Optimization of current and future therapy for autoimmune diseases

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There are multiple immune-based therapeutics available for some of the most prevalent autoimmune diseases, but for others, there are few or no approved immune therapies. This dichotomy poses discrete challenges. First, for diseases in which multiple therapy choices exist, a rational decision tree is required to select the best therapy. Second, we must devise new strategies for the autoimmune diseases that have the highest unmet clinical need. This commentary outlines new strategies for designing more efficient and selective approaches for immune therapy of autoimmune diseases.

In many diseases considered to be autoimmune, including rheumatoid arthritis, psoriasis and multiple sclerosis, there have been numerous successes in developing targeted immune therapies over the past two decades that have led to an improved range of choices for clinical treatment[1–3]. However, there are still unmet clinical needs in these diseases. First-line therapies are not effective in all patients with autoimmune diseases, and the choice of which medication to prescribe poses challenges for physicians who are largely left to choose among treatments with distinctly different mechanisms without having a great deal of mechanistic information about the biology of an individual patient.

As of the writing of this commentary, there are no approved clinical tests that are effective at predicting the therapeutic success or toxicity of treatments for autoimmune diseases. Therefore, treating physicians are unable to give a patient optimal, evidence-based advice on the best therapeutics available to arrest their disease that have the least risk of adverse events. However, promising tests for predicting these outcomes are emerging, as we discuss below[4,5]. Predictive tests would be beneficial for patients in that they would allow these individuals to receive the most efficacious therapy at the earliest possible time. These tests would also probably provide economic benefits, as such tests would help avoid treatments that do not work and would mitigate known complications and long-term comorbidity.

Such tests would guide future therapeutic trials designed to compare an experimental drug with an approved therapeutic, allowing some of the outstanding issues to be addressed, such as whether the experimental drug would work as well as the approved drug in the same biomarker-stratified population or whether the experimental drug would complement the approved drug and work in individuals who did not respond to the approved therapeutic. Biomarkers could thus become outcomes *per se* if their predictive value was sufficiently strong, thereby enhancing the efficiency and ethical acceptability of future clinical trial designs. An additional crucial factor concerns the strategic use of agents already available; substantial recent advances have been made to optimally use immune-modifying agents for some autoimmune diseases[6,7]. An ideal strategy would comprise the combined use of multiple representative biomarkers for disease activity, allowing for the optimization of a therapeutic regimen.

In contrast to the autoimmune diseases for which there are multiple therapeutic options, there are currently no therapies approved for some other autoimmune conditions, including type 1 diabetes (T1D), neuromyelitis optica (NMO), pemphigus vulgaris and myasthenia gravis. Somewhat surprisingly, these are the four diseases for which our understanding of the immune pathology is perhaps the greatest, particularly regarding the characterization of the targets of the adaptive immune response, where the major targets of T cell and antibody responses are known. However, inducing antigen-specific tolerance to the dominant immune responses driving these diseases has previously been attempted only on rare occasions or not at all, despite the promising results that have been obtained using this approach in pre-clinical models.

The development of new therapeutics is costly, and the diseases for which there exists the most detailed knowledge of the adaptive immune response tend to be orphan diseases. The development of pharmaceutical treatments for orphan diseases has not typically been considered to be a cost effective endeavor, although recently there has been more enthusiasm in the pharmaceutical sector for research into such targets[8]. We propose that a reawakening of efforts to test antigen-specific tolerance could be a major opportunity for therapeutic advances in these diseases in which a great deal is known about the characteristics of adaptive immunity to key autoantigens.

In contrast, at present, many of the approaches in the treatment of these autoimmune diseases continue to use the 'big hammers', therapeutics originally developed for avoidance of rejection of transplants or for ablative therapy in cancer. These big hammers

