The problems and promises of research into human immunology and autoimmune disease

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Translational research in autoimmunity is hampered by a number of hurdles, including a lack of knowledge regarding initiating and pathologically relevant autoantigens, the low frequency of autoreactive pathogenic B and T cells, difficulty in accessing the affected tissue, differences between self-reactive and pathogen-specific lymphocytes, a lack of etiologically relevant preclinical animal models and the heterogeneity of disease presentation. Given the need for biomarkers and new therapeutics, it is imperative that these hurdles be surmounted.

From rodent models to the clinic

Preclinical animal models of human autoimmune diseases have been invaluable for the discovery of key immune processes, basic disease mechanisms and candidate immune-targeting strategies with clinical utility. Preclinical studies in mice have resolved issues of dosing, the impact of environmental factors on disease and, at times, clarified the potential causes of failed human trials. These findings point to the potential usefulness of animal models, provided they are tested and interpreted properly.

In multiple sclerosis, several treatments were first identified as effective in the animal model of disease, experimental autoimmune encephalomyelitis (EAE), before entering the clinic. A well known example of one of these treatments is the highly effective, albeit somewhat risky (given the risk of developing progressive multifocal leukoencephalopathy), biologic natalizumab, which blocks the cellular adhesion molecule α4 integrin and is given to patients with multiple sclerosis with a very active course. In multiple sclerosis research, initial limitations of the animal model (involving induction of EAE in Lewis rats) led to the development of more sophisticated experimental designs. Different disease courses, namely a remitting course compared to a progressive course, can be induced in mice using different disease strains and distinct protocols. Neuronal pathology, a hallmark of multiple sclerosis, can be mimicked and investigated in EAE models. These models can be used to identify underlying mechanisms relevant to individuals with multiple sclerosis. In addition, spontaneous disease models are available, which provide the opportunity to study disease onset. Humanized animal models are currently being generated and improved on in different laboratories and may be implemented in future studies.

Although these models have proven useful in identifying and validating mechanistic predictions, the inability to translate preclinical leads in clinical trials has led to a reassessment of the causes of these failures. Some may have been caused by an inappropriate experimental design of the animal studies and not the animal model itself. For example, the treatments from two thirds of the studies in autoimmune diabetes that were then tested as therapeutic interventions were examined preclinically in a preventive setting. Most studies are also terminated too early, with only 7% of studies in non-obese diabetic (NOD) mice being followed up beyond 32 weeks. Ultimately, an animal model is only as good as the questions asked of it.

The inability to predict the likelihood of success of therapies in humans using animal models may be related to the distinct differences in the immune systems of mice and humans. Any immune phenotype or immunological process or mechanism may have species-specific features that preclude direct extrapolation or comparison between mice and humans. Marked differences between the immune systems of mice and humans include key discrepancies in both innate and adaptive immunity. In a published list of more than 80 of these differences, some examples include the differential expression of regulatory T (Treg) cell markers (notably, forkhead box P3 (Foxp3)), variations in the balance of leukocyte subsets, defects in antigen-presenting cells, dysregulation of thymic selection, differences in the role played by cells that produce interleukin-17 (IL-17) in disease, inflammation and immune regulation and complement deficiencies. This large number of disparities between mice and humans has a considerable impact on the differences in the immune processes that drive the development of autoimmune disorders in the two species.

The time has come to challenge the notion that immunologic processes in mice are similar to those in humans. Any given animal model...
will not be representative of the entire breadth of molecular and clinical heterogeneity present in human populations but, rather, the animal model more likely corresponds to specific aspects of the complex disease manifestations in humans observed in the clinic. This hypothesis has implications for the truisms entertained by many institutional review boards, the US Food and Drug Administration and the European Medicines Agency that any immune intervention therapy should first be validated using preclinical animal models. The key issue here should be how well the animal model reproduces the specific immunologic characteristics of human disease. To address this, we must first understand the unique immunologic alterations present in each human autoimmune disease. This approach will allow for the use of relevant models to test safety and assess clinical efficacy so that drugs that perform well at the preclinical stage will be more likely to be efficacious in humans. Mechanistic studies in mice can only be tested in humans. In these cases, a proper experimental design assessing the breadth of the immune response using whole-blood assays (rather than peripheral blood mononuclear cells) from human donors may be used to screen for potential adverse events (for example, the cytokine storm that results from treatment of patients with the CD28 super agonist\textsuperscript{13}) that could not be foreseen using animal models.

