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Computational Nanomedcine, Immunoinformatics and the Discovery of Vaccines

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For infectious disease at least, vaccination and sanitation are seemingly without doubt the most cost-effective and the most efficient prophylactic treatments currently available. If we think of the years directly prior the First World War, human mortality was primarily causedby influenza, pneumonia, enteritis, anddiarrhoea. At the same time, the great killers of today, cancer and heart disease, killed no more than one in ten. Think back another two hundred years - to the last days of the seventeenth century - and average life expectancy seldom exceeded four decades. Death was again mainly caused by contagious disease: tuberculosis, smallpox, yellow fever, malaria, and dysentery, affecting infants, children, and adult all alike. In the early years of the 21st century, things are radically different. Infectious disease is responsible for less than 2% of deaths, while chronic disease now causes over 60% of deaths. Disease, particularly infectious disease, has largely been beaten. Many factors have conspired to effect this – improved water quality, better precautionary hygiene, improved nutrition, decreased overcrowding – as well as many interventionary measures, principally antibiotic therapy and vaccines.

The first hundred years of the vaccine story was solely concerned with smallpox. As recently as the late 1960s, some 10-12 million cases of smallpox were recorded in 31 countries, with annual deaths of 2 million. Yet today smallpox has, with the exception of a few well-guarded stockpiles, been completely eradicated: there have been no new cases for 30 years. The story of smallpox is thus the high point of the vaccination story; no other disease has been eradicated. Polio or Poliomyelitis is the next nearest to full eradication, having long been targeted by a systematic, coordinated, worldwide eradication campaign. Today we can count cases worldwide in the tens or hundreds instead of the hundreds of thousands or millions.

Yet, vaccine-preventable disease still kills millions. Infectious and contagious disease cause approximately 25% of world mortality, particularly in children under five. In the While in developed countries, mortality for diseases such as diphtheria, polio, or measles is less than 0.1%, in other parts of the world deaths from such infectious diseases is significant. Pertussis, tetanus, Influenza, Hib, Hepatitis B are all responsible for deaths that number in the hundreds of thousands. Perhaps the most execrable situation is Measles, which accounts for 70000 (over 5) and 540000 (under 5). However, the leading global causes of death worldwide remain tuberculosis, diarrhoeal illnesses, especially rotaviruses; HIV/AIDS; and malaria. In 2010, 8.8 million contracted TB and 1.4 million died. Disturbing though these numbers may seem, they nonetheless represent a significant reversal or a once ever-escalating trend when compared with the recent past. The number with latent TB peaked in 2005 at 9 million, while deaths from TB reached their peak at 1.8 million in 2003.

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

Many viral infections remain recalcitrant threats of the first order. 350 million people are infected with hepatitis B, 170 million by Hepatitis C, and 40 million by human immunodeficiency virus type 1 (HIV-1). This dire situation is further compounded by the threat from the thirty-five new or previously unknown infectious diseases identified in the past thirty years: HIV, West Nile fever, Ebola, Dengue fever, SARS, and potentially pandemic H5N1 influenza. Every year, between 5 and 15% of the global population becomes infected with a new influenza strain, causing upwards of half-a-million deaths.

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

Vaccination also works to greatly reduce the morbidity of disease, often imbuing lifetime protection; this is particularly important for benign yet economically important infections, particularly the so-called common cold. Diverse sporadic or epidemic infections of the human respiratory track - as caused by in excess of 200 distinct viruses, such as RSV or, more properly, respiratory syncytial virus, coronaviruses, influenza A and B, rhinoviruses, para-influenza virus, and cytomegalovirus - remain a principal cause of hospitalisation, community morbidity with an estimated 60% of GP referrals associated with such infections, and this causes an enormous numbers of working days to be lost in developed countries.

No unbiased, right-thinking individual, looking back across the last 200 years, would wish to argue seriously with the contention that the design and development of vaccines is an innately labour intensive process. The processes deployed to meet the objective of creating new and better vaccines are in desperate need of change. This change must

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be radical if we hope to simplify such processes. Simple processes are hopefully also fast and efficient processes. Part of the answer is computational, embodied primarily in computational, as opposed to thereotical immunology, also known as immunoinformatics.

