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Antigen-chip technology for accessing global information concerning the state of the body

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INTRODUCTION

Until now immunologists have been able to focus on one or a few antibodies, cytokines or T cell clones at a time; immunology has lacked the comprehensive view of the immune system needed to optimize diagnosis, prognosis, drug development, treatment choice, patient stratification, and monitoring. Microarray technology combined with advanced informatic technologies has opened new opportunities for approaching the vast information stored in the immune system. As a first step in developing an antigen chip we have studied arrays of antigens using standard ELISA microtitre plates, and have applied a clustering algorithm (originally developed for DNA chips) to detect patterns of antibody reactivity within the sera of healthy persons and those with various diseases[1]. We have used this algorithm to analyse global antibody patterns and can successfully discriminate various conditions. For example, we have successfully discriminated between type 1 diabetic persons and healthy controls with 95% sensitivity and 90% specificity. The global patterns of antibodies are clearly more efficient in stratifying patients than any one-to-one antigen–antibody reaction. We have now developed an antigen microarray (antigen chip) that makes it possible to profile the global state of the immune system by analysing the patterns of autoantibodies to hundreds of different self antigens[2]. The present architecture of the chip can accommodate thousands of different antigens.

IMMUNE SYSTEM COMPUTER

The immune system is the key player in body maintenance. The immune system expresses both the genetic endowment of the individual and the life experience of the individual; the immune system deals with post-genomic adaptation to life. Like the central nervous system, the immune system is *self-organizing*: it

begins with genetically coded, primary instructions, to which it adds information culled from the individual's experience with the environment in health and disease. Just as each person develops a unique brain, each person develops an individualized immune system. If we need to know about the past immune history and the future susceptibility of the individual, it would be very useful to be able to consult the immune system.

The output of the immune system is a complex series of processes termed *inflammation*. Inflammation is initiated, regulated and terminated by cytokines, chemokines, adhesion molecules, antibodies and other immune molecules produced by macrophages, dendritic cells, B cells, T cells and other immune system cells. These cells and molecules orchestrate wound healing, blood vessel growth, connective tissue formation, apoptosis, tissue regeneration and much else that is needed for body maintenance and defence against invaders. The task of the immune system is to append the right type of inflammatory response dynamically over time according to the shifting needs of the tissues. Immune system cells patrol the body continuously and sense the defence and maintenance needs of the individual. The immune system integrates enormous amounts of information, stores the information in its antibody and lymphocyte repertoires and uses this information to express the type and grade of inflammation needed at each site and at each moment. The immune system records and knows the body's most critical secrets; the immune system uses what has been called the immunological homunculusn[3]. In short, the immune system functions as the *bio-informatic computer, defence force* and *public works department* of the body (Figure 1).

BIOMEDICAL APPLICATION

Theoretically, the immune system computer should be able to provide us with both the history and potential of its activity, which in practical terms translates into vital diagnostic and prognostic information. To learn about the state of an individual's body we only need consult the immune system computer. In practice, however, it has been feasible up until now to view only a very limited part of the computational output of the immune system. Traditionally, immunological diagnosis has been based on an attempt to correlate each disease with a specific immune reactivity, such as an antibody or a T cell response to a single antigen specific for the disease entity. This approach has been largely unsuccessful for three main reasons. First, a specific antigen or antigens may not have been identified in the disease (for example, Behçet's disease, rheumatoid arthritis, and others). Secondly, immunity to multiple self-antigens, and not to a single self-antigen, is manifest in various patients suffering from a single disease (for example, a dozen different antigens are associated with type 1 diabetes). Thirdly, a significant number of healthy persons may manifest antibodies or T cell reactivities to self-antigens targeted in autoimmune diseases, such as insulin, DNA, myelin basic protein, thyroglobulin and others. For this reason false-positive tests are not uncommon. Hence, there is a real danger of making a false diagnosis based on the determination of a given immune reactivity. Novel approaches, therefore, are needed to support