**Disease heterogeneity and classification**

Each individual with an autoimmune disorder presents with unique clinical features. Currently, autoimmune diseases are classified by the simultaneous presence of a number of clinical symptoms, but there is a lack of understanding of the specific immune deviations underlying these symptoms. The majority of these etiologic differences may result in a modest phenotype, however, some of the differences may have a robust impact on immunological processes that lead to autoimmunity and therefore influence the patient’s responsiveness to immune-modulating therapy. This suggests that we need to better assess genetic and immunologic alterations in larger patient cohorts, examining both the natural history of disease and each patient’s response to therapy. In this way, we can understand how each of these features contributes to disease risk, initiation and progression, and we can use these features to differentiate patients into subsets with respect to their predicted response to therapy.

This method is exemplified by findings in rheumatoid arthritis showing that the disease represents two main syndromes: autoantibody-positive and autoantibody-negative disease\textsuperscript{14,15}. These two syndromes are often clinically indistinguishable, certainly at a patient’s first visit, but are etiologically distinct, with different underlying genetic and environmental risk factors and different histologies\textsuperscript{14–16}. These differences alter a patient’s susceptibility to immune-modulating therapy. Phase 3 clinical trials assessing the efficacy of teplizumab (a humanized monoclonal antibody to CD3)\textsuperscript{17} and abatacept\textsuperscript{18} in diabetes further underscore the heterogeneity of autoimmune diseases and the differing responses to immunotherapy among patients\textsuperscript{19}.

Recent studies point to a suite of genetic risk factors that are shared across various diseases but that have differing clinical presentations\textsuperscript{20}, implying shared mechanisms of disease pathogenesis and the possibility that immunomodulatory strategies in one autoimmune disease may be effective in another seemingly unrelated disease. Although the role of B cells in the pathogenesis of type 1 diabetes (T1D) remains unresolved\textsuperscript{21}, immune intervention with rituximab, a monoclonal antibody targeting B lymphocytes expressing CD20, temporarily delayed a progressive loss in β cell function in newly diagnosed T1D\textsuperscript{22}, was beneficial in multiple sclerosis and is an accepted therapy for rheumatoid arthritis\textsuperscript{23}.

To investigate immune processes and disease mechanisms in human tissue, there is a need for large repositories of biological samples from both affected patients and healthy subjects (Table 1). Researchers in the field of autoimmunity have access to a number of repositories, including birth cohorts to study the natural history of inflammation and disease, blood samples of patients before and after diagnosis to study disease progression after clinical manifestation and repositories of disease intervention

### Table 1 Barriers and opportunities in translational research in human autoimmunity

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<td>Define peripheral immune correlates acting as surrogate markers of inflammatory tissue lesions</td>
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is thereafter followed by a rather stereotypic sis, the relapsing-remitting course of disease become increasingly clear. In multiple sclerosis, progression and response to therapy has prediction of therapeutic response Biomarkers: diagnosis, prognosis and predictive utility of MRI scans (for example, acute inflammation or relapse are also not pos-
ture spreading by the time of disease onset. Finally, the antigen driving the disease may be unknown or there may not be a specific driving antigen present at all (for example, in psoriasis and inflammatory bowel disease), which poses additional challenges for the immune monitoring of disease progression and therapeutic intervention. Biomarkers can also be used to predict or monitor a patient’s response to therapy. The presence of autoantibodies against thyroid peroxidase (TPO) in T1D islet allograft recipients before transplantation can predict the development of Graves’ disease upon the tapering of immune suppression after loss of allograft function.11. Cellular immunological biomarkers are more challenging to accurately determine, but baseline autoreactive CD4+ or CD8+ T cell responses predict the clinical efficacy of β cell replacement therapy, whereas their recurrence in circulation mirrors recurrent islet autoimmunity and loss of islet graft function.33. In rheumatoid arthritis, the efficacy of methotrexate treatment can be modeled by measuring a set of laboratory, genetic and clinical parameters. In multiple sclerosis, immunological surrogate markers of the therapeutic response to interferon β35–36 and glatiramer acetate37 have also been described. The inaccessibility of the affected tissue in some diseases makes it difficult to monitor disease progression and a patient’s response to therapy. The imaging of pathological lesions is the ideal way to monitor the efficacy of a therapy, but this imaging is not feasible using non-invasive methods in many autoimmune diseases. Therefore, studies of the immune response to therapy in human immunology are often confined to assessments of peripheral blood components that may act as surrogate markers of the pathology in the target organs. Although there are examples of immune responses in peripheral blood that associate with lesional autoreactivity38–39 or that correlate with disease progression or recurrence, associations between lesional and peripheral immune correlates have not been confirmed.40

