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# Autoantibody repertoires, natural biomarkers, and system controllers

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**The immune system is composed of networks of interacting cells and molecules; therefore, to understand and control immune behavior we need to adopt the thinking and tools of systems immunology. This review describes the use of an antigen microarray device and informatics to profile the repertoires of autoantibodies in health and disease. Autoantibody profiling provides an insight into the biomarkers used by the immune system in its dialog with the body. Heat shock protein 60 (HSP60) and HSP70 are cited as examples of key hubs in physiological regulatory networks; HSP molecules and peptides can be viewed as natural regulators because the immune system itself deploys them to modulate inflammatory reactions. The discovery of such natural biomarkers paves the way towards natural control.**

## Frustrating reduction

Immunology has succeeded splendidly in dissecting the immune system into its component genes, transcription factors, signal transducers, receptors, ligands, cytokines, chemokines, adhesion molecules, molecular effectors, and various innate and adaptive cell types. Moreover, immunology has characterized the processes emerging from the interactions of these components as they are initiated, regulated, and organized by the immune system to suit the dynamic and changing needs of the individual and the species [1]. The information and knowledge immunology has gathered about the immune system is astounding.

In the middle years of the 20th century, medical students could be taught the entire field of immunology in two or three lectures; immunity resulted from antibodies raised in response to an infection or to simple vaccines, and that was that. There was a paucity of knowledge, and there was no confusion.

Today, we are much less ignorant about the immune system, but we seem to suffer more from confusion and frustration. Why can we not devise effective vaccines against HIV, tuberculosis or malaria? Why can we not harness immune responses to reject tumors or at least deprive them of their immune supporters? Why are autoimmune diseases flourishing and therapies failing? Why can we not induce tolerance to allografts when our bodies are already tolerant to the trillions of foreign organisms living in our microbiome [2]? Indeed, what prevents the gut microbiome from activating inflammatory bowel disease [3]?

We have reduced the immune system to its most fundamental component parts; even the human genome has been deciphered. Knowledge has exploded and the data are overwhelming, but, alas, we still lack understanding [4]. One might say that we are still ignorant, albeit at a much deeper level. Our success in taking the system apart has not generated a commensurate degree of understanding; knowledge of all the pieces has not empowered us to control the system to our satisfaction. The reductionist program has not yet delivered the goods. This frustration has led us in the direction of systems immunology. This review discusses profiling the autoantibody repertoire in health and disease as a systems immunology approach for discovering safe and effective ways to modulate the immune response.

## The quest for a systems immunology

The concept of a systems biology, of which systems immunology is a subdivision, is based on the idea that analytical reduction of biological complexity alone cannot tell us how a complex living system functions as a whole entity; analysis alone does not empower us to understand the healthy function of a system or its diseases [4–6]. Analysis, nevertheless, serves as the only sound basis for the next challenge: synthetic reconstruction of the essential processes that form the system. Having taken the machine apart, we can now attempt to put it back together again, and successful synthesis should lead to control. Control is important for immunology because the immune system generally works to keep us healthy and sometimes makes us sick; immunology is a medical science. How then are we to deal with the masses of data that obscure understanding, and how are we to gain control? There are at least two ways: intelligent design and discovery of natural controllers.

## Intelligent immune design

The intelligent design approach is to integrate, using advanced informational technologies, all of the knowledge we have gained from genomics combined with the knowledge we hope to gain from epigenomics, transcriptomics, proteomics, metabolomics, interactomics, and all the other omic domains of biology [7–13]. Theoretically, after we have comprehensive knowledge, we could consider deploying computer-assisted integrations and simulations that will identify and characterize the networks of interactions that control the system as a whole [14]; on the basis of this information, one might achieve a degree of understanding that would enable the intelligent design of therapeutic

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control. This approach amounts to a quantum leap in what, in the early days, we used to call immune physiology.