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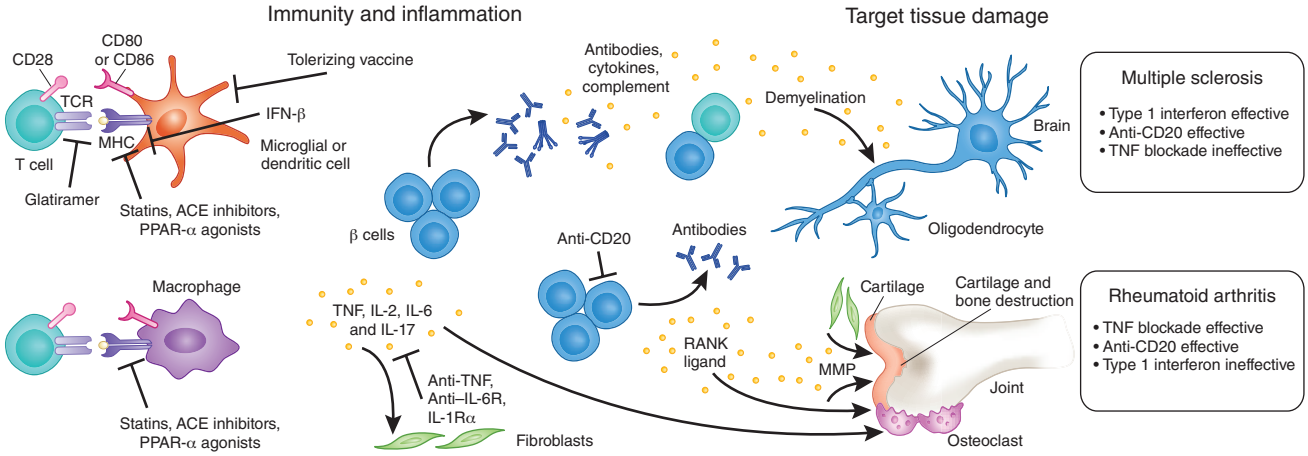

Figure 1 The same therapies can have different effects in different autoimmune diseases. The therapeutic immune modulation of autoimmune diseases requires an appreciation of the underlying cellular pathways and of how these differ according to the disease. For example, multiple sclerosis cannot be thought of as 'rheumatoid arthritis of the brain', so different therapeutic strategies are required for multiple sclerosis and rheumatoid arthritis. TNF blockade is a successful strategy for treating rheumatological diseases, but this treatment can worsen RRMS. Although TNF is crucial for mediating the migration of T cells into the brain, it also has a role in the survival and turnover of oligodendrocytes, which are required for myelin formation. Similarly, although IFN-β therapy is successful in treating RRMS, it is ineffective in rheumatological diseases. This could be because type 1 IFNs such as IFN-β drive TNF expression under T_H1 conditions, thus exacerbating the effects of TNF in the pathology of rheumatoid arthritis. PPAR-α, peroxisome proliferator–activated receptor α.

are often cytotoxic monoclonal antibodies that kill wide swaths of the normal immune system in order to delete the rare pathogenic cell. This divergence between the perceived wisdom (that antigen-specific therapy has a place in the therapeutic armamentarium for autoimmune disease) and actual clinical practice (the sequential trials of drugs that prevent allorejection) regarding autoimmune diseases has many complex origins but could be bridged by a better and earlier engagement between academia and the pharmaceutical industry[9,10].

A wealth of approved therapies for many autoimmune diseases

Five percent of adults in Europe and North America, two thirds of whom are female, suffer from an autoimmune disease[11]. Among the most prevalent autoimmune diseases are rheumatoid arthritis, autoimmune thyroiditis, various forms of psoriasis, inflammatory bowel disease, lupus and multiple sclerosis. We now have multiple choices for approved therapies for some of these diseases[1,2]. Other diseases, such as the two major forms of autoimmune thyroiditis, Hashimoto's disease and Graves disease, are generally treated with replacement therapy (for Hashimoto's disease) or a combination of ablative therapy and thyroxine replacement (for Graves disease)[12,13].

In relapsing remitting multiple sclerosis (RRMS), there are three major forms of approved first-line therapy: the type 1 interferon (IFN) IFN-β; glatiramer, a random polymeric peptide comprised of glutamate, lysine, tyrosine and alanine; and a recently approved small-molecule analog of spingosine phosphate, fingolimod[14–16]. There are variations in patient responses to these drugs and in the effectiveness of the drugs, for example, IFN-β and glatiramer have therapeutic benefit in approximately 50% of patients, reduce the relapse rate in treated patients by about 30% and have some benefit in reducing progression of disability, whereas fingolimod reduces the relapse rates by 50% in treated patients as well as reducing progression of disability by about 25%[14–16]. In addition, there are many new and promising treatments currently in the late stages of clinical trials for RRMS, such as alemtuzumab, an infused monoclonal antibody that targets CD52 on lymphocytes, monocytes and dendritic cells[17], and the oral drugs dimethylfumarate[18], terifluonimide and laquinimod[19,20]. In RRMS, a potent second-line therapy is available with natalizumab (a monoclonal antibody to α4 integrin), however, this drug, like many other potent immune suppressants, has the particularly serious adverse side effect of progressive multifocal leucoencephalopathy (PML), which poses challenges to its use[21,22].

