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Immunomics: discovering new targets for vaccines and therapeutics

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T-cell-epitope mapping has emerged as one of the most powerful new drug discovery tools for a range of biomedical applications. Initially, T-cell-epitope discovery was applied to the development of vaccines for infectious diseases and cancer. T-cell-epitope-mapping applications have now expanded to include reengineering of protein therapeutics (a process now called deimmunization), as well as the fields of autoimmunity, endocrinology, allergy, transplantation and diagnostics. Research employing T-cell-epitope mapping falls within the realm of immunomics, a new field that addresses the interface between host and (pathogen) proteome, bridging informatics, genomics, proteomics, immunology and clinical medicine. This review highlights aspects of recent immunomics research that are related to the discovery of the T-cell immunome.

Immunomics: a brave new science

The marriage of a new technology with almost any field of inquiry is guaranteed to result in exciting new discoveries. For example, the marriage of bioinformatics (computational biology) with molecular biology and the emergence of their progeny, genomics, have radically transformed science in the past two decades. Bioinformatics tools that identify putative genes, aligning and comparing gene sequences and seeking out proteins of particular types, are now considered requisite tools for the 'sea chest' of a savvy scientist.

Genomics has successfully emerged as an area of research, distinct from other fields of scientific inquiry. However, the same cannot yet be said about immunomics – the field of inquiry related to the interface between the host immune system and proteins derived from pathogens or from self. Scientific investigation of that interface usually involves searching for the antigens and mapping the epitopes that stimulate an immune response. In the past, scientists isolated proteins from whole cells and then digested the proteins (antigens) to find smaller fragments, known as epitopes, that stimulated the T-cell and B-cell response.

By contrast, immunomics tools, such as T-cell- and B-cell-epitope-mapping algorithms, are only slowly being integrated into the mainstream of scientific inquiry. Is this because the internal workings of these algorithms are obscure? In fact, most T-cell-epitope-mapping algorithms are based on straightforward mathematical analyses of the patterns of amino acids that occur in peptides bound to (and presented in the context of) human leukocyte antigen (HLA) by antigen-presenting cells (Box 1). Because the epitope peptide is bound in a linear form to HLA, the interface between ligand and T cell can be modeled with breathtaking accuracy. Several T-cell-epitope-mapping algorithms have already been developed, and the T-cell-epitope-mapping approach, outlined in Figure 1, has been successfully integrated into the field of drug discovery in several research laboratories, allowing these groups to accelerate their discovery programs dramatically. However, not all epitope-mapping tools are equivalent. For reviews of T-cell-epitope-mapping tools, see De Groot and Berzofsky [1], the accompanying issue of Methods [2] and the list of independently developed, validated tools in Table 1.

B-cell-epitope-mapping algorithms have lagged behind T-cell-epitope-mapping algorithms. Recent examples of B-cell-epitope-mapping algorithms include 3DEX [3] and CEP [4]. Many such algorithms have been created and used to analyze existing datasets *in silico* [5] but only a handful of these tools have been used in prospective research studies, and validated using *in vitro* and/or *in vivo* methods. Few B-cell-epitope-mapping algorithms are in current use. Consequently, this article will focus on the applications of T-cell-epitope-mapping tools in the discovery of the T-cell immunome.

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BOX 1

Immunomics terminology

Antigen: usually a protein (sometimes a glycolipid or carbohydrate) that causes the immune system to produce antibodies or a T-cell response targeted against it. This is caused by the presence of epitopes that engage T cells or B-cell receptors.
Epitope: the defined group of amino acids derived from a protein antigen that interacts with the B-cell receptor (immunoglobulin) or the T-cell receptor, thereby activating an immune response.
HLA: proteins on the surface of leukocytes involved in the body's response to foreign substances. In humans, these MHC proteins present T-cell epitopes to T cells.
Immunomics: the field of inquiry related to the interface between the host immune system and proteins derived from pathogens or from self.
Immunomics tools: comprising immunoinformatics (*in silico* analysis) and also bench-based immunology techniques, such as HLA binding assays, ELISpot assays and MHC-tetramers.
Immunome: the set of epitopes derived from a proteome (human or pathogen) that are presented to the host immune system in the context of MHC class I and class II molecules or that engage antibodies, engendering a protective immune response.
Immunodominant protein: a protein that has been identified as the dominant antigen in terms of the number of T cells that respond to the antigen or the number of individual responses.
MHC: two classes of molecules on cell surfaces (class I for cytotoxic T lymphocytes and class II for T helper cells) primarily responsible for the graft versus host rejection, also involved in signaling between lymphocytes and antigen-presenting cells.

Perhaps immunomics is to immunology as the spherical shape of the earth was to early cartographers – something that could not be fully conceived at first. Indeed, despite decades of experience with T-cell-epitope-mapping algorithms and the publication of >2000 articles on the topic, many immunologists appear to prefer traditional approaches to mapping T-cell epitopes, believing that epitopes identified using 'hands on' peptide-by-peptide *in vitro* assays are somehow more substantive than epitopes selected by *in silico* methods. And yet, epitope mapping is not, as some would have it, unsubstantiated. Instead, T-cell-epitope-mapping research is uncovering dogma and revealing new truths, as will be illustrated in examples given herein.

