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Combining antigen and immunomodulators: Emerging trends in antigen-specific immunotherapy for autoimmunity ☆

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ABSTRACT

A majority of current therapies for autoimmune diseases are general immunosuppressants, which can compromise patient response to opportunistic infection and lead to adverse events. Using antigen-specific immunotherapy (ASIT) to selectively disarm autoimmune diseases, without suppressing the global immune response, would be a transformative therapy for patients. ASIT has been used historically in allergy hyposensitization therapy to induce tolerance to an allergen. Similar strategies to induce immune tolerance toward autoantigens responsible for autoimmune disease have been attempted but have yielded limited clinical success. Recent studies of ASIT for autoimmunity have explored combination therapy, combining the disease-causing autoantigen with an immunomodulatory compound. ASIT combination therapy may direct the immune response in an antigen-specific manner, potentially reversing the root cause of autoimmunity while limiting side effects. This review analyzes recent advances in ASIT applied to autoimmune diseases, emphasizing current combination therapies and future strategies.

Contents

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1. Introduction

Antigen-specific immunotherapy (ASIT) has been used in the clinic for over a century to induce antigen-specific immune responses. Vaccines were the first approach to direct an antigen-specific immune response, utilizing disease-causing antigens in order to induce prophylactic protective immune responses against specific foreign pathogens. Treatments with specific allergy-inducing antigens have also been useful for the induction of antigen-specific immune tolerance for allergy desensitization. Clinical treatment of autoimmune diseases, however, still relies primarily on global immune suppression through the use of potent small molecule immunomodulators. Within the last decade, scientists have more deeply explored combinations of immunomodulators and autoantigens in the hope of creating effective ASIT for the treatment of autoimmune diseases, a strategy that could substantially improve clinical outcomes without compromising the entire immune system.

One of the most successful strategies in ASIT for inducing immune tolerance has been the use of hyposensitization therapy in the treatment of allergies. Hyposensitization therapy has been used since the early 1900s as a means to desensitize patients to specific allergens [1]. In the seminal papers published by Noon [2] and Freeman [3] in 1911, pollen extracts were injected subcutaneously using an increasing-dose schedule in order to relieve symptoms from grass pollen allergy and hay fever [1]. The current "gold standard" for hyposensitization therapy is surprisingly similar to these techniques described over a century ago [1]. Although hyposensitization for allergies has been effective in many cases, several disadvantages have yet to be remedied. The dosing schedule is often difficult for patients to complete due to the frequency and length of the therapy [1]. The majority of hyposensitization therapy is given via subcutaneous injections and needs to be administered by a trained professional over a period of years [1,4]. Sublingual ASIT may ultimately increase treatment convenience; however, the most important consideration, safety, may remain an issue [4]. Unfortunately, in some cases, hyposensitization therapy can become life threatening as anaphylaxis can occur following treatment of severe allergies, reinforcing the requirement for administration by a trained professional in a clinical setting [1]. Additionally, the mechanisms whereby hyposensitization therapy induces therapeutic immune tolerance or anaphylactic shock are still not completely understood [5].

Using approaches similar to allergen hyposensitization therapy, ASIT for the treatment of autoimmune diseases using only disease-causing autoantigen has been explored with minimal clinical success. Although these therapies often work in animal models, translation to humans has not shown the same level of efficacy [6–8]. Efforts to induce tolerance in autoimmune patients often use repeat administration of low doses of autoantigen or altered peptide ligands, but thus far, these approaches have suffered from poor long-term clinical effectiveness and variable outcomes [6,9,10].

Most of the currently approved autoimmune therapies are immunomodulators; the majority of these immunomodulators non-specifically cause immune suppression (i.e., immunosuppressants). As our understanding of immunology has improved, many therapeutic molecules once thought to act as specific immunosuppressants have recently been shown to have multiple mechanisms of action with numerous downstream effects. For example, rapamycin (Sirolimus) has traditionally been considered an immunosuppressant drug; however, recently, it has been discovered that the mammalian target of rapamycin (mTOR) pathway is essential in maintaining the balance between tolerance and inflammation [11]. Immunomodulation in the treatment of autoimmunity, therefore, extends far beyond immunosuppression and can involve shifting the immune response toward tolerance through a variety of mechanisms (Table 1). Unfortunately, the lack of antigen-specificity in immunomodulation can lead to undesired side effects and potentially increase the risk of opportunistic infections in patients taking these immunosuppressive therapies.

One promising strategy in the creation of ASIT for autoimmunity is combination therapy of antigen and immunomodulator. This strategy mimics the successful "antigen-adjuvant" model used in the creation of vaccines. Adjuvants are immunomodulators used in vaccines to enhance the antigen-specific immune response, increasing the potency of the vaccine. Applying this paradigm for treating autoimmune disease, the combination of antigen and immunomodulator may be able to direct the immune response toward tolerance to autoantigen.

This review highlights recent work combining immunomodulators with autoantigen either by co-administration or co-delivery to induce tolerance in autoimmune disease. We present a thorough background on the immunological processes involved in autoimmunity and tolerance, along with an examination of currently approved therapies. Recent experimental work utilizing co-administration and co-delivery techniques, combining antigen and immunomodulator, have shown exciting new promise in autoimmune therapy. ASIT combination therapies have also shown promise in the clinic. With the recent advances in ASIT, the potential to induce antigen-specific tolerance to treat, prevent, or possibly cure a subset of autoimmune diseases in humans may be on the horizon.

Table 1
Mechanisms of action for autoimmune therapies.

Mechanism of action	Drug example
Cell cycle interference	
Prevent cell division	Lefunomide
Inhibition of DNA synthesis	Methotrexate
Controlling pro-inflammatory cytokines	
Prevent cytokine production	Ciclosporin
Inhibit receptor binding	Tocilizumab
Induction of neutralizing antibodies	Interferon-β
Inhibiting transport of auto-reactive cells	
Preventing cell adhesion	Natalizumab
Trap cells in lymphatics	Finglomod
Inhibiting T-cell activation	
Blocking B7 Co-stimulation	Abatacept
Blocking other co-stimulation pathways	Alemtuzumab
Competitive inhibition of MHC binding	Glatiramer acetate
B-cell depletion	
Antibody-dependent cell cytotoxicity	Rituximab
Other proposed mechanisms to improve therapies	
Auto-antibody deletion	
Ex vivo antigen-specific immune cell activation	
Anergy of auto-reactive cells	
Inducing regulatory cell proliferation	
Antigen-specific interruption of T-cell activation	
Inducing antigen presentation with co-inhibitory signals	

2. Introduction to autoimmune diseases

2.1. Immune tolerance and regulatory responses

The protective response of the immune system is deeply rooted in the selective recognition of foreign substances, or non-self-antigens, and the absence of a reaction to native antigens; the latter can be defined as immunological self-tolerance. The loss of this tolerance to self-antigens may result in an immune response directed toward "self" and is defined as an autoimmune response. While the origin of many autoimmune diseases still remains unclear, it is thought that a lapse in tolerance to autoantigens is a key step in the progression of the autoimmune response [12]. In order to understand how autoimmune diseases may develop in an individual, it is important to first assess the ways in which the body maintains tolerance toward autoantigens. The processes through which the immune system attempts to achieve and maintain tolerance toward autoantigens can be classified into two categories: central and peripheral.

Central tolerance involves the presentation of autoantigen to T-cells and B-cells in the thymus and bone marrow. This process is commonly referred to as negative selection and includes inducing apoptosis in developing lymphocytes which may recognize autoantigens or preventing their expansion and release into systemic circulation. Inevitably, some lymphocytes that recognize autoantigens are able to bypass the mechanism of central tolerance [13]. Fortunately, the immune system contains a variety of mechanisms to prevent activation of these potentially autoreactive lymphocytes in peripheral tissue, known as peripheral tolerance. These mechanisms include physical separation of auto-reactive T-cells from cells presenting autoantigens via the major histocompatibility complex (MHC) [14,15]. Naive T-cells are contained primarily to lymphoid peripheral tissues and blood, and as a result, their encounters with autoantigen presentation by non-lymphoid tissue cells are limited in healthy individuals [14]. In addition to antigen presentation via MHC restriction, T-cell activation requires the presence of surface expressed secondary context signaling (co-stimulatory) receptors, examples include CD80 (B7-1), CD86 (B7-2), CD40L, CD70, OX40L, and many others [16,17]. Failure to provide the appropriate stimulatory context signals may result in functional inactivation of the lymphocyte, known as anergy. Besides these co-stimulatory signals, secondary context receptors exist which are capable of inducing anergy in T-cells, also known as "co-inhibitory" receptors, and include cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed death-1 (PD-1) [16]. Ligation of these receptors has been shown to inhibit T-cell activation [18]. Conversely, mice lacking the co-inhibitory receptor CTLA-4 develop lymphoproliferative disorders leading to death, suggesting a highly dependent regulatory component of these receptors [19]. The combination of these factors help support peripheral tolerance to maintain T-cell-dependent self-tolerance.

The immune system also regulates antigen presentation in order to control peripheral T-cell responses. In the periphery, antigen-presenting cells (APCs), particularly dendritic cells (DCs), are major contributors to the initiation and regulation of downstream immune responses. In addition to antigen uptake, processing, and presentation capability, DCs express a variety of co-stimulatory and co-inhibitory receptors and are responsive to their local external environment. For example, DCs can respond to signals elicited by pattern recognition receptors (PRRs) binding pathogen associated molecular patterns (PAMPs) [20]. Encounters with many PAMPs can result in up-regulation of co-stimulatory surface receptors, overexpression of MHC, and secretion of inflammatory cytokines, a microenvironment that can stimulate activation of naive T-cells [20,21]. It is important to note that in the absence of these inflammatory signals, immature DCs can present antigen and induce tolerance in naive T-cells, providing another means for regulation of autoimmune responses [14].

A third mechanism of peripheral tolerance is the presence of regulatory T-cells (Tregs). Tregs suppress immune responses in an antigen-specific manner through cytokine secretion, metabolic disruption, and alteration of DC function [22]. It has been shown that secretion of cytokines such as interleukin (IL)-10, transforming growth factor (TGF)-β, and IL-35 plays a role in the suppression of immune response; however, the importance of these cytokines in the overall function of Tregs is still a point of debate [22]. Furthermore, it is hypothesized that Treg populations are capable of inducing apoptosis through deprivation of IL-2, a pro-inflammatory cytokine, due to the high expression of CD25, although the mechanisms are still not yet understood [22]. In addition to the previously mentioned influences on the local environment, it is believed that regulatory T-cells also act to alter the function of DCs upon antigen-MHC recognition and CTLA-4 ligation. Studies indicate that Tregs are capable of up-regulating the expression of indoleamine 2, 3-dioxygenase (IDO) in DCs, an enzyme that has been found to limit the inflammatory response and induce a tolerogenic response [23]. Additionally, studies have indicated that Treg interactions with DCs may downregulate the expression of B7 (CD80/CD86) limiting DC function in activating T-cells [22].

2.2. Immunology of autoimmunity

In general, autoimmune diseases develop upon failure of the numerous regulatory pathways mentioned previously; however, ongoing studies are continuously evaluating and exploring new mechanisms whereby self-tolerance is disrupted. Breakdown of tolerance toward autoantigen is often thought to be a result of both genetic and environmental risk factors, including exposure to infection by particular pathogens [24]. Multiple hypotheses have been generated to explain the downstream processes by which immune responses against autoantigen may occur upon exposure to an infectious pathogen including molecular mimicry of endogenous protein antigens, epitope spreading, and bystander activation; however, the exact mechanisms whereby autoimmune disease develops are still not well understood [24].

2.3. Autoimmune diseases

There are currently over 80 autoimmune diseases identified by the National Institute of Allergy and Infectious Diseases (NIAID) affecting an estimated 20 million Americans [25]. Some of the most common autoimmune diseases include type 1 diabetes (T1D), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), inflammatory bowel disease, psoriasis, and multiple sclerosis (MS). The discovery of a disease-causing antigen or epitope is vital to the development of ASIT for autoimmunity; however, identifying such antigens is not a simple task, particularly in systemic autoimmune diseases such as SLE, for which there may be multiple antigenic targets [26]. The majority of current research in ASIT is focused on RA, T1D, and MS as they all have robust animal models, allowing for a greater understanding of autoimmune pathogenesis and the identification of disease-causing autoantigens.