In rheumatoid arthritis, Crohn's disease and psoriasis, blockade of tumor necrosis factor (TNF) is highly beneficial in about two thirds of patients[1,2]. There is also evidence for therapeutic effects of rituximab, abatacept and tocilizumab in rheumatoid arthritis used either alone or in combination with conventional agents such as methotrexate. These agents have equivalent efficacy if they are given to individuals who did not show disease improvement after methotrexate treatment alone and to individuals who have been pre-exposed to TNF inhibitors, although the efficacy of these agents diminishes if the individual has previously failed to show a therapeutic response to TNF inhibition[3,23,24]. Likewise, therapeutics targeting interleukin-12 (IL-12) p40 have shown promise in rheumatoid arthritis, Crohn's disease and psoriasis, and a monoclonal antibody to IL-12 p40 has been approved for the treatment of psoriasis[25–27]. In multiple sclerosis, rheumatoid arthritis, Crohn's disease and psoriasis, the numerous choices for therapy impose a considerable challenge: in which patients will a given therapy work best and in which will it not work at all?

One target does not fit all diseases

Although biologics targeting TNF and its receptors in rheumatoid arthritis, Crohn's disease and psoriasis may be highly effective for many patients, TNF blockade can exacerbate RRMS, and this effect has led to the addition of a warning label on such therapeutic agents[2] (**Fig. 1**). Even though the pathogenic role of TNF in such conditions is undeniable, the marked worsening of disease seen with attempts at TNF blockade in multiple sclerosis requires us to consider whether there is another dimension for the role of TNF in demyelinating diseases. TNF has a key role in the migration of T cells into the brain, and factors relating to T cell immunology have been shown to be central to the pathogenesis of multiple sclerosis. TNF induces vascular cell adhesion molecule 1 (VCAM-1), which has recently been geneti-

cally associated with susceptibility to multiple sclerosis[28]. VCAM-1, along with osteopontin, binds α4 integrin[29]. These interactions are crucial for the transport of T cells out of blood vessels and into the brain. So, one might think that blockade of TNF or its receptors would improve the symptoms of multiple sclerosis. However, TNF is also crucial for the survival and turnover of oligodendrocytes, which are the key myelin-forming cells[2,30]. It is not known whether TNF inhibitors could be used in combination with agents that might ameliorate their adverse effects in multiple sclerosis without interfering with their potential beneficial effects.

Conversely, therapy with IFN-β has been successful in about 50% of patients with RRMS but has been unsuccessful in treating rheumatoid arthritis[2] (**Fig. 1**). Recent studies in other forms of demyelinating disease, for example, psoriasis and ulcerative colitis, might help to explain why blockade of TNF or the administration of type 1 IFNs are effective in certain autoimmune diseases but not in others. Of note, type 1 IFNs drive TNF expression under conditions that favor the development of type 1 T helper (T_H1) cells[31]. This might explain why type 1 IFNs are not therapeutic in rheumatoid arthritis, where they might upregulate TNF, a key pathologic mediator, whereas they are beneficial in multiple sclerosis[31,32], where increasing TNF concentrations may have some therapeutic benefit. In addition, paradoxical psoriaform eruptions after TNF blockade probably reflect manifestations of the same type I IFN pathway in individuals who are predisposed to develop psoriasis.

Taken together, these outcomes illustrate that a single therapeutic strategy is probably not suitable for all autoimmune diseases or even for individual subsets of patients within one diagnostic category, as there may be heterogeneous biology underlying some of these clinical entities. These confounding results point to the need for a better understanding of the downstream effects of powerful mediators such as type 1 IFNs and TNF and an improved characterization of a patient's individual biology before attempting large-scale trials in humans.

Clinical trial design and biomarkers

Mechanistic biomarkers are needed to select appropriate treatments and guide their dosing to optimize efficacy and mitigate safety risks. Using biomarkers would have benefits in improving trial designs as well as in the clinic. The story of natalizumab provides an example of how a biomarker might be used to offset the serious risks of a highly effective drug. Natalizumab, a humanized immunoglobulin G4 (IgG4) monoclonal antibody that binds α4 integrin, was approved in 2004 for the treatment of RRMS on the basis of 1 year of results in a 2-year phase 3 trial of 942 patients with RMMS[21]. However, within 3 months of approval, the drug was voluntarily withdrawn from the market after three patients developed PML, resulting in two fatalities[22]. To date, over 80,000 patients have been treated with natalizumab, with 145 cases of PML reported as of July 15, 2011 resulting in 29 deaths. The risk of developing PML is 1.94 per 1,000 patients who have been on natalizumab for between 2 and 3 years.