Positive confirmation of the predictive accuracy of epitope-mapping algorithms and their utility in drug development has come from a range of disciplines. Initially, T-cell-epitope discovery focused on the development of vaccines for infectious diseases [2]. More recently, the number of epitope-mapping applications expanded to include reengineering of protein therapeutics [6], autoimmunity [7,8], endocrinology [9], allergy [10,11], transplantation [12] and diagnostics [13]. Even more-potent applications await the scientific adventurer.

Beyond the horizon: new applications

T-cell-epitope-mapping algorithms can be applied to a wide range of protein datasets, yielding exciting new discoveries. For example, if scientists were to apply an epitope mapping analysis to the entire proteome of a pathogen, thereby uncovering peptides that stimulate responses in the human host, the resulting data could be used to develop new diagnostic tests (such as the ELISpot assay kit, which differentiates tuberculosis infection from Bacille Calmette–Guerin, BCG, vaccination [14]), new vaccines (based on the antigens that are discovered [15]), and new means of comparing a pathogen under examination with other pathogens (by comparing genes that stimulate immune responses). If it is possible to examine the human immunome (i.e. the set of peptides that interacts with the human immune system) and to compare these peptides with those presented by pathogens or by allergens, then it should also be possible to reveal new truths about autoimmunity [7]. A few of the many scientific questions that could be answered using T-cell-epitope-mapping tools are listed in Box 2 and discussed in more detail in the next sections.

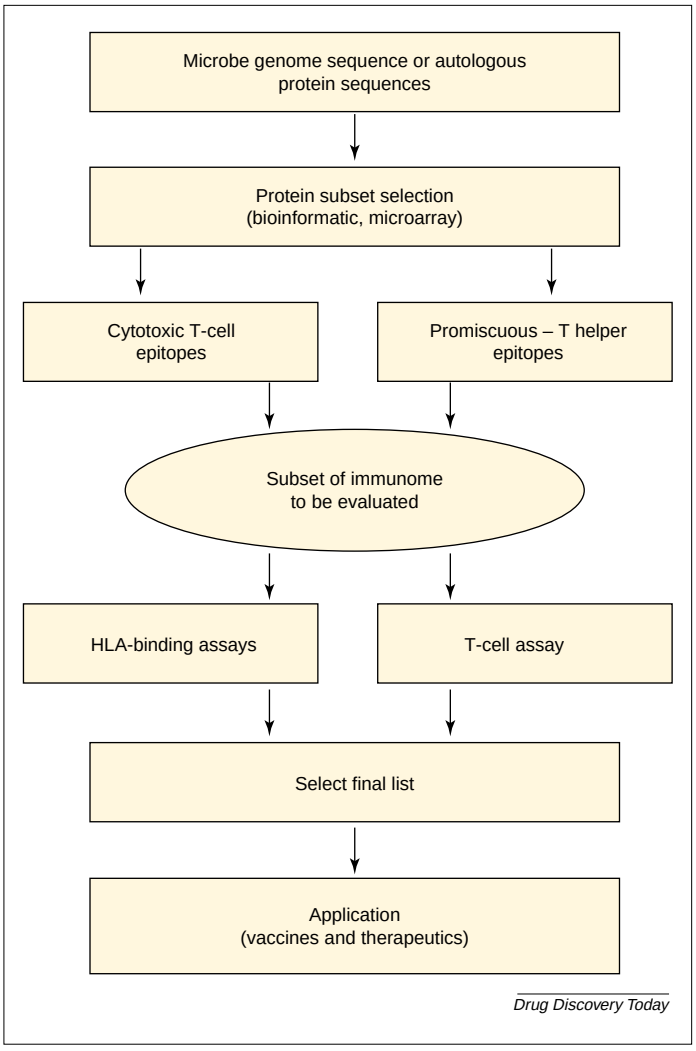

FIGURE 1

T-cell-immunome-discovery flow chart. Bioinformatics tools for selecting protein subsets (by searching for motifs corresponding to secretion signals or transmembrane domains) combined with molecular tools, such as microarrays, allow the selection of a subset of genes from genomic sequences for further *in silico* screening. Epitope-mapping tools allow the selection of the ensemble of epitopes within these proteins that could interact with the host cellular immune system. Confirmation of the immunogenicity of these epitopes can be obtained *in vitro* (using HLA binding assays and/or T-cell assays) or *in vivo*, in HLA transgenic mice.