RA is typified by infiltration of the synovium by $CD4^+$ T-cells, B-cells, and macrophages resulting in inflammation in joints. In recent years, the focus of RA pathogenesis has shifted to the study of autoantibodies including anti-IgG rheumatoid factors (RFs) and anti-citrullinated protein antibodies (ACPAs), as these autoantibodies have been found to reliably predict disease progression [27]. Further research is required to determine the relevance of these autoantibodies and others to subsets of RA patients and disease progression.

Recently, disease-specific targets for the treatment of T1D have also been identified including preproinsulin (PPI), glutamic acid decarboxylase (GAD65), and heat shock protein 60 (HSP60) [28]. T1D involves the destruction of insulin-producing pancreatic β-cells, resulting in the loss of the body's ability to produce insulin and failure to control blood glucose levels. As such, clinical studies are commonly performed in early onset T1D patients in order to retain β-cell function and provide the greatest benefit to the patient. Each of these antigens has been identified to play a role in the non-obese diabetic (NOD) mouse model for

T1D and have recently been explored in clinical trials for antigen-specific therapies [29–31].

Similarly, potential disease-causing autoantigens have been identified in MS including myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP), and myelin-associated glycoprotein (MAG) [32]. MS is characterized by inflammation of the central nervous system (CNS) due to immune cell-mediated degradation of myelin proteins, resulting in neurological complications. In the most common form of MS, symptoms follow a relapsing-remitting form, and these symptoms may vary from one relapse period to another depending upon the location of the CNS inflammation [33]. A commonly employed animal model for MS is experimental autoimmune encephalomyelitis (EAE), which is usually induced in healthy mice by vaccination with MBP, MOG, or PLP autoantigens, allowing for insight into the pathogenesis of demyelinating autoimmune diseases [34].

3. Current therapies for autoimmunity

Although autoimmune diseases are diverse in both cause and progression, most of the current therapies fall into a few distinct categories; general immunosuppressants, mobility and transport inhibitors, immune cell activation inhibitors, and antigen mimics (shown in Fig. 1). The downfall of the majority of current autoimmune therapies is the lack of antigen specificity. Many therapies inhibit or modify the global immune response hindering the patient's ability to fight off foreign pathogens. In order to decrease unwanted side effects and increase efficacy, treatments that induce antigen-specific tolerance are needed for autoimmune diseases. Recent advances in combinational ASIT may hold the key to improved therapeutics and will be discussed in a later section.

3.1. General immunosuppressants

Autoimmune diseases have traditionally used immunosuppressant medications that globally suppress the immune response. Immunosuppressants are highly effective for many patients and therefore remain the current "gold standard" of autoimmunity treatment [35]. In many immunosuppressant therapies, the benefits can be counterbalanced by toxicity or severe adverse events. In fact, current treatments for the autoimmune disease RA fail in up to 50% of patients due to adverse side effects [36].

Immunosuppressants used in autoimmune treatment consist of both small molecules and biologics, such as proteins and antibodies, and can elicit their effect through several different mechanisms. Many anti-inflammatory compounds act by inhibition of immune cell proliferation, such as lefunomide (Arava) for RA and teriflunomide (Aubagio) for MS, which block synthesis of DNA necessary for cell division [37]. Chemotherapeutics including mitoxantrone (Novantrone) and methotrexate have also been used in treating autoimmunity due to their ability to inhibit DNA synthesis [37,38]. Inhibition of cellular proliferation inhibits the rapid expansion of auto-reactive immune cells that can cause tissue damage and further inflammation thereby reducing disease symptoms.

Another common mechanism whereby immunosuppressant drugs act is via control of the cytokine response. Cytokines act as soluble messengers of the immune system; creating inflammatory or tolerogenic responses depending on the type and quantity of cytokines that are secreted in the local microenvironment. Autoimmune therapies have tried to leverage the complexity of the cytokine response by inhibiting the production and action of pro-inflammatory cytokines. Small molecule immunosuppressant compounds such as ciclosporin, used in the

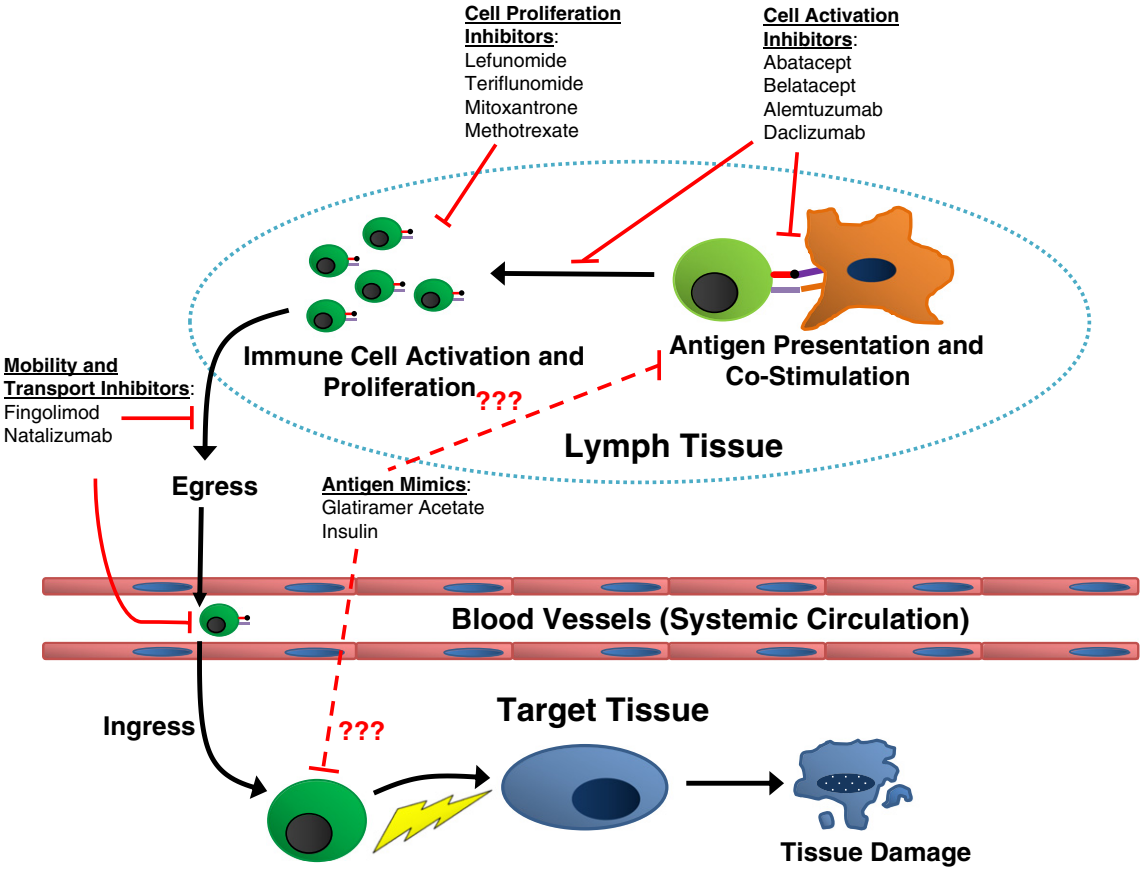

Fig. 1. Current therapies for autoimmunity fall into distinct categories, including immunosuppressants that inhibit cell proliferation, mobility and transport inhibitors, immune cell activation inhibitors, and antigen mimics. These therapies act throughout the immune response, in the lymph tissue, in systemic circulation, and in the diseased tissue to suppress autoimmune disease symptoms. Therapies often act at several locations and the mechanism of action of many of them, particularly those that fall under the category of antigen mimics, are not well understood.

treatment of RA and T1D, act by disrupting the pathway by which the pro-inflammatory cytokine IL-2 is produced [38]. Additionally, several different biologics inhibiting cytokine binding are approved for use to treat RA including tocilizumab (Actemra) and etancercept (Enbrel), which inhibit pro-inflammatory cytokine signaling by IL-6 and tumor necrosis factor (TNF)-α, respectively [38,39]. In the treatment of MS, interferon (IFN)-β therapy has been shown to decrease IFN-γ production through induction of neutralizing antibodies which help to decrease relapse rates in relapsing-remitting MS [37]. Although cytokine-targeted therapies have had successes in the clinic, the fact remains that cytokines are important in protection against invading pathogens, thus disruption of cytokine production or action can increase susceptibility to infection [12].

The mechanisms for many immunosuppressants currently used to treat autoimmunity are not well understood. Glucocorticoids, mainly prednisone and prednisolone, are commonly given to patients with SLE and RA. These drugs have been shown to have numerous pleiotropic immunosuppressant effects but may act somewhat by their ability to reduce the expression of cellular receptors needed for robust immune responses [40]. Dimethyl fumarate (Tecfidera) was approved by the FDA for treatment of MS in 2013 and is believed to work by preventing oxidative stress via activation of the Nrf2 transcriptional pathway; however, its influence on the immune response is still debated [37,41].

Unfortunately, a common theme among all immunosuppressants is their lack of specificity. These therapies must often be used long-term in order to suppress the immune response to self and do not cure the underlying disease condition but rather mitigate symptoms by reducing tissue damage and inflammation. Due to their long-term use and lack of specificity, severe toxicity issues associated with global immunosuppression are common [38,42].

3.2. Mobilitiy and transport inhibitors

Autoimmune diseases require the mobility of auto-reactive immune cells or antibodies to migrate to their site of action. Mobility and transport inhibitors attempt to prevent this process. Similar to general anti-inflammatory molecules, severe side effects are often associated with these therapies, as they can restrict the movement of immune cells that are necessary to fight off foreign pathogens. One such therapy, natalizumab (Tysabri), is a humanized antibody-targeting vascular cell adhesion molecule-1 (VCAM-1) for the treatment of MS. Natalizumab reduces leukocyte trafficking across the blood brain barrier by inhibiting binding to the necessary cell adhesion molecules, thereby decreasing the number of auto-reactive T-cells in the CNS tissue [43]. Unfortunately, soon after it was approved by the FDA in 2004, natalizumab was found to be associated with an increased incidence of progressive multifocal myeloencephalopathy (PML), a fatal viral disease of the CNS [44]. It was found that there were several risk factors associated with PML, most notably the presence of JC virus antibodies in MS patients. Upon implementation of PML risk mitigation strategies, including testing for JC virus antibodies before beginning therapy, natalizumab was reapproved in 2006 for MS patients un-responsive to other therapies [44]. Another mobility blocking therapy, efalizumab (Raptiva), an anti-LFA-1 antibody, met with a similar fate as natalizumab. Efalizumab was found to reduce the severity of chronic psoriasis, an autoimmune disease of the skin, but it was withdrawn from clinical use in all cases after several PML cases in patients [16].

Another FDA-approved drug, fingolimod (Gilenya), is a small molecule mobility inhibitor used for the treatment of MS. Fingolimod acts by internalization of S1P-receptors on immune cells to prevent them from egressing from lymph nodes and trafficking to the CNS [37]. Unlike natalizumab and efalizumab, fingolimod has not been shown to result in PML and can therefore be used in patients that test positive for the JC virus. Nevertheless, mobility and transport inhibitors are often not prescribed until an MS patient presents with aggressive disease and CNS lesions [37].

3.3. Immune cell activation inhibitors

As previously discussed, both antigen presentation and a co-stimulatory context signal are needed to activate immune cells in an antigen-specific immune response. Recent evidence suggests that a change or lack of co-stimulation can prevent immune activation and even skew the response toward tolerance [16,45,46]. Due to the importance of co-stimulation in directing the antigen-specific immune response, several co-stimulatory pathways have been investigated in the induction of tolerance and treatment of autoimmunity.