A predictive test based on the detection of antibodies against the John Cunningham virus (JCV) that causes PML is now approved in Europe, with approval pending in the United States, and it is hoped that this test will mitigate the risk of developing PML[33,34]. Using this test, nearly 100% of pre-PML sera (sera collected from individuals who eventually went on to develop PML) test positive for antibodies to JCV. In a pivotal study, all 17 samples (100%) of pre-PML sera were positive for antibody to JCV, which was significantly different from the 53.6% seropositivity observed in the overall multiple sclerosis study population ($P < 0.0001$)[34].

The presence of antibodies to JCV indicates exposure to the virus and greatly increases the risk of developing PML after treatment with natalizumab for more than 2 years. It also indicates that having such antibodies is not protective against PML in patients taking natalizumab, implying that cellular immunity within the brain is crucial in preventing PML. Natalizumab impairs this process by blocking immune-cell traffic into the brain. This emphasizes the importance of the absence of cellular immunity to JCV within the brain in the development of PML and, perhaps counterintuitively, highlights the fact that patients without antibodies to JCV are far less likely to develop PML after being treated with natalizumab[34]. Thus, cellular immunity to JCV and the capacity of immune cells that recognize this virus to home to the brain seems to be far more crucial than the presence of antibodies to JCV in the pathogenesis of PML. It will be interesting to see whether similar approaches in the context of B cell–depleting therapies in autoimmune diseases, where PML has also been observed, will be similarly necessary and effective.

Biomarkers may provide predictive value for autoimmune diseases other than multiple sclerosis as well. Monitoring the concentration of the cytokine IL-21 might be useful for clinically mitigating one of the toxicities of a powerful and promising drug for RRMS, alemtuzumab, a monoclonal antibody directed against CD52 (refs. 17,35). Patients treated with alemtuzumab have a marked reduction in relapse rate and disease progression, however, approximately one third of individuals who are treated with this drug develop a new autoimmune condition, usually thyroiditis[17], although immune thrombocytopenic purpura and Goodpasture's syndrome have also been observed in some of these patients. Patients who went on to develop a new autoimmune disease after treatment with alemtuzumab had twofold higher concentrations of IL-21 at baseline compared to those individuals who did not develop this complication after treatment[35].

The use of multiplex measurements of cytokines and antibodies has proven valuable in delineating biomarkers that predict responses to therapies in rheumatoid arthritis[36–38]. For example, a signature consisting of a combination of 24 cytokines and autoantibodies was tested for its ability to predict outcomes in response to a TNF blocker, etanercept, in three independent cohorts with positive predictive values of 58–72% and negative predictive values of 63–78%[38]. Another mechanistic biomarker is currently showing promise in trials of antibodies to CD20 for treatment of rheumatoid arthritis. CD20 is not present on plasmablasts, and high concentrations of a marker for antibody-secreting plasmablasts, IgJ, predict non-responsiveness to rituximab and to another antibody to CD20, ocrelizumab[39]. Combining the detection of high concentrations of IgJ with the detection of low concentrations of another biomarker, Fc receptor-like 5 (FCRL5), improved the prediction of therapeutic responses to antibodies to CD20 (ref. 39).

A T_H17 profile, particularly in regards to the production of the T_H17-associated cytokine IL-17, might also provide a useful biomarker to determine whether to treat an individual who has an autoimmune disease with IFN-β. Studies in the experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis have indicated that IFN-β improved the clinical outcome in T_H1-associated EAE but worsened disease in T_H17-associated EAE[31]. Concordantly, individuals with high concentrations of IL-17F in their serum did not respond to treatment with IFN-β[31]. In NMO, which is characterized by high concentrations of IL-17 in the spinal fluid, several trials with IFN-β indicated that it worsened the disease outcome[40–43]. A recent small trial of IFN-β in ulcerative colitis showed that two thirds of patients in the small trial benefited from treatment, with a quarter of the patients achieving remission. Patients classified as 'non-responders' were characterized by high IL-17 production in their lamina propria

mononuclear cells[44]. Further refinements in predicting patients as responders and non-responders to IFN-β in RRMS have also been defined by combining measurements of IL-7 with those of IL-17F. IL-7 is a major driver of T_H1 responses in mouse and human T cells[5], and it was found that the presence of high concentrations of IL-7 and low concentrations of IL-17F in the serum before beginning therapy predicted a patient's responsiveness to IFN-β[5].