Central to Immunoinformatics and computational immunology is the capacity to make accurate predictions. Yet, obtaining routes to prediction that are accurate, robust, and dependable continually eludes us. Immunoinformatics deals with empirical, data-dependent methods. The success and utility of such approaches methods depends very much on the data used to propagate and parameterise them; they cannot escape the severe limitations imposed by the data used to create them. The data from which we build models forms a complex phase space of structural and property variation, which can be extremely multi-dimensional, with a high degree of inter-dimensional correlation. When the data we work with is reliable and our knowledge of it is complete, then we can create useful models by applying standard methods from computer science to build accurate and predictive models relating observed biological activity to underlying measurable or predictable properties. Usually, such approaches are also much superior when used to interpolate than they are when used to extrapolate. We need complete and thorough datasets effectively and efficiently able to explore the complex relationships between structure and function, necessitating continuous improvement in all aspects of data quality.

So let us look at three different areas of prediction: the identification of protective antigens as candidate subunit vaccines, the discovery of small molecule adjuvants, and the use of immunoinformatic tools to design epitope ensembles as candidate vaccines.

Adjuvants are defined as any chemical which is able to enhance an immune response when applied simultaneously with a vaccine and thus improve the efficacy of vaccination. It is possible that some adjuvants act as immune potentiators, triggering an early innate immune response that enhances the vaccine effectiveness by increasing the vaccine uptake. Adjuvants may also enhance vaccination by improving the depot effect, the co-localisation of the antigen and immune potentiators by delaying the spread of the antigen from the site of infection so that absorption occurs over a prolonged period. Aluminium hydroxide or Alum is the only adjuvant currently licensed in humans. Aluminium-based adjuvants prolong antigen persistence due to the depot effect, as well as stimulating the production of IgG1 and IgE antibodies and triggering the secretion of interleukin-4. There are also several small-molecules, drug-like adjuvants, particularly imiquimod, resiquimod, and other imidazoquinolines. Other small molecules that have been investigated for adjuvant properties include Monophosphoryl-Lipid A, muramyl dipeptide, QS21, PLG and Seppic ISA-51. In many cases, the adjuvant molecules have displayed toxic properties or showed poor adsorption making them unsuitable for use: thus, the enormous demand for new adjuvants.

Techniques used routinely by the pharmaceutical industry can also be used to discover small molecule adjuvants. Three-dimensional or structure-based virtual screening (SB-VS), which utilises automated protein docking (APD), is an effective means of identifying ligands with high celerity:it docks enormous numbers of ligand molecules into a defined binding site. In this way, large small-molecule databases are screened rapidly and accurately; particularly if we target compound collections containing subsets enriched through pre-selection for a defined target or targets. SB-VS can be exceptionally logistically efficient, saving time, labour, and resource. Months, years, or decades of robotically-mediated experimentation, bedevilled as it is by signal-to-noise issues, are replaced by weeks of computational analysis, albeit complemented by a mere handful of reliable, hand-crafted assays. By using SB-VS, tens or hundreds of thousands of molecules can be reduced in number by several orders of magnitude. Thevery smallset left can then be docked against the target structure. This yields at most a few hundred molecules that can be readily tested. This handful is put through a hierarchical cascade of highly specific and informative assays in vitro, with actives then tested for their whole system adjuvant properties in vivo. Many examples of VS have shown even the simplest, most parsimonious, most unsophisticated of approaches may prove successful: this is a key strength of the VS technology. In this way, lead adjuvant molecules are found with unmatched cost-effectiveness and efficiency; all without requiring highly expensive and often misleading HTS.

In the context of adjuvant discovery, we exemplify such assertions by echoing our recent application of VS to the discovery of antagonists of the CCR4 Chemokine receptor. These molecules function as effective adjuvants, acting via the regulatory mechanisms of the cellular arm of the adaptive immune system. CCR4 is a chemokine receptor which is expressed by Th2-type CD4+ T cells. It has been linked to allergic inflammation conditions including allergic rhinitis, atopic dermatitis, and asthma. Chemokine receptors are a large and important subfamily, comprising 18 distinct proteins, of the G-protein coupled receptors or GPCRs, which transduces leukocyte chemo-attractant chemokines, which are secreted by cells when activated by inflammatory stimuli. GPCRs are a well-known and well-understood superfamily of transmembrane proteins that transduce a variety of extracellular but endogenous signals into an intracellular response. Activation of chemokine receptors induces an inflammatory response by triggering migration of leukocytes from circulation to the point of injury or infection.

Inhibiting CCR4 receptors may give rise to adjuvantism as the receptor is expressed by regulatory T cells (also-known-as Tregs) that normally suppress immune responses, inhibit maturation of DCs, and down-regulate co-stimulatory molecule expression. Inhibiting CCR4 function, and thus blocking interaction of DC with Tregs at vaccination, is anticipated to exacerbate vaccine responses, and thus an effective CCR4 antagonist should prove to be an effective adjuvant. By combining experimental validation with VS, we have identified several potential adjuvants, acting through the apparent inhibition of Treg proliferation [2]. These molecules behave appropriately in a variety of in vitro assays, and increase the levels of various correlates of protection in vaccinated mice, and even show some enhancement in related challenge models[3]; also showing activity against potential cancer antigens [4].

