterization of key antigens and epitopes.

TRADITIONAL VACCINOLOGY

Pathogens with complex life cycles, complex genomes, proteomes and transcriptomes, and correspondingly complex immunomes, represent a great challenge for the development of effective vaccines. The *Plasmodium* parasite which causes malaria exemplifies this challenge. Such pathogens express a broad repertoire of antigens and epitopes that could be available for recognition by the host immune system. In some cases, effective immune responses directed against only a subset of these antigens and epitopes are sufficient for competent protection. This is demonstrated by the effectiveness of subunit vaccines such as the recombinant HBsAg antigen based vaccine for hepatitis B (Arnon, 2011; Schetgen, 2014). However, for many pathogens, subunit vaccines based on only one or a few antigens have proved poorly effective (Meeusen *et al*. 2007; Foged *et al*. 2014). For many vaccines that are currently in use worldwide, for example the highly effective whole-organism based Bacillus Calmette-Guérin (BCG) vaccine, the mechanism of protective immunity remains unknown (Kaufmann *et al*. 2014). This gap in knowledge highlights the difficulties in assessing clear interactions between host and pathogens. Due to the poor efficacy of most subunit vaccines, until very recently almost all licensed vaccines were based on the whole organism, typically either live-attenuated pathogens or inactivated/killed pathogens (Grimm and Ackerman, 2013). In the case of malaria where extensive efforts directed towards subunit vaccines have thus far failed (Schwartz *et al*. 2012), a resurgence of effort towards development of a whole-organism vaccine has occurred (Hoffman *et al*. 2010; McCarthy and Good, 2010; Good, 2011; Mikolajczak *et al*. 2014). A genetically attenuated *Trypanosoma cruzi* parasite is also being considered for vaccination against Chagas disease (Sanchez-Valdez *et al*. 2015). However, these whole-organism vaccines are associated with number of problems. In some cases, they have been associated with reversion to virulence, causing a proportion of vacinees to develop some form of the disease they had been vaccinated against (Bonanni and Santos, 2011). Furthermore, whole-organism vaccines are almost exclusively restricted to pathogens that can be cultured *in vitro* and have relatively low antigen variability, which can be difficult for pathogens like *Plasmodium* (malaria), *Mycobacterium tuberculosis* (tuberculosis) or *Schistosoma* (schistosomiasis) (Doolan *et al*. 2014).

Reverse vaccinology for rational vaccine design

An alternative strategy to culture-based *in vitro* approaches for the development of an effective vaccine against complex pathogens is that of reverse vaccinology, pioneered by Rappuoli in 2001 (Rappuoli, 2001). This approach analyses the entire

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genome of a pathogen to rapidly identify putative protective antigens and predict potential vaccine candidates (Rinaudo *et al*. 2009; Heinson *et al*. 2015). Reverse vaccinology studies were among the first to harness the wealth of information generated by genome sequencing for vaccine development. The proof of concept for this approach was established by the screening of surface-exposed proteins in the *Neisseria meningitides* genome, a causative agent of meningococcal disease, and resulted in a phase III vaccine trial after 40 years of setbacks using conventional methods (Pizza *et al*. 2000; Serruto *et al*. 2012). Briefly, the sequence of the *N. meningitides* virulent strain MC58 was analysed using bioinformatics algorithms for surface-exposed proteins, which were then recombinantly expressed in *E. coli*, purified and tested in mice for their potential to induce bactericidal antibodies. Humoral response was analysed by Western blot and surface localization of the target protein was confirmed by enzyme-linked immunosorbent assay (ELISA) and flow cytometry. Of the 91 proteins found to be positive in the bioinformatic screening, 28 were able to induce antibodies with bactericidal activity and were prioritized based on their ability to induce broad protection. Ultimately five proteins were combined in a multicomponent vaccine named 4CMenB (Pizza *et al*. 2000; Serruto *et al*. 2012). Very recently, two multicomponent vaccine formulations based on sequencing of the whole meningococcal genome to identify surface antigens of the meningococcal strains, called Bexsero® (Novartis) and Trumemba™ (Pfizer), were made available for clinical immunization against invasive group B meningococcal disease, although still subject to additional monitoring; however, unfavourable cost-effectiveness ratios for application of these vaccines have been reported (Christensen *et al*. 2013; Pouwels *et al*. 2013; Leca *et al*. 2015; Tirani *et al*. 2015).

Reverse vaccinology has limitations; in particular, it cannot predict polysaccharides, lipids or glycolipids which may be active compounds for a vaccine (Kanampalliwar *et al*. 2013; Bertholet *et al*. 2014). It is also unlikely that a reverse vaccinology approach by itself to be able to predict a good correlate of protective immunity (Bertholet *et al*. 2014).