Given the dearth of reliable, robust and confirmed immune correlates of disease progression and intervention in autoimmune disease, there is a need for new technologies that allow for the monitoring of immune or autoimmune responses in relation to disease prediction and progression and the efficacy of intervention.41

These technologies must deal with several particular features of autoimmune responses, such as the discordance between autobody titers and disease severity, the intrinsic characteristics of autoreactive T cells (low TCR avidity and epitopes that bind with low affinity to HLA), the low frequency of autoreactive T cells in the circulation33,44 and the presence of immune regulatory processes that mask autoimmunity in vitro and ex vivo assays, such as T cell proliferation assays, enzyme-linked immunosorbent spot and intracellular cytokine staining, which have proven very effective in monitoring immune responses to pathogens, viruses and tumors48, seem to be limited in their ability to detect specific autoreactive T cells.

Flow cytometry has been a fundamental tool for the discovery and definition of rare cell subsets in the immune system. Technological improvements in flow cytometry and cell sorting, including the introduction of new fluorophores such as quantum dots, have increased our knowledge of disease processes and intracellular features, including transcription, signaling, apoptosis and the cell cycle, which has aided in the development of cytokmetry-based clinical diagnostics.43,45–47. Classical in vitro and ex vivo assays, such as T cell proliferation assays, enzyme-linked immunosorbent spot and intracellular cytokine staining, which have proven very effective in monitoring immune responses to pathogens, viruses and tumors, seem to be limited in their ability to detect specific autoreactive T cells.

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of diabetes epitopes. T cells specific to each of the epitopes were defined by the binding of major histocompatibility complex multimers with a unique combination of two quantum dots, allowing for a more accurate discrimination of low-avidity autoreactive TCRs from the background. Transition element isotopes that are not normally found in biological systems can be used as chelated antibody tags in atomic mass spectrometric analyses of single cells. Detailed signatures and response profiles of human leukocytes to drugs can be generated that permit the simultaneous measurement of up to 100 cellular parameters. Likewise, multiplex technologies allowing for the simultaneous detection of many different autoantibodies of differing reactivity are currently under development, which may lead to more refined stratification and analysis of patient subgroups and the response of patients in each of these groups to therapy.

Combination therapy

The complex etiology of autoimmune diseases suggests that successful treatment of these diseases may need to be directed toward multiple targets, each with differing modes of action. In rheumatoid arthritis, combination therapies outperform most single treatments in isolation. The advantages of such a strategy include the ability to realize synergies to enhance treatment efficacy while using modest drug dosages to minimize toxicities. In T1D, these types of combinations have not been tested, however, single agents have not been able to preserve islet function durably in T1D. A major challenge in testing combination therapies will be clinical trial design, as the current lack of immune correlates that define early efficacy of immune or disease-modifying monotherapy limits our ability to test the effectiveness of different combinations of drugs. These benefits notwithstanding, combination therapy can also increase the likelihood of adverse outcomes caused by greater immunosuppression, the development of opportunistic infections or adverse interactions between therapeutics where one agent decreases the effectiveness of the other (as is the case with thymoglobulin and sirolimus).