Obviously, in order to have an effective vaccine, one requires the biologic component that the immune system will be trained to recognise and which it will respond to in times of infection. One way to do this is by using anodyne versions of whole pathogens or through the use of epitope ensembles, which we discuss below. Currently, of course, the principal biological component of vaccines is the single or combined protein antigen. We have developed a new approach to identify antigens, and thus potential vaccine candidates, using more alignment-free sequence representations [5-7]. Our approach utilises data on protective antigens derived from diverse pathogens to create statistical models capable of predicting whole-protein antigenicity [8]. Our alignment-independent method for antigen identification uses the auto cross covariance transformation to transform protein sequences

into uniform vectors. In our method, amino acid residues are represented by z descriptors, which characterise the hydrophobicity, molecular size, and polarity of residues.

We initially applied our approach to groups of known viral, bacterial, and tumour antigens, developing models capable of identifying antigen. Extra models were subsequently added for fungal and parasite antigens. For bacterial, viral and tumour antigens, models had prediction accuracies in the 70% to 89% range [5-7]. For the parasite and fungal antigens, models had good predictive ability with 78% to 97% accuracy. These models were incorporated into a server for protective antigen prediction called VaxiJen [6] (URL: http://www.darrenflower.info/VaxiJen).VaxiJen is an imperfect but encouraging start; in its turn, Vaxijen has spawned imitators, see for example the work by Bowman and co-workers [9], andis, encouragingly, starting to be used by experimentalists to help identify real-world disease targets [10].

For all or nearly all in computational studies addressing real-world problems, there is a pressing need for experimental validation. Certain recent studies combine immunoinformatic-driven vaccine design with experimental validation in various animal models, giving strong credence to their computational results [11,12]. For example, Wieser et al. [11] identified computationally immunodominant epitopes from six virulence-associated E coli antigens: Usp, Iha, FyuA, ChuA, IreA, and IroN; creating two wholly artificial genes were created, each encoding eight extended peptide epitopes, which when expressed in a recombinant fashion, resulting in a vaccine active against pathogenic but not benign E. coli in the gut. The most important featureof this work was that this study, led by experimentalists, was validated experimentally [11]. However, an increasing number of papers describing *in silico* analyses of genomes and proteomes, producing epitope ensembles as putative candidate vaccines, are now being published [13-19]. Most seem to be sound and workmanlike efforts; yet their value cannot easily be quantified. As a consequence, their significance is questionable. Prediction without validation will exert little influence and convince no one. Continuing to publish papers which have not been verified experimentally is almost counterproductive in this context. Why? Because it generates a swathe of papers that many experimentalists will see as unproven and thus insubstantial, which in their turn distract both from experimentally validated computation and from analytical immunoinformatics papers, which will stand I their own right. It will also undermine the standing of the field as a whole, since the technical simplicity of such protocols, implemented via web-based servers, is such that they can be undertaken by almost anyone.

It is important to realize what can be done and what cannot be done, what is useful and what is not. Risk is associated with predictions, but there should not be any significant risk associated with an analysis. To put it rather simply: prediction is about making informed, educated guesses about uncertain, untested events, while analysis is about identifying relationships amongst known, certain data. However, despite the steady increase in studies reporting the real-world use of prediction algorithms, there is still an on-going need for truly convincing validations of the underlying approach.

Clearly, the overall veracity and reliability of any in silicoreverse vaccinology exercise is strongly dependent on the accuracy of prediction, which in turn necessitates a robust definition of which proteins are potential antigens and which are not. Such a definition is not readily forthcoming. Instead, it has been necessary to devise more approximate definitions capturing some, but not all, necessary information to discriminate properly between antigens and non-antigens. There are limits to what computational vaccinology can achieve as well as immense opportunities to exploit its potential.

Prediction, like all forms of forecasting, is prone to error and is seldom fool-proof. The same, however, is also true of all human activities, experimental science included. Predictions made by informatics are seldom perfect, but neither are predictions about the weather or stock market forecasts. What immunoinformatics can offer is tools and methods that form part of a wider experimental and clinical endeavour. It offers a set of techniques replete with utilitarian value which can be leveraged by computational vaccinology to facilitate the design and discovery of vaccines.

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