



Antigen-specific tolerance to self-antigens in protein replacement therapy, gene therapy and autoimmunity

Lawrence Steinman, Peggy P Ho, William H Robinson,
Paul J Utz and Pablo Villoslada

Trials of antigen-specific tolerance have been undertaken in the clinic for over fifty years and the results of these antigen-specific clinical trials are described in this review. Antigen-specific tolerization of the immune system in protein replacement therapy for hemophilia A is an accepted treatment. Clinical trials are ongoing for autoimmune conditions such as type 1 diabetes, multiple sclerosis, neuromyelitis optica, and rheumatoid arthritis with various antigen-specific strategies. Trials for tolerization in celiac disease aim for antigen specific tolerance to gluten, an environmental trigger, which may then halt the progression to autoimmunity targeting a self-antigen, tissue transglutaminase. Although many promising approaches have been demonstrated in pre-clinical models, this review will focus primarily on clinical trials of antigen-specific tolerance that have been taken to the clinic and with initial results reported in the peer reviewed literature. A separate article on approaches with CAR-T cells appears in this volume.

Address

Stanford University School of Medicine, Stanford, CA 94305, United States

Corresponding author: Steinman, Lawrence (steinman@stanford.edu)

Current Opinion in Immunology 2019, **61**:46–53

This review comes from a themed issue on **Autoimmunity**

Edited by **Ignacio Sanz** and **Frances Lund**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 30th August 2019

<https://doi.org/10.1016/j.coi.2019.07.011>

0952-7915/© 2019 Elsevier Ltd. All rights reserved.

Introduction

Almost all approved therapies for autoimmune disease target cytokines, checkpoint molecules, molecules involved in the traffic of immune cells, or key signaling pathways in inflammation. There are no approved therapies as yet involving antigen-specific tolerance (AST). This review will not include research on tolerance to extrinsic foreign antigens, commonly called allergens. Celiac disease will be granted an exception due to the spread of the immunopathology from gliadin, an

exogenous antigen in wheat, to encompass self-antigens, such as tissue transglutaminase, as shall be described.

In a metaphorical sense the classic lines from an epic American poem now 102 years old, *The Road Not Taken*, still ring true about the development of therapies based on antigen-specific tolerance to self: ‘Two roads diverged in a yellow wood, And sorry I could not travel both [1].’ Most activities in drug development for autoimmune diseases have taken the ‘well-traveled road’. Great success has come with antibodies to specific cytokines. Targeted and massive depletion of all peripheral CD20+ B cells or large-scale elimination of myeloid cells expressing CD52 both result in massive perturbations of immune function following deletions of major components of the immune system. Blocking lymphocyte homing with a monoclonal antibody to $\alpha 4$ integrin, or impeding lymphocyte egress from lymph nodes with sphingosine-1-phosphate (S1P) modulators exposes patients to risks of immune suppression as the mobile immune system is stifled from reaching the point of attack against unwanted microbial infection. Comparatively fewer efforts have been devoted to approaches involving antigen-specific tolerance.

Here we review the subject of AST, where an impressive number of clinical trials have been undertaken for autoimmune conditions. In addition, AST has become a ‘standard of care’ in protein replacement therapy for hemophilia A. AST may play an important role in gene therapy for not only the various hemophilia-related diseases, but may play key roles in blocking unwanted immune responses in other single gene diseases as well.

In hemophilia A for example, an individual with this X-linked recessive disease does not produce wild type full-length Factor VIII. Administration of recombinant Factor VIII leads to antibodies that inhibit coagulation and can be life threatening [2–4]. Tolerization to Factor VIII has proven effective in abrogating the antibody and T cell responses that inhibit clotting. The restoration of immune tolerance after exposure to high doses of a self-protein is in agreement with mathematical models of the immune system that predict how an optimal range of concentrations of antigen would be critical for triggering an effective immune response, while extreme concentrations of antigen might suppress the immune response, providing versions of low-zone and high-zone tolerance [2,3,5].

In autoimmune diseases such as type 1 diabetes, there are immune responses to islet antigens including proinsulin and glutamic acid decarboxylase which characterize the disease and are presumed to play pathogenic roles [6,7^{••},8,9,10^{••}]. Autoimmunity to islet antigens emanates in the destruction of the pancreatic beta cell. In other autoimmune diseases such as multiple sclerosis (MS), neuromyelitis optica (NMO), and rheumatoid arthritis, many candidate antigens have been described that likely play key roles in pathogenesis. If one is going to attempt antigen-specific tolerance, it is best done in a condition where an antigen-specific autoimmune response is likely to be a key aspect in the pathogenesis of disease. This is true whether we are dealing with an autoimmune disease, where the target antigen has been well-characterized, or in protein replacement therapy where an immune response to the wild-type protein is likely to occur in an individual who never made the wild-type protein, as in hemophilia A.