Measuring the dimensions of the human T-cell response to pathogens

One application of T-cell-epitope mapping might be to measure the dimensions of the human immune response or, more specifically,

TABLE 1[a]

An overview of validated T-cell-epitope-mapping tools[b]

Tool	Chief scientist(s)	Company or research institution	HLA class I available? (number)	HLA class II available? (number)	Type	Website
EpiMatrix®	De Groot, Martin	EpiVax	Yes (24)	Yes (74)	Matrix-based and pocket profile	www.epivax.com
TEPITOPE	Sturniolo, Tiziana Hammer, Juergen	Vaccinome	No (0)	Yes (51)	Pocket profile method (matrix-based)	www.vaccinome.com
SYFPEITHI	Rammensee, Hans-Georg Stevanovic, Stefan	University of Tuebingen, Germany	Yes (6)	Yes (6)	Extended-anchor method	www.syfpeithi.de
Predict	Brusic, Vladimir	Kent Ridge Digital Laboratories	Yes (unknown)	Yes (unknown)	ANN (artificial neural networks)	http://research.i2r.a-star.edu.sg/fimm
MHC Thread	Margalit, Hannah	Hebrew University, Israel	Yes (7)	No (0)	Threading	www.csd.abdn.ac.uk/~gjlk/MHC-Thread
MHC Pred	Flower, Darren	Jenner Institute	Yes (11)	Yes (3)	Partial least-squares-based multivariate statistical method	www.jenner.ac.uk/MHCPred
NetMHC	Buus, Soren Brunak, Soren	Institute of Medical Microbiology and Immunology	Yes (120)	No (0)	ANN	www.cbs.dtu.dk/services/NetMHC
LpPep	Weng, Zhiping	Boston University, USA	Yes (1)	No (0)	Linear programming	http://zlab.bu.edu/zhiping/lppep.html
Bimas	Parker, Kenneth	NIH	Yes (18)	No (0)	Matrix-based	http://bimas.dcrt.nih.gov/molbio/hla_bind

[a]Table adapted and updated from Methods Volume 34 (entire volume devoted to computational tools for vaccine development, including epitope-mapping tools) [2].
[b]Validated T-cell-epitope-mapping tools include those that have been substantiated by *in vitro* or *in vivo* studies.
[c]EpiMatrix® is available for use within the context of collaborative agreements; contact the author of this review for additional information.

the breadth and diversity of human T cells that recognize a pathogen. Consider for a moment the interface between the human host's immune system and the proteome of the tuberculosis pathogen (*Mycobacterium tuberculosis*, MTB). The dynamics of that interface have puzzled researchers for years. Researchers have been disassembling it, protein-by-protein, searching for the genes and proteins that are associated with MTB-infection control, as well as trying to determine the genes and proteins associated with MTB virulence. They have examined the interface between pathogen and host using 2D gel electrophoresis [16] and human sera to screen expressed gene fragments [17]. However, the MTB proteome, the etiologic agent of tuberculosis, contains ~4000 proteins. Evaluating every protein using human serum and gel electrophoresis could take decades even if proteomics tools are added to the mix [18]; because the process of searching for epitopes using these methods is labor- and time-intensive. *In silico* methods can significantly accelerate this process.

Fishing for antigens using epitopes as bait

Several methods for identifying candidate antigens for vaccines directly from pathogen-genome sequences have been developed. Rappuoli and colleagues [19] identified novel targets for meningitis-vaccine development using an approach they termed reverse vaccinology. They expressed *Neisseria meningitis* proteins in *Escherichia coli* and then used human sera to screen for proteins that might be relevant to the immune response. The reverse vaccinology method permits rapid and comprehensive assessment of any pathogen's surface-protein repertoire; novel protein antigens identified in this manner are subsequently tested in animal models for their potential to serve as vaccine candidates.

A related approach, termed the whole genome approach, employs T-cell-epitope-mapping tools for finding new protein candidates for vaccines and for diagnostic tests. This approach involves analyzing the pathogen's entire proteome *in silico*, using T-cell-epitope-mapping tools and further *in vitro* evaluation of the resulting sets of peptides. The peptides identified can then be used to test whether pathogen-infected human subjects have T cells that respond to the peptides *in vitro*. Such a response implies that the protein, from which the peptide was derived, was expressed during infection, processed and presented to the immune system in the course of a 'natural' immune response. Using this method, measuring the immune response to an epitope reveals an antigen. The concept has been described as fishing for antigens using epitopes as bait.

BOX 2

Applications of T-cell-epitope mapping

- Design of new vaccines – for cancer and infectious diseases.
- Design of new protein therapeutics – deimmunized with respect to T-cell-epitope content.
- Antigen discovery – by 'fishing for antigens' using T-cell epitopes as 'bait', using whole genomes or cancer proteomes as a point of departure.
- Autoimmunity – discovery of crossreactive T-cell epitopes, comparing self and heterologous proteins.
- Transplantation – discovery of immunogenic determinants of self and association with graft antigens leading to rejection and scoring transplants for possible rejection, based on HLA immunogenicity.
- Allergy – determination of T-cell epitopes associated with IgE response.