Early intervention

Preventive therapy before the loss of immune tolerance is ideal, but no genetic or immunological markers exist that predict this early preclinical phase of many autoimmune diseases. Furthermore, self-reactivity without the development of clinical symptoms is not uncommon. In several inflammatory diseases, including rheumatoid arthritis, inflammatory bowel disease and T1D, early and aggressive intervention may effectively slow disease progression. The effectiveness of early interventions may be due in part to the increasing damage inflicted by the inflammatory lesions later in the course of the disease (for example, bone erosion or loss of hormone-producing cells) and a reduced ability to define clinical efficacy once the disease is in its later stages. In multiple sclerosis, investigations into early aggressive therapies are still lacking, but are warranted, and may be easier to conduct once a prognostic marker for this disease is available. Options to intervene early in, or, preferably, prevent, autoimmune diseases depend on how risk is defined, the safety and side effects of the therapy compared to the disease burden and its complications and the burden and risks of an autoimmune disease once it is established. Unfortunately, most genetic risk markers and biomarkers have insufficient specificity and sensitivity for accurate prediction, and the relatively low prevalence of these conditions (on the order of a few percent of the population) means that genetic prediction (beyond family history) is probably not a realistic option.

In some autoimmune diseases, the predictive power of biomarkers, mainly disease-specific autoantibodies in subjects at risk, might be used to design clinical trials aimed at preventing the precipitation of disease. For example, pemphigus is one of the most clearly defined autoimmune diseases that is mediated by autoantibodies. The involvement of epitope spreading in the pathogenesis of pemphigus has been shown in pemphigus foliaceous, as subjects in the preclinical stage of this disease have antibodies that recognize different epitopes of desmoglein 1 than those present in subjects after disease onset. Likewise, in several instances of pemphigus, intermolecular epitope spreading has occurred along with alterations in the pemphigus phenotype, indicating that such autoantibodies might serve as markers to initiate preventive treatment. Likewise, an association between epitope spreading and the development of rheumatoid arthritis has been described. Although in these cases, no defined epitope could be identified, a broadening of the antigen repertoire recognized by autoantibodies, the presence of autoantibodies or an increase in the amount of such autoantibodies could be used to design clinical trials to test preventive treatments. Indeed, the identification of such markers has led to the first clinical interventions aimed at preventing rheumatoid arthritis at a very early stage. In T1D, intervention therapy in pre-diabetic subjects has been unsuccessful so far despite large international efforts that screened hundreds of thousands of relatives of individuals with T1D. This failure may have been a result of a lack of predictive biomarkers and our inability to define disease modification and immune deviation. The only intervention trial in T1D showing disease remission (defined as insulin independence) as a primary endpoint involved autologous hematopoietic stem cell therapy after chemotherapy. All other intervention trials in T1D relied on markers of β cell function and glycemic control, which may not accurately reflect preservation of β cell mass. None of these trials considered immune correlates, even as secondary endpoints of clinical efficacy, despite the autoimmune nature of T1D. This underscores the great demand for accurate and robust biomarkers in autoimmune diseases.

The emerging impact of genetics studies

Genome-wide association studies (GWASs) have revolutionized the genetic analysis of complex diseases, with hundreds of associations having already been identified and with more emerging all the time. In autoimmunity, these hypothesis-free screening approaches have consistently implicated immunological genes, sweeping aside any lingering doubts that inflammation may be a secondary consequence rather than a driving force of autoimmune disease. The extensive overlap in regions implicated in GWASs is striking but under this overlap is complex, with associated alleles increasing the risk of some diseases and reducing the risk of others.

The fact that the findings of the GWASs so far only account for a fraction of the observed heritability in autoimmune diseases and are individually rarely able to conclusively implicate a particular causal mechanism or even a specific gene in these diseases has somewhat detracted from the discoveries they have made. Unlike the genetic claims of the past, GWAS associations provide unbiased, statistically indisputable insight into disease etiology, regardless of the individual odds ratios associated with disease status. It is clear that detailed fine mapping and extensive functional analyses will be necessary to fully understand these new data, but new insights as a result of these data are already becoming apparent.

It is widely accepted that extensive fine mapping in very large cohorts will be necessary to separate out the functionally relevant variants driving these associations from the enormous number of correlated but neutral variations that characterize these findings. Such efforts are already underway for many of the loci identified and can be expected to deliver catalogs of associated variants at each site.