We describe here tolerizing approaches that have been tested in clinical trials with administration of whole proteins and with peptide epitopes. In addition, plasmid-based DNA therapy has been attempted using DNA transfection to deliver proteins that can tolerate to autoantigens in type 1 diabetes and in multiple sclerosis. Dendritic cells engineered to induce tolerance to self-antigens have been tested in clinical trials in multiple sclerosis, neuromyelitis optica and rheumatoid arthritis, and these approaches will be reviewed here (Figure 1). We shall discuss later some other approaches whose initial results have not yet been reported using nanoparticles to deliver self-antigens or peptides. We include in this review recent promising work in celiac disease. The antigen-specific approach aims to tolerate to the environmental trigger, gluten, which may then block autoimmunity to self-antigens such as transglutaminase.

Antigen-specific therapy with low zone and high zone tolerance to Factor VIII in hemophilia A

The concepts of high and low-zone tolerance for suppressing the immune response to a soluble protein date to a 1924 publication [2]. It is well to remember that in addition to providing the latest and most interesting publications, at times it is a benefit to working scientists to actually see where the foundations of current work actually originate. Mitchison [3] writing in a paper entitled, "The Dosage Requirements for Immunological Paralysis by Soluble Proteins" stated, "That the choice of the immunological response between paralysis and immunity can be controlled by antigen dosage was first noted by Glenny and Hopkins [2]." In hemophilia A, deletions in the gene encoding clotting Factor VIII are responsible for defective coagulation and bleeding. The advent of recombinant Factor VIII has vastly improved the lives of those with hemophilia A, but the

administration of the wild type full-length Factor VIII leads to an immune response to the administered product, inhibiting clotting and leading to bleeding episodes with high morbidity and even fatalities.

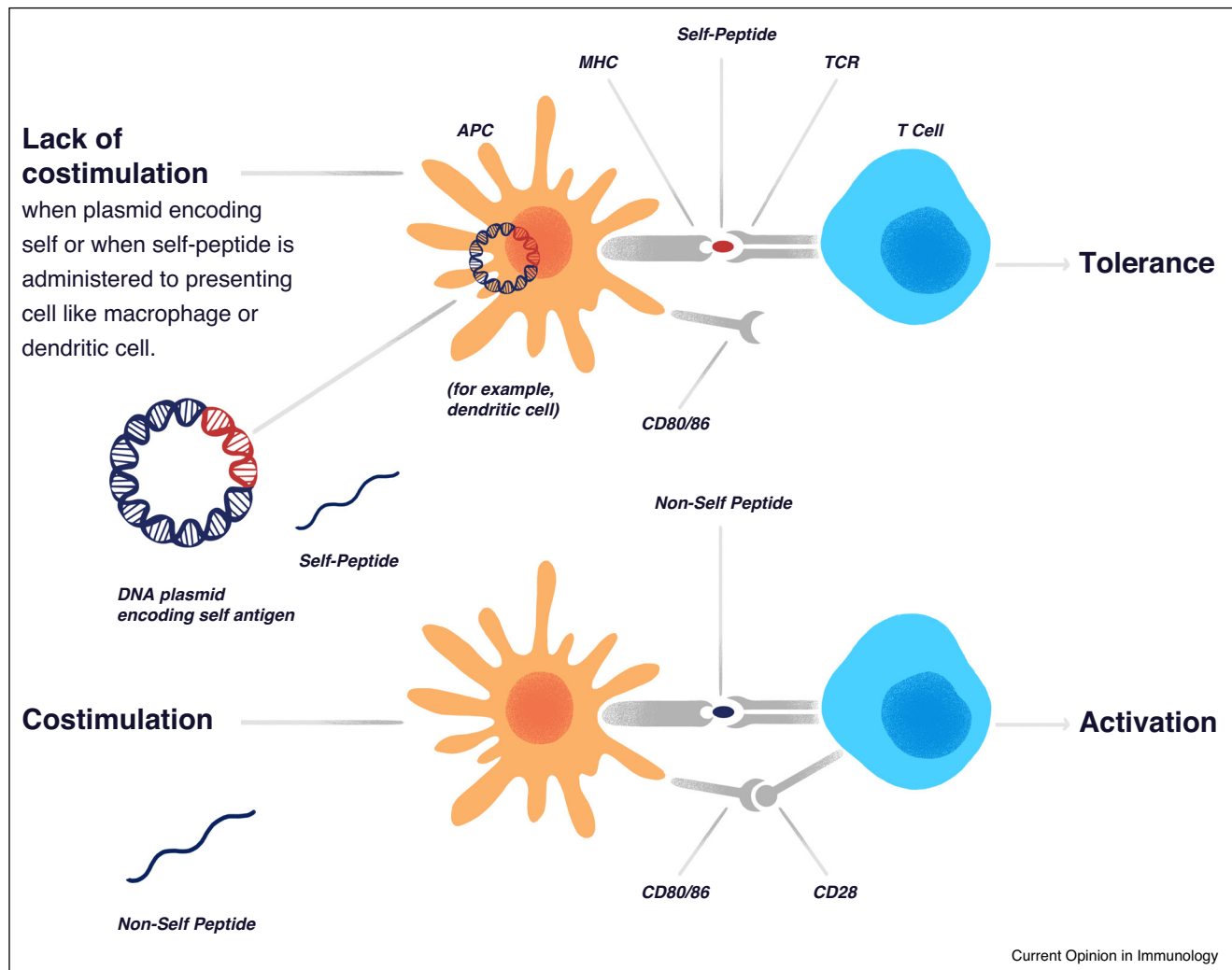
High-zone administration of massive amounts of Factor VIII in those with hemophilia A has been used to avert massive bleeding episodes. A 1977 paper showed that very high doses of factor VIII, ultimately self-administered reduced bleeding episodes: 'There have been a few bleeding episodes, mostly in one bad elbow but sometimes more general. . . . He has been able to do progressive more active physiotherapy and is making great progress. He has passed another examination and is able to use his typewriter again' [4]. We ask the younger readers of this review to research for their own education, the concept of a typewriter!