There are, unfortunately, at least three fundamental problems with implementing such an intelligent design strategy. The first problem lies in available resources. It will take much time and money to complete all of the omics we need for a comprehensive picture of the immune system and its interactions with the body; even if we were to complete the omic inquiry, we would probably lack the computational power to integrate all of the domains of omic data within a reasonable time frame. Second, there are problems associated with kinetics. We lack at present the instruments needed to measure the continuous dynamic fluxes that characterize biologic systems; omic determinations are static, assume steady-state kinetics, or are of limited dynamic range. Complexity is the third challenge associated with the intelligent immune design approach. Living systems are intrinsically pleiotropic and redundant [1]; no single molecule, cell, or organism performs only one function, and, despite the existence of single lethal mutations, relatively few biological processes are dependent on only one element in the system. Simply too many alternatives account for most of the emergent properties of the living system; how can we model such a slippery system? We will need to develop new concepts of system design to accommodate the nonlinear, pleiotropic and counter-intuitive networking that generates overt immune behavior. As a consequence, even if we had at our disposal unlimited time and funding, we would probably continue to be frustrated by unforeseeable side effects emerging from our most intelligently designed interventions – tumor necrosis factor-α, for example, has been discovered to function as a major hub in the networks that control inflammation [15], but anti-tumor necrosis factor-α antibody treatments make rheumatoid arthritis better (in some patients [16]) and multiple sclerosis worse (in some patients [17]).

## Natural controllers of inflammation

There is an opportunistic alternative to brute force omics and the intelligent design of pharmaceutical control; the idea here would be to discover how the system has evolved to control itself naturally. Rather than impose the logic of human engineering on immune system control, we might follow the lead of nature and uncover the control elements that are intrinsic to the immune system's dialog with the body. The discovery of natural controllers is a way to exploit evolution's insights; we do not have to know everything that goes on within the immune system, we only need attend to what the system can tell us about its interactions with the body. Profiling autoantibodies might enable a shortcut to control.

To maintain health, the immune system has to diagnose whether particular tissues of the body are healthy or in need of immune attention; if the immune system detects that something is wrong, it then has to decide what inflammatory processes are needed to solve the problem. Immune decisions are complicated by dynamic changes in states of health, disease, and repair; consequently, immune responses are continuously evolving with varying kinetics [4]. Indeed, the tasks of the immune system demand daunting complexity.

Classically, it had been assumed that the immune system had a single aim and a single response: it existed only to sense and destroy foreign invaders. To achieve this aim, the system just had to be purged of antigen receptors that could possibly respond to self-components [18]. Any antigen detected by a clone of receptor-bearing lymphocytes by definition had to be foreign to the organism. The lymphocytes proliferated automatically and made specific antibodies that neutralized or killed the foreign entity, and the responding lymphocyte clones went on to differentiate into memory cells that could detect their specific antigen and respond to it with greater speed and vigor on secondary contact [18]. The immune system was controlled, in a similar way to a chemical reaction, by the concentrations of its reactants: the numbers of clones bearing specific antigen receptors and the concentration of the antigen were all one needed to know (no wonder the story needed only a few lectures).

By now we have learned that the immune system is composed of diverse populations of cells bearing innate receptors or bearing both innate receptors and somatically generated adaptive receptors; immune cells detect antigens that bind to adaptive receptors and respond to myriads of molecules that are ligands for diverse innate receptors. Moreover, these receptors, adaptive and innate, recognize body molecules. The immune system not only fights invading pathogens, it also manages the microbiome; heals wounds and generates scars and connective tissues; rids the body of tumors and aged or aberrant cells; induces angiogenesis and regulates blood vessels; triggers cell regeneration; sculpts connective tissues; and manages the inflammatory response in its varied forms [1]. Indeed, the immune system is now known to function in sensing and modulating the metabolic state of the body [19].

To carry out its critical functions in maintaining the body, the immune system, in a similar way to a competent physician, senses and records the state of the body. The diagnosis proceeds in a speedy and efficient manner and is linked to a fitting therapeutic response. Each threat or maintenance problem requires an inflammatory response tailored to the evolving circumstances: infection, symbiosis, injury, neoplasia, or degeneration. The immune system, in a similar way to the good physician, is able to make speedy diagnoses and adjustments to treatment using informative biomarkers [20]. Neither the physician nor the immune system can know, and does not need to know, everything going on inside the patient – carefully selected biomarkers integrate and simplify complex information into an accessible form. Medical science discovered its biomarkers (such as fever, white blood count, physical examination, and laboratory tests) with the evolution of human culture and medical practice, whereas the immune system evolved its own informative biomarkers over millions of years of co-evolution with the multicellular body [20,21]. Quite simply, the evolving immune system learned through trial and error which body molecules could provide the safest and most reliable information about the immune state. The immune–body dialog is based on natural biomarkers in a continuous, never-ending cycle of activity – heat shock proteins (HSPs), cytokines, chemokines, and selected self-antigens alert immune cells and guide their