The B7 (CD80/86) signaling pathway is one of the most well-characterized co-stimulatory pathways in T-cell activation and has therefore been a major target in T-cell-mediated autoimmune diseases. The B7 pathway consists of two main molecular interactions, B7:CD28 binding leading to immune-stimulation and B7: CTLA-4 binding leading to immunosuppression or tolerance to the presented antigen [47]. Since CTLA-4 acts as a co-inhibitory signal in T-cell activation, it has been the key focus in targeting the B7 pathway for autoimmune therapy. Three immunomodulatory biologics approved by the FDA in the past 10 years either target or are derived from CTLA-4; with two primarily used in the treatment of autoimmunity [48]. Abatacept (Orencia), a CTLA-4 IgG1 fusion protein, was the first biologic targeting the B7 pathway approved to treat autoimmunity. It was initially approved to treat RA in 2005 and is currently under investigation in the treatment of other T-cell-mediated autoimmune diseases including T1D, psoriasis, and SLE [48]. Another CTLA-4 IgG1 fusion protein, belatacept (Nulojix), was created to improve binding affinity to B7 as compared to abatacept. Belatacept was approved to treat organ transplant rejection in 2011 and is currently in clinical trials for the treatment of RA and T1D [48]. Although these B7 pathway inhibitors show promise in the treatment of autoimmunity, they are not antigen-specific in their immune modulation.

Several other therapies target cell surface markers involved in activation of the immune response. Alemtuzumab (Lemtrada) is an anti-CD52 antibody approved for the treatment of MS [37,39]. CD52 is found on a variety of immune cells and, although its exact function is still unknown, it is believed to be involved in co-stimulation as its cross-linking leads in T-cell activation [37]. Another antibody targeting T-cell receptors, daclizumab (Zenapex), is approved to prevent organ transplant rejection and is currently under investigation as a treatment for MS. Daclizumab binds to CD25, which is expressed on activated T-cells and Tregs. Ongoing phase III clinical studies indicate that in addition to blocking T-cell activation, daclizumab also works to expand regulatory natural killer (NK) cells to treat MS [49].

In addition to targeting T-cell activation, with our increasing understanding of the role of B-cells in autoimmunity, there has been investigation into the use of B-cell-targeted therapies in the treatment of autoimmune diseases such as RA, MS, and SLE. Rituximab (Rituxan), a chimeric IgG1 anti-CD20 monoclonal antibody, is often administered alongside methotrexate to RA patients who are unresponsive to more common treatments such as anti-TNF agents [50]. Rituximab has also been successful in clinical trials investigating the effectiveness of B-cell depletion in the treatment of MS [51] and SLE [52]. Recently, other human antibodies targeting CD20 such as ocrelizumab, veltuzumab, ofatumumab, and TRU015 have been clinically investigated for treatment of autoimmune diseases [53].

3.4. Antigenic mimics

Use of antigen mimics, or “decoys,” is a strategy aimed at inducing an antigen-specific immune response while avoiding potential anaphlaxysis that may be associated with the native antigen. Insulin and insulin analogs used in the treatment of T1D are some of the most widely used antigen mimics for autoimmune therapy; however, until just recently, insulin was considered a hormonal therapy that had little to no effect on the immune response. Recent evidence suggesting that insulin is

the initiating antigen in the development of T1D has led researchers to revisit insulin therapy [54]. Although better understanding of the immune response offers the potential to enhance T1D treatment, so far clinical trials have failed to improve upon current insulin therapy [54].

Another form of antigen mimics, altered peptide ligands (APLs), is created through substituting different amino acid for those in the antigenic epitope. APLs of antigenic epitopes in MBP with varying affinity for MHC class II molecules have been synthesized and studied for the induction of immune tolerance to treat EAE. Results indicate a correlation between APL affinity for MHC class II molecules and EAE disease prevention, with APLs of higher affinity displaying a shift in cytokine secretion toward IL-10 and greater suppression of T-cell proliferation [55]. Due to the heterogeneity of the antigen-specific T-cell populations involved in an autoimmune response, it may be necessary to design an APL capable of inducing tolerance across a wide range of T-cell receptor (TCR) affinities in order to produce a lasting effect [56,57].

Glatiramer acetate (Copaxone) is an altered polymeric version of the MS-associated antigen MBP. Many immunomodulatory mechanisms have been proposed for glatiramer acetate including competitive binding to MHC class II molecules, a shift toward a T-helper type 2 immune response, and TCR antagonism in MBP-specific T-cells [57,58]. The multiple mechanism of action would suggest that glatiramer acetate may act through both antigen-specific and non-specific pathways to alter autoimmune responses; however, further studies are required to determine the relevance of each of these mechanisms.

3.5. Current combination therapies for autoimmune disease

In many cases, combinations of drugs from the therapeutic categories discussed previously are used in order to enhance efficacy. One example of a combination therapy currently prescribed to RA patients is treatment with both a small molecule immunosuppressant, methotrexate, and a TNF-α inhibitor in order to achieve a synergistic effect [38]. This synergistic effect is not found in all combinations of therapies utilizing two biologics. For example, a TNF-α inhibitor and co-stimulation inhibitor, abatacept, did not achieve additional clinical benefits in the treatment of RA but rather caused toxicity from immunosuppression complications [38]. Although this combination approach has shown promise, it is still missing the antigen specificity needed to reduce side effects and increase long-term efficacy.

4. Combination strategies for ASIT in autoimmunity

With the clinical inefficacy of many antigen-only therapies for autoimmunity, recent research has focused on combination therapy containing antigen and immunomodulator to enhance efficacy. Combination therapy can be accomplished by either co-administration (dosing in a similar time-frame, often via the same route) or co-delivery (utilizing a vehicle to physically or chemically keep the antigen and immunomodulator in close physical proximity) (Table 2 and Fig. 2). By applying the "antigen-adjuvant" combination paradigm of vaccines to the treatment autoimmunity, it may be possible skew the immune response toward antigen-specific tolerance.

Table 2
Definitions of types of ASIT delivery.

Term	Definition	Example
Mono-therapy	Single component therapy	Immunomodulatory drug alone, or antigen alone
Combination therapy	Multiple components given together in either the same time and/or same space	Encompasses both co-administration and co-delivery
Co-administration	Multiple components given together at the same time but not in the same space	Injection of antigen and immunomodulatory drug together but not held together either chemically or physically
Co-delivery	Multiple components given together in the same time and same space	Antigen and immunomodulatory drug are linked, co-encapsulated, or held together another way either chemically or physically

4.1. Co-administration

Many of the initial studies done with antigen and immunomodulators in the mid-2000s utilized co-administration to create ASIT combination therapy (Table 3). Dosing antigen and immunomodulator together without a co-delivery vehicle offers the flexibility of delivering the compounds via different routes. Also, the lack of a vehicle needed to co-encapsulate or connect the components may be more economically feasible and allow for ease of manufacturing and formulation; factors that may help accelerate the transition into to the clinic. Using co-administration in ASIT also has the disadvantage of producing similar side effects as many current therapies; since when the antigen and immunomodulator are separated the immunomodulator may produce a general immunosuppressive response rather than an antigen-specific response.

4.1.1. Co-administration with small molecule immunosuppressants

Small molecule immunosuppressants are commonly prescribed for autoimmune disease treatment. In order to reduce global immunosuppression, recent studies have investigated the co-injection of autoantigen, or DNA encoding autoantigen, simultaneously with a small molecule immunosuppressant. Kang and colleagues pioneered the use of the term "tolerogenic adjuvant" in their 2008 paper involving the co-administration of dexamethasone and autoantigen to induce antigen-specific tolerance in a model of autoimmunity [59]. Co-injection of dexamethasone and OVA resulted in long-term antigen-specific tolerance as well as the proliferation of OVA-specific regulatory T-cells. Similar antigen-specific tolerogenic responses were also seen using a T1D murine model [59]. In a subsequent paper, Kang and colleagues demonstrated that co-injection of a different "tolerogenic adjuvant," the immunosuppressant FK-506 (Tacrolimus), with a plasmid DNA encoding autoantigen, rather than the antigen itself, also results in expansion of Tregs and suppression of autoimmunity in mice [60].

4.1.2. Co-administration with biological molecules

Unlike the monoclonal antibodies that dominate the clinically approved biologics for autoimmunity, the majority of co-administration research for ASIT has focused on the use of plasmid DNA as the biological delivery platform. Co-administration of plasmid DNA encoding autoantigen and a plasmid containing immunomodulatory gene have been studied by several research groups. The injection of two plasmids, with autoantigen on one and immunomodulator on the other classifies these studies as co-administration rather than co-delivery.

In 2001, Garren and colleagues published a paper examining DNA vaccination using two plasmids, one encoded with interleukin (IL)-4, a cytokine associated with immunosuppression in MS, and the second encoded with an MS-associated autoantigen [61]. The co-vaccination strategy was tested in EAE mice with both PLP- and MOG-induced models. In both models, co-administration of IL-4 and autoantigen encoding plasmids was found to suppress EAE disease compared to treatment with each gene individually. Interestingly, the MOG and IL-4 DNA vaccination was able to reverse established disease when given after EAE symptoms were present [61]. In a similar study, Glinka and colleagues investigated the use of DNA vaccination to co-administer autoantigen and a co-stimulation blocker for the treatment of NOD mice [62]. The study used a plasmid encoding for a fusion construct of PPI and GAD65 for induction of autoantigen expression, along with a plasmid encoding a mutant B7 molecule known to bind CTLA-4 and block co-stimulation during T-cell activation. This approach was successful in decreasing disease symptoms and stimulating the tolerogenic response following co-administration of the plasmids [62].

Although co-administration of DNA has been successful in animal models, it may be difficult to control dosage kinetics and gene

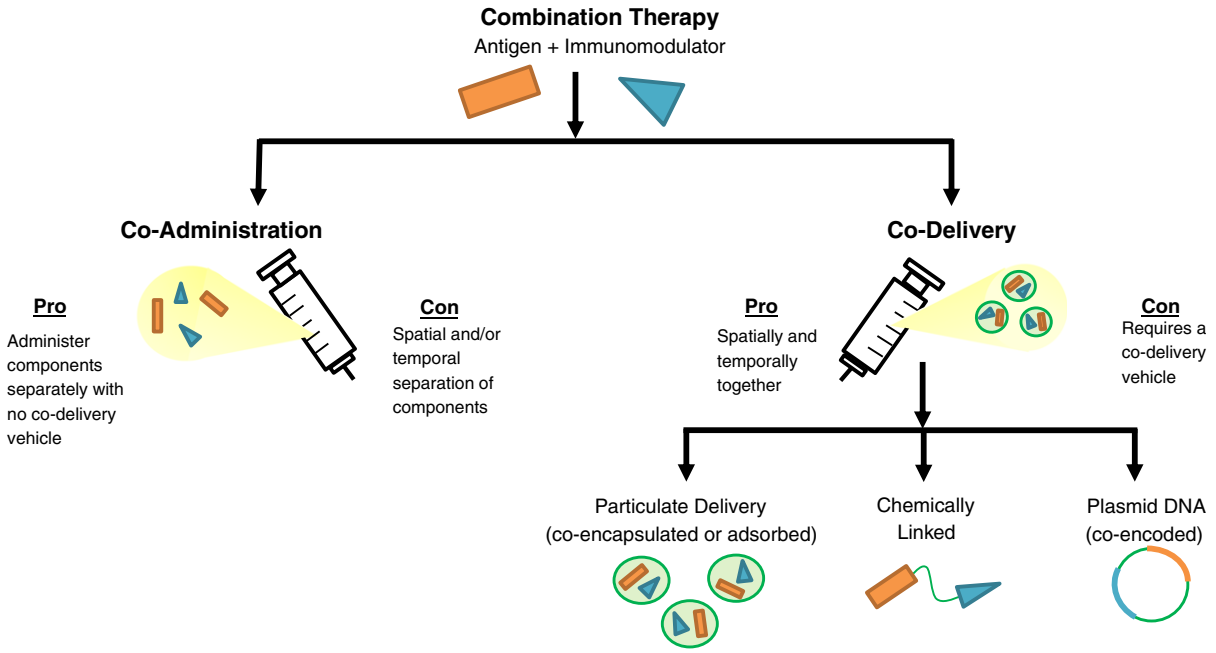

Fig. 2. Combination therapy for ASIT can be accomplished by either co-administration or co-delivery of antigen and immunomodulator. With the increasing diversity of antigen and immunomodulatory molecules that could be used for ASIT, each independent combination therapy will have to be rationally designed to fit appropriate formulation parameters. Several experimental technologies exist to temporally and/or spatially link antigen with immunomodulatory molecules. These include 1) co-administration or formulation of independent components into a single injection solution and 2) co-delivery or physical linkage of the antigen and immunomodulatory molecule. Both methods have shown positive ASIT data; however, long-term clinical benefit has not been established for each of these ASIT formulation approaches.

expression, limiting its clinical potential. The combination of protein immunomodulators with antigens has gained interest for easier clinical translation. In a recent study, MOG_{35-55} and the tolerogenic cytokine IL-10 were encapsulated into separate PLGA nanoparticles for the treatment of EAE [63]. Both prophylactic and therapeutic treatment regimens co-administering particles containing MOG and particles containing IL-10 significantly suppressed EAE symptoms [63]. Using a similar strategy, Lewis and colleagues created PLGA microparticles each containing a single component; insulin B autoantigen, GM-CSF, vitamin D3, or TGF-β1. When these 4 different microparticles were mixed at a 1:1:1:1 ratio, they were found to significantly prevent the incidence of T1D in NOD mice [64]. These successful experimental studies suggest the feasibility of a prophylactic or therapeutic co-administration platform to treat autoimmune disease.