IMMUNOMICS-BASED VACCINOLOGY

In a field of study as complex as vaccinology, where the intricacy of the human immune system is evident from the cohorts of non-homogenous groups where disease phenotype and molecular profile (among other elements) have immense variation (Falus, 2008), elegant approaches are required to decipher the host immune response to pathogens. Immunomics facilitates such a rational, systematic and comprehensive approach to antigen selection and prioritization for vaccine development. The wealth of information that immunomics draws on, namely large-scale genomic, proteomic and transcriptomic datasets, can be accessed via large public-access databanks such as GenBank (http://www.ncbi.nlm.nih.gov/genbank) or UniProt (http://www.uniprot.org/); pathogen-specific databases such as PlasmoDB (www.PlasmoDB.org) or TriTrypDB (http://tritrypdb.org/tritrypdb); or immunology based databases such as the Immune Epitope Database (http://www.iedb.org/) or the Innate Immune Database (http://www.innatedb.com). The available information in these databases is used as primary input for epitope prediction and therefore it is crucial that sequences are verified, annotated and curated. Moreover, immunomics also faces the challenges associated with computational predictive algorithms, which are subject to a series of conditional instructions for weightings and outputs dependent on 'learned' behaviour or characteristics. If those conditional instructions are flawed, the error is magnified exponentially, resulting in inaccurate data.

However, the multifactorial nature of protective immunity (Pulendran and Ahmed, 2006), the large number of antigenic determinants or epitopes that can be recognized by the cells of the immune system as immunodominant or subdominant epitopes (Sette and Sundaram, 2006), the broad range of putative epitopes restricted by multiple human leukocyte antigen (HLA) alleles prevalent in the human population (del Guercio *et al*. 1995) combined with the high variation in the frequencies of different major histocompatibility complexes (MHC) alleles in different ethnicities (Sidney *et al*. 2010), precludes analysing such large and complex datasets without the use of computation. The need for accurate prediction of biologically relevant epitopes for rational vaccine design is therefore crucial.

Computational predictive methods

The prediction of peptide epitopes from primary protein sequences is not a modern achievement. In fact, reports from as early as 1987 show that peptide epitopes could be predicted from protein sequence by consideration of hydrophobicity and amphipathic helices, and these sequences could be synthesized to enable further study (Cease *et al*. 1987; Gotch *et al*. 1987; Margalit *et al*. 1987). Subsequently, it was established that T cell epitopes are generally linear and continuous. MHC class I molecules typically bind peptides that are 8–15 amino acids long (Rammensee *et al*. 1999), while MHC class II molecules usually bind longer peptides of 12–25 amino acids in length (Jardetzky *et al*. 1996). These features provided the foundation for improved prediction algorithms that considered the affinity of binding of a specific peptide sequence to a given MHC molecule. Using experimental

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affinity data deposited in public databases as training data, researchers developed statistical methods to take the early prediction algorithms to a new level. Widely used algorithms include those in the Immune Epitope DataBase (Kim *et al*. 2012; Vita *et al*. 2015) such as average relative binding (ARB) (Bui *et al*. 2005), or alternate algorithms such as support vector machine for prediction of MHC-binding peptides (SVMHC) (Donnes and Kohlbacher, 2006) or NetMHCII-2·2 (Nielsen and Lund, 2009). More recent prediction algorithms consider additional features, such as proteosomal cleavage sites and transporter associated with antigen processing (TAP)-binding patterns, further enhancing accuracy (Tenzer *et al*. 2005; Antonets and Bazhan, 2013). Current computational models consider quantitative matrices, artificial neural networks, hidden Markov models, support vector machines (SVMs), quantitative structure activity relationship and molecular docking simulations (Brusic *et al*. 2004; Desai and Kulkarni-Kale, 2014). These improvements are crucial given the high degree of MHC polymorphism and complexity of generation and presentation of T cell epitopes (Desai and Kulkarni-Kale, 2014). Overall, *in silico* epitope predictions represent a more targeted, cost- and time-effective strategy as compared with more traditional approaches such as screening pools of overlapping peptide pools by enzyme-linked immunospot (ELIspot) or intracellular cytokine staining (ICS), or other epitope identification methods like X-ray crystallography and nuclear magnetic ressonance (NMR) techniques (Sun *et al*. 2013).