behaviors, and these molecular signals enable the immune system to adjust its evolving responses to the state of the body [22]. The bottom line is that the state of the immune system mirrors the state of the body. The opportunity is to mine from the immune system the information that it uses to diagnose and treat the body – autoimmune repertoires provide one such opportunity.

## The antigen microarray

Antigen microarrays can be used to profile autoantibody repertoires that disclose the ongoing responses of the immune system to the body [23]. As we shall see, some of the self-antigens marked by the natural autoimmune repertoire can serve as biomarkers, not only to the immune system but also to us for diagnosis and treatment [20]. Furthermore, immune biomarker reactions can guide us to intrinsic control elements deployed by the immune system itself.

Antigen microarrays are prepared by using a robotic apparatus to spot replicates of hundreds to thousands of selected molecules on a chemically derivatized glass slide, each spot with a known address (Figure 1). The molecules – which are defined as antigens if they bind antibodies – are crosslinked to the surface to keep them in place. This can be used to successfully detect antibodies to various molecular classes, including proteins and protein fragments, synthetic peptides, lipids, carbohydrates, and nucleic acids. Fluid to be tested for antibodies – such as serum, plasma, milk, saliva, urine, cerebrospinal fluid, or culture medium – is incubated on the slide (microliter volumes suffice). After washing, the antibodies that bind to the spotted antigens are detected by secondary antibodies – anti-IgG, anti-IgM, and anti-IgA isotypes – labeled with fluorescence-sensitive dyes; the relative amount of antibody binding to each spot is detected by a laser reader. The antibody binding reactions are analyzed
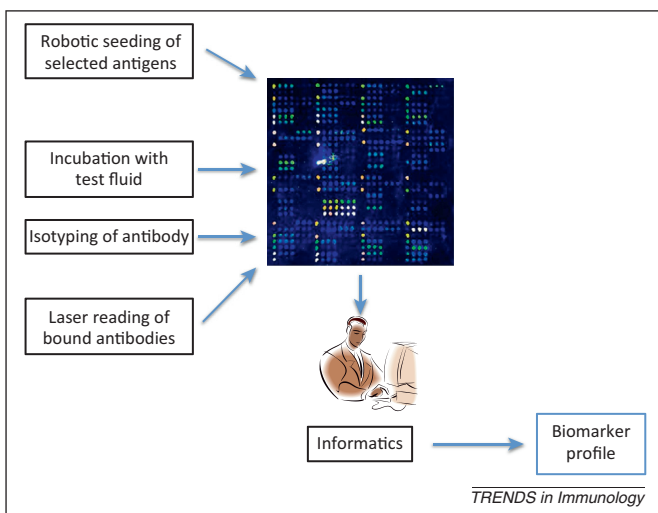
bioinformatically to identify informative antibody profiles that serve as signatures for the test groups; validation of informative profiles is obtained by testing new groups of samples that were not used to develop the initial test algorithms. The antigen microarray and its attendant informatic analysis provide a useful step towards a diagnostic systems immunology.

## Unknowns and caveats

The antigen microarray is subject to a number of confounding factors. The measured antibody reactivities result from polyclonal mixtures of antibodies and cross-reactivities in the test fluid; as we are not dealing with separated antibodies, we cannot know the original immunogenic stimulus. Antigen molecules bound to the surface of the chip, even when they are small synthetic peptides, are mixtures of conformational epitopes; the antigens, like the antibodies, are not single species. The choice of antigens for spotting is critical to the success of microarray profiling; the literature and our experience regarding relevant pathways and networks, along with a measure of good fortune, are needed to build an informative antigen microarray. Selecting informative antigens is an evolving process. Different batches of slides, antigens, and other reagents can differ substantially; care must be taken to standardize and validate results. Different types of informatic analyses will highlight different features of the data, so sufficiently large groups of samples must be used to obtain significant results. Informative antibody profiling requires carefully selected control and test groups that must be matched to isolate the specific characteristics under study. Gender, age, genotypes, geography, lifestyle, and other factors have to be considered. Samples must be carefully annotated to enable accurate interpretation of the data.