4.1.3. Drawbacks of co-administration

While the co-administration approach has shown potential in animal models of autoimmunity, the lack of a formulation keeping the autoantigen and immunomodulator in the same microenvironment opens the door for non-specific immunosuppression or complete lack of efficacy upon separation of the components following dose administration. In fact, not delivering antigen and immunomodulator together both temporally and spatially can result in induction of an inflammatory response rather than a tolerogenic response. In a recent study, it was found that autoantigen co-administered with rapamycin, a small molecule immunosuppressant, resulted in expansion of autoantigen-specific T-cells and inhibition of Tregs, the opposite of the desired tolerogenic response [65].

Table 3
Co-administration examples in ASIT combination therapy.

Immunomodulator	Antigen	Disease model	Reference
Dexamethasone	$OVA_{323-339}$ peptide	Allergy	[59]
Dexamethasone	Insulin-derived B:9-23 peptide	T1D	[59]
FK-506	Plasmid encoding MOG_{35-55} peptide	MS	[60]
Plasmid encoding IL-4	Plasmid encoding $PLP_{139-151}$ peptide	MS	[61]
Plasmid encoding IL-4	Plasmid encoding MOG protein	MS	[61]
Plasmid encoding mutant B7-1 (B7-1wa)	Fusion plasmid of PPIns-GAD65 proteins	T1D	[62]
Recombinant IL-10	MOG_{35-55} peptide	MS	[63]
GM-CSF, vitamin D_3, and TGF-β1	Insulin-derived B:9-23 peptide	T1D	[64]

4.2. Co-delivery

Unlike co-administration, co-delivery ensures that the antigen and immunomodulator are delivered at the same time and presented in the same environment to auto-reactive immune cells (Table 2). Many investigators have examined the effect of delivering encapsulated immunomodulators or autoantigen alone for autoimmune therapy [66–70]; however, only recently have studies focused on the effect of co-delivering these components via a variety of different delivery vehicles (Table 4 and Fig. 2).

4.2.1. Co-delivery with small molecule immunosuppressants

Building upon previous literature using co-administration of autoantigen and small molecule immunosuppressant, several research groups have investigated the possibility of co-delivering these components. Various delivery vehicles have been employed with results that suggest that dosing compounds together both spatially and temporally may enhance treatment efficacy. Liposomes, dextran microparticles, and gold and PLGA nanoparticles have all been used in recent studies in order to co-deliver autoantigen and immunosuppressant for ASIT in animal models of autoimmunity [65,71–73]. In each of these examples, the two components were either co-encapsulated or co-adsorbed to the delivery vehicle to ensure simultaneous delivery of the components to immune cells [65,71–73].

Peine and colleagues extrapolated work by Kang and colleagues by co-encapsulating autoantigen and dexamethasone in microparticles [59,71]. Dexamethasone was co-encapsulated into acid-sensitive acetylated dextran microparticles with the MS-antigen MOG and used in the

Table 4
Co-delivery examples in ASIT combination therapy.

Immunomodulator	Antigen(s)	Disease model	Co-delivery vehicle	Reference
Dexamethasone	MOG_{35-55} peptide	MS	Acetylated dextran microparticles, co-encapsulated	[71]
ITE	MOG_{35-55}, $PLP_{139-151}$, and PLP_{18-191} peptides	MS	Gold nanoparticles loaded on surface and stabilized by PEG	[72]
Rapamycin	OVA protein and $OVA_{323-339}$ peptide	Allergy	PLGA nanoparticles, co-encapsulated	[65]
Rapamycin	$PLP_{139-151}$ peptide	MS	PLGA nanoparticles, co-encapsulated	[65]
Rapamycin	$FVIII_{74-89}$, $FVIII_{1723-1737}$, $FVIII_{2191-2210}$ peptides	Hemophilia	PLGA nanoparticles, co-encapsulated	[65]
NF-κB inhibitor (curcumin, quercetin, or Bay 11-7082)	OVA protein	Allergy	Co-encapsulated in liposomes	[73]
Curcumin	Methylated BSA protein	RA	Co-encapsulated in liposomes	[73]
LABL peptide ($CD11a_{237-247}$)	$GAD65_{208-217}$ peptide	T1D	Linked via spacer peptide	[74]
LABL peptide ($CD11a_{237-246}$)	$PLP_{139-151}$ peptide	MS	Linked via spacer peptide	[75]
LABL peptide ($CD11a_{237-246}$)	$PLP_{139-151}$ peptide	MS	Mulivalently linked to same hyaluronic acid polymer backbone	[77]
B7 pathway targeting peptide (B7AP, CD80-CAP, or sF2)	$PLP_{139-151}$ peptide	MS	Mulivalently linked to same hyaluronic acid polymer backbone	[78]
Plasmid encoding IL-4	Plasmid encoding GAD65-IgG Fc fusion protein	T1D	Encoded on same plasmid	[79]
Plasmid encoding BAX	Plasmid encoding GAD65	T1D	Encoded on same plasmid	[80]
Plasmid encoding BTLA	TAT_{49-57} MOG_{35-55} peptide	MS	Self-assembled nanoparticles of peptide and plasmid; used to treat DCs that were injected into mice	[81]

treatment of EAE. The co-delivery of the components significantly decreased clinical disease score as compared to mixtures of dexamethasone and MOG, demonstrating the importance of delivering both components concurrently to immune cells [71].

Yeste and colleagues investigated co-delivery of MOG and the small molecule immunosuppressant 2-(1′*H*-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) [72]. Both MOG and ITE were co-loaded onto the outside of gold nanoparticles and were found to induce tolerogenic DCs and FoxP3 + Tregs in naïve primary cells. The nanoparticles significantly suppressed EAE disease symptoms as compared to the components given without use of the co-delivery vehicle [72]. In a unique experiment, Yeste and colleagues also demonstrated that their co-delivery system was effective even after epitope spreading, by utilizing two different epitopes of autoantigen to suppress EAE [72]. Using a similar approach, Maldonado and colleagues delivered both autoantigen and rapamycin co-encapsulated in PLGA nanoparticles to treat a number of autoimmune models [65]. The antigen–rapamycin nanoparticles were able to induce antigen-specific tolerance in EAE, in a model of hypersensitivity, and in a model of the genetic disease hemophilia [65]. In both studies, encapsulating the immunosuppressant alone was found to suppress disease; however, the autoantigen-containing nanoparticle did not [65,72].

In addition to the possible use of different types of delivery vehicles to produce antigen-specific tolerance, Capini and colleagues demonstrated that different immunosuppressant drugs could also be effective [73]. Their study examined the effects of three different NF-κB inhibitors: curcumin, quercetin, and Bay11-7082. When co-encapsulated into liposomes with autoantigen, each of the three compounds was able to induce antigen-specific Treg responses and decrease disease severity in a mouse model of RA, antigen-induced inflammatory arthritis (AIA) [73].

4.2.2. Co-delivery with peptides

Peptides targeting immune cell adhesion or co-stimulation molecules have been conjugated with autoantigen epitopes to enable co-delivery. Siahaan and colleagues have published a number of papers on bi-functional peptide inhibitors (BPIs) that link a peptide autoantigen with an immune cell inhibitor targeting the cell adhesion molecule ICAM-1. BPIs have suppressed disease in animal models of both T1D and MS [74,75]. The originally developed BPI co-delivery vehicle contained the MS epitope $PLP_{139-151}$ linked to the peptide LABL, derived from α_L integrin, for the treatment of EAE. This unique ASIT was found to significantly decrease the severity of EAE disease as compared to each peptide alone or mixed [75,76]. Interestingly, the BPI decreased the rate of anaphylaxis in mice as compared to PLP alone, suggesting that autoimmune treatments containing immunomodulators may offer improved safety as compared to autoantigen alone [75].

Building off of Siahaan's work, Berkland and colleagues have published several papers on a multivalent approach known as Soluble Antigen Arrays (SAgAs). SAgAs consist of antigenic peptides and immunomodulator peptides that are co-delivered via a hyaluronic acid backbone. Peptides inhibiting cell adhesion (via ICAM-1) and the B7 (CD80/CD86) pathway have shown efficacy in EAE when co-delivered with MS antigen using SAgAs [77,78].

4.2.3. Co-delivery with biological molecules

As previously discussed, DNA vaccines have successfully co-administered two plasmids, separate autoantigen and immunomodulator, to treat autoimmunity. In attempt to improve upon this technique, autoantigen and immunomodulator were encoded on a single plasmid. A couple of recent studies investigated this strategy for the treatment of TID in NOD mice with plasmids containing GAD65 and a secondary molecule, the cytokine IL-4 or the pro-apoptotic protein BAX, respectively [79,80]. In both studies, the plasmid containing both autoantigen and immunomodulator was able to prevent and suppress disease [79,80].

Another study utilized the co-delivery of a plasmid encoding the B- and T-lymphocyte attenuator protein (BTLA) and MOG antigen to pre-treat DCs before using those DCs to treat EAE mice [81]. These pre-treated DCs were found to decrease the severity of EAE when injected prophylactically; however, this approach may be too complex for clinical application [81].

5. Clinical trials of ASIT for autoimmunity

5.1. Antigen-only ASIT clinical trials

Similar to allergy hyposensitization, the introduction of a disease-causing autoantigen to a patient with autoimmunity can result in undesired and potentially life-threatening adverse events [82,83]. A Phase II clinical study of an altered peptide ligand of MBP, an antigen associated with MS, had to be halted due to three of the eight patients suffering worsening symptoms, resulting in an increase in CNS lesions up to 2.4 times the amount before therapy and leaving one patient unable to walk. In other cases, administration of a slightly altered autoantigen to treat autoimmunity did not directly aggravate the disease but instead resulted in an allergic response to the antigen [83]. Fortunately, in the majority of clinical trials, these adverse events were avoided; however, ASIT did not result in any benefit compared to placebo [84].

5.2. Combination ASIT clinical trials

Recently, trials of combinations of antigen and immunomodulator for ASIT have shown promise. In several clinical studies for MS, the FDA-approved drug glatiramer acetate (GA) was used as a mimic of

the disease-associated antigen MBP and was co-administered with immunomodulators to study the effects of combination therapy. In one study, improved success in both decreasing CNS symptoms and lowering the risk of disease relapse was seen in combination of GA and the antibiotic minocycline, as compared to GA alone [85]. In another study, GA and natalizumab co-therapy was found to be safe and suppressed CNS lesions in MS compared to GA alone [86]. Unfortunately, the majority of combination trials with GA did not contain a control group with immunomodulator alone; however, in comparing therapy outcomes to those seen with natalizumab, it was found that the combination therapy did not improve efficacy [86]. A few studies have had success with a modified dosing schedule, where patients undergo short-term use of immunosuppressant therapy, either with mitoxantrone or methylprednisolone, with GA before starting on GA alone [87,88]. In addition to improving clinical outcomes compared to GA alone, it was also found that the short-term therapy limited the adverse effects associated with long-term immunosuppressive use [87].

Although ASIT using only antigen has been successful in allergies, recent clinical trials with co-administration of an immunomodulator have been found to be more effective than allergen alone. In several clinical studies, omalizumab (Xolair), an anti-IgE antibody, was added to allergen hyposensitization therapy in the hope of decreasing the chance of dangerous side effects, such as anaphylaxis [89]. This combination therapy was found to increase efficacy as compared to the allergy-associated antigen alone [89,90].

