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PAPER

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

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Traditionally, immunologic 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. The state of the body, however, appears to be encoded by the immune system in collectives of reactivities and not by single reactivities. Here we describe our use of microarray technology and informatics to develop an antigen chip capable of detecting global patterns of antibodies binding to hundreds of antigens simultaneously. The patterns fashion diagnostic signatures. *Lupus* (2006) **15,** 428–430.

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The immune system: an individual bio-informatic computer

To endure the hazards of existence, the individual needs to be defended, and the immune system defends us against infectious agents, foreign cells and molecules, and abnormal cells arising within our own bodies. But defense is not enough. The integrity of the body also demands maintenance.

The immune system is the key player in body maintenance.[1] The immune system expresses both the genetic endowment of the individual and the life experience of the individual; the immune system deals with post-natal adaptation to life. Like the central nervous system, the immune system is self-organizing:[2] 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.

The output of the immune system is a complex series of processes termed inflammation.[3] 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 defense 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 defense 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. In short, the immune system functions as the bio-informatic computer, defense force and public works department of the body.

Immune diagnosis

If we need to know about the past immune history, the present state of the body and the future susceptibility of the individual, it would be very useful to be able to consult the immune system. 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. In practice, however, it has

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been feasible up until now to view only a very limited part of the computational output of the immune system. Traditionally, immunologic 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 (eg, Behçet's disease, rheumatoid arthritis and others). Second, 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;[4] dozens of different autoantibodies have been linked to lupus.[5] Third, 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.[6] 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.

Immunological homunculus

Indeed, many natural antibodies detected in healthy subjects are autoantibodies; they bind to self-molecules.[6,7] The functions of natural autoantibodies are not clear, but the specific self-molecules recognized by these autoantibodies appear to form clinically defining signatures: some autoantibodies create a pattern that heralds susceptibility to a future autoimmune disease,[8,9] while a different autoantibody pattern can mark resistance to the disease.[9]

It has been proposed that natural autoantibodies and auto-reactive T cells in healthy individuals may be directed to a specific and limited set of self-molecules; this selective autoimmunity has been termed the immunological homunculus[10–12] or immunculus[13] – the immune system's internal representation of the body. From this point of view, the emergence of an autoimmune disease is not caused by the emergence of an autoimmune lymphocyte clone – healthy people are filled with them – but rather the disease emerges from a defect or weakening of the natural regulation of homuncular autoreactivity.[1,10] Thus, it would seem that a subject's immunological homunculus – the global pattern of autoreactivity organized and expressed within the individual's immune system – can inform us about the individual's state of health or disease – past and present, with some predictive power for the future. How then can we get the homunculus to talk?

The antigen chip

A full description of the immunological homunculus would require information about an individual's T-cell antigen specificities (repertoire) and frequencies of T-cell functional types (Th1, Th2, Th3, CTL, Treg and so forth), their B-cell repertoire, and B-cell types, autoantibody repertoire and antibody isotypes, and innate immune cells (macrophages, dendritic cells, neutrophils and so forth). Most of this information is not accessible – in fact, much of it is not characterized in detail, but known only in general terms. Nevertheless, antibodies are precisely measurable and the patterns of one's global repertoire of autoantibodies in blood and body fluids is accessible. The autoantibody homunculus, at least, can be consulted. Moreover, microarray technology combined with advanced informatic technologies has opened new opportunities for approaching the vast information stored in antibody repertoires. We set out to develop an antigen chip to mine this information.