In addition to identifying predictive biomarkers, selecting the appropriate end points, phenotypes and allowable background treatments will also be crucial for improving the trial design for complex autoimmune diseases. For example, some therapies for systemic lupus erythematosus (SLE), such as antibodies to CD20, did not meet their prespecified endpoints in clinical trials[45]. However, exploratory analyses have indicated that if the endpoint criteria are refined or subsets of patients with higher disease activity or those on less aggressive background treatments are evaluated, a treatment effect might be observed.

In a *post hoc* analysis of one trial of antibodies to CD20 in which reduction in the number of severe flares was tested as an endpoint, rituximab was observed to have an effect regardless of the severity of illness or the background treatments in the trial subsets[45,46]. Such an analysis using more extreme outcomes has proven powerful in analyzing many sets of complex biological data[47] and may be a useful principle to consider in clinical trial design to test therapies for heterogeneous autoimmune diseases. Studies using more severely affected patients, patients with less background treatment and/or patients with extreme outcomes as the key analytic parameters can be powered with fewer participants[47]. Misclassification of imprecise phenotypes such as 'flare' in SLE or 'relapse' in RRMS is a substantial confounder in clinical trials[5,26,45,46], and, thus, refining the criteria for the endpoints and trial outcomes according to phenotypes that can be determined more precisely may allow for more robust testing of experimental drugs. It is here that laboratory-guided, biologic coverage of an intended immunologic target could become extremely useful.

Recycling combinations of approved drugs

Another strategy for improving the treatment of autoimmune disease will be to explore potentially synergistic combination therapies. Repurposing drugs approved for other conditions has a certain logic, as these drugs may have properties that allow them to be beneficial for multiple diseases. Combining therapies aimed at different molecular targets might enable blockade of more of the key molecular pathways involved in the pathogenesis of complex autoimmune diseases (**Fig. 2**). However, the use of a strategic, biomarker-driven process in testing such combinations would still afford better refinement of treatment than is achieved with the standard, global immune suppressant approaches currently being used. Optimally, the most effective combinations could even be selected based on the biology of individual patients. Testing a single therapy in isolation may ultimately constrain the efficacy of any single approach, as, like cancer, autoimmune diseases involve pathological perturbations in multiple molecular pathways[2,48].

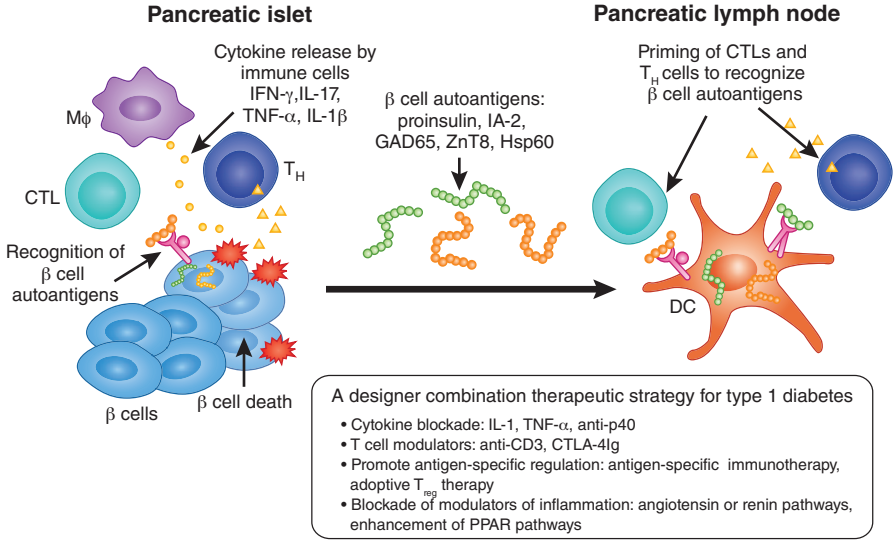

Figure 2 Optimizing combination strategies, using T1D as an example. Based on the knowledge of their underlying mechanisms, rational combination strategies for autoimmune diseases may be devised to target various aspects of the pathogenic process. For example, in T1D, an effective combination therapy could consist of blocking cytokines, modulating T cell function, promoting antigen-specific regulation and blocking inflammatory pathways.