A slight variation of the use of "tolerogenic adjuvants" in ASIT for autoimmunity is the success of traditional vaccine adjuvants co-administered with antigen for improved allergy hyposensitization therapy and ASIT for asthma [91]. When using traditional adjuvants for autoimmune therapy, the key to creating a successful ASIT for autoimmunity may reside in finding the appropriate patient population. Attempts to co-administer a T1D antigen GAD65 with a traditional vaccine adjuvant, alum, met with limited success [31]. Upon further trials, it was found that this treatment was successful in suppressing T1D but only in children and adolescents with recent onset of the disease [30]. In another successful clinical study, human B-chain of insulin was given to patients with incomplete Freund's adjuvant. Although the focus of this study was safety, it was also found that patients had a robust antigen-specific Treg response even 2 years after finishing the treatment [92]. With the success of immunomodulator co-administration in recent literature, it is possible that the addition of an immunosuppressant to traditional adjuvants combined with antigen may allow for even broader patient efficacy.

6. Challenges for the future of ASIT for autoimmunity

6.1. Human translation of pre-clinical successes

Although there are promising results in animal models of autoimmune diseases, most successes in these models have not been translatable to humans. One of the most promising methods for ASIT autoimmunity that had success in animal models was the administration of oral antigen to treat MS, T1D, or RA. When attempted in humans, no therapeutic benefits were found [7,8]. While it is difficult to interpret these negative findings, discrepancies in immune tolerance and autoimmunity between humans and animal models, such as mice, have been noted. These include differential expression of Treg markers such as FoxP3, variations in the balance of leukocyte subsets, dysregulation of central tolerance such as thymic selection, and different roles played by cells that produce IL-17, among others [93]. Directly linked to these immunological differences, the development of the disease in animals is often unrelated to that in humans. Often animal models of autoimmunity require induction with an immunogenic antigen, such as in the majority of EAE models of MS [94]. A few disease models do exist where the autoimmune disease can occur spontaneously such as NOD and some versions of EAE, which in some instances may offer better understanding of the human disease than inducible models [93,94].

Acute animal models of autoimmunity also may not be predictive for the treatment of chronic human immune disorders. The majority of animal studies conducted treat the disease before symptoms appear, whereas human therapies will mostly be given years after onset of the pathogenic process [8,94]. Many animal studies are terminated too early to see any long-term issues that may arise. For example, only 7% of all studies with NOD mice are followed up beyond 32 weeks, which does not reflect the lifelong duration of T1D in humans [93]. Additionally, the complete disease-causing mechanisms are not completely understood in humans. For example, it has been hypothesized from studies of identical twins that while there is a genetic component to many autoimmune disorders, there are also additional "environmental" components that affect the disease that are not reflected in highly controlled pre-clinical studies and may offer limited applicability to human trials [95].

Treatment safety and tolerability, which is immensely important in human therapies, is also often overlooked or difficult to assess in animal models [8]. Some safety issues may only arise in humans, and using cells from human donors in combination with animal models may help prevent toxic compounds from reaching the clinic [93]. For example, the production of a cytokine storm in humans using a CD28 agonist was not foreseen using animal models [93].

Nevertheless, these animal models have helped make important discoveries in the treatment of human autoimmunity. The EAE model of MS has helped identify four recently approved therapies; glatiramer acetate (Copaxone), mitoxantrone (Novantrone), natalizumab (Tysabri), and fingolimod (Gilenya) [7]. Improved animal models and better understanding of the immunology of human autoimmunity may increase the clinical success of experimental therapies.

6.2. Antigen identification and epitope spreading

For many autoimmune diseases, the animal model is not the rate-limiting step to developing ASIT; rather, the immunodominant disease-causing antigen(s) may not be identified. SLE, for example, can manifest symptoms in many different organs and the disease-causing autoantigen may vary greatly between SLE patients [96]. Even diseases that have relatively well-characterized disease-causing antigens, such as MS, a single antigen for ASIT can be difficult to determine due to epitope spreading [97]. A few recent trials in MS have shown promise by using multiple antigens to elicit the antigen-specific response; however, they are still in the early stages of human testing [98,99].

In allergy hyposensitization therapy, diagnostics, such as the skin prick test, determine the most important allergen in specific patients [100]. If this type of "personalized medicine" could be applied to autoimmunity, it may greatly improve outcomes. The analysis of peripheral blood for immunodominant autoantigens may allow for ASIT to be tailored to the individual patient, increasing the possibility for therapeutic success [93]. Emerging diagnostic practices such as component-resolved testing, high-throughput antibody repertoire analysis, and indirect T-cell recognition assays may improve the ability to determine the correct autoantigen for personalized ASIT [101–103].

Autoimmune diseases with only one known immunodominant antigen, including myasthenia gravis and neuromyelitis optica, may provide better targets for ASIT [104]. Recent studies using an antibody against the disease-causing antigen in neuromyelitis optica, aquaporin 4, have shown success in animals and will soon enter clinical trials [105].

6.3. Immunomodulator optimization

While antigen(s) for ASIT are defined by the disease, there is a wide-array of immunomodulators to choose from when exploring combination therapy. To date, the immunomodulator chosen for co-administration or co-delivery with antigen has been ad hoc at best.

The majority of studies focus on a single immunomodulator in combination therapy for ASIT. A few recent studies have attempted to determine the best tolerance-inducing immunomodulator by measuring the induction of Tregs by various small molecule immunosuppressants [106,107]. However, a successful immunomodulator in one autoimmune disease may not be appropriate for another, and therefore, immunomodulator screening may need to occur on a disease-by-disease basis. Additionally, recent successes of combinations of immunomodulators for autoimmune treatment may indicate that multiple immunomodulators may be more effective for the induction of antigen-specific tolerance [108,109].

Recent clinical successes have been achieved utilizing traditional adjuvants as immunomodulators in ASIT [30,92]. Unfortunately, the viability of this method for the treatment of autoimmunity is still hotly debated as conflicting studies have shown that combinations of traditional adjuvants and antigen can both induce and treat autoimmune disease in rodent models. Immunologists have only recently started unraveling mechanisms such as immune cell "exhaustion" in autoimmunity and immune tolerance pathways in cancer, both of which may have direct implications for ASIT combination therapy [110,111].

6.4. Co-delivery vehicle

Co-delivery adds an extra layer of complexity to the creation of ASIT for autoimmunity due to the need for the correct delivery vehicle, yet mounting evidence suggests that co-delivery may do more to enhance the antigen-specific tolerance than co-administration of separate components. The determination of the correct co-delivery vehicle is important to ensure both antigen and immunomodulator interact with the immune response at the same time and in the same space. Numerous vehicles for co-delivery were utilized for combination therapy in ASIT reviewed here, including microparticles, nanoparticles, liposomes, direct chemical linkage, multivalent presentation on polymers, and plasmid DNA (Fig. 2) [65,71–75,77–80]. Each of these approaches offers its own unique benefits, challenges, and potential.

The particulate delivery systems (e.g. microparticles, nanoparticles, and liposomes) most directly mimic the delivery systems currently employed in vaccines. Vaccines commonly utilize aluminum salts, which are particulate in nature, to deliver the antigen of interest [112]. Recent studies with antigen conjugated to micro- and nanoparticles have successfully suppressed disease in EAE [113,114]. These particulate systems are often intended to be immunologically inert; however, the material, size, and shape of the particles can promote immune responses [115,116]. Particulate delivery systems are unique in that the antigen and immunomodulator may be on the surface of the particle, encapsulated, or both. Particles with surface-conjugated antigens may be preferred when targeting B-cells [116]. Surface antigens may also target T-cells when displayed in the context of MHC [117]. Alternatively, encapsulation of antigen is often used when uptake by APCs is preferred as much higher concentrations of antigen per particle can be achieved with encapsulation in contrast to surface modification [116]. Encapsulation of antigen and/or immunomodulator can also improve pharmacokinetic properties; for example, encapsulation of antigen can decrease rapid dilution and clearance that is associated with many injected biologics [116].

Multivalency may also influence the immune response, as it has been shown that the valency and the size of multivalent scaffolds play an important role in immunomodulation [115]. Dintzis and colleagues developed a number of "rules" exploring the effect of multivalency on the immunogenicity or tolerogenic properties of linear polymeric delivery systems [115,118]. They found that polymers with a molecular weight greater than 100 kDa and a valency greater than 20 compounds per polymer were more immunogenic, while systems under 100 kDa tended to be more tolerogenic [115]. Both particulate systems with surface-bound materials and linear polymers displaying antigens have utilized multivalency as an approach to ASIT combination therapy for autoimmunity [72,77,78].

Plasmid DNA delivery systems have also been investigated for combination therapy, as both antigen and immunomodulator can be encoded onto a single plasmid[79,80]. Antigen-specific treatments utilizing DNA have been shown to have benefits over whole protein or peptide antigens such as increased intercellular persistence due to stable expression from transduced genes [119]. Recent clinical trials utilizing a plasmid DNA encoding proinsulin demonstrated positive results in antigen-specific tolerance in T1D patients [120].

Finally, a very unique delivery system of utilizing cells themselves as delivery vehicles for ASIT has emerged with the potential to induce antigen-specific tolerance in autoimmunity. In studies spanning several decades, Miller and colleagues have shown that chemically coupling antigen to apoptotic cells can be used to induce antigen-specific tolerance [121,122]. Antigens coupled to apoptotic splenocytes, peripheral blood leukocytes, or erythrocytes have had positive results in animal models of autoimmunity [122–124]. Additionally, these antigen-coupled cells have been tested in humans and have shown promising results in a Phase 1 clinical trial [125]. Another innovation utilizes cells treated with ASIT *ex vivo*. In these systems, DCs are obtained from the bone marrow of genetically similar animals and treated with antigen and immunomodulator [81,126]. The cells treated with the combination therapy are then injected into the autoimmune animal model to induce tolerance [81,126]. These studies benefit from utilizing a delivery system capable of removing the "middle-man" of cellular uptake by APCs and co-delivery of immunomodulator, since cells are treated *ex vivo*. Unfortunately, cell-based methods for ASIT are still relatively young and experimental. Furthermore, the complexity of these systems may create difficulty in widespread clinical application due to challenges associated with manufacturing, high cost, and patient accessibility[125].

6.5. Route of administration

Recently published studies in animals have used a variety of different routes of administration including intravenous (IV), intramuscular (IM), and subcutaneous (SC), with over-arching success. When translating these therapies to humans and larger animal models, the route of administration will certainly play an import role in clinical outcomes. The route of administration dictates the barriers the therapy will face before reaching the site of action. For example, oral therapies must migrate through the GI track and often undergo first-pass metabolism in the liver before entering circulation, whereas IV therapies bypass these barriers. The route of administration in animals may not be translatable to humans, such as the use of oral antigen administration for autoimmunity that was found to have minimal clinical efficacy [7,8].

Many of the ASIT strategies utilize the interaction of immune cells in the lymphatic system in order to skew the immune response toward tolerance. It has been demonstrated that efficient delivery of vaccine components to the lymph nodes is critical to mounting an effective antigen-specific response [127]. By optimizing delivery vehicle size, drainage to lymph nodes has been achieved from multiple different injection sites [128]. Nanoparticles ranging in size from 10 to 200 nm have been found to drain to the lymph nodes following injection [128]. SC delivery has been effective in both passive drainage and active transport by peripheral macrophages from the site of injection to the lymph node [115]. IM injection may be more likely to utilize active transport as immune cells are often recruited to the depot at the injection site [115]. A unique route of administration, intranodal injection, bypasses the transport step. Intranodal administration in allergy hyposensitization has been shown to safely promote antigen-specific tolerance while reducing dose size by up to 1000× the dose delivered via conventional routes [129].

Allergy hyposensitization strategies have explored sublingual, intranasal, and oral routes of administration [1]. Sublingual treatment has yielded the greatest success as it increases convenience while maintaining the efficacy of the traditional SC therapy [4]. Recently, three sublingual hyposensitization therapies have been approved by

the FDA to treat grass and ragweed allergies [130]. Historically, intranasal administration of hyposensitization therapy had suffered from a high number of local adverse events [131]. A new approach utilizing strips coated with dust-mite allergens for transdermal delivery at the nasal septum reported positive outcomes in a recent clinical trial [132]. Oral hyposensitization to food allergens has also had some clinical success; however, there is still concern about serious adverse reactions, which could be addressed via combination therapy strategies proposed here [131].

