talk should be considered a criminal offence. The knowledge about bioweapons is everywhere, but requiring terrorists to

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navigate the intricacies of scientific data is quite different from providing them with ready-to-use ideas! We should refrain from speaking about these matters, except with the appropriate representatives of the people, who may implement policies to protect our fellow citizens. And of course, we should support more work in microbiology to defeat those irresponsible persons, who condemn us to live with all kinds of plagues.

All this is easy to say, but, unfortunately, our 'democratic' system dictates otherwise. We will find an infinite variety of reasons to allow everybody to act as they please in the name of 'freedom'. But this is not freedom. Freedom always entails duties and responsibilities. This means that any act must be seen in its social context—after all, we are social animals, and this is the basis of democracy—and in its biological context, because our behaviour necessarily affects all other living organisms.

But many scientists have an ego so inflated that it is simply impossible for them to think of responsibility when it is

their own moment of glory and the fashion for 'Impact Factors' and a lot of pseudo-scientific measurement of science quality supports this attitude. If we want to fight terrorism, we first must uproot it from our own behaviour, simply by recognising that we have to *share* freedom, not to impose our freedom on others. This means that scientists must think of the consequences of what they are doing and, sometimes, refrain from speaking publicly or even from trying to know more. I do not believe that any God will help us or redeem us of our deeds, and so the responsibility rests entirely with us. Our knowhow is such that, if we do not

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adopt a more responsible attitude, we may ultimately destroy humanity. It is time to take the steps needed to construct a better future for Man and many other living species, be it at the price of ignorance.

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Still waiting for the end

The promise of genomics and proteomics for autoimmune diseases • by *Dimitrios Balomenos, Carla Carvalho-Pinto and Carlos Martínez-A.*

Autoimmune diseases are fairly common in the human population, affecting ~5–10% of people in the developed world. They are serious diseases as well: as vital organs or cells are slowly destroyed by the immune system, the quality of life for patients gradually decreases, leading in many cases to premature death. Some are devastating diseases, such as multiple sclerosis (MS) or rheumatoid arthritis (RA), and cannot

be cured at all; others, most notably type 1 diabetes, can be managed with life-long therapeutic intervention. In any case, as

on the individuals affected as well as on health care systems. Although autoimmune diseases are not among the lead-

As many autoimmune diseases require costly long-term treatment, they place a burden on the individuals affected as well as on health care systems

many autoimmune diseases require costly long-term treatments, they place a burden

ing diseases in the developed world, it is nevertheless more than justified to work

towards understanding their aetiology and to design effective therapies.

Most known autoimmune diseases—currently about 70—share three important features: aggression of the immune system toward the body's own cells, termed 'loss of tolerance'; the effect of sex steroids on disease propensity—more than 90% of patients of some autoimmune disorders are women (Whitacre, 2001); and the gradual onset and non-specific nature of early manifestations. This last

characteristic often leads to delayed or incorrect diagnosis, rendering timely therapeutic intervention more difficult. The most common and well-studied autoimmune syndromes include RA, type 1

Many excellent reviews have been written on the mechanisms involved in the acquisition of immune tolerance and

with respect to disease characteristics (Theofilopoulos and Dixon, 1985). In one strain of mice—as for human SLE—disease development depends on female hormones, while disease propensity in another strain is linked to the Y chromosome.

Studies of identical twins provided early evidence for the role of inherited factors in autoimmune diseases—SLE and IDDM show the greatest genetic predisposition, with concordance levels of up to 50% (Wanstrat and Wakeland,

A gene expression reference constitutes a starting point from which current methodology leads to understanding and treatment of autoimmune disease.

The non-specific nature of early manifestations often leads to delayed or incorrect diagnosis, rendering timely intervention more difficult

diabetes, also known as insulin-dependent diabetes mellitus (IDDM), systemic lupus erythematosus (SLE), MS, myasthenia gravis and thyroiditis.

Despite considerable efforts in this field of research, a thorough understanding of autoimmune disease has so far been elusive. This is due mainly to the complexity of these disorders, which involve many genetic traits as well as environmental factors. Nevertheless, some progress has been made in the last decade: analysis of rodent autoimmune disease models and the more recent use of knockout technology and linkage analysis of disease susceptibility loci have contributed towards a better comprehension of autoimmune disorders. Furthermore, the potential of new technologies such as the sequencing of the human genome, as well as advances in genomics and proteomics, now provides immunologists with a new box of tools with which to study these diseases and eventually devise effective therapies.

their failure, as well as on the role of environmental and genetic risk factors in the development of autoimmune diseases (Mackay, 2000). But despite the accumulated information, the reason that the

immune system turns against cells of the body remains a mystery. A number of mechanisms—complement lysis, responses to cell receptors, preferential T-cell polarisation, cytolysis and recruitment of inflammatory leukocytes—are being discussed as responsible for auto-reactivity. From the genetic point of view, the underlying cause of autoimmunity is still unknown; there is evidence that environmental factors such as microbial infections may contribute to disease onset, but the data available are not yet sufficient to support this hypothesis.