Research teams at Brown University, USA and EpiVax have begun to apply this new approach to MTB research and have already reported on the remarkable diversity of human immune responses to MTB proteins that have yet to be ascribed a function [20]. These studies appear to suggest that the immune response to epitopes from many different MTB proteins could be important in controlling infection. An association between the breadth of the T-cell response and the control of infection has previously been demonstrated for Hepatitis C infection [21]. Further studies of the breadth of immune responses to pathogens, studies that are now possible using immunomics (B- and T-cell-epitope mapping) tools, could demonstrate that previously discovered immunodominant proteins represent the tip of the iceberg, in terms of the number of antigens that are recognized in the course of the human immune response to infectious diseases and cancer.

In some cases, rather than screening whole proteomes for T-cell epitopes indiscriminately, researchers have chosen to use bioinformatics tools to select for families of proteins that could be associated with effective infection control. For example, Gennaro and colleagues [22] used proteomics tools to select putative secreted proteins, expressing selected genes in *E. coli*. They used this rapid approach to identify proteins that could be used for vaccine development, or for the design of diagnostic tests. One can also combine the whole genome epitope-mapping approach (previously described) and the selected protein family approach (used by Gennaro) to: (1) target proteins using bioinformatics methods; (2) refine the search using the epitope-mapping method.

The genome-derived epitope-driven approach provides a novel means of probing the human immune response and might allow researchers to uncover the true breadth of immune responses to a proteome from any pathogen, leading to improved understanding of pathogenesis. The reverse vaccinology approach provides a means for rapid expression and *in vitro* screening of novel vaccine antigens. Both approaches are equally valuable means of identifying novel antigens and hold promise for accelerating the development of new vaccines in the next few decades.

Comparing epitopes with self: autoimmunity

Immunomics tools could also be used to examine epitopes for uniqueness or for any similarity with those from other pathogens, or for homology with any known alloantigen (foreign protein) or with self (the human proteome). In fact, startling discoveries might ensue. Although comparisons between genes and genomes have been carried out for several pathogens, including *Listeria monocytogenes* [23] and several *Plasmodium* (malaria) species [24], to date few researchers have attempted to compare T-cell epitopes from different pathogens. This approach might unveil significant differences between pathogens regarding the absolute number of T-cell epitopes that interact with the human immune system. Perhaps this type of analysis will reveal that the proteomes of commensal pathogens (e.g. *Staphylococcus epidermis*, a skin commensal) contain few T-cell epitopes (enabling the commensal pathogen to hide from the host's immune response), whereas many epitopes will be evident following the analysis of the proteomes of accidental pathogens (e.g. *Borrellia bergdoferi*, the agent of Lyme disease) that interface with the host less-often and would, therefore, not be under selective pressure to become less immunogenic.

Immunome analyses might also uncover new methods that pathogens use to camouflage themselves from immune attack. For example, similarities between self (human) and T-cell epitopes for *Helicobacter pylori* have been uncovered. *H. pylori* is a human pathogen that has been associated with dyspepsia, atherosclerosis and a range of autoimmune diseases such autoimmune gastritis, idiopathic thrombocytopenic purpura (ITP), acne rosacea and idiopathic chronic urticaria. Similarities between autologous proteins and *H. pylori* proteins were initially uncovered using sera from subjects who had autoimmune gastritis [25]. Subsequently, T-cell clones that recognized autoantigens and crossreacted with *H. pylori* proteins were identified [26], a process that might have been accelerated by a comparison between T-cell epitopes of the *H. pylori* genome and self.

Epitope mapping could also help explain food intolerance. For example, certain human proteins have been found to contain regions that resemble proteins from wheat (wheat gliadin). This observation raised the question: whether T cells responding to the epitopes derived from wheat could play a role in the development of celiac disease (an autoimmune disease that is exacerbated by exposure to wheat products). Following an analysis of the entire human genome, eleven gut-expressed proteins with high T-cell-epitope homology to known celiac disease epitopes were identified. Although peptides derived directly from these homologous human gene sequences did not stimulate immune responses, mutated peptides containing single amino acid modifications (deamidations) stimulated immune responses at levels exceeding the positive control (wheat gliadin) [27].

These data appear to support the hypothesis that T cells reacting to some human peptides escape negative selection in the thymus (the natural process by which tolerance is induced). This theory is supported by experimental studies of mutated peptides in the hen-egg-lysozyme model of autoimmunity [28]. Peptides containing deamidated epitopes were able to escape tolerance and induce an autoimmune response.

Bacteria and viruses are believed to use tolerance to their advantage by modifying their own epitopes so that they resemble epitopes contained in self (autologous) proteins, because humans are generally tolerant to self. When this mechanism is successful it is called epitope mimicry (in reference to the protective effect of mimicry on the survival of the pathogen). When human immune responses are triggered, reaction to the exogenous epitope can trigger autoimmunity as well.