Recently, bioinformatic tools have been used to probe the gene expression profiles in publically available databases from inflammatory bowel disease (IBD) experiments and to make comparisons with gene expression profiles from a compendium of 164 drug compounds tested on cell lines. Ideal matches were obtained when the profiles were most 'dissimilar', in other words, when the drug suppressed the pathways that were aberrantly elevated in the disease[48]. In that study, the gamma aminobutyric acid (GABA) agonist topiramate was identified as a potential treatment for IBD[48]. Before these experiments were performed, other researchers had identified signatures of both gene and protein expression for the enzymes involved in GABA metabolism in brain tissue taken from individuals with acute multiple sclerosis lesions[49]. GABA is the major inhibitory transmitter in the central nervous system, and it also has inhibitory effects on the immune system[49]. Topiramate is widely used to treat epilepsy but was also beneficial in the EAE model. However the efficacy of topiramate in inflammatory diseases such as multiple sclerosis and IBD can only be proven through clinical trials. In addition, such drugs may also be effective when combined with current therapies.

As another example, the angiotensin-converting enzyme (ACE) is elevated in acute multiple sclerosis lesions[50,51], and ACE inhibitors and angiotensin-receptor blockers were shown to reverse paralysis in EAE. These drugs are currently used in millions of people to treat hypertension, and testing of these drugs is scheduled for multiple sclerosis. In a large clinical study of people with hyperlipidemia, it was found that those individuals on statins had a lower incidence of rheumatoid arthritis[52]. Observations that statins reduce circulating concentrations of C-reactive protein and related pro-inflammatory molecules, combined with evidence that statins are effective in many pre-clinical models of autoimmune disease, have kindled enthusiasm for testing these widely used cholesterol-lowering drugs in autoimmune diseases[52]. Statins work by blocking the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, but this blockade has more effects than just lowering cholesterol. Statin-mediated blockade of this enzyme reduces isoprenoid production, and therefore post-translational modifications of key inflammatory molecules are diminished, thus providing a rationale for the use of statins in some inflammatory condi-

tions[53], although the effects of this blockade on autoimmune diseases in two pre-clinical models of type 1 diabetes and SLE may be less impressive[54,55].

Because many therapies that might be repurposed for the treatment of autoimmune conditions are generic, profits from these treatment options will probably be minimal, which diminishes the incentive for funding traditional large-scale trials. More efficient experimental designs are appropriate and necessary if we expect to test approved drugs for new indications. Many clinical trials have incorporated the internet and other contemporary tools into data management, quality assurance and the training of international teams of investigators. However, it is crucial to avoid compromising the safety and well being of human volunteers while looking for smaller trials and more efficient approaches to larger trials. The pharmaceutical industry is already spearheading efforts to collaborate in some open-source ventures for discovering biomarkers[56] and in the collection of post-marketing safety information. In addition, the US National Institute of Allergy and Infectious Diseases has developed global bioinformatic capabilities for optimizing therapies to treat the worldwide human immunodeficiency virus epidemic[57]. Like any data that might be acquired more informally, hypotheses generated from such approaches will need to be confirmed and critiqued using appropriate methodologies.

The promise of antigen-specific therapy

Instead of targeting and killing large populations of normal immune cells with antibodies to key receptors such as CD20 (refs. 2,45,46) or CD52 (ref. 17), or instead of blocking major lymphocyte homing mechanisms by targeting α4 integrin[2,21,22] or spingosine phosphate receptors[16], some researchers are attempting to modulate only the key adaptive immune responses in certain autoimmune diseases. The idea is to modulate only pathogenic cells and leave the rest of the immune system intact. Those diseases for which we have perhaps the most extensive understanding of the key adaptive immune response include myasthenia gravis, T1D, NMO, pemphigus vulgaris and celiac disease. In each of these diseases, attempts to downregulate the immune response to the key autoantigen seem warranted. Several early studies have also been attempted in SLE using agents that target specific autoantibody-antigen interactions. Although they are theoretically compelling, to date, these approaches must be considered to be unproven in SLE[58–60].