Physicochemical properties associated with the T cell receptor also critically influence effective cell mediated immune responses (Osuna *et al*. 2014, Madura *et al*. 2015). Very recent studies have shown that the T cell receptor (TCR) undergoes conformational changes upon engagement with a peptide, allowing for discrimination between peptides. This conformational change causes the peptide to diverge its amino terminus partly away from the MHC peptide binding groove, forming a higher affinity interface with the TCR than is formed with the MHC groove (Dyson, 2015; Madura *et al*. 2015). This discovery suggests that future epitope predictions strategies that consider details such as antigen TCR-MHC affinity may result in improved accuracy of epitope predictions and consequently reduced time for validation studies.

With regard to B-cell epitope predictions, most of the computational methods and databases currently available focus on continuous or linear B cell epitopes (Ansari and Raghava, 2010). However, many B-cell epitopes are conformational and discontinuous (Braga-Neto and Marques, 2006; Ansari and Raghava, 2010), corresponding to the tridimensional features on the surface of the antigen where recognition by the immune system occurs (Braga-Neto and Marques, 2006). This creates difficulties for the bioinformatic prediction of B-cell epitopes. Ideally, predictive algorithms would use tridimensional surface models of the protein antigens and measure surface energy interactions of variable regions of the immunoglobulins that correlate with B-cell activation. However, due to the computational complexity associated with analysing tridimensional interactions and the limited number of known antibody-antigen complex structures, only a limited number of prediction methods exist for discontinuous epitopes [reviewed in (Yao *et al*. 2013)]; most are considered to perform poorly (Sun *et al*. 2013). It has been suggested that combining multiple classifiers for B-cell epitope definition, as an ensemble, could improve the performance of computational B-cell epitope prediction tools (El-Manzalawy and Honavar 2014), but further multidisciplinary understanding of conformational epitopes may also contribute to the improving performance of these predictive algorithms.

Enabling technologies

Immunomics-based approaches can enhance our understanding of key features of the immune system in health and disease. Classical methods of assessing immune responses have typically focused only on the frequency and magnitude of a single immune parameter, e.g. antibody titre. Immunomics, on the other hand, allows for a multifactorial view of the response and considers the relevant biological outcome, e.g. protective immunity, by effectively taking into account pertinent and appropriate elements, such as particular epitope combinations, the cytokine response measured, and the T cell population used as the target. Multiple experimental methods, with different strengths, must also be used, and consequent results integrated. For example, using an immunomics approach to a disease model, Quintana and his team have shown that immunomics can be used to predict future disease (Quintana *et al*. 2004). Moreover, it has been computationally shown how the immune response to an epitope modulates the behaviour of an immune network, providing evidence for immunomic regulatory networks from immunomic microarray data (Braga-Neto and Marques, 2006).

The quality of the immune response is at least as important as the quantity, as quality can be a key determinant of protection (Zepp, 2010; Doolan *et al*. 2014). Some of the more conventional methods for assessment of immune responses as a measure of T- or B-cell reactivity include immunoassays such as the Jerne plaque assay (Jerne and Nordin 1963), the splenic focus assay (Klinman and Aschinazi 1971), ELISA (Engvall and Perlmann 1971), interferon-γ (IFN-γ) ELIspot (Czerkinsky *et al*. 1983),

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fluorescence-activated cell sorting (Hayakawa *et al*. 1987; McHeyzer-Williams *et al*. 1993), peptide-induced ICS (Ozen *et al*. 1998), and tetramer staining (Altman *et al*. 1996; Skinner *et al*. 2000). More recently, technological and conceptual advances have resulted in the development and application of novel methods to comprehensively assess immune responses, including approaches to simultaneously examine a large number of cell functions and phenotypic markers, including at the single cell level. Such methods include, but are not limited to, the analysis of gene expression using Nanostring (Geiss *et al*. 2008) or Fluidigm (Spurgeon *et al*. 2008), as well as mass spectrometry based methods such as CyTOF technologies (Cheung and Utz, 2011). The quality of those responses can be assessed via phenotypic markers, differentiation state, profile of secreted cytokines, avidity, affinity and repertoire diversity (Siegrist, 2013). If used singularly, none of the methods cited above can describe the complete set of characteristics that define an antigen-specific response. Immunomics can address such problems by integrating different fields of study, thus providing a platform for combining the strengths and compensating the flaws of different methods and approaches to vaccine development.

Particularly transformative are multidisciplinary computational and mathematical methods developed to cope with multiplex data, including host–pathogen interactions (Raman *et al*. 2010), for which experimental analysis may be costly and laborious if the pathogen is complex. These mathematical and bioinformatic platforms may also support other aspects of vaccinology such as adjuvant discovery (Schellhammer and Rarey, 2004; Sollner *et al*. 2010).