Some of our current insights are based on early mouse models that correspond to diverse autoimmune syndromes. These immediately suggested the role of various immune cell types, cytokines and the involvement of apoptosis, but they also hinted at the overall complexity of these syndromes. For example, mouse models of lupus all eventually develop anti-DNA antibodies and glomerulonephritis, but they show considerable heterogeneity

2001). Further progress in linkage analysis of man and mouse has allowed systematic examination of the genes involved. These studies showed that major histocompatibility complex genes play a critical role in the development of autoimmune diseases, which was suggested earlier by mouse models of IDDM and SLE arthritis.

Linkage analyses nonetheless failed to discover those genes that actually cause autoimmune diseases, and the multifactorial nature of autoimmune disease genetics certainly complicates their identification. However, it has revealed the role of genetic heterogeneity by which

From the genetic point of view, the underlying cause of autoimmunity is still unknown

distinct genetic defects may lead to a similar disease (Wanstrat and Wakeland, 2001), and that of epistatic interactions by which one genetically altered locus may affect another. The combination of two weak susceptibility loci may thus result in greater disease susceptibility than would be predicted for each defect alone; a specific locus may also have a suppressive effect on a genetic background otherwise sufficient for disease development. Identification of disease genes is further hindered by environmental and stochastic

events that influence disease development. Although linkage analysis has not been as productive as originally hoped, it has helped to understand the nature of inherited traits in autoimmunity. And new tools, including a database for single-nucleotide polymorphisms, will facilitate

(Sakaguchi *et al.*, 2001). These cells probably play an important role not only in control of autoimmunity, but also in governing tumour immunity and transplantation tolerance. The mechanisms underlying the regulatory effect are not yet clear, but it is postulated that to exert

determining mRNA levels—an array can include tens of thousands of probes and can therefore measure the expression of an equivalent number of genes in a single experiment (Lockhart and Winzler, 2000). Since the result of gene expression is the production of proteins that constitute the targets for therapeutic approaches, the field of proteomics is complementary to that of genomics (Pandey and Mann, 2000).

Microarray technology is also expected to initiate a new era in genetic linkage analysis that will facilitate the study of phenotypes of patients and their relatives, providing new impetus and strategies for mapping disease traits. Based on the analysis of expression phenotypes, it will provide tools for early diagnosis before disease onset and provide independent, objective definitions of the pathological process (Wanstrat and Wakeland, 2001). Although immunological analysis of autoimmune disease patients indicates common features in some diseases (Tsokos and Kammer, 2000), linkage analysis suggests that genetic aetiology varies. Genomics and proteomics could classify each patient's phenotype and define the potential severity of the disease as well as the response to therapy, on the basis of which individualised treatment could be designed.

Basic immunological studies and analysis of knockout mice indicated the critical role of cytokines in autoimmune disease, making these molecules potential targets for therapeutic intervention (Kroemer and Martínez-A., 1992). The most outstanding example is the use of anti-TNF treatment in RA, despite incomplete understanding of its immune aetiology. Using an antibody to this cytokine, or blocking the action of its receptor, has shown solid

Immunological studies, genomic linkage analysis and knockout technology have not yet clarified the pathway of disease development for any autoimmune disorder

linkage analysis in this complex research area. The sequencing of the human and the mouse genome will further aid in finding markers and candidate genes, identifying disease genes and helping to understand the diverse pathways that lead to autoimmune diseases.

Quite often, new concepts in science derive from unpredicted sources. A new insight into the causes of autoimmune diseases has come from analysing mice deficient in molecules that were not obviously related to autoimmunity. One example is that knockout mice lacking DNase 1 or the serum amyloid P component, which is linked to Alzheimer's disease, indicated that defective clearance of debris that resulted from programmed cell death—apoptosis—may lead to SLE (Maekawa and Yasutomo, 2001). Also, lupus-related characteristics were unexpectedly observed in mice lacking the cell cycle inhibitor p21, prompting us to suggest that regulators of the cell cycle have a unique role in regulating the immune response (Balomenos and Martínez-A., 2000), since lymphocytes cycle in an intense, prolonged and repeated manner to establish an immune response and generate immunological memory. The production of mice deficient in key immunological molecules has also been helpful in providing clues as to the origins of autoimmune disease, and may eventually contribute to the design of medical treatments.