7. Conclusion

Creation of an antigen-specific immune response has long been the cornerstone of vaccines, arguably one of the most important healthcare-related inventions. Mechanisms based on prototypical vaccine design have been effectively adapted for producing antigen-specific tolerance for allergies (i.e., hyposensitization therapy); however, clinical advancement of effective experimental ASIT therapeutics to treat autoimmunity has not been as successful. As outlined above, the approach of vaccines, which utilize both antigen and immunomodulator (i.e., adjuvant), may hold the key to developing successful ASIT for autoimmune disorders and potentially to improve current hyposensitization therapies. Researchers have seen promising results in an array of experimental models of autoimmunity by both co-administration and co-delivery of autoantigen and immunomodulator as an enhanced ASIT treatment. Future work should emphasize the effects of each component alone and together in combination therapies to enhance our understanding of the mechanisms by which tolerance is induced. As these strategies and experimental therapies evolve and move into the clinic, the outcomes of these studies may vastly change the way that autoimmune therapy is approached, especially with the potential to increase efficacy, diminish side effects, and reduce the lengthy dosing schedule of current hyposensitization therapy. With several recent successful proof-of-principle studies, there is increased hope that ASIT combination therapy may hold the potential to cure autoimmune diseases, rather than just treat and/or prevent disease symptoms.

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References

[1] M.T. Krishna, A.P. Huissoon, Clinical immunology review series: an approach to desensitization, Clin. Exp. Immunol. 163 (2011) 131–146.
[2] L. Noon, Prophylactic inoculation against hay fever, Lancet 1 (1911) 1572–1573.
[3] J. Freeman, Further observations on the treatment of Hay fever by hypodermic inoculations of pollen vaccine, Lancet 2 (1911) 814–817.
[4] C.B. Smarr, P.J. Bryce, S.D. Miller, Antigen-specific tolerance in immunotherapy of Th2-associated allergic diseases, Crit. Rev. Immunol. 33 (2013) 389–414.
[5] M. Larche, C.A. Akdis, R. Valenta, Immunological mechanisms of allergen-specific immunotherapy, Nat. Rev. Immunol. 6 (2006) 761–771.
[6] C.A. Sabatos-Peyton, J. Verhagen, D.C. Wraith, Antigen-specific immunotherapy of autoimmune and allergic diseases, Curr. Opin. Immunol. 22 (2010) 609–615.
[7] A.H. Badawi, T.J. Siahaan, Immune modulating peptides for the treatment and suppression of multiple sclerosis, Clin. Immunol. 144 (2012) 127–138 (Orlando, Fla.),.
[8] M. Feldmann, L. Steinman, Design of effective immunotherapy for human autoimmunity, Nature 435 (2005) 612–619.
[9] D.C. Wraith, Therapeutic peptide vaccines for treatment of autoimmune diseases, Immunol. Lett. 122 (2009) 134–136.
[10] P.O. Anderson, B.A. Manzo, A. Sundstedt, S. Minaee, A. Symonds, S. Khalid, M.E. Rodriguez-Cabezas, K. Nicolson, S. Li, D.C. Wraith, P. Wang, Persistent antigenic stimulation alters the transcription program in T cells, resulting in antigen-specific tolerance, Eur. J. Immunol. 36 (2006) 1374–1385.
[11] S.P. Cobbold, The mTOR pathway and integrating immune regulation, Immunology 140 (2013) 391–398.
[12] T.J. Kindt, R.A. Goldsby, B.A. Osborne, J. Kuby, Kuby Immunology, 6th ed. W.H. Freeman and Company, New York, 2007.
[13] G.Y. Liu, P.J. Fairchild, R.M. Smith, J.R. Prowle, D. Kioussis, D.C. Wraith, Low avidity recognition of self-antigen by T cells permits escape from central tolerance, Immunity 3 (1995) 407–415.
[14] D.L. Mueller, Mechanisms maintaining peripheral tolerance, Nat. Immunol. 11 (2010) 21–27.
[15] L.S. Walker, A.K. Abbas, The enemy within: keeping self-reactive T cells at bay in the periphery, Nat. Rev. Immunol. 2 (2002) 11–19.
[16] C. Chittasupho, T.J. Siahaan, C.M. Vines, C. Berkland, Autoimmune therapies targeting costimulation and emerging trends in multivalent therapeutics, Ther. Deliv. 2 (2011) 873–889.
[17] L. Chen, D.B. Flies, Molecular mechanisms of T cell co-stimulation and co-inhibition, Nat. Rev. Immunol. 13 (2013) 227–242.
[18] B.T. Fife, J.A. Bluestone, Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways, Immunol. Rev. 224 (2008) 166–182.
[19] P. Waterhouse, J.M. Penninger, E. Timms, A. Wakeham, A. Shahinian, K.P. Lee, C.B. Thompson, H. Griesser, T.W. Mak, Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4, Science (New York, N.Y.) 270 (1995) 985–988.
[20] J.P. Mackern-Oberti, F. Vega, C. Llanos, S.M. Bueno, A.M. Kalergis, Targeting dendritic cell function during systemic autoimmunity to restore tolerance, Int. J. Mol. Sci. 15 (2014) 16381–16417.
[21] D. Wang, B. Sun, M. Feng, H. Feng, W. Gong, Q. Liu, S. Ge, Role of scavenger receptors in dendritic cell function, Hum. Immunol. 76 (2015) 442–446.
[22] D.A. Vignali, L.W. Collison, C.J. Workman, How regulatory T cells work, Nat. Rev. Immunol. 8 (2008) 523–532.
[23] A.L. Mellor, D.H. Munn, IDO expression by dendritic cells: tolerance and tryptophan catabolism, Nat. Rev. Immunol. 4 (2004) 762–774.
[24] A.M. Ercolini, S.D. Miller, The role of infections in autoimmune disease, Clin. Exp. Immunol. 155 (2009) 1–15.
[25] T.A.D.C. Commitee, Progress in Autoimmune Diseases Research, Report to Congress, U.D.o.H.a.H, Bethesda, MD, 2005.
[26] L.A. Casciola-Rosen, G. Anhalt, A. Rosen, Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes, J. Exp. Med. 179 (1994) 1317–1330.
[27] L.I. Sakkas, D.P. Bogdanos, C. Katsiari, C.D. Platsoucas, Anti-citrullinated peptides as autoantigens in rheumatoid arthritis-relevance to treatment, Autoimmun. Rev. 13 (2014) 1114–1120.
[28] K.T. Coppieters, L.C. Harrison, M.G. von Herrath, Trials in type 1 diabetes: antigen-specific therapies, Clin. Immunol. 149 (2013) 345–355 (Orlando, Fla.).
[29] V.A. Huurman, P.E. van der Meide, G. Duinkerken, S. Willemen, I.R. Cohen, D. Elias, B.O. Roep, Immunological efficacy of heat shock protein 60 peptide DiaPep277 therapy in clinical type I diabetes, Clin. Exp. Immunol. 152 (2008) 488–497.
[30] J. Ludvigsson, M. Cheramy, S. Axelsson, M. Pihl, L. Akerman, R. Casas, G.A.D.S.G.i.S, Clinical, GAD-treatment of children and adolescents with recent-onset type 1 diabetes preserves residual insulin secretion after 30 months, Diabetes Metab. Res. Rev. 30 (2014) 405–414.
[31] J. Ludvigsson, D. Krisky, R. Casas, T. Battelino, L. Castano, J. Greening, O. Kordonouri, T. Otonkoski, P. Pozzilli, J.J. Robert, H.J. Veeze, J. Palmer, U. Samuelsson, H. Elding Larsson, J. Aman, G. Kardell, J. Neiderud Helsingborg, G. Lundstrom, E. Albinsson, A. Carlsson, M. Nordvall, H. Fors, C.G. Arvidsson, S. Edvardson, R. Hanas, K. Larsson, B. Rathsman, H. Forsgren, H. Desaix, G. Forsander, N.O. Nilsson, C.G. Akesson, P. Keskinen, R. Veijola, T. Talvitie, K. Raile, T. Kapellen, W. Burger, A. Neu, I. Engelsberger, B. Heidtmann, S. Bechtold, D. Leslie, F. Chiarelli, A. Cicognani, G. Chiumello, F. Cerutti, G.V. Zuccotti, A. Gomez Gila, I. Rica, R. Barrio, M. Clemente, M.J. Lopez Garcia, M. Rodriguez, I. Gonzalez, J.P. Lopez, M. Oyarzabal, H.M. Reeser, R. Nuboer, P. Stouthart, N. Bratina, N. Bratanic, M. de Kerdanet, J. Weill, N. Ser, P. Barat, A.M. Bertrand, J.C. Carel, R. Reynaud, R. Coutant, S. Baron, GAD65 antigen therapy in recently diagnosed type 1 diabetes mellitus, N Engl J Med 366 (2012) 433–442.
[32] J. Fraussen, N. Claes, L. de Bock, V. Somers, Targets of the humoral autoimmune response in multiple sclerosis, Autoimmun. Rev. 13 (2014) 1126–1137.
[33] L. Steinman, Immunology of relapse and remission in multiple sclerosis, Annu. Rev. Immunol. 32 (2014) 257–281.
[34] A.P. Robinson, C.T. Harp, A. Noronha, S.D. Miller, The experimental autoimmune encephalomyelitis (EAE) model of MS: utility for understanding disease pathophysiology and treatment, Handb. Clin. Neurol. 122 (2014) 173–189.
[35] M.D. Rosenblum, I.K. Gratz, J.S. Paw, A.K. Abbas, Treating human autoimmunity: current practice and future prospects, Sci. Transl. Med. 4 (2012) (125sr121).
[36] F. Wolfe, K. Michaud, B. Stephenson, J. Doyle, Toward a definition and method of assessment of treatment failure and treatment effectiveness: the case of leflunomide versus methotrexate, J. Rheumatol. 30 (2003) 1725–1732.
[37] A.H. Cross, R.T. Naismith, Established and novel disease-modifying treatments in multiple sclerosis, J. Intern. Med. 275 (2014) 350–363.
[38] M. Her, A. Kavanaugh, Advances in use of immunomodulatory agents-a rheumatology perspective, Nat. Rev. Gastroenterol. Hepatol. 12 (2015) 363–368.
[39] Z. Rosman, Y. Shoenfeld, G. Zandman-Goddard, Biologic therapy for autoimmune diseases: an update, BMC Med. 11 (2013) 88.
[40] G. Ruiz-Irastorza, A. Danza, M. Khamashta, Glucocorticoid use and abuse in SLE, Rheumatology 51 (2012) 1145–1153.
[41] P. Albrecht, I. Bouchachia, N. Goebels, N. Henke, H.H. Hofstetter, A. Issberner, Z. Kovacs, J. Lewerenz, D. Lisak, P. Maher, A.K. Mausberg, K. Quasthoff, C. Zimmermann, H.P. Hartung, A. Methner, Effects of dimethyl fumarate on neuroprotection and immunomodulation, J. Neuroinflammation 9 (2012) 163.
[42] S.D. Miller, D.M. Turley, J.R. Podojil, Antigen-specific tolerance strategies for the prevention and treatment of autoimmune disease, Nat. Rev. Immunol. 7 (2007) 665–677.