Several examples of T-cell-epitope mimicry that have gone awry have been published, this usually occurs in association with autoimmune pathology. Examples include crossreactive T-cell recognition of epitopes derived from microbial pathogens and from proteins contained in atherosclerotic plaque [29], and crossreactivity between epitopes derived from cardiac myosin and epitopes contained in proteins of *Trypanosoma cruzi*, the causative agent of Chagas disease [30]. Is *H. pylori* one of many human pathogens that use epitope mimicry as a means of evading an immune response? Is it possible that more epitope mimicry occurs than can be measured, simply because autoimmune manifestations represent a measurable aberrant response to crossreactive epitopes and tolerance to crossreactive epitopes is harder to detect? One means of investigating this question would be to map the entire immunome of these pathogens, searching for overlaps

between potential HLA ligands, derived from the pathogen and from self.

Uncovering virulence by comparative immunome analysis

Given the range of accurate, validated epitope-mapping tools that are now easily available on the internet and through collaborations (Table 1), T-cell immunome studies need not be limited to the intersection of a pathogen's immunome and the human proteome. Potentially exciting comparisons include the similarity between the immunome of MTB and that of *Mycobacterium leprae*, a related but less-virulent *Mycobacterium*, similarities that might reveal whether the basis for the difference in virulence is attributable not only to the lack of virulence genes, but also, perhaps, to the number of potential T-cell epitopes presented to the human immune system. Further, researchers could consider comparing the immunomes of *M. leprae* and MTB with the immunome of *Mycobacterium bovis*, the attenuated *Mycobacterium* that is used to prevent both of the previously mentioned *Mycobacterium* diseases (i.e. in the BCG vaccine). Presumably, proteins or epitopes that are found in MTB, but not in the BCG vaccine, could be ripe for exploitation as vaccine antigens. These and other pathogen comparisons are likely to bear valuable fruit, in terms of novel candidates for vaccines and even diagnostic tools.

Perhaps equally as important as discovering new candidate diagnostics, immunome research is likely to reveal potential antigenic relationships between unrelated (but immunologically similar) pathogens that might preset an individual immune response, for example, downregulating or deviating from the immune response from cellular immunity to humoral immunity. At least one investigator has suggested that previous exposure to a particular pathogen could increase or decrease the immune response to another pathogen [31]. Similarly, exposure to heterologous T-cell epitopes from pathogens can prime crossreactive T cells that impair tolerance to transplantation [32,33]. Thus, improved understanding of the dimensions of the human immunome (by mapping the human immune response to a wide range of heterologous proteins) might allow us to define vaccine candidates more accurately and to uncover new means for averting or avoiding undesirable immune responses.

New discoveries about the human immune response to self

Let us now consider the response of the human immune system to autologous proteins. Advances in molecular biology have led to a dramatic increase in the number of biological therapeutics that are recombinant human protein products, such as cytokines, growth factors and monoclonal antibodies, available for use in clinical settings [34,35]. Unfortunately, the use of these products in clinical practice is often associated with the development of antibodies directed against the therapeutic protein. These antitherapeutic protein antibodies neutralize or otherwise compromise the clinical effect of protein therapeutics and can also be associated with serious adverse events, such as autoimmunity. These events might occur even though the therapy involves a recombinant human protein or a humanized monoclonal antibody, agents that should not, in theory, breach tolerance.

Several mechanisms for the induction of antibodies against therapeutic proteins have been described [36]. These have included: (1) inadvertent formulation of the protein therapeutic with proinflammatory contaminants or adjuvants; (2) formation of immunogenic protein aggregates that crosslink B-cell receptors; and (3) T-cell-dependent antibody formation [37]. Therapeutic drug manufacturers have developed methods for addressing the first two problems, including improved purification of the recombinant protein and careful formulation of the therapeutic product. The third mechanism, which involves interaction between the cellular and humoral immune systems and the presentation of epitopes bound to HLA derived from the therapeutic protein, remains a challenge to therapeutic protein developers. The activation of T helper cells by T-cell epitopes (derived from the HLA-bound therapeutic protein) contributes to the development of high-affinity antibodies (targeting therapeutics), even when the therapeutic closely resembles an autologous (self-derived) protein. Thus, mapping epitopes that are contained in recombinant, autologous and therapeutic proteins could allow their identification and subsequent modulation, thereby downregulating the immune response. This approach (termed deimmunization* by epitope modification) is also being pursued as a method for developing new, improved therapeutic proteins [38].

Natural deimmunization of self

If the T-cell response contributes to autoimmunity even when tolerance is intact [39], it stands to reason that, in the course of human evolution, some human proteins could have been deimmunized already (modified so as to reduce the total number of T-cell epitopes). Setting aside for a moment the well-established concept of self-tolerance, one could use T-cell-epitope-mapping tools to evaluate each of the autologous proteins that interface with the human immune system and rank them in order of epitope content. Alternate concepts about self-tolerance could be investigated by applying this T-cell-epitope-mapping process to proteins of interest. Researchers at EpiVax have established preliminary evidence that selected self proteins (specifically those that are abundant in serum) have lower inherent epitope content than random proteins and known antigens. Are these proteins naturally deimmunized?