A trial comparing high zone to low zone tolerance showed that both were equivalent in achieving the primary endpoint of 'success', with a negative anti-Factor VIII titer [11]. Bleeding was absent in 8 of 58 in the low dose tolerance group versus 21 of 57 in the high zone tolerance group ($P < 0.0085$) [11]. In the future it will be interesting to see whether Emicizumab, a bispecific monoclonal antibody that bridges activated factor IX and factor X, and that overcomes the need for delivering Factor VIII proves to be efficacious [12[•]]. Other approaches to AST have shown promise for protein replacement therapy in hemophilia A. For example, a recombinant Factor VIII Fc Fusion protein has shown promise in patients with hemophilia A with high levels of anti-Factor VIII antibody [13]. For immunologists reading papers in this field, the hematologists refer to Factor VIII antibody as 'inhibitor'. The jargon in one field might be opaque to experts in another field, so we call this to the attention of the likely audience for this review, we immunologists.

Tolerization in Gene and protein replacement therapy

There have been promising strategies that are being tested in the clinic for treatment of unwanted immune responses to adeno-associated virus (AAV) vectors in gene therapy. Nanoparticles encapsulating rapamycin, co-administered with AAV vectors, prevented the induction of anti-capsid humoral and cell-mediated responses in pre-clinical studies in mice and nonhuman primates [14^{••}]. Another approach using an engineered DNA plasmid encoding a truncated dystrophin gene, was tested in an animal model of Duchenne Muscular Dystrophy. The DNA plasmid was engineered so that the non-coding region had reduced CpG content. CpG nucleotides stimulate innate immunity via the Toll-like receptor 9 [15^{••},16]. The CpG hexanucleotides were replaced by a competitive GpG hexanucleotide, which stoichiometrically competes to block the CpG motifs [15^{••},16].

Figure 1



Many approaches to antigen-specific tolerance, including tolerization with peptides, tolerization with engineered dendritic cells, and tolerization with engineered DNA plasmids, involve presentation of antigen to the immune system without effective co-stimulation.

The engineered plasmid reduced anti-dystrophin antibody and T-cell responses, and also reduced immune responses to AAV. Moreover, the decreased immunogenicity of dystrophin, led to increase in muscle strength in two electrophysiologic tests [15[•],16]. Both protein replacement therapy and gene therapy will likely improve, if the inherent immunogenicity of a native protein is reduced. Remember protein replacement therapy and gene therapy are used in individuals who have mutations that prohibit them from ever producing full-length native protein. When such a protein is replaced, an immune response is likely.

Antigen-specific tolerance in multiple sclerosis and neuromyelitis optica

The subject of AST in MS and NMO was reviewed in detail four years ago, and the readers are referred to a

more detailed publication, focusing on MS [17]. Here we review further progress since that publication in 2015.

A quarter of a century ago, Weiner *et al.* attempted AST in relapsing remitting MS (RRMS) with oral administration of bovine myelin preparations. Early stage Phase 2 clinical studies showed promise, but a pivotal trial was negative [18,19]. A pivotal trial of a native MBP peptide in secondary progressive MS was negative [20].

Around the turn of the century altered peptide ligands were tested in RRMS, with conflicting results. At higher doses weekly administration of an altered peptide to myelin basic protein worsened MS [21]. At lower doses weekly administration of the same altered peptide to myelin basic protein, attenuated magnetic resonance activity, but allergic reactions were seen beginning about

8 weeks into the regimen, as the immune response to myelin proteins took on a Th2 phenotype, with production of IL-4 [22,23]. These studies led us to see that allergic responses to self-molecules can occur, a version of Ehrlich's Horror Autotoxicus [24].