Consider myasthenia gravis pathogenesis, for which both human and mouse studies have shown that the major autoimmune response is directed against the acetylcholine receptor (AChR)[61,62]. Thus, it would be logical to diminish the response to antibodies to AChR while leaving the rest of the immune system intact[63,64]. A second minor autoantigen in myasthenia gravis is muscle-specific kinase (MuSK), so a therapeutic strategy to suppress antibodies to AChR and MuSK would cover all the known adaptive immune responses in this disease[63].

In NMO, an immune response to the water channel aquaporin-4 (AQP4) is crucial for diagnosis of the disease[65]. Antibodies to AQP4 and complement mediate the destruction of astrocytes in the optic nerve and spinal cord in NMO[66]. Recombinant monoclonal antibodies to AQP4 have been generated from patients with NMO and can then be engineered to lack the Fc domains that induce complement-mediated cytotoxicity in these individuals. These non-pathogenic antibodies could be used as a therapy in NMO, as they would compete with the pathogenic antibodies for binding to AQP4. Pre-clinical trials of this approach are being undertaken[67–69], and such an approach could also be applied to other antibody-mediated diseases such as pemphigus vulgaris[70] or myasthenia gravis.

In T1D, the production of antibodies to key components of pancreatic beta cells, including insulin, glutamic acid decarboxylase (GAD), the tyrosine phosphatase-like protein ICA512 (IA-2) and the zinc transporter (ZnT8), is crucial in the development of disease[71,72]. Other proteins, such as the heat shock proteins, may also have a key role in disease pathogenesis[73]. A number of antigen-specific therapeutic approaches are currently being investigated for T1D. A phase 3 clinical trial of GAD in alum adjuvant did not meet its key endpoints[74]. However, alum is a powerful activator of innate immunity, and therefore the use of this immunogenic adjuvant may have contributed to the failure of this agent[75]. A key problem in attempting to induce antigen-specific tolerance is the requirement of targeting a central mechanism in the disease and showing a suppression of immunity by the mechanism in both pre-clinical and human experiments. The use of alum (which, in this case, was used with the aim of reverting pathogenic T_H1 autoimmunity by inducing the countering effect of T_H2 autoimmunity) raises issues about whether the tolerance induction concept can be generalized and whether this approach might have increased adaptive immunity to GAD rather than tolerizing or suppressing such immunity. Tolerizing adjuvants have not previously been a major area of interest in drug research, but this may need to change if the power and potential of antigen-specific therapy is to be realized.

Strategies to reduce the immune response to proinsulin are in progress: for example, clinical trials are near completion with a DNA vaccine encoding proinsulin. The DNA vaccine was engineered with a backbone modified to exclude immunogenic CpG hexanucleotide sequences[76,77]. The proinsulin sequence was modified so that the protein remained intracellular by inserting a chimeric intron upstream of the proinsulin gene sequence[76,77]. In non-obese diabetic mice, this DNA vaccine prevented diabetes when administered before the onset of hyperglycemia and could also reverse hyperglycemia after onset. There were also changes in the immune responses of the mice after the vaccine was administered; the mice had a reduction in the number of T cells reactive to a major insulin epitope, B9-23, and a reduction in the amount of autoantibodies in their pancreatic islets[77]. Delivering oral insulin to the immune system has also shown promising results in clinical trials conducted during the earliest stages of autoimmunity in T1D[78], and it is probable that this approach can be refined through small-scale mechanistic studies that use biomarkers to optimize the dose and frequency of insulin administration, both of which have a key impact on outcome[79].

Another approach is to identify key immunogenic epitopes in T1D. To date, insulin $B_{10–18}$, IA-$2_{797–805}$, $IGRP_{265–273}$ and the proinsulin epitope C19-A3 have been identified as key in the development of the disease[80]. Intradermal administration of the C19-A3 epitope, which is restricted to the MHC cell surface receptor human leukocyte antigen (HLA)-DR4 was shown to be safe and did not induce allergic hypersensitivity or exacerbate disease[80]. Moreover, peptide-specific T cells secreting the immune suppressive cytokine IL-10 were observed at 3 months after treatment with C19-A3 in 4 of 18 patients in the low-dose group, showing that this key suppressive cytokine is induced during dosing with the potentially tolerizing peptide. A trial of nasal insulin for 1 year reduced the IFN-γ response to proinsulin[81]. Nasal insulin had no effect on C-peptide in this trial. In addition, a successful phase 3 trial using a peptide from heat shock protein 60 (HSP60)[73] has recently been reported, with the initial analysis of the data showing the preservation in the amounts of C-peptide and the maintenance of glycosylated hemoglobin after 1 year of therapy[82].