As a first step in developing the antigen chip, we studied arrays of antigens using standard ELISA microtitre plates, and applied this approach to characterize repertoires in mice. Despite the technical limitations of limited wells of antigens and large amounts of reagents, we were encouraged to discover that the known susceptibilities to experimental autoimmune diseases of inbred stains of mice were evident in their natural homuncular repertoires; mice express disease-associated autoantibody reactivities without being intentionally immunized to the particular antigens.[8,14] Immunization induces the clinical expression of the disease imprinted in the homuncular repertoire. We even succeeded in confirming the unsuspected susceptibility of NOD mice to experimental autoimmune myasthenia gravis based on their natural repertoire.[15]

The variability of outbred humans required bioinformatics to discover meaningful repertoire patterns. We applied clustering algorithms to successfully detect patterns of antibody reactivities indicative of persons with type 1 diabetic.[16] Additional bioinformatics analysis demonstrated that we could discern discriminating diagnostic repertoire patterns not only in autoimmune diseases such as type 1 diabetes and multiple sclerosis, but also in Behçet's disease, an inflammatory condition of unknown etiology and in type 2 diabetes, a metabolic disease.[17]

On this basis, we went on to develop the antigen microarray chip.[9] A robotic apparatus is used to spot molecules of choice – proteins, peptides, sugars, lipids, nucleic acids – to a coated glass slide, and the molecules are covalently linked to the surface (see Figure 1); a drop of blood or other body fluid can be tested for antibodies binding to hundreds to thousands of these antigen spots. The subject's bound antibodies are detected using

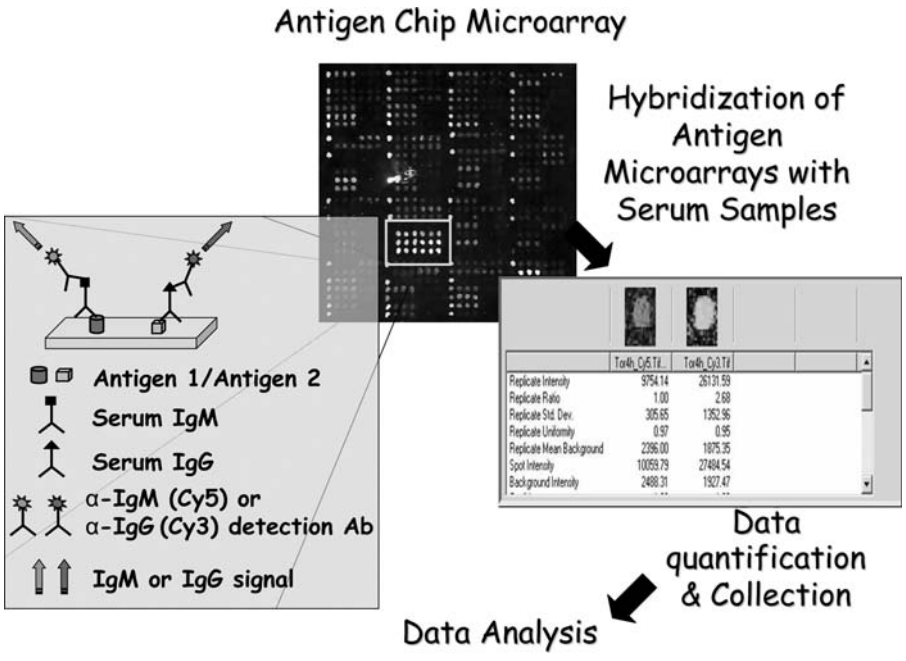

Figure 1 A schematic representation of the antigen microarray chip.

fluorescence-labelled second antibodies specific to human isotypes (IgG, IgM, IgA), and the reactions are developed by laser activation. In addition to being able to measure thousands of antibodies in a few microlitres of fluid, the microarray device is considerably more sensitive and has a much greater window of reactivity than the standard ELISA/microtitre assay; meaningful fluorescence intensities of a single spot may range from 0.01 to 65 000 units using the present, first-generation chip.

The autoantibody reactivities simultaneously generate an immune system signature that profiles the individual's state of health or disease. We are in the process of writing for publication the results of several studies in human diseases, including autoimmune diseases such as lupus and tumours. We have also used the antigen chip to characterize the primordial immunological homunculus made in utero by the developing human fetus (unpublished data). Notably, a study in mice shows that antigen chip technology and informatics makes it possible to predict future diabetes in a defined mice model by analysing the patterns of autoantibodies to hundreds of self antigens.[9] Thus, a proof-of-concept has been achieved; patterns of a system's reactivity can be more informative than any of the single reactivities that form the system.[1]

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