Tracking these clues returned the study of immune regulation, one of the classic issues in immunology, to the forefront of research. There is accumulating evidence that regulatory lymphocytes (Treg cells) contribute to the maintenance of self-tolerance, which is at the very core of autoimmune diseases. Sakaguchi and co-workers showed that a reduction in Treg cells caused by environmental or genetic factors leads to autoaggression

suppression of self-reactive T cells, Treg cells require low antigen concentrations and are more effectively activated by self-mimicking molecules, preventing auto-aggression. Alternatively, Treg cell may compete efficiently with self-reactive T-cells for interaction with antigen-presenting cells. Furthermore, they may restrain autoreactive T-cells and subsequently prevent development of autoimmunity (Sakaguchi *et al.*, 2001).

This knowledge is promising, as it unveils some of the subtle mechanisms involved in turning on an immune response against self. As pointed out above, classical immunological studies, genomic linkage analysis and knockout technology together have yielded only partial information on autoimmunity, as they have not yet clarified the pathway of disease development for any autoimmune disorder. These approaches attempt to fish out key molecules involved in disease development from a myriad of unknown genes; the strategy was more systematic in the case of genomic linkage, but the majority of genes implicated remain unknown.

Fortunately, new approaches such as genomics and proteomics are now available and may yield new information on autoimmune syndromes and other

Genomics and proteomics could classify each patient's phenotype and define the potential severity of the disease as well as response to therapy

diseases by providing a reference point for gene expression. Gene activation and variation in mRNA expression levels depending on the cellular state or phenotype is an evolving research area, which was initiated by northern blot analysis, RT-PCR, differential display and serial analysis of gene expression (SAGE). Functional genomics is much more powerful than other techniques in

results in RA treatment, and further approaches in these therapies are currently being investigated (Feldmann, 2001). IFN- γ is another key cytokine implicated in autoimmune disease; it is involved in autoantibody generation in mouse models of SLE and myasthenia gravis, as well as in regulating the inflammatory process in glomerulonephritis development. IFN- β and CTLA-4 may

also be appropriate targets for treatment of MS and psoriasis, respectively. The chemokines are a group of inflammatory cytokines regulating immune cell migration that are now being explored as therapeutic targets, and prospects for

We believe that a combination of new and established technologies will eventually resolve the causes of autoimmune diseases and lead to the design of novel therapies

treatment may be within our grasp (Godessart and Kunkel, 2001).

In the last few years, scientists have placed emphasis on gene therapy to improve the effectiveness of cytokine- and chemokine-based treatments. Although there are still problems related to gene delivery methods and in achieving local effects to avoid systemic problems, this method has been effective in the treatment of autoimmune diseases in animal models, and clinical trials to treat RA in humans are under way (Tarner and Fathman, 2001). Cytokine-targeted treatments do not constitute the only therapeutic strategy. Immunological treatments have also been proposed and are being tested, taking advantage of our knowledge of the immune cells participating in each disease, the association of co-stimulatory molecules, the process of apoptosis or cell cycling, and the newly re-emerged concept of Treg cells.

As new therapeutic targets are being discovered and gene therapy becomes a reality, the effective treatment of autoimmune diseases is looking more promising. Recent advances in tissue repair engineering also have the potential to revolutionise classical treatment of autoimmune disease by combining immune-based therapies with replacement of damaged tissue. Such a strategy could be applied, for instance, to replace islet cells lost through diabetes, or to substitute joint cartilage after RA, with possible applications to other autoimmune diseases. Following interaction with appropriate growth factors, stem

cells from the blood or bone marrow could be programmed to differentiate into these tissues. But although scientists expect many advances in this area, considerable progress is still needed before this field will provide applications for autoimmune diseases.

The allure of the new genomic and proteomic technologies lies not only in the vast amounts of information that these technologies are expected to generate, but in their synergy with classical immunological studies and the technical advances of the last decade. We believe that the combination of these approaches will resolve the causes of autoimmune diseases, and lead to the design of novel therapeutic treatments. This is perhaps an optimistic vision of what may be expected in the future; from the sceptic's point of view, the cost of applying these technologies and the need for more specialised researchers could impede rapid progress in this field. But investment in this research will certainly pay dividends in lower health care costs, longer life expectancy and improved quality of life for those who develop one of the many autoimmune diseases.

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