As shown in Figure 2, the immunogenicity scores of abundant serum proteins (such as albumin) and the constant domain of immunoglobulin (between them contributing to almost 90% of serum-protein content) were lower than expected, based on an analysis of the protein sequence. By contrast, antigenic proteins from pathogens such as influenza, tetanus and allergens generally scored much higher. Based on this analysis, it would appear that some prevalent human proteins contain significantly fewer T-cell epitopes than random proteins and common antigens. These discoveries could dramatically change existing ideas about the nature of tolerance.

Potential applications of T-cell-epitope mapping in the field of tolerance and transplantation include measuring graft versus host immune responses following transplantation, improving allograft matches and monitoring T-cell responses to opportunistic pathogens following transplantation. Finally, large swaths of unexplored

*Deimmunization is the term now used in the field of protein therapeutics for the process of reengineering proteins so as to diminish or abrogate an immune response. The process involves modification of the T-cell epitopes in the protein. Alternative approaches, such as pegylation and humanization, use alternate methodology to achieve the same effect.

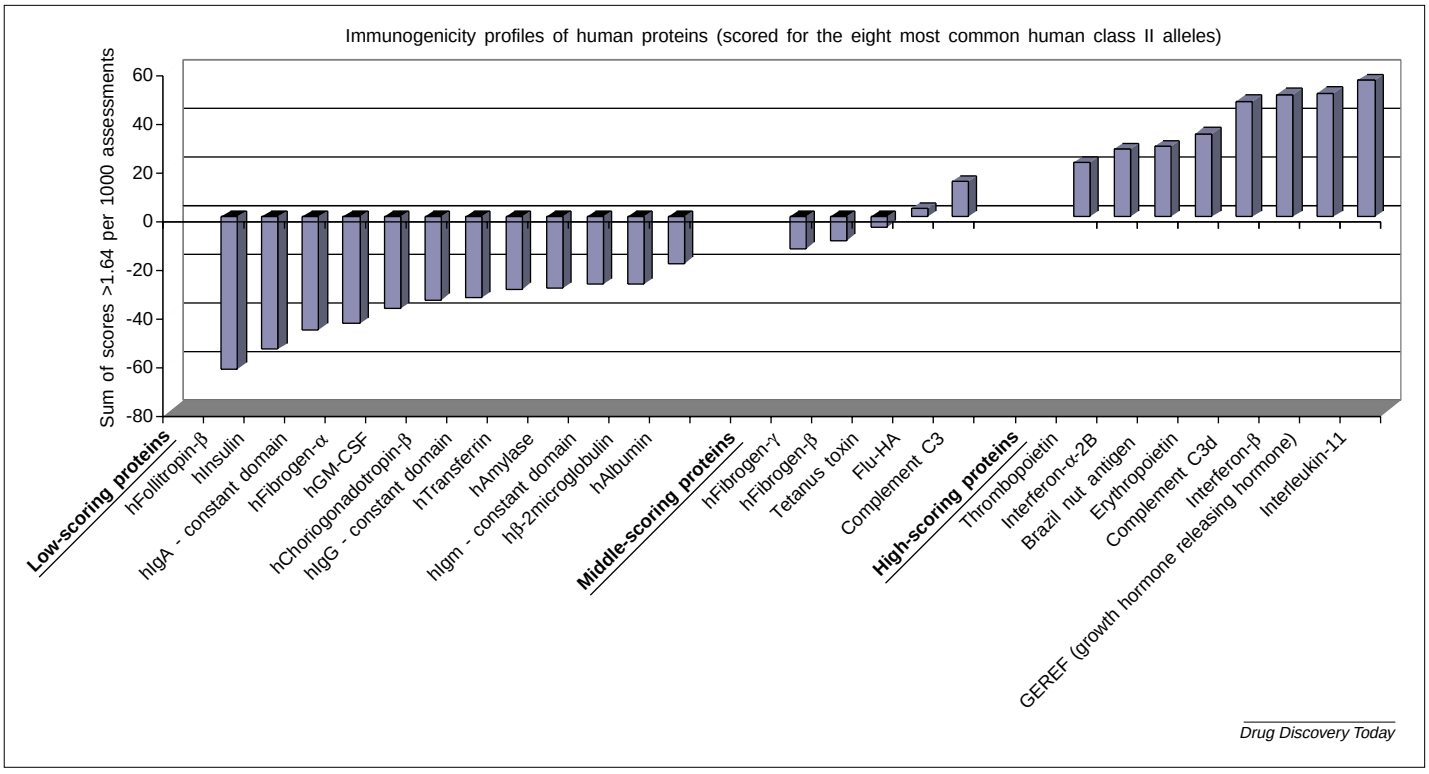

FIGURE 2

Inherent immunogenicity of human proteins. To perform this analysis, we used the EpiMatrix® tool, which is a matrix-based algorithm for T-cell-epitope mapping. This tool is standardized so that comparisons can be made across predictions for different HLA alleles. In this case we used the EpiMatrix® prediction matrices for eight MHC class II alleles that are representative of >98% of human populations. First, we measured the number of potential T helper epitopes that would occur in random-sequence pseudo proteins (composed of amino acids) at their naturally occurring frequencies and computed the mean epitope score – per 1000 assessments of 0.5, with SD ±7.9. We then compared proteins by summing the total number of EpiMatrix® scores for each protein that scored above an accepted cutoff for immunogenicity (>1.67). The difference between the scores for random proteins and test proteins was calculated.

territory remain – relating to the role of regulatory T-cells, which are also epitope-specific, in the development and maintenance of tolerance. Perhaps T-cell-epitope-mapping tools can help us find the way.