More recently there have been further trials of native myelin peptides. For example, Wraith and colleagues described open label trials with delivery of myelin peptides intradermally (Study 1) or subcutaneously (Study 2) [25]. The proprietary mixture of peptides included four native regions of myelin basic protein (MBP), MBP30-44, MBP 83-99; MBP 131-145 and MBP 140-154. [26]. 'In study 1, there was a significant decrease in new/persisting T1 gadolinium-enhanced (GdE) lesions in cohort 1 from baseline to week 16, returning to baseline values at week 48. In study 2, the number of T1 GdE lesions were significantly reduced on treatment and remained reduced at study completion. Safety results were unremarkable in both studies.' [25].

Selmaj *et al.* have further pioneered the transdermal delivery of native myelin peptides in clinical trials of MS [27,28]. Antigen-specific tolerance to the delivered myelin peptides was noted, with diminution in antigen-specific gamma interferon production and increases in IL-10. Magnetic resonance imaging (MRI) activity and relapse activity were modulated: 'Compared with placebo, treatment with a myelin peptide skin patch (1 mg) showed a 66.5% reduction in the cumulative number of Gd + lesions ($p = 0.02$) during the 12 months of the study. The annual relapse rate in patients treated with a mixture of myelin peptides (1 mg) was significantly lower compared with the placebo group (0.43 versus 1.4; $P = 0.007$)' [27].

Cell therapeutic approaches with myelin peptides have been attempted in MS and in NMO. Myelin peptides chemically coupled to autologous lymphocytes were tested in patients with RRMS. The peptides coupled were MOG1-20, MOG35-55, MBP13-32, MBP83-99, MBP111-129, MBP146-170 and PLP139-154. Safety was generally good and antigen-specific reduction of T cell responses was observed: 'Four patients given higher doses of autologous cells showed reduction of the proliferation response to some or all of the antigens tested.' [29].

Dendritic cells engineered to be tolerogenic were tested in a first-in-human trial in patients with MS and with NMO (30). In tissue culture the dendritic cells were exposed to IL-4 and GM-CSF for a week. At day 3, dexamethasone was added to the cells to induce the tolerogenic phenotype. At day 6, a mixture of cytokines including IL-1 β , IL-6, TNF- α , and prostaglandin E2 was added. Finally, the tolerogenic dendritic cells were pulsed with the following peptides: MBP13-32 (MBP1), MBP83-99 (MBP2), MBP11-129 (MBP3), MBP146-170 (MBP4), MOG1-20 (MOG1), MOG35-55 (MOG2), PLP139-154 (PLP1), and AQP463-76.

We reported that 'Patients remained stable clinically in terms of relapse, disability, and in various measurements using imaging. We observed a significant increase in the production of IL-10 levels in PBMCs stimulated with the peptides as well as an increase in the frequency of a regulatory T cell, designated Tr1, by week 12 of follow-up. In this phase 1b trial, we concluded that the intravenous administration of peptide-loaded dendritic cells is safe and feasible.' We observed that there was an increase in IL-10 production, without a concomitant increase in γ -interferon production by peptide-specific T cells in MS and NMOSD patients. The shift from pro-inflammatory γ -interferon production to suppressive IL-10 cytokine production serves to indicate that a key regulatory response in tolerance, characterized by the Tr1 T cell, is activated with these engineered dendritic cells. Further larger clinical trials are planned [30**].

In addition to myelin antigens other molecules are targeted via the adaptive immune system in MS. The small heat shock protein (HspB5), known as α B crystallin (CRYAB) is one of the strongest targets of the adaptive immune system in MS [31,32]. Administration of CRYAB reduced inflammatory disease in the brain in various pre-clinical models of MS [33]. This finding was taken forward into a Phase 2 clinical trial in RRMS: 'In a 48-week randomized, placebo-controlled, double-blind Phase IIa trial, three bimonthly intravenous injections of 7.5, 12.5 or 17.5 mg HspB5 were found to be safe and well tolerated in RR-MS patients. While predefined clinical endpoints did not differ significantly between the relatively small groups of MS patients treated with either HspB5 or placebo, repeated administration especially of the lower doses of HspB5 led to a progressive decline in MS lesion activity as monitored by MRI, which was not seen in the placebo group. Exploratory linear regression analysis revealed this decline to be significant in the combined group receiving either of the two lower doses, and to result in a 76% reduction in both number and total volumes of active MRI lesions at 9 months into the study.' [34].