To date, the most extensive attempts at antigen-specific tolerance have been in multiple sclerosis. Three trials have attempted to target a single myelin protein, myelin basic protein (MBP). One trial targeting one epitope on MBP was unsuccessful in phase 3 (ref. 83). Early stage trials with four MBP peptides

are currently in progress, with no results yet reported[84]. A phase 1 trial of fixed autologous peripheral blood leukocytes coupled with a cocktail of seven myelin peptides has been completed in RRMS, and the results on the safety of this treatment method will soon be reported[85,86]. The most advanced progress to be reported for this approach to treat multiple sclerosis used a modified plasmid to encode MBP and has been tested in a phase 2 trial of 267 patients. There was a reduction of 50% in the median rate of new gadolinium-enhancing lesions between weeks 28 and 48 of treatment compared to individuals treated with placebo ($P < 0.07$), which was the primary endpoint of that study[87]. There was also a reduction in antibodies to myelin, including antibodies to MBP in the cerebrospinal fluid, and a reduction in T_H1 cells reactive to myelin antigens, including MBP, after 1 year of treatment compared to individuals treated with placebo[87,88]. Using a prospectively defined endpoint in this study, individuals who were grouped in the 50% of subjects with the highest concentrations of antibodies to MBP in their cerebrospinal fluid at baseline had the greatest reductions in gadolinium-enhancing lesions compared to individuals treated with placebo ($P < 0.02$)[87].

These results suggest that patients might be stratified on the basis of high and low immunity to a specific antigen targeted by the therapy being tested. Patients who have the highest immune response to an autoantigen may be expected to benefit most from an antigen-specific reduction of their immune responses. It is still early for such approaches to be used in autoimmune disease, but the proof of concept that multiple antigenic peptides can induce adaptive immunoregulatory pathways has already been provided in the allergy field[89,90].

Future therapeutic directions

Clinical trials to develop new therapies of autoimmune diseases in the next few years will be a very expensive proposition. The development of predictive biomarkers may make testing these new therapies more economical by allowing the selection of patients who are most likely to respond to a given treatment. Controlled clinical trials are required to use a comparator group, and trial designs range from using true placebo treatments to using comparisons or add on therapies as compared to the control groups receiving the current standard of care. Improving the ability to identify subsets of patients who are more likely to respond biologically to a given agent would allow a more robust comparison between a treatment and a control group, expose less patients inappropriately to a test treatment and increase the efficiency and reduce the costs of clinical trials. Drugs with apparently equivalent efficacy in an entire population may show particular benefits in different subsets of these populations.

Big hammers like antibodies to CD20 and CD52 will probably prove useful across a range of disorders, leading to difficult choices about which of these powerful immune therapeutics are best to prescribe to individual patients. The first approved therapy for lupus, belimumab, is one of these big hammers, and its initial success emphasizes the importance of using such powerful immune suppressants at this stage of our understanding of such complex diseases[91–93]. Belimumab is a B-lymphocyte stimulator (BLyS)-specific inhibitor and has been shown to reduce disease activity and the occurrence of severe flares[91]. However, it also reduces the concentrations of circulating $CD19^+$, $CD20^+$, naive and activated B cells, plasma cells and plasmacytoid cells[91].

Combinations of these big hammers and other more precise drugs will be required to maximize their benefit and minimize their side effects, but establishing and optimizing these treatments will require a highly integrated approach[94]. Predictive tests might help to improve the rationality of such treatment choices and may help to predict which patients might have the least chance of certain adverse effects. In the meantime, testing already approved therapeutics for new indications is an appealing idea, especially for old drugs that are now generic and inexpensive. Statins, ACE inhibitors and GABA modulators are examples of treatments that may find new roles as immune therapies. Additionally, for those diseases where the adaptive immune response seems to have a central role in pathogenesis, it would be worthwhile to continue to pursue research and clinical trials on antigen-specific tolerance.

The current standard of care is the suppression of large portions of the immune system to target the very few cells that are actually involved in the pathogenesis of an autoimmune disease, and in the future, this approach may well appear to have been an illogical strategy. However, collateral damage to large populations of normal cells has been an acceptable practice over the last few decades given the seriousness and sometimes life-threatening nature of unchecked autoimmunity. It is hoped that such practices will be relegated to the archives of medical history when the study and practice of medicine can begin to approach the scope and promise of current technologies.

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The authors declare no competing financial interests.

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