Conclusion: new territories to explore

The moment has arrived for scientists to begin to use imagination as a compass and immunomics tools as sextants for discovering the new world of the human immunome. This approach has potential to make dramatic advances and to improve human health. To begin with, in this review, the author suggests that scientists measure the breadth of several immunomes that should be representative of commensal, opportunist and accidental human pathogens (such as MTB, *Pneumococcus* and *Legionella pneumophilia*).

BOX 3

Immunomics resources

International Immunomics Society (IIMMS)
The society promotes research and fellowship in immunomics. One meeting is scheduled annually and the proceedings are published (www.research.i2r.a-star.edu.sg/IIMMS/goals.html).

Immunome Research
Immunome Research is a journal published by the IIMMS. The journal aims to provide a focal point for the field of immunomics (www.immunome-research.com).

The breadth of the immune response to these pathogens might lead to the development of new approaches to vaccines and therapeutic interventions.

Perhaps scientists should also begin by evaluating the interface between self proteins (such as abundant proteins, autoantigens and proteins located in privileged sites, for example the eye, the brain and the testis) and the human immune system. We suggest that *in silico* analyses be matched with *in vitro* assays (binding studies, MHC-tetramers and ELISpot assays). Furthermore, these discoveries should be validated *in vivo*, using HLA transgenic mice.

Finally, scientists should integrate immunomics into the scientific research discovery process more rapidly. T-cell-epitope-mapping tools, to be specific, are not less-accurate than bioinformatics tools that are already used on a daily basis. Many of the T-cell-epitope-mapping tools that are commonly available on the internet and by collaboration can now identify T-cell epitopes with breathtaking accuracy. As with all bioinformatics tools, *in vitro* and *in vivo* confirmations of *in silico* observations are necessary; however, validated T-cell-epitope-mapping tools can now be used with confidence to begin new scientific explorations. It is time to set aside timeworn approaches to immunology and set a confident, new course.

Once we set sail, we can anticipate that T-cell-epitope mapping and other immunomics tools will contribute to the discovery of entirely new perspectives in a wide range of fields. This article lists just a few of the new directions immunomics researchers are

pursuing. Immunomics visionaries are already searching for new horizons for the benefit of medicine and science (Box 3).