In addition to protein and peptide-based therapies, as well as cell-based approaches to AST, attempts at antigen-specific tolerization in MS have been taken through Phase 2 with an engineered DNA plasmid encoding myelin basic protein [35,36]. This approach has also been taken into the clinic in type 1 diabetes, which will be discussed in the section on type 1 diabetes [6].

A Phase 2 trial was undertaken in 267 patients with RRMS with the engineered plasmid encoding myelin basic protein [36]. 'RRMS patients were randomized 1:1:1 into three groups: placebo, 0.5 mg BHT-3009, or 1.5 mg BHT-3009, given intramuscularly at weeks 0, 2, 4, and every 4 weeks thereafter until week 44. The primary endpoint was the 4-week rate of occurrence of new GdE

lesions on brain MRI from weeks 28 to 48. Protein microarrays were used to measure levels of anti-myelin autoantibodies. No major safety issues were seen. The primary endpoint was nearly attained with a reduction in GdE lesions at 44 weeks, $p < 0.07$ at the 0.5 mg dose, and several secondary endpoints were attained on reduction of GdE lesions, $p < 0.05$. At the 0.5 mg dose 23 anti-myelin antibodies including anti-MBP epitopes were reduced in the CSF, again demonstrating the contraction of epitope spreading of the antibody response, similar to what was seen in the Phase 1 trial' [17,35].

Antigen-specific tolerance in Type 1 diabetes

Several clinical trials of antigen-specific therapy in type 1 diabetes have been reported in the past decade [10^{••},37]. Positive outcomes have been associated with tolerization to proinsulin in early stage trials.

Peakman and colleagues [7^{••}] gave intradermal injections of an DR4(DRB1*0401)-restricted immunodominant proinsulin peptide every 2 or 4 weeks for 6 months in newly diagnosed type 1 diabetes patients. Various parameters including C-peptide measured by a mixed meal tolerance test, and insulin usage were assessed 'Placebo subjects showed a significant decline in stimulated C-peptide (measuring insulin reserve) at 3, 6, 9, and 12 months versus baseline. There were no safety signals of concern. The lower frequency of injections was more effective than the higher frequency. The placebo group's daily insulin use increased by 50% over 12 months but remained unchanged in the intervention groups.' Changes in HgbA1c were not significant, though 'there was a trend for HbA1c levels to increase over time in the placebo group, whereas in the treatment groups, there was a trend for values to decline and then stabilize after 6 months. The peptide treatment stimulated an IL-10 cytokine response in CD4 positive T cells reactive to proinsulin. This was particularly notable in responders, defined as those who had any increase over baseline, C-peptide [10^{••}].

Prevention trials have been undertaken in type 1 diabetes. One prevention trial aimed at an antigen-specific approach: A trial of prevention of type 1 diabetes in relatives with autoantibody responses to islet antigens was undertaken with daily oral dosing of insulin. Results were disappointing and the regimen did not delay or prevent the development of diabetes over 2.7 years [38]. Another prevention trial used Teplizumab, an antibody to CD3 [39[•]]. Teplizumab had previously failed to meet its primary endpoint in phase 3 in an attempt to modulate type 1 diabetes early after diagnosis, though there were many promising aspects of the outcome of this trial [40,41]. For example, '5% (19/415) of patients in the Teplizumab groups were not taking insulin at 1 year, compared with no patients in the placebo group at 1 year ($p = 0.03$).' [40]. In the recent phase 2 trial of Teplizumab

the primary endpoint was met: 'The median time to the diagnosis of type 1 diabetes was 48.4 months in the Teplizumab group and 24.4 months in the placebo group; the disease was diagnosed in 19 (43%) of the participants who received Teplizumab and in 23 (72%) of those who received placebo.' There are safety issues with this approach including a transient activation of Epstein Barr virus with rash and transient lymphopenia [39[•]]. Whether Teplizumab should be categorized as antigen-specific therapy can be debated, but the results are noteworthy and are included in this review. Teplizumab was first developed as an immune suppressive biologic to treat transplant rejection and targets all CD3⁺ T cells [42].

A phase 2 trial [6] was reported in individuals over 18 years of age with type 1 diabetes who were diagnosed within the past five years. Weekly injections of a DNA plasmid encoding proinsulin were given for 12 weeks. The plasmid was heavily engineered with the signal recognition sequence within the coding region of proinsulin modified to prevent secretion, and with the non-coding backbone engineered so that immunostimulatory CpG sequences were excised and replaced with GpG sequences that suppress inflammation, and compete with CpG nucleotides for binding to the Toll-like Receptor 9 [43]. The primary endpoint in the trial was levels of C-peptide relative to baseline. The investigators reported the following: 'No serious adverse events related to BHT-3021 occurred. C-peptide levels improved relative to placebo at all doses, most notably at 1 mg at 15 weeks (+19.5% BHT-3021 versus -8.8% BHT-placebo, $P < 0.026$). Proinsulin-reactive CD8⁺ T cells, but not T cells against unrelated islet or foreign molecules, declined in the BHT-3021 arm ($P < 0.006$).' [6].