[43] J.M. Fletcher, S.J. Lalor, C.M. Sweeney, N. Tubridy, K.H. Mills, T cells in multiple sclerosis and experimental autoimmune encephalomyelitis, Clin. Exp. Immunol. 162 (2010) 1–11.
[44] R. Rudick, C. Polman, D. Clifford, D. Miller, L. Steinman, Natalizumab: bench to bedside and beyond, JAMA Neurol. 70 (2013) 172–182.
[45] L. Steinman, Blocking adhesion molecules as therapy for multiple sclerosis: natalizumab, Nat. Rev. Drug Discov. 4 (2005) 510–518.
[46] J.R. Podojil, D.M. Turley, S.D. Miller, Therapeutic blockade of T-cell antigen receptor signal transduction and costimulation in autoimmune disease, Adv. Exp. Med. Biol. 640 (2008) 234–251.
[47] A.H. Sharpe, G.J. Freeman, The B7-CD28 superfamily, Nat. Rev. Immunol. 2 (2002) 116–126.
[48] S. Yao, Y. Zhu, L. Chen, Advances in targeting cell surface signalling molecules for immune modulation, Nat. Rev. Drug Discov. 12 (2013) 130–146.
[49] R. Milo, The efficacy and safety of daclizumab and its potential role in the treatment of multiple sclerosis, Ther. Adv. Neurol. Disord. 7 (2014) 7–21.
[50] J.C. Edwards, G. Cambridge, B-cell targeting in rheumatoid arthritis and other autoimmune diseases, Nat. Rev. Immunol. 6 (2006) 394–403.
[51] S.L. Hauser, E. Waubant, D.L. Arnold, T. Vollmer, J. Antel, R.J. Fox, A. Bar-Or, M. Panzara, N. Sarkar, S. Agarwal, A. Langer-Gould, C.H. Smith, B-cell depletion with rituximab in relapsing-remitting multiple sclerosis, N. Engl. J. Med. 358 (2008) 676–688.
[52] M.J. Leandro, J.C. Edwards, G. Cambridge, M.R. Ehrenstein, D.A. Isenberg, An open study of B lymphocyte depletion in systemic lupus erythematosus, Arthritis Rheum. 46 (2002) 2673–2677.
[53] T. Dorner, A. Radbruch, G.R. Burmester, B-cell-directed therapies for autoimmune disease, Nat. Rev. Rheumatol. 5 (2009) 433–441.
[54] V. Brezar, J.C. Carel, C. Boitard, R. Mallone, Beyond the hormone: insulin as an autoimmune target in type 1 diabetes, Endocr. Rev. 32 (2011) 623–669.
[55] L. Gabrysova, D.C. Wraith, Antigenic strength controls the generation of antigen-specific IL-10-secreting T regulatory cells, Eur. J. Immunol. 40 (2010) 1386–1395.
[56] D. McCue, K.R. Ryan, D.C. Wraith, S.M. Anderton, Activation thresholds determine susceptibility to peptide-induced tolerance in a heterogeneous myelin-reactive T cell repertoire, J. Neuroimmunol. 156 (2004) 96–106.
[57] M. Larche, D.C. Wraith, Peptide-based therapeutic vaccines for allergic and autoimmune diseases, Nat. Med. 11 (2005) S69–S76.
[58] R. Aharoni, The mechanism of action of glatiramer acetate in multiple sclerosis and beyond, Autoimmun. Rev. 12 (2013) 543–553.
[59] Y. Kang, L. Xu, B. Wang, A. Chen, G. Zheng, Cutting edge: immunosuppressant as adjuvant for tolerogenic immunization, J. Immunol. 180 (2008) 5172–5176.
[60] Y. Kang, J. Zhao, Y. Liu, A. Chen, G. Zheng, Y. Yu, J. Mi, Q. Zou, B. Wang, FK506 as an adjuvant of tolerogenic DNA vaccination for the prevention of experimental autoimmune encephalomyelitis, J. Gene Med. 11 (2009) 1064–1070.
[61] H. Garren, P.J. Ruiz, T.A. Watkins, P. Fontoura, L.T. Nguyen, E.R. Estline, D.L. Hirschberg, L. Steinman, Combination of gene delivery and DNA vaccination to protect from and reverse Th1 autoimmune disease via deviation to the Th2 pathway, Immunity 15 (2001) 15–22.
[62] Y. Glinka, Y. Chang, G.J. Prud'homme, Protective regulatory T cell generation in autoimmune diabetes by DNA covaccination with islet antigens and a selective CTLA-4 ligand, Mol. Ther. 14 (2006) 578–587.
[63] G. Cappellano, A.D. Woldetsadik, E. Orilieri, Y. Shivakumar, M. Rizzi, F. Carniato, C.L. Gigliotti, E. Boggio, N. Clemente, C. Comi, C. Dianzani, R. Boldorini, A. Chiocchetti, F. Reno, U. Dianzani, Subcutaneous inverse vaccination with PLGA particles loaded with a MOG peptide and IL-10 decreases the severity of experimental autoimmune encephalomyelitis, Vaccine 32 (2014) 5681–5689.
[64] J.S. Lewis, N.V. Dolgova, Y. Zhang, C.Q. Xia, C.H. Wasserfall, M.A. Atkinson, M.J. Clare-Salzler, B.G. Keselowsky, A combination dual-sized microparticle system modulates dendritic cells and prevents type 1 diabetes in prediabetic NOD mice, Clin. Immunol. 160 (2015) 90–102.
[65] R.A. Maldonado, R.A. LaMothe, J.D. Ferrari, A.H. Zhang, R.J. Rossi, P.N. Kolte, A.P. Griset, C. O'Neil, D.H. Altreuter, E. Browning, L. Johnston, O.C. Farokhzad, R. Langer, D.W. Scott, U.H. von Andrian, T.K. Kishimoto, Polymeric synthetic nanoparticles for the induction of antigen-specific immunological tolerance, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) E156–E165.
[66] M. Look, W.M. Saltzman, J. Craft, T.M. Fahmy, The nanomaterial-dependent modulation of dendritic cells and its potential influence on therapeutic immunosuppression in lupus, Biomaterials 35 (2014) 1089–1095.
[67] J.M. Gammon, L.H. Tostanoski, A.R. Adapa, Y.C. Chiu, C.M. Jewell, Controlled delivery of a metabolic modulator promotes regulatory T cells and restrains autoimmunity, J. Control.Release 210 (2015) 169–178.
[68] A.A. Belogurov Jr., A.V. Stepanov, I.V. Smirnov, D. Melamed, A. Bacon, A.E. Mamedov, V.M. Boitsov, L.P. Sashchenko, N.A. Ponomarenko, S.N. Sharanova, A.N. Boyko, M.V. Dubina, A. Friboulet, D.D. Genkin, A.G. Gabibov, Liposome-encapsulated peptides protect against experimental allergic encephalitis, FASEB J. 27 (2013) 222–231.
[69] K.J. Kauffman, N. Kanthamneni, S.A. Meenach, B.C. Pierson, E.M. Bachelder, K.M. Ainslie, Optimization of rapamycin-loaded acetalated dextran microparticles for immunosuppression, Int. J. Pharm. 422 (2012) 356–363.
[70] N. Schweingruber, A. Haine, K. Tiede, A. Karabinskaya, J. van den Brandt, S. Wust, J.M. Metselaar, R. Gold, J.P. Tuckermann, H.M. Reichardt, F. Luhder, Liposomal encapsulation of glucocorticoids alters their mode of action in the treatment of experimental autoimmune encephalomyelitis, J. Immunol. 187 (2011) 4310–4318.
[71] K.J. Peine, M. Guerau-de-Arellano, P. Lee, N. Kanthamneni, M. Severin, G.D. Probst, H. Peng, Y. Yang, Z. Vangundy, T.L. Papenfuss, A.E. Lovett-Racke, E.M. Bachelder, K.M. Ainslie, Treatment of experimental autoimmune encephalomyelitis by codelivery of disease associated Peptide and dexamethasone in acetalated dextran microparticles, Mol. Pharm. 11 (2014) 828–835.
[72] A. Yeste, M. Nadeau, E.J. Burns, H.L. Weiner, F.J. Quintana, Nanoparticle-mediated codelivery of myelin antigen and a tolerogenic small molecule suppresses experimental autoimmune encephalomyelitis, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 11270–11275.
[73] C. Capini, M. Jaturanpinyo, H.I. Chang, S. Mutalik, A. McNally, S. Street, R. Steptoe, B. O'Sullivan, N. Davies, R. Thomas, Antigen-specific suppression of inflammatory arthritis using liposomes, J. Immunol. 182 (2009) 3556–3565.
[74] J.S. Murray, S. Oney, J.E. Page, A. Kratochvil-Stava, Y. Hu, I.T. Makagiansar, J.C. Brown, N. Kobayashi, T.J. Siahaan, Suppression of type 1 diabetes in NOD mice by bifunctional peptide inhibitor: modulation of the immunological synapse formation, Chem. Biol. Drug Des. 70 (2007) 227–236.
[75] N. Kobayashi, H. Kobayashi, L. Gu, T. Malefyt, T.J. Siahaan, Antigen-specific suppression of experimental autoimmune encephalomyelitis by a novel bifunctional peptide inhibitor, J. Pharmacol. Exp. Ther. 322 (2007) 879–886.
[76] R. Ridwan, P. Kiptoo, N. Kobayashi, S. Weir, M. Hughes, T. Williams, R. Soegianto, T.J. Siahaan, Antigen-specific suppression of experimental autoimmune encephalomyelitis by a novel bifunctional peptide inhibitor: structure optimization and pharmacokinetics, J. Pharmacol. Exp. Ther. 332 (2010) 1136–1145.
[77] J.O. Sestak, B.P. Sullivan, S. Thati, L. Northrup, B. Hartwell, L. Antunez, M.L. Forrest, C.M. Vines, T.J. Siahaan, C. Berkland, Codelivery of antigen and an immune cell adhesion inhibitor is necessary for efficacy of soluble antigen arrays in experimental autoimmune encephalomyelitis, Mol. Ther. Methods Clin. Dev. 1 (2014).
[78] L. Northrup, J.O. Sestak, B.P. Sullivan, S. Thati, B.L. Hartwell, T.J. Siahaan, C.M. Vines, C. Berkland, Co-delivery of autoantigen and b7 pathway modulators suppresses experimental autoimmune encephalomyelitis, AAPS J. 16 (2014) 1204–1213.
[79] R. Tisch, B. Wang, D.J. Weaver, B. Liu, T. Bui, J. Arthos, D.V. Serreze, Antigen-specific mediated suppression of beta cell autoimmunity by plasmid DNA vaccination, J. Immunol. 166 (2001) 2122–2132.
[80] A.F. Li, J. Hough, D. Henderson, A. Escher, Co-delivery of pro-apoptotic BAX with a DNA vaccine recruits dendritic cells and promotes efficacy of autoimmune diabetes prevention in mice, Vaccine 22 (2004) 1751–1763.
[81] B. Yuan, L. Zhao, F. Fu, Y. Liu, C. Lin, X. Wu, H. Shen, Z. Yang, A novel nanoparticle containing MOG peptide with BTLA induces T cell tolerance and prevents multiple sclerosis, Mol. Immunol. 57 (2014) 93–99.
[82] B. Bielekova, B. Goodwin, N. Richert, I. Cortese, T. Kondo, G. Afshar, B. Gran, J. Eaton, J. Antel, J.A. Frank, H.F. McFarland, R. Martin, Encephalitogenic potential of the myelin basic protein peptide (amino acids 83-99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand, Nat. Med. 6 (2000) 1167–1175.
[83] L. Kappos, G. Comi, H. Panitch, J. Oger, J. Antel, P. Conlon, L. Steinman, Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial. The Altered Peptide Ligand in Relapsing MS Study Group, Nat. Med. 6 (2000) 1176–1182.
[84] M.S. Freedman, A. Bar-Or, J. Oger, A. Traboulsee, D. Patry, C. Young, T. Olsson, D. Li, H.P. Hartung, M. Krantz, L. Ferenczi, T. Verco, M.-. Investigators, A phase III study evaluating the efficacy and safety of MBP8298 in secondary progressive MS, Neurology 77 (2011) 1551–1560.
[85] L.M. Metz, D. Li, A. Traboulsee, M.L. Myles, P. Duquette, J. Godin, M. Constantin, V.W. Yong, G.A.m.s. investigators, Glatiramer acetate in combination with minocycline in patients with relapsing–remitting multiple sclerosis: results of a Canadian, multicenter, double-blind, placebo-controlled trial, Mult. Scler. 15 (2009) 1183–1194.
[86] A.D. Goodman, H. Rossman, A. Bar-Or, A. Miller, D.H. Miller, K. Schmierer, F. Lublin, O. Khan, N.M. Bormann, M. Yang, M.A. Panzara, A.W. Sandrock, G. Investigators, GLANCE: results of a phase 2, randomized, double-blind, placebo-controlled study, Neurology 72 (2009) 806–812.
[87] T. Vollmer, H. Panitch, A. Bar-Or, J. Dunn, M.S. Freedman, S.K. Gazda, D. Campagnolo, F. Deutsch, D.L. Arnold, Glatiramer acetate after induction therapy with mitoxantrone in relapsing multiple sclerosis, Mult. Scler. 14 (2008) 663–670.
[88] N. De Stefano, M. Filippi, C. Hawkins, g. study, Short-term combination of glatiramer acetate with i.v. steroid treatment preceding treatment with GA alone assessed by MRI-disease activity in patients with relapsing-remitting multiple sclerosis, J. Neurol. Sci. 266 (2008) 44–50.
[89] M.V. Kopp, E. Hamelmann, S. Zielen, W. Kamin, K.C. Bergmann, C. Sieder, S. Stenglein, S. Seyfried, U. Wahn, D.s. group, Combination of omalizumab and specific immunotherapy is superior to immunotherapy in patients with seasonal allergic rhinoconjunctivitis and co-morbid seasonal allergic asthma, Clin. Exp. Allergy 39 (2009) 271–279.
[90] J. Kuehr, J. Brauburger, S. Zielen, U. Schauer, W. Kamin, A. Von Berg, W. Leupold, K.C. Bergmann, C. Rolinck-Werninghaus, M. Grave, T. Hultsch, U. Wahn, Efficacy of combination treatment with anti-IgE plus specific immunotherapy in polysensitized children and adolescents with seasonal allergic rhinitis, J. Allergy Clin. Immunol. 109 (2002) 274–280.
[91] N. Mothes, M. Heinzkill, K.J. Drachenberg, W.R. Sperr, M.T. Krauth, Y. Majlesi, H. Semper, P. Valent, V. Niederberger, D. Kraft, R. Valenta, Allergen-specific immunotherapy with a monophosphoryl lipid A-adjuvanted vaccine: reduced seasonally boosted immunoglobulin E production and inhibition of basophil histamine release by therapy-induced blocking antibodies, Clin. Exp. Allergy 33 (2003) 1198–1208.
[92] T. Orban, K. Farkas, H. Jalahej, J. Kis, A. Treszl, B. Falk, H. Reijonen, J. Wolfsdorf, A. Ricker, J.B. Matthews, N. Tchao, P. Sayre, P. Bianchine, Autoantigen-specific regulatory T cells induced in patients with type 1 diabetes mellitus by insulin B-chain immunotherapy, J. Autoimmun. 34 (2010) 408–415.
[93] B.O. Roep, J. Buckner, S. Sawcer, R. Toes, F. Zipp, The problems and promises of research into human immunology and autoimmune disease, Nat. Med. 18 (2012) 48–53.