Immunomics for rational vaccine design

The use of an immunomics based approach to vaccinology is a promising alternative for efficacious vaccine design. Unlike reverse vaccinology, immunomics also considers the immune system. It provides a means to systematically identify the antigens and epitopes that interact with the host immune system (Sette *et al*. 2005; De Groot, 2006). The premise of an immunomics-based, rational vaccine design is a consistent induction of the desired immune response against the key pathogen antigen(s) or epitopes which are targeted by protective immune responses (Barbosa and Barral-Netto 2013; Doolan *et al*. 2014; Slifka and Amanna, 2014).

Immunomics of viruses. A number of immunomics approaches have been applied to viruses. In the case of HIV, an effective vaccine might require the design or discovery of immunogens which elicit good neutralizing antibodies against circulating strains of the virus (Kwong *et al*. 2011). For many years, only a few neutralizing monoclonal antibodies against the virus were known; the most intensely studied have been the antibodies directed against the glycoproteins gp120 and gp41 (Burton *et al*. 2012). With the advent of immunomics, more neutralizing monoclonal antibodies, some with unexpected epitopes, have been identified (see Burton *et al*. 2012). Most recently, Gallerano *et al*. (2015) have analysed polyclonal antibody responses of HIV-infected persons to overlapping peptides covering the complete amino acid sequences of the gp120 and gp41 proteins, and identified major epitopes that can be recognized by antibodies.

Another immunomics study has prospected the influenza A (H1N1) virus immunome and transcriptome to show a complex host response pathway to the virus, and unravelled interactions between virus and host (Dimitrakopoulou *et al*. 2014). A different study has used immunomics to better understand the repertoire of T cell specificities for H1N1, with the objective of developing an universal vaccine for influenza virus to combat the continuous antigenic drift of the virus (Assarsson *et al*. 2008). This study used over 4000 peptides from a panel of 23 influenza A virus strains based on predicted high-affinity binding to HLA class I or class II and high conservancy levels. Peripheral blood mononuclear cells (PBMCs) from healthy human donors were tested for reactivity against HLA-matched peptides by using IFN-γ ELIspot. One epitope, called PB1, was found to be the major target for both $CD4^+$ and $CD8^+$ T cell responses; 54 other non-redundant epitopes (38 class I and 16 class II) were also identified, and provide a potential base for the development of a universal influenza vaccine (Assarsson *et al*. 2008).

Immunomics of bacteria. Immunomics has been also successfully applied to bacteria. In one example, a *Francisella tularensis* protein microarray was generated and probed with serum from experimentally immunized mice to identify 11 of the 12 antigens previously discovered using traditional methods plus an additional 31 new antigens (Eyles *et al*. 2007); this study further demonstrated an IgG subclass bias towards IgG2a in protected animals.

T cell based approaches have also been pursued to advance bacterial vaccine development. Moise *et al*. (Moise *et al*. 2012) evaluated the *Helicobacter pylori* genome for $CD4^+$ T cell epitopes by using the predictor algorithm EpiMatrix; resulting epitopes were experimentally validated for MHC binding and T cell reactivity in p27 knockout mice infected with the mouse-adapted *H. pylori* strain. The immunoreactive epitopes were assembled into a multi-epitope vaccine that induced a broad immune response as determined by IFN-γ production in ELIspot assays (Moise *et al*. 2012).

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For *Mycobacterium tuberculosis*, $CD4^+$ T cells are crucial for controlling the infection (Woodworth *et al*. 2014). Recently, a genome-wide screening for $CD4^+$ T cell reactivity against *M. tuberculosis* (Arlehamn *et al*. 2013) identified a number of novel epitopes and antigens that may represent a potential vaccine candidates. This immunomics-based study screened the sequences from five complete *M. tuberculosis* genomes available from the National Center for Biotechnology Information (NCBI) database and protein sequences were parsed into 15-mer peptides, which were then ranked by consensus percentile of HLA binding for the 22 alleles most commonly present in the general population. For each protein, no less than two of the best-predicted binders were selected for synthesis, creating a synthetic library of over twenty thousand peptides that were tested by IFN-γ ELIspot against T cells from latent TB-infected donors. The reactive epitopes were ranked on the basis of magnitude of response and mapped to individual bacterial antigens using a reference genome. The results revealed a very heterogeneous response to infection: 82 antigens were recognized by more than 10% of donors. Hundreds of novel epitopes recognized by the human immune system have been identified, attesting to the potential of genome-wide screening strategy (Arlehamn *et al*. 2013; Arlehamn and Sette, 2014).