In the trial with the engineered DNA plasmid to proinsulin, other outcome measures including HgbA1c and insulin usage were stable during dosing and increased after dosing was discontinued at 12 weeks [6]. Antigen-specific reduction in CD8 T cells to proinsulin, thought to be one of the main drivers of beta cell destruction, is a strong indicator that antigen-specific tolerance was achieved. The actual increases in C-peptide might indicate that dormant beta cells were producing insulin again, or that there was some regeneration of islet cells.

Attempts at modulating ongoing type 1 diabetes with subcutaneous injections of alum-GAD (glutamic acid decarboxylase) failed to reach their primary endpoint in phase 2 and phase 3 trials [8,9]. More recently the injection of the alum-GAD formulation into lymph nodes was shown to elicit a Th2-like immunomodulation to GAD dominated by the Th2 cytokine IL-13 [44]. Induction of Th2 responses to myelin proteins were discontinued due to Th2 driven allergic reactions [22,23]. Induction of anaphylaxis to myelin peptides has been described in mice after Th2 modulation [24]. Severe anaphylaxis to

GAD has been reported in pre-clinical models after repeated immunization with GAD peptides [45].

Antigen-specific therapy with peptides, altered peptides and proteins delivered via engineered DNA plasmids and tolerogenic dendritic cells, is based in part on modulating antigen presentation via altering normal co-stimulatory signaling, see Figure 1. The presentation of self-antigen by APCs without adequate co-stimulation leads to energy or tolerance of T cells, because of the lack of interaction between CD28 with the B7 molecules, CD80 or CD86 [46–48].

There are a number of promising strategies for AST in type 1 diabetes that have not yet reported results in clinical trials. These approaches include the delivery of tolerogenic antigen-major histocompatibility products with nanoparticles as well as the delivery of engineered erythrocytes or erythrocyte proteins like the cell surface marker glycoprotein A covalently linked to antigenic peptides. These pre-clinical studies are described in the detailed review (Ref. [37]) and in peer-reviewed manuscripts [49,50]. Because of space considerations only approaches that have been tested in the clinic are reported in this review for *Current Opinions*.

Antigen-specific tolerance in celiac disease

The pathogenesis of celiac disease is directed to the exogenous gliadin antigens, but spreads to include autoimmune responses to transglutaminase [51]. Early stage trials attempting to tolerize to gliadin peptides via intradermal immunization have shown promise. Anderson and colleagues have developed a protocol called NexVax2 with dose escalation of gliadin peptides that are targeted in celiac patients. The protocol reduces adverse reactions to these peptides. They have reported that there are no elevations in plasma IL-8, IL-2, MCP-1, IL-6, IL-10, and IP-10, with the dose escalation protocols [52,53**]. A recent news item on the internet on NexVax2 [54] stated that: ‘The results from an interim analysis revealed Nexvax2 did not provide statistically meaningful protection from gluten exposure for celiac disease patients when compared with placebo. Similar to earlier Phase 1 results, Nexvax2 was found to be safe and generally well tolerated. There were no concerning safety issues identified during the study.’

The future for antigen-specific tolerance

The pace of clinical trials involving antigen-specific tolerance in autoimmune disease and in protein replacement and gene therapy is increasing [55]. Some of the studies show very promising results. The application of antigen-specific tolerance is still largely in early stage clinical trials. However, in the future, in diseases where the pathogenesis is restricted to unwanted adaptive immunity to known antigens, application of antigen-specific tolerance might become the preferred therapy. We might look back some day on current ‘blunt hammer’

treatment regimens for autoimmune disease, with some degree of amazement of the types of therapies used in the first part of the 21st century. Administration of the potent immune suppressive drugs that often emerged from blocking rejection in organ transplant, or targeting lymphomas, might someday be a chapter of history in a review on the evolution of precision medicine for autoimmune diseases.

The studies reported in this review, make such a prediction plausible, and give some encouragement to the concept that someday antigen-specific tolerance will be a norm in immune therapy. There are other diseases where we know the key antigenic targets of the autoimmune response, and as AST gains traction, individualized therapy tolerizing the immune system to specific unwanted responses may be the common and logical way to intervene in a future where medicine is highly specific not only for the individual -so called personalized medicine- but becomes laser focused to stop a particular pathogenic adaptive immune response.

Conflict of interest statement

Lawrence Steinman is a founder of Tolerion, where he is a shareholder and patent holder for work on antigen-specific tolerance. Steinman is also on the Board of Directors at Tolerion.

William Robinson and PJ Utz are co-founders of Tolerion, where they are shareholders and patent holders for work on antigen-specific tolerance.