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References

1 De Groot, A.S. and Berzofsky, J.A. (2004) From genome to vaccine – new immunoinformatics tools for vaccine design. *Methods* 34, 425–428
2 *Methods* (2004) *Bioinformatics in Vaccine Design*. 34 (Entire volume devoted to computational tools for vaccine development including epitope mapping tools)
3 Schreiber, A. *et al.* (2005) 3D-Epitope-Explorer (3DEX): localization of conformational epitopes within three-dimensional structures of proteins. *J. Comput. Chem.* 26, 879–887
4 Kulkarni-Kale, U. *et al.* (2005) CEP: a conformational epitope prediction server. *Nucleic Acids Res.* 33, 168–171
5 Batori, V. *et al.* (2005) An *in silico* method using an epitope motif database for predicting the location of antigenic determinants on proteins in a structural context. *J. Mol. Recognit.* 19, 21–29
6 De Groot, A.S. *et al.* (2005) De-immunization of therapeutic proteins by T cell epitope modification. *Dev. Biol. (Basel)* 122, 171–194
7 Santori, F.R. *et al.* (2002) Genomics-based identification of self-ligands with T cell receptor-specific biological activity. *Immunol. Rev.* 190, 146–160
8 Klee, L. and Zand, R. (2004) Probable epitopes: relationships between myelin basic protein antigenic determinants and viral and bacterial proteins. *Neuroinformatics* 2, 59–70
9 Vali, M. *et al.* (2000) Thyroglobulin as autoantigen: structure–function relationships. *Rev. Endocr. Metab. Disord.* 1, 69–77
10 Westritschnig, K. and Valenta, R. (2003) Can we genetically engineer safer and more effective immunotherapy reagents? *Curr. Opin. Allergy Clin. Immunol.* 3, 495–500
11 Walden, P. and Sterry, W. (2004) New and emerging vaccination strategies for prevention and treatment of dermatological diseases. *Expert Rev. Vaccines* 3, 421–431
12 Stevanovic, S. (2005) Antigen processing is predictable: from genes to T cell epitopes. *Transpl. Immunol.* 14, 171–174
13 Mallone, R. and Nepom, G.T. (2004) MHC Class II tetramers and the pursuit of antigen-specific T cells: define, deviate, delete. *Clin. Immunol.* 110, 232–242
14 Ferrand, R.A. *et al.* (2005) Interferon-gamma responses to ESAT-6 in tuberculosis patients early into and after anti-tuberculosis treatment. *Int. J. Tuberc. Lung Dis.* 9, 1034–1039
15 Mustafa, A.S. (2002) Development of new vaccines and diagnostic reagents against tuberculosis. *Mol. Immunol.* 39, 113–119
16 Dobos, K.M. *et al.* (2004) Proteomic approaches to antigen discovery. *Methods Mol. Med.* 94, 3–17
17 Hendrickson, R.C. *et al.* (2000) Mass spectrometric identification of mtb81, a novel serological marker for tuberculosis. *J. Clin. Microbiol.* 38, 2354–2361
18 Arthur, J.W. and Wilkins, M.R. (2004) Using proteomics to mine genome sequences. *J. Proteome Res.* 3, 393–402
19 Pizza, M. *et al.* (2000) Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. *Science* 287, 1816–1820
20 McMurry, J. *et al.* (2005) Analyzing *Mycobacterium tuberculosis* proteomes for candidate vaccine epitopes. *Tuberculosis (Edinb.)* 85, 95–105
21 Lauer, G.M. *et al.* (2004) High resolution analysis of cellular immune responses in resolved and persistent hepatitis C virus infection. *Gastroenterology* 127, 924–936
22 Ben Amor, Y. *et al.* (2005) Immunological characterization of novel secreted antigens of *Mycobacterium tuberculosis*. *Scand. J. Immunol.* 61, 139–146
23 Trost, M. *et al.* (2005) Comparative proteome analysis of secretory proteins from pathogenic and nonpathogenic Listeria species. *Proteomics* 5, 1544–1557
24 Carlton, J.M. *et al.* (2001) Profiling the malaria genome: a gene survey of three species of malaria parasite with comparison to other apicomplexan species. *Mol. Biochem. Parasitol.* 118, 201–210
25 Claeys, D. *et al.* (1998) The gastric H+,K+-ATPase is a major autoantigen in chronic *Helicobacter pylori* gastritis with body mucosa atrophy. *Gastroenterology* 115, 340–347
26 Amedei, A. *et al.* (2003) Molecular mimicry between *Helicobacter pylori* antigens and H+, K+–adenosine triphosphatase in human gastric autoimmunity. *J. Exp. Med.* 198, 1147–1156
27 Kumar, R. *et al.* (2002) Human genome search in celiac disease: mutated gliadin T-cell-like epitope in two human proteins promotes T-cell activation. *J. Mol. Biol.* 319, 593–602
28 McAdam, S.N. *et al.* (2001) T cell recognition of the dominant I-A(k)-restricted hen egg lysozyme epitope: critical role for asparagine deamidation. *J. Exp. Med.* 193, 1239–1246
29 Benagiano, M. *et al.* (2005) Human 60-kDa heat shock protein is a target autoantigen of T cells derived from atherosclerotic plaques. *J. Immunol.* 174, 6509–6517
30 Iwai, L.K. *et al.* (2005) T-cell molecular mimicry in Chagas disease: identification and partial structural analysis of multiple cross-reactive epitopes between Trypanosoma cruzi B13 and cardiac myosin heavy chain. *J. Autoimmun.* 24, 111–117
31 Kim, S.K. *et al.* (2005) Private specificities of CD8 T cell responses control patterns of heterologous immunity. *J. Exp. Med.* 201, 523–533
32 Ely, L.K. *et al.* (2005) Antagonism of antiviral and allogeneic activity of a human public CTL clonotype by a single altered peptide ligand: implications for allograft rejection. *J. Immunol.* 174, 5593–5601
33 Wu, Z. *et al.* (2004) Homeostatic proliferation is a barrier to transplantation tolerance. *Nat. Med.* 10, 87–92
34 Sibilia, J. and Griscelli, C. (2005) Immunotherapy of inflammatory diseases, 2005. *Expert Opin. Biol. Ther.* 5 (*Suppl. 1*), 1–13
35 Reichert, J.M. *et al.* (2005) Monoclonal antibody successes in the clinic. *Nat. Biotechnol.* 23, 1073–1078
36 Rosenberg, A.S. (2003) Immunogenicity of biological therapeutics: a hierarchy of concerns. *Dev. Biol. (Basel)* 112, 15–21
37 Zubler, R.H. (2001) Naive and memory B cells in T-cell-dependent and T-independent responses. *Springer Semin. Immunopathol.* 23, 405–419
38 Tangri, S. *et al.* (2005) Rationally engineered therapeutic proteins with reduced immunogenicity. *J. Immunol.* 174, 3187–3196
39 Marleau, A.M. and Sarvetnick, N. (2005) T cell homeostasis in tolerance and immunity. *J. Leukoc. Biol.* 78, 575–584