[94] D. Baker, W. Gerritsen, J. Rundle, S. Amor, Critical appraisal of animal models of multiple sclerosis, Mult. Scler. 17 (2011) 647–657.
[95] L. Wang, F.S. Wang, M.E. Gershwin, Human autoimmune diseases: a comprehensive update, J. Intern. Med. 278 (2015) 369–395.
[96] T.N. Marion, A.E. Postlethwaite, Chance, genetics, and the heterogeneity of disease and pathogenesis in systemic lupus erythematosus, Semin. Immunopathol. 36 (2014) 495–517.
[97] C.L. Vanderlugt, S.D. Miller, Epitope spreading in immune-mediated diseases: implications for immunotherapy, Nat. Rev. Immunol. 2 (2002) 85–95.
[98] H.B. Streeter, R. Rigden, K.F. Martin, N.J. Scolding, D.C. Wraith, Preclinical development and first-in-human study of ATX-MS-1467 for immunotherapy of MS, Neurol. Neuroimmunol. Neuroinflamm. 2 (2015), e93.
[99] A. Walczak, M. Siger, A. Ciach, M. Szczepanik, K. Selmaj, Transdermal application of myelin peptides in multiple sclerosis treatment, JAMA Neurol. 70 (2013) 1105–1109.
[100] J.A. Lieberman, S.H. Sicherer, Diagnosis of food allergy: epicutaneous skin tests, in vitro tests, and oral food challenge, Curr. Allergy Asthma Rep. 11 (2011) 58–64.
[101] K. Skamstrup Hansen, L.K. Poulsen, Component resolved testing for allergic sensitization, Curr. Allergy Asthma Rep. 10 (2010) 340–348.
[102] B.J. DeKosky, G.C. Ippolito, R.P. Deschner, J.J. Lavinder, Y. Wine, B.M. Rawlings, N. Varadarajan, C. Giesecke, T. Dorner, S.F. Andrews, P.C. Wilson, S.P. Hunicke-Smith, C.G. Willson, A.D. Ellington, G. Georgiou, High-throughput sequencing of the paired human immunoglobulin heavy and light chain repertoire, Nat. Biotechnol. 31 (2013) 166–169.
[103] L. Tong, C. Schuhmacher, M. Assenmacher, K. Zanker, P. Jahn, Multiplex and functional detection of antigen-specific human T cells by ITRA–indirect T cell recognition assay, J. Immunol. Methods 404 (2014) 13–23.
[104] L. Steinman, The road not taken: antigen-specific therapy and neuroinflammatory disease, JAMA Neurol. 70 (2013) 1100–1101.
[105] L. Tradtrantip, H. Zhang, S. Saadoun, P.W. Phuan, C. Lam, M.C. Papadopoulos, J.L. Bennett, A.S. Verkman, Anti-aquaporin-4 monoclonal antibody blocker therapy for neuromyelitis optica, Ann. Neurol. 71 (2012) 314–322.
[106] T. Wu, L. Zhang, K. Xu, C. Sun, T. Lei, J. Peng, G. Liu, R. Wang, Y. Zhao, Immunosuppressive drugs on inducing Ag-specific CD4(+)CD25(+)Foxp3(+) Treg cells during immune response in vivo, Transpl. Immunol. 27 (2012) 30–38.
[107] R. Mao, W. Xiao, H. Liu, B. Chen, B. Yi, P. Kraj, J.X. She, Systematic evaluation of 640 FDA drugs for their effect on CD4(+)Foxp3(+) regulatory T cells using a novel cell-based high throughput screening assay, Biochem. Pharmacol. 85 (2013) 1513–1524.
[108] D. De Cock, K. Van der Elst, S. Meyfroidt, P. Verschueren, R. Westhovens, The optimal combination therapy for the treatment of early rheumatoid arthritis, Expert. Opin. Pharmacother. 16 (2015) 1615–1625.
[109] A.H. Cross, R.S. Klein, L. Piccio, Rituximab combination therapy in relapsing multiple sclerosis, Ther. Adv. Neurol. Disord. 5 (2012) 311–319.
[110] O. Leavy, Autoimmunity: benefits of exhaustion, Nat. Rev. Immunol. 15 (2015) 468.
[111] K. Minton, Tumour immunology: stressed DCs can't handle T cells, Nat. Rev. Immunol. 15 (2015) 465.
[112] L.J. Peek, C.R. Middaugh, C. Berkland, Nanotechnology in vaccine delivery, Adv. Drug Deliv. Rev. 60 (2008) 915–928.
[113] D.R. Getts, A.J. Martin, D.P. McCarthy, R.L. Terry, Z.N. Hunter, W.T. Yap, M.T. Getts, M. Pleiss, X. Luo, N.J. King, L.D. Shea, S.D. Miller, Microparticles bearing encephalitogenic peptides induce T-cell tolerance and ameliorate experimental autoimmune encephalomyelitis, Nat. Biotechnol. 30 (2012) 1217–1224.
[114] Z. Hunter, D.P. McCarthy, W.T. Yap, C.T. Harp, D.R. Getts, L.D. Shea, S.D. Miller, A biodegradable nanoparticle platform for the induction of antigen-specific immune tolerance for treatment of autoimmune disease, ACS Nano 8 (2014) 2148–2160.
[115] B.L. Hartwell, L. Antunez, B.P. Sullivan, S. Thati, J.O. Sestak, C. Berkland, Multivalent nanomaterials: learning from vaccines and progressing to antigen-specific immunotherapies, J. Pharm. Sci. 104 (2015) 346–361.
[116] D.J. Irvine, M.A. Swartz, G.L. Szeto, Engineering synthetic vaccines using cues from natural immunity, Nat. Mater. 12 (2013) 978–990.
[117] S. Tsai, A. Shameli, J. Yamanouchi, X. Clemente-Casares, J. Wang, P. Serra, Y. Yang, Z. Medarova, A. Moore, P. Santamaria, Reversal of autoimmunity by boosting memory-like autoregulatory T cells, Immunity 32 (2010) 568–580.
[118] H.M. Dintzis, R.Z. Dintzis, B. Vogelstein, Molecular determinants of immunogenicity: the immunon model of immune response, Proc. Natl. Acad. Sci. U. S. A. 73 (1976) 3671–3675.
[119] M.A. Kutzler, D.B. Weiner, DNA vaccines: ready for prime time? Nat. Rev. Genet. 9 (2008) 776–788.
[120] B.O. Roep, N. Solvason, P.A. Gottlieb, J.R. Abreu, L.C. Harrison, G.S. Eisenbarth, L. Yu, M. Leviten, W.A. Hagopian, J.B. Buse, M. von Herrath, J. Quan, R.S. King, W.H. Robinson, P.J. Utz, H. Garren, B.H.T. Investigators, L. Steinman, Plasmid-encoded proinsulin preserves C-peptide while specifically reducing proinsulin-specific CD8(+) T cells in type 1 diabetes, Science Translational Medicine 5 (2013) 191ra182.
[121] S.D. Miller, R.P. Wetzig, H.N. Claman, The induction of cell-mediated immunity and tolerance with protein antigens coupled to syngeneic lymphoid cells, J. Exp. Med. 149 (1979) 758–773.
[122] D.R. Getts, D.P. McCarthy, S.D. Miller, Exploiting apoptosis for therapeutic tolerance induction, J. Immunol. 191 (2013) 5341–5346.
[123] X. Luo, K.L. Pothoven, D. McCarthy, M. DeGutes, A. Martin, D.R. Getts, G. Xia, J. He, X. Zhang, D.B. Kaufman, S.D. Miller, ECDI-fixed allogeneic splenocytes induce donor-specific tolerance for long-term survival of islet transplants via two distinct mechanisms, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 14527–14532.
[124] D.M. Turley, S.D. Miller, Peripheral tolerance induction using ethylenecarbodiimide-fixed APCs uses both direct and indirect mechanisms of antigen presentation for prevention of experimental autoimmune encephalomyelitis, J. Immunol. 178 (2007) 2212–2220.
[125] A. Lutterotti, S. Yousef, A. Sputtek, K.H. Sturner, J.P. Stellmann, P. Breiden, S. Reinhardt, C. Schulze, M. Bester, C. Heesen, S. Schippling, S.D. Miller, M. Sospedra, R. Martin, Antigen-specific tolerance by autologous myelin peptide-coupled cells: a phase 1 trial in multiple sclerosis, Sci. Transl. Med. 5 (2013) (188ra175).
[126] M.I. Iruretagoyena, S.E. Sepulveda, J.P. Lezana, M. Hermoso, M. Bronfman, M.A. Gutierrez, S.H. Jacobelli, A.M. Kalergis, Inhibition of nuclear factor-kappa B enhances the capacity of immature dendritic cells to induce antigen-specific tolerance in experimental autoimmune encephalomyelitis, J. Pharmacol. Exp. Ther. 318 (2006) 59–67.
[127] C.M. Jewell, S.C. Lopez, D.J. Irvine, In situ engineering of the lymph node microenvironment via intranodal injection of adjuvant-releasing polymer particles, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 15745–15750.
[128] H. Liu, D.J. Irvine, Guiding principles in the design of molecular bioconjugates for vaccine applications, Bioconjug. Chem. 26 (2015) 791–801.
[129] J.I. Andorko, K.L. Hess, C.M. Jewell, Harnessing biomaterials to engineer the lymph node microenvironment for immunity or tolerance, AAPS J. 17 (2015) 323–338.
[130] N. Pleskovic, A. Bartholow, D.A. Gentile, D.P. Skoner, The future of sublingual immunotherapy in the United States, Curr. Allergy Asthma Rep. 15 (2015) 545.
[131] T.B. Casale, J.R. Stokes, Immunotherapy: what lies beyond, J. Allergy Clin. Immunol. 133 (2014) 612–619 (quiz 620).
[132] E.C. Liao, J.J. Tsai, Clinical effectiveness of Tyrophagus putrescentiae allergy by local nasal immunotherapy using strips of Dermatophagoides pteronyssinus, J. Asthma 48 (2011) 957–964.