Peggy Ho and Lawrence Steinman hold patents on antigen-specific tolerance licensed to Stanford University.

Pablo Villoslada has no conflicts of interest in this work.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Frost R: *Mountain Interval*. New York: H. Holt and Company; 1916.
2. Glenny AT, Hopkins BE: **Duration of passive immunity**. *J Hyg (Lond)* 1923, **22**:208–221.
3. Mitchison NA: **The dosage requirements for immunological paralysis by soluble proteins**. *Immunology* 1968, **15**:509–530.
4. Brackmann HH, Gormsen J: **Massive factor-VIII infusion in haemophiliac with factor-VIII inhibitor, high responder**. *Lancet* 1977, **2**:933.
5. Iranzo J, Villoslada P: **Autoimmunity and tumor immunology: two facets of a probabilistic immune system**. *BMC Syst Biol* 2014, **8**:120.
6. Roep BO, Solvason N, Gottlieb PA, Abreu JRF, Harrison LC, Eisenbarth GS, Yu L, Leviten M, Hagopian WA, Buse JB *et al.*: **Plasmid-encoded proinsulin preserves C-peptide while specifically reducing proinsulin-specific CD8(+) T cells in type 1 diabetes**. *Sci Transl Med* 2013, **5** 191ra.

7. Alhadj Ali M, Liu YF, Arif S, Tatovic D, Shariff H, Gibson VB, Yusuf N, Baptista R, Eichmann M, Petrov N *et al.*: **Metabolic and immune effects of immunotherapy with proinsulin peptide in human new-onset type 1 diabetes.** *Sci Transl Med* 2017, **9**.
- Peptide-based tolerance to proinsulin peptide showing preservation of C-peptide during exposure to proinsulin peptides with stimulation of an IL-10 cytokine response.
8. Wherrett DK, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Gottlieb PA, Greenbaum CJ, Herold KC, Marks JB *et al.*: **Antigen-based therapy with glutamic acid decarboxylase (GAD) vaccine in patients with recent-onset type 1 diabetes: a randomised double-blind trial.** *Lancet* 2011, **378**:319-327.
9. Ludvigsson J, Krisky D, Casas R, Battelino T, Castano L, Greening J, Kordonouri O, Otonkoski T, Pozzilli P, Robert JJ *et al.*: **GAD65 antigen therapy in recently diagnosed type 1 diabetes mellitus.** *N Engl J Med* 2012, **366**:433-442.
10. Roep BO, Wheeler DCS, Peakman M: **Antigen-based immune modulation therapy for type 1 diabetes: the era of precision medicine.** *Lancet Diabetes Endocrinol* 2019, **7**:65-74.
- Detailed review of antigen-specific tolerance focusing on type 1 diabetes.
11. Hay CR, DiMichele DM, International Immune Tolerance S: **The principal results of the International Immune Tolerance Study: a randomized dose comparison.** *Blood* 2012, **119**:1335-1344.
12. Carcao M, Escuriola-Ettingshausen C, Santagostino E, Oldenburg J, Liesner N, Nolan B, Batorova A, Haya S, Young G: **Future of immunotolerance treatment G: the changing face of immune tolerance induction in haemophilia A with the advent of emicizumab.** *Haemophilia* 2019.
- Recent advances in tolerizing in Hemophilia A aiming to bypass strategy of high zone tolerance.
13. Carcao M, Shapiro A, Staber JM, Hwang N, Druzgal C, Lieu W K, Belletrutti M, Thornburg CD, Ahuja SP, Morales-Arias J *et al.*: **Recombinant factor VIII Fc fusion protein for immune tolerance induction in patients with severe haemophilia A with inhibitors-A retrospective analysis.** *Haemophilia* 2018, **24**:245-252.
14. Meliani A, Boisgerault F, Hardet R, Marmier S, Collaud F, Ronzitti G, Leborgne C, Costa Verdera H, Simon Sola M, Charles S *et al.*: **Antigen-selective modulation of AAV immunogenicity with tolerogenic rapamycin nanoparticles enables successful vector re-administration.** *Nat Commun* 2018, **9**:4098.
- Use of nanoparticles with rapamycin to reduce immunogenicity of AAV viral vectors.
15. Ho PP, Lahey LJ, Mourikioti F, Kraft PE, Filareto A, Brandt M, Magnusson KEG, Finn EE, Chamberlain JS, Robinson WH *et al.*: **Engineered DNA plasmid reduces immunity to dystrophin while improving muscle force in a model of gene therapy of Duchenne dystrophy.** *Proc Natl Acad Sci U S A* 2018, **115**:E9182-E9191.