Immunomics of parasites. We and others have had a particular interest in the immunomics of *Plasmodium* spp. (Doolan, 2011). In early studies which served as proof of concept for the protein microarray platform, 250 genes representing putative proteins from *P. falciparum* were selected from a genomic sequence database according to the pattern of stage-specific gene or protein expression, subcellular localization, secondary structure, and known immunogenicity or antigenicity in human and animal models (Doolan *et al*. 2008). Each of these 250 sequences was then printed onto a protein microarray which was probed with human sera from individuals differing in immune status. This study showed that the protein microarray platform could be successfully applied to identify antigens recognised as serodominant by individuals naturally or experimentally exposed to malaria. A more comprehensive array of 2320 protein fragments representing 23% of the *P. falciparum* proteome was then fabricated; the protein selection considered stage-specific transcription or protein expression, subcellular localization, secondary protein structure, and documented immunogenicity in humans or animal models at the time of antigen selection, as indicated by multidimensional protein identification technology. Subsequent studies with this array identified a signature of 16 proteins that were associated with the sterile immunity induced by experimental immunization with radiation attenuated sporozoites (Trieu *et al*. 2011), and a signature of 49 antigens associated with the anti-disease immunity induced by natural exposure to malaria (Crompton *et al*. 2010). Those data provide experimental support for a multivalent vaccine. These and other studies identified a number of antigens that had not been previously described as immunologically reactive (reviewed in (Davies *et al*. 2015)). Our group has also shown that immunomics-based approaches can be applied to the study of cellular responses against *Plasmodium*. In the proof of concept demonstration of an epitope-based T cell screening approach in the *P. falciparum* model, the parasite genomic sequence was scanned to identify and prioritize a set of genes representing antigens potentially expressed in the sporozoite and intrahepatic stage of the parasite life cycle. A total of 27 proteins putatively expressed in the sporozoite proteome were selected according to their level of expression in the sporozoite proteome as determined by MudPIT, as well as stage specificity. This panel included 10 antigens expressed only in sporozoites, and 17 antigens common to other stages of the parasite life cycle. Evaluation of these proteins has shown that 16 of them were reproducibly recognised by peripheral blood mononuclear cell (PBMC)s from irradiated sporozoite immunized volunteers but not by naive controls, and nine of these antigens were more antigenic than other well-characterized antigens considered as leading vaccine candidates (Doolan *et al*. 2003). Subsequently, we have applied this strategy to the complete pre-erythrocytic stage proteome and shown that only approximately 30% of the proteome is recognized, and identified the set of antigens that are highly reactive for T cell responses (Proietti & Doolan, in preparation).

With regard to immunomics for other parasites, *Schistosoma* proteome and transcriptome have been mined to identify surface-derived proteins, a subset of which were then expressed and printed on a protein microarray (Driguez *et al*. 2010; Loukas *et al*. 2011). These arrays have been probed with specimens from humans representing distinct clinical categories as well as experimentally immunized rodents. Several proteins which are predicted to be good potential vaccine targets have been identified (Loukas *et al*. 2011; McWilliam *et al*. 2012). These include the tetraspanin SmTSP-2, the tegumental antigen Sm29, and the very low-density lipoprotein-binding protein SjSVLBP as well as other novel proteins (reviewed in (McWilliam *et al*. 2012). More recently, these protein microarrays have been screened for IgE and multiple IgG subclasses responses using sera from resistant or susceptible individuals (Gaze *et al*. 2014). The resultant antibody profiles could distinguish between protected *vs* non-protected cohorts and allowed identification of antigens that might represent excellent vaccine candidates (Gaze *et al*. 2014). The same

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study also identified protein associated with potentially deleterious hypersensitivity responses if used as subunit vaccines in endemic populations.

CONCLUSION

Infectious diseases continue to pose a major threat to public health worldwide and the need for prophylactic or therapeutic vaccines is urgent. Many of the diseases with high indexes of mortality or morbidity are caused by pathogens with large complex genomes and multistage life cycles, which present substantial challenges for the development of an effective vaccine. Immunomics, by focusing on the key components of host–pathogen interactions, provides a sound foundation to systematically search for critical determinants of immunity, namely key target antigens and epitopes, which could form the base of rationally designed new generation vaccines. Provided that immunomics continues to exploit state-of-the-art techniques and technologies and is able to respond to the inherent challenges associated with large datasets, this approach offers, in our opinion, an enormous potential as a 21st century solution to the challenge of rationally designing vaccines which are highly effective against complex pathogens such as the causative agent of malaria.

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