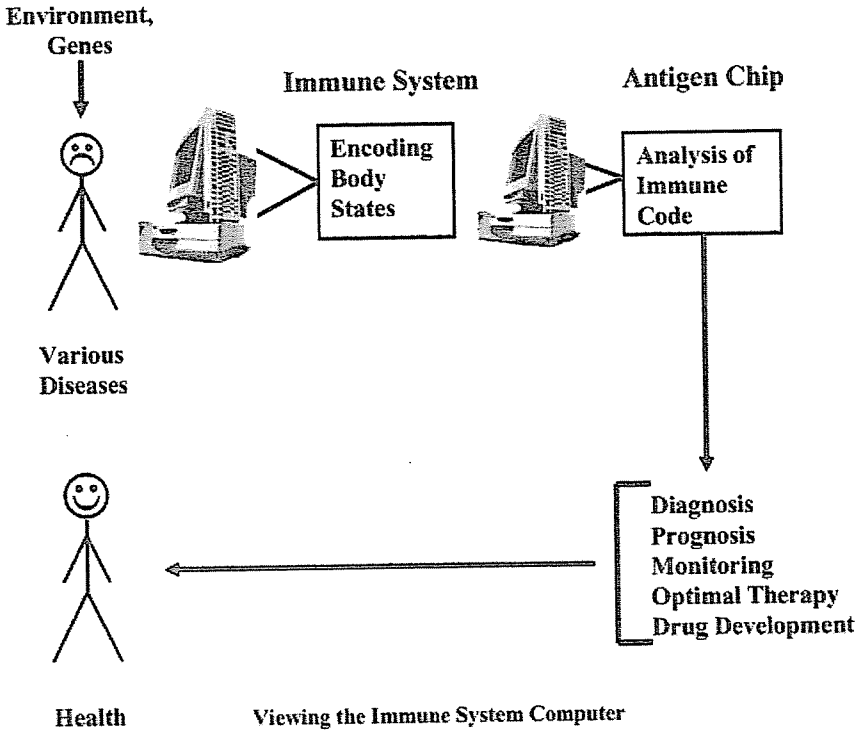

Figure 1 Viewing the immune system computer. The states of the immune system encode the various states of the body in disease. The antigen chip by viewing, as it were, the 'computer screen' system, can report to us on the state of the body

the diagnoses of specific diseases in a way that would justify specific therapeutic interventions. Because immunologists till now have been able to focus on one or a few antibodies, cytokines or T-cell clones at a time, we have lacked the comprehensive view of the immune system needed to facilitate diagnosis, prognosis, drug development, treatment optimization, patient stratification, prediction of response to treatment, and monitoring. Our approach is unique in that it gathers data from the global repertoire of antibodies reactive with key molecules of the body. Our antigen chip will provide a unique tool for post-genomic monitoring and management of human health and disease.

DISEASES

Antigen chips would appear to provide access to the immune system's bio-informatic computer, and thus constitute a platform for the development of numerous chip applications.

Autoimmune diseases are an obvious target for the use of antigen chips. We have used a global antibody assessment guided by bio-informatics to successfully separate various healthy and autoimmune disease populations[2]. *Infectious diseases*, too, require better diagnostic discrimination between persons who will be susceptible to a particular vaccination and persons who will not respond. Certain infections can trigger autoimmune responses, and it is important to be able to diagnose persons who are destined to develop autoimmune diseases. Hepatitis viruses are notorious for inducing chronic inflammation that may lead to hepatocellular carcinoma. The antigen chip could be used to screen for potentially dangerous changes in the autoimmune antibody repertoire resulting from such infections.

Immunotherapy of cancer is another situation in which it would be advantageous to classify persons with different types of immune reactivities to self-antigens; many, if not most tumour-associated antigens are self-antigens. Thus, it could be important in the design of therapeutic tumour vaccines to know what kind of immune potential is present in the patient.

Monitoring: assays for monitoring the state of the immune system are needed. Various immunologic therapies are now being developed. There is a critical need to develop markers that will enable the physician to monitor the response of the immune system to various treatments designed to modulate or arrest chronic inflammation and autoimmune diseases, vaccinate against infectious agents, or effect the immunotherapy of cancer. Furthermore, the monitoring of the overall breadth of the *recovering* immune system becomes crucially important in individuals who have received chemotherapy and stem cell transplants for leukaemias and other cancers. An immune system with a broader repertoire reflects one with more potential to combat infections.

Medicine badly needs predictive markers to stratify subjects and design trials based on 'inside information' provided by the immune system regarding the response of the test subjects to treatments. Immunomodulatory and anti-inflammatory drugs have focused on the disease as the only endpoint, and have failed to monitor the cause of the disease. The immune system can provide us with the inside information needed to optimize effectiveness and save time in arriving at dosing and other variables. Indeed, the patterns of reactivity detected using microarray chips will make it possible to profile subjects and identify those who are more likely or less likely to respond to a particular treatment. The new informatics can be expected to provide a new outlook on important medical problems; the antigen chip provides a voice for the immunological homunculus to tell us about the individual's body state[3].

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