- Tolerization with a microdystrophin encoding plasmid in animal model of Duchenne Dystrophy with AAVgene therapy. Immunogenicity of microdystrophin was reduced with increase in muscle strength.
16. Parks RJ, Gussoni E: **Building immune tolerance through DNA vaccination.** *Proc Natl Acad Sci U S A* 2018, **115**:9652-9654.
17. Steinman L: **The re-emergence of antigen-specific tolerance as a potential therapy for MS.** *Mult Scler* 2015, **21**:1223-1238.
18. Faria AM, Weiner HL: **Oral tolerance.** *Immunol Rev* 2005, **206**:232-259.
19. Weiner HL, Mackin GA, Matsui M, Orav EJ, Khoury SJ, Dawson DM, Hafler DA: **Double-blind pilot trial of oral tolerization with myelin antigens in multiple sclerosis.** *Science* 1993, **259**:1321-1324.
20. Freedman MS, Bar-Or A, Oger J, Traboulsee A, Patry D, Young C, Olsson T, Li D, Hartung HP, Krantz M *et al.*: **A phase III study evaluating the efficacy and safety of MBP8298 in secondary progressive MS.** *Neurology* 2011, **77**:1551-1560.
21. Bielekova B, Goodwin B, Richert N, Cortese I, Kondo T, Afshar G, Gran B, Eaton J, Antel J, Frank JA *et al.*: **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* 2000, **6**:1167-1175.
22. Kappos L, Comi G, Panitch H, Oger J, Antel J, Conlon P, Steinman L: **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* 2000, **6**:1176-1182.
23. Genain CP, Zamvil SS: **Specific immunotherapy: one size does not fit all.** *Nat Med* 2000, **6**:1098-1100.
24. Pedotti R, Mitchell D, Wedemeyer J, Karpuz M, Chabas D, Hattab EM, Tsai M, Galli SJ, Steinman L: **An unexpected version of horror autotoxicus: anaphylactic shock to a self-peptide.** *Nat Immunol* 2001, **2**:216-222.
25. Chataway J, Martin K, Barrell K, Sharrack B, Stolt P, Wraith DC, Group A-MS: **Effects of ATX-MS-1467 immunotherapy over 16 weeks in relapsing multiple sclerosis.** *Neurology* 2018, **90**:e955-e962.
26. Streeter HB, Rigden R, Martin KF, Scolding NJ, Wraith DC: **Preclinical development and first-in-human study of ATX-MS-1467 for immunotherapy of MS.** *Neurol Neuroimmunol Neuroinflamm* 2015, **2**:e93.
27. Jurynczyk M, Walczak A, Jurewicz A, Jesionek-Kupnicka D, Szczepanik M, Selmaj K: **Immune regulation of multiple sclerosis by transdermally applied myelin peptides.** *Ann Neurol* 2010, **68**:593-601.
28. Walczak A, Siger M, Ciach A, Szczepanik M, Selmaj K: **Transdermal application of myelin peptides in multiple sclerosis treatment.** *JAMA Neurol* 2013, **70**:1105-1109.
29. Lutterotti A, Yousef S, Sputtek A, Stürner KH, Stellmann JP, Breiden P, Reinhardt S, Schulze C, Bester M, Heesen C *et al.*: **Antigen-specific tolerance by autologous myelin peptide-coupled cells: a phase 1 trial in multiple sclerosis.** *Sci Transl Med* 2013, **5**:188ra.
30. Zubizarreta I, Florez-Grau G, Vila G, Cabezon R, Espana C, Andorra M, Saiz A, Llufríu S, Sepúlveda M, Sola-Valls N *et al.*: **Immune tolerance in multiple sclerosis and neuromyelitis optica with peptide-loaded tolerogenic dendritic cells in a phase 1b trial.** *Proc Natl Acad Sci U S A* 2019, **116**:8463-8470.
- Tolerization in multiple sclerosis and neuromyelitis optica patients with peptide loaded engineered dendritic cells.
31. van Noort JM, van Sechel AC, Bajramovic JJ, el Ouagmiri M, Polman CH, Lassmann H, Ravid R: **The small heat-shock protein alpha B-crystallin as candidate autoantigen in multiple sclerosis.** *Nature* 1995, **375**:798-801.
32. Steinman L: **Multiple sclerosis. Presenting an odd autoantigen.** *Nature* 1995, **375**:739-740.
33. Ousman SS, Tomooka BH, van Noort JM, Wawrousek EF, O'Connor KC, Hafler DA, Sobel RA, Robinson WH, Steinman L: **Protective and therapeutic role for alphaB-crystallin in autoimmune demyelination.** *Nature* 2007, **448**:474-479.
34. van Noort JM, Bsibsi M, Nacken PJ, Verbeek R, Venneker EH: **Therapeutic intervention in multiple sclerosis with Alpha B-crystallin: A Randomized Controlled Phase IIa Trial.** *