In addition to these purely DNA-based efforts,
biological sample repositories are developing (for example, the Cambridge BioResource and the Genotype and Phenotype (GAP) registry) to meet the challenges inherent in identifying the functional consequences of these associated genetic variants. Given that the correlation between genetic variation and disease status is inevitably only a shadow of the correlation between such genetic variation and the intermediate biologically meaningful phenotypes, for example, gene expression and cellular activity, these functional studies will be substantially more powerful but will need to include individuals with uncommon genotypes and consider relevant target tissues. It seems inevitable that the study of genetics will radically improve our understanding of the immune system, both unperturbed and in the context of autoimmune disease. GWASs have already identified genetic variants that cluster in particular immune pathways, and the functional implications of these variants are now being identified through studies in healthy control individuals as well as in affected patients.86-88.

The study of genetics also has the potential to identify factors influencing disease course and severity, although for the field of genetics to succeed in this area, the clinical heterogeneity in the natural course of the disease needs to be reliably measured and separated from the confounding influence of treatment and the environment, which has probably not yet been achieved.89

Linkage and interaction in common autoimmune diseases is rare, with Crohn's disease providing a notable exception.31-33. It therefore seems unlikely that we will see many rare highly penetrant alleles that are responsible for monogenic forms of common autoimmune diseases. However, less common variations (with alleles frequencies in the range of 0.5–5%) have largely been unexplored and are starting to yield new insights.87,88. Similarly, it seems likely that the extent of missing heritability has been overestimated and that many associated common variants remain to be identified,88 and it is hoped that we will see larger studies and comprehensive meta-analyses in the future. For some common variants, particular alleles, such as HLA, associate with particular types of autoimmune responses (autoantibodies compared to autoreactive T cells) or types of autoantibodies.89. In rheumatoid arthritis, distinct genetic risk factors are associated with distinct phenotypes, implying that different immunological and pathogenic mechanisms underlie rheumatoid arthritis that is positive for antibodies to citrullinated proteins and rheumatoid arthritis that is negative for these antibodies.14

Pharmacogenomics is another area in which genetics seems highly likely to deliver results. Although this is a relatively unexplored area in autoimmunity, it seems inevitable that response rates and sensitivity to adverse reactions to treatments will be altered by gene variations in receptor function, drug metabolism pathways and the signaling response to biologicals (fragment C receptor (FcR) polymorphisms) and other agents such as methotrexate.34,35

Integrating genetic risk factors with relevant environmental effects also seems likely to increase our understanding of disease pathogenesis, although this may prove to be the most challenging aspect of clinical immunology given how difficult it is to establish the relevance of individual environmental effects. Animal models have provided compelling evidence of the importance of environmental factors in disease etiology,12 which is in keeping with what has been seen in human epidemiology.90

**Moving forward**

Despite considerable progress in our understanding of the etiology of autoimmunity diseases and the development of new therapeutics to treat them, multiple barriers to our understanding of these diseases exist. The ultimate goal is to define disease mechanisms, develop therapeutics targeted to these mechanisms and develop strategies to evaluate the safety and efficacy of these therapies. The hurdles that must be overcome before this goal can be met include the inability to define the heterogeneity of human subjects at the molecular level, to develop models that faithfully reflect human disease or to assess the immune system at the site of disease pathogenesis, as well as the difficulties in assessing therapeutic outcome early in the course of disease. Recognition of these hurdles has led to new approaches in the study of autoimmune diseases. Biorepositories of clinically well characterized subjects, longitudinal sampling during clinical trials and tissue banks are beginning to allow researchers to directly address immunologic questions in the context of disease. Studies in animal models are being complemented with *in vitro* and *ex vivo* studies in human tissue to identify new mechanisms of disease and to test therapeutic approaches. Newly developed technologies have enhanced our ability to interrogate the immune system. GWASs have increased our understanding not only of the complexity of autoimmunity but have also identified pathways that participate in the disease process. Most notably, the introduction and success of biologics has encouraged the further development of immune-targeted therapeutics and raised the possibility of the use of combination therapeutics that are aimed at slowing the progression of disease and providing targeted therapy for patients based on particular immunological alterations.