Antigen microarrays have been used to characterize antibody profiles in healthy individuals and in those with various diseases. The following sections summarize some of the lessons learned from autoantibody profiling.

## Natural autoantibody repertoires in sera of mothers and their newborns

Natural autoantibody repertoires have been characterized in the cord bloods of healthy newborn infants and in the sera of their mothers [24]. The results shed light on the development of systems immunology in health.

### Congenital autoantibody repertoires are shared by different newborns

Maternal antibodies of the IgG isotype only are actively transported across the placenta; as expected, IgG antibody reactivities in maternal and cord bloods are highly correlated [24], both those binding to self-antigens and those binding to foreign antigens. By contrast, maternal IgM and IgA antibodies are not transferred to the fetus, so any IgM or IgA antibodies present in cord blood must have been produced by the developing fetus before birth. Surprisingly, all cord bloods contain IgM and IgA autoantibodies, and these cord-blood reactivities show a high correlation among different, unrelated babies, and a much lower correlation with their mothers' IgM and IgA repertoires. Thus, humans begin life with a common set of autoantibody reactivities to major



**Figure 1**. Antigen microarray chip for profiling antibody repertoires. Selected antigens are seeded on a coated glass slide and fixed by crosslinking. Test fluids suspected of containing antibodies to the antigens are incubated in microliter volumes on the surface of the slide, and antibodies binding to each antigen spot are detected and quantified to a relative degree using isotype-specific antibodies marked with fluorescence probes. A laser reader is used to score each spot, and the results are determined by bioinformatics analysis. Comparison of test and control samples enables the elucidation of a biomarker profile on the basis of a subset of informative antigen reactivities.

self-antigens. These shared autoantibodies constitute a so-called 'congenital immunological homunculus': that is, an image of body molecules mirrored in healthy immune system repertoires [25]. Some shared but currently unknown mechanism must positively select for such homuncular autoantibodies in different fetuses, starting before birth.

*Healthy autoantibody repertoires diverge after birth*
It is reasonable to conclude that the mothers too began their postnatal immune development with a shared autoantibody repertoire not unlike that of their own neonates. Because the IgM and IgA repertoires of these women differ from one another and from those of their newborns [24,26], we can conclude that the congenital autoimmune repertoire evolves in response to individual immune experience – infections, vaccinations, and individualized microbiomes probably account for healthy postnatal variation in the individual repertoire.

*Amounts of autoantibodies compare with the amounts of antibodies binding to foreign antigens*
The degrees of reactivity to many self-antigens are comparable to those measured to foreign antigens associated with vaccines, viruses, and bacteria; thus, some natural autoantibodies seem to be as prominent as the antibodies produced naturally in response to stimulation by foreign antigens [24,26].

*Natural autoantibodies bind self-antigens associated with autoimmune diseases*
The particular self-antigens that bind autoantibodies in healthy mothers and newborns can be seen in various publications [24,26–29]; the point here is that many of these self-antigens are also associated with overt autoimmune diseases such as multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, type 1 diabetes, and others. Autoimmune diseases are usually associated with effector T cells or high-titer autoantibodies that target many of the same self-antigens as normal autoantibodies in healthy subjects. This suggests that at least some autoimmune diseases might emerge from a pathogenic shift in the phenotype of previously existing natural autoimmunity [25,27]. In other words, the emergence of an autoimmune disease might not require a new autoimmunization but rather a loss of control of existing autoimmunity. An extension of this reasoning would suggest that an autoimmune disease could arise from some lapse in the function of natural control mechanisms exercised by the immune system in healthy people. This way of thinking is the basis for the quest for natural controllers that might restore the healthy autoimmune phenotype (see below).

*Autoantibody network architecture and influential self-antigens*
Autoantibody reactivities to certain sets of self-antigens seem to be correlated; if a person has autoantibodies to self-antigen $j$, for example, he or she is likely to have autoantibodies to self-antigens $i$ and $k$. Thus, particular autoantibody repertoires seem to be connected by networks of autoantibodies to sets of self-antigens [28]. Moreover, autoantibody networks are dominated by key reactivities: for example, autoantibodies to HSP60 form hubs of network connectivity to other antibodies to many different self-antigens [29]. The immunological homunculus manifests organized autoantibody repertoires [25,27].

### The structures of autoantibody repertoires provide diagnostic biomarkers
Several groups [30] have used antigen microarrays to uncover biomarker autoantibody profiles that are characteristic of diseases such as multiple sclerosis [31,32] and systemic lupus erythematosus [33–35], and allograft rejection also manifests biomarker profiles [36,37]. Indeed, autoantibody profiles can make fine distinctions within a disease; different profiles distinguish different clinical types of multiple sclerosis [31] and can distinguish between cerebrospinal fluid and blood autoantibodies [32]. In a mouse model of tumor development, differences have been detected in autoantibody profiles between locally growing and metastatic tumors, and these differences can be used to follow the effects of tumor resection [38]. Thus, autoantibody repertoires provide an opportunity for mining immune information about various states of health and disease. Furthermore, and no less important, autoantibody repertoires can guide us to natural controllers used by the immune system to control itself.

### The autoantibody repertoire discloses natural controllers of inflammation: the case of HSP60 and HSP70
HSP60 and HSP70 are examples of immune biomarker molecules. Antigen microarray studies show that human cord blood contains IgM autoantibodies to various peptide epitopes of both HSP60 and HSP70 [24,28,29]; thus, natural autoantibodies to HSP60 and HSP70 seem to be part of the healthy immune system. Nevertheless, amplification of autoimmune effector responses to these molecules is associated with autoimmune diseases such as type 1 diabetes [39], arthritis [40,41], and atherosclerosis [42,43]. These findings demonstrate the complex relationship between natural autoimmunity and autoimmune disease: some forms of autoimmunity to HSP60 or HSP70 seem to be associated with health, whereas other forms of autoimmunity to these same self-antigens seem to be related to disease.

An antigen microarray experiment was designed to gain insight into the roles of HSP60 and HSP70 autoimmunity in type 1 diabetes. Male non-obese diabetic (NOD) mice, compared to female NOD mice, are relatively resistant to the spontaneous development of type 1 diabetes; indeed, about 50% of male NOD mice are resistant to an accelerated form of type 1 diabetes induced by the toxic compound cyclophosphamide [44]. The cyclophosphamide-induced model was used to ask whether autoantibody repertoires could mark resistant male mice and distinguish them from susceptible male mice before the induction of accelerated disease with cyclophosphamide [45]. Individual male NOD mice were bled to obtain a serum sample, and the mice were then exposed to the toxin before being monitored for type 1 diabetes; the question was whether the susceptible and resistant mice bore discriminating autoantibody biomarkers before they were manipulated experimentally. It was found that the mice bearing natural IgG and IgM
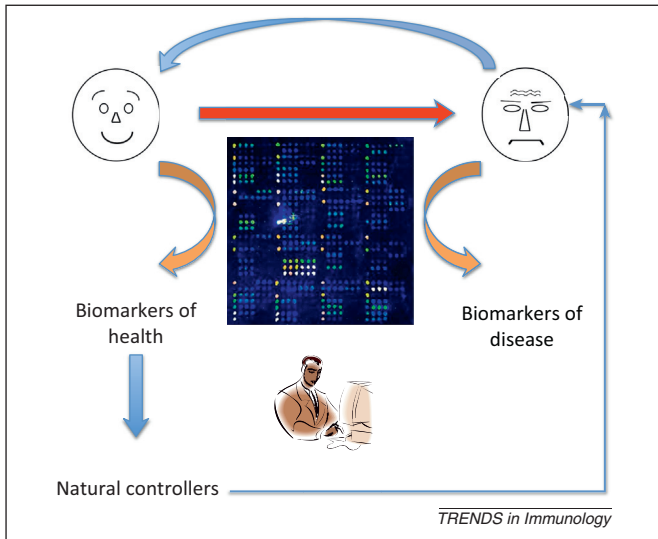
**Figure 2**. Natural controllers of inflammatory disease can be discovered by profiling immune system biomarkers. A state of health (the smiling face to the left) may be diverted to a state of inflammatory disease (the red arrow leading to the frowning face to the right) by a lapse in natural regulation of an otherwise benign inflammatory response. The two states of health and disease can be probed and discriminated using antigen-microarray technology and informatics to discern immune biomarkers [23]. Biomarkers of health, such as heat shock protein 60 (HSP60) or HSP70 peptides, which are used by the immune system to manage its interactions with the body [45], can also be used therapeutically as natural controllers to reinstate health (as marked by the blue arrows) [46–49].

autoantibodies to HSP60 and HSP70 epitopes were the mice that resisted induction of type 1 diabetes; the susceptible mice lacked these autoantibodies. Thus, natural autoantibodies to HSP60 and HSP70 epitopes served as biomarkers for predicting future resistance to type 1 diabetes [45].

This antigen microarray finding was also compatible with our studies showing that administration of an HSP60 peptide epitope termed p277 could serve to arrest β-cell destruction in NOD mice [46] and in humans with recent-onset type 1 diabetes [47]. In mice, a single injection of 100 μg sufficed, whereas humans required 1 mg injected subcutaneously several times a year. Likewise, injections of HSP60 or of a different HSP60 peptide, Hu3, were found to arrest arthritis [48]. Additionally, a peptide of HSP70 is recognized by regulatory T cells that are effective in treating arthritis in rodents [49].

The mechanisms by which HSP molecules and their peptides downregulate autoimmune diseases involve activation of regulatory T cells [49,50] and other components of the self-antigen network [51], but the details are beyond the scope of this review. The important points here are that antigen-microarray profiling of autoantibody repertoires can identify immune biomarkers of disease [20] and that some of these biomarker molecules are controllers that activate the immune system to regulate itself [52] (Figure 2). Organic controllers such as HSP60 and HSP70 seem to be effective and are certainly much safer than the intelligently designed pharmacological treatments widely used at present to suppress autoimmune diseases.

## Concluding remarks

The immune response is the expression of a system, hence immunology is by necessity systems immunology.

Immunologists, to really understand immunity, will have to think about systems and system design, work with systems people, and use computational technologies; even doing immune genetics has become an informational enterprise. The question is how to turn information into understanding. One proposal is to harness computer science to simulate *in silico* a complete, functioning organism indistinguishable from the real thing [53]; such a grand challenge, even if achieved, would provide a model with a complexity that would approach that of a real organism. Understanding, in the meantime, would probably be better served by realistic simulations focused on more defined questions [54,4]. The ultimate tests of understanding are prediction and control [55]; thus, as discussed here, the aims of systems immunology should include elucidation of the hubs of information deployed by the immune system to regulate itself. The reward will be getting away from pharmaceutical design and into, theoretically at least, safer and more effective natural control.

## References

1 Cohen, I.R. (2000) *Tending Adam's Garden: Evolving the Cognitive Immune Self,* Academic Press
2 Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. *Nature* 487, 207–214
3 Bakhtiar, S.M. *et al.* (2013) Implications of the human microbiome in inflammatory bowel diseases. *FEMS Microbiol. Lett.* 342, 10–17
4 Cohen, I.R. and Harel, D. (2007) Explaining a complex living system: dynamics, multi-scaling and emergence. *J. R. Soc. Interface* 4, 175–182
5 Purvis, J.E. and Lahav, G. (2013) Encoding and decoding cellular information through signaling dynamics. *Cell* 152, 945–956
6 Vainas, O. *et al.* (2011) Reactive animation: from piecemeal experimentation to reactive biological systems. *Autoimmunity* 44, 271–281
7 Idris, S.F. (2013) The role of high-throughput technologies in clinical cancer genomics. *Expert Rev. Mol. Diagn.* 13, 167–181
8 Teperino, R. *et al.* (2013) Bridging epigenomics and complex disease: the basics. *Cell. Mol. Life Sci.* 70, 1609–1621
9 Choi, D.S. *et al.* (2013) Proteomics, transcriptomics and lipidomics of exosomes and ectosomes. *Proteomics* http://dx.doi.org/10.1002/pmic.201200329
10 Russell, C. *et al.* (2013) Application of genomics, proteomics and metabolomics in drug discovery, development and clinic. *Ther. Deliv.* 4, 395–413
11 Putri, S.P. *et al.* (2013) Current metabolomics: practical applications. *J. Biosci. Bioeng.* 115, 579–589
12 Derouiche, A. *et al.* (2012) Protein phosphorylation from the perspective of systems biology. *Curr. Opin. Biotechnol.* 23, 585–590
13 Rogne, M. and Taskén, K. (2013) Cell signalling analyses in the functional genomics era. *N. Biotechnol.* 30, 333–338
14 Efroni, S. *et al.* (2003) Toward rigorous comprehension of biological complexity: modeling, execution, and visualization of thymic T-cell maturation. *Genome Res.* 13, 2485–2497
15 Sade-Feldman, M. *et al.* (2013) Tumor necrosis factor-α blocks differentiation and enhances suppressive activity of immature myeloid cells during chronic inflammation. *Immunity* 38, 541–554
16 Herenius, M.M. *et al.* (2013) Anti-TNF therapy reduces serum levels of chemerin in rheumatoid arthritis: a new mechanism by which anti-TNF might reduce inflammation. *JAMA* 309, 887–895
17 Gregory, A.P. *et al.* (2012) TNF receptor 1 genetic risk mirrors outcome of anti-TNF therapy in multiple sclerosis. *Nature* 488, 508–511
18 Burnet, F.M. (1959) *The Clonal Selection Theory of Acquired Immunity,* Cambridge University Press
19 Brooks-Worrell, B. *et al.* (2012) Biomarkers and immune-modulating therapies for type 2 diabetes. *Trends Immunol.* 33, 546–553
20 Cohen, I.R. (2007) Biomarkers, self-antigens and the immunological homunculus. *J. Autoimmun.* 29, 246–249
21 Cohen, I.R. (2009) Biomarker vaccines. *Hum. Vaccin.* 5, 637–639

22 Cohen, I.R. (2007) Real and artificial immune systems: computing the state of the body. *Nat. Rev. Immunol.* 7, 569–574

23 Quintana, J.F. *et al.* (2006) Antigen-chip technology for accessing global information about the state of the body. *Lupus* 15, 428–430

24 Merbl, Y. *et al.* (2007) Newborn humans manifest autoantibodies to defined self molecules detected by antigen microarray informatics. *J. Clin. Invest.* 117, 712–718

25 Cohen, I.R. (1992) The cognitive paradigm and the immunological homunculus. *Immunol. Today* 13, 490–494

26 Madi, A. *et al.* (2012) The natural autoantibody repertoire in newborns and adults: a current overview. *Adv. Exp. Med. Biol.* 750, 198–212

27 Cohen, I.R. and Young, D.B. (1991) Autoimmunity, microbial immunity and the immunological homunculus. *Immunol. Today* 12, 105–110

28 Madi, A. *et al.* (2009) Organization of the autoantibody repertoire in healthy newborns and adults revealed by system level informatics of antigen microarray data. *Proc. Natl. Acad. Sci. U.S.A.* 106, 14484–14489

29 Madi, A. *et al.* (2011) Network theory analysis of antibody-antigen reactivity data: the immune trees at birth and adulthood. *PLoS ONE* 6, e17445

30 Robinson, W.H. *et al.* (2002) Autoantigen microarrays for multiplex characterization of autoantibody responses. *Nat. Med.* 8, 295–301

31 Quintana, F.J. *et al.* (2008) Antigen microarrays identify unique serum autoantibody signatures associated with different clinical forms and pathologic subtypes of multiple sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 105, 18889–18894

32 Quintana, F.J. *et al.* (2012) Antigen microarrays identify CNS-produced autoantibodies in RRMS. *Neurology* 78, 532–539

33 Graham, K.L. *et al.* (2004) High-throughput methods for measuring autoantibodies in systemic lupus erythematosus and other autoimmune diseases. *Autoimmunity* 37, 269–272

34 Fattal, I. *et al.* (2010) An antibody profile of systemic lupus erythematosus detected by antigen microarray. *Immunology* 130, 337–343

35 Li, Q.Z. *et al.* (2005) Identification of autoantibody clusters that best predict lupus disease activity using glomerular proteome arrays. *J. Clin. Invest.* 115, 3428–3439

36 Hagedorn, P.H. *et al.* (2010) Chronic rejection of a lung transplant is marked by a profile of specific autoantibodies. *Immunology* 130, 427–435

37 Hagedorn, P.H. *et al.* (2010) Integrative analysis correlates donor transcripts to recipient autoantibodies in primary graft dysfunction after lung transplantation. *Immunology* 132, 394–400

38 Merbl, Y. *et al.* (2009) A systems immunology approach to the host-tumor interaction: large-scale patterns of natural autoantibodies distinguish healthy and tumor-bearing mice. *PLoS ONE* 4, e6053

39 Elias, D. *et al.* (1991) Vaccination against autoimmune mouse diabetes with a T-cell epitope of the human 65-kDa heat shock protein. *Proc. Natl. Acad. Sci. U.S.A.* 88, 3088–3091

40 van Eden, W. *et al.* (1988) Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. *Nature* 331, 171–173

41 Quintana, F.J. *et al.* (2004) Inhibition of adjuvant-induced arthritis by DNA vaccination with the 70-kd or the 90-kd human heat-shock protein: immune cross-regulation with the 60-kd heat-shock protein. *Arthritis Rheum.* 50, 3712–3720

42 Almanzar, G. *et al.* (2012) Autoreactive HSP60 epitope-specific T-cells in early human atherosclerotic lesions. *J. Autoimmun.* 39, 441–450

43 Rizzo, M. *et al.* (2011) Heat shock protein-60 and risk for cardiovascular disease. *Curr. Pharm. Des.* 17, 3662–3668

44 Ablamunits, V. *et al.* (1999) Acceleration of autoimmune diabetes by cyclophosphamide is associated with an enhanced IFN-γ secretion pathway. *J. Autoimmun.* 13, 383–392

45 Quintana, F.J. *et al.* (2004) Functional immunomics: microarray analysis of IgG autoantibody repertoires predicts the future response of mice to induced diabetes. *Proc. Natl. Acad. Sci. U.S.A.* 101 (Suppl. 2), 14615–14621

46 Elias, D. and Cohen, I.R. (1994) Peptide therapy for diabetes in NOD mice. *Lancet* 343, 704–706

47 Raz, I. *et al.* (2001) β-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. *Lancet* 358, 1749–1753

48 Quintana, F.J. *et al.* (2003) DNA fragments of the human 60-kDa heat shock protein (HSP60) vaccinate against adjuvant arthritis: identification of a regulatory HSP60 peptide. *J. Immunol.* 171, 3533–3541

49 van Herwijnen, M.J. *et al.* (2012) Regulatory T cells that recognize a ubiquitous stress-inducible self-antigen are long-lived suppressors of autoimmune arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 109, 14134–14139

50 Zanin-Zhorov, A. *et al.* (2006) Heat shock protein 60 enhances CD4+CD25+ regulatory T cell function via innate TLR2 signaling. *J. Clin. Invest.* 116, 2022–2032

51 Quintana, F.J. and Cohen, I.R. (2011) The HSP60 immune system network. *Trends Immunol.* 32, 89–95

52 Cohen, I.R. (2002) Peptide therapy for type I diabetes: the immunological homunculus and the rationale for vaccination. *Diabetologia* 45, 1468–1474

53 Harel, D. (2005) A Turing-like test for biological modeling. *Nat. Biotechnol.* 23, 495–496

54 Efroni, S. *et al.* (2007) Emergent dynamics of thymocyte development and lineage determination. *PLoS Comput. Biol.* 3, e13

55 Efroni, S. *et al.* (2005) A theory for complex systems: reactive animation. In *Multidisciplinary Approaches to Theory in Medicine (Studies in Multidisciplinarity, Vol. 3)* (Paton, R. and McNamara, L., eds), pp. 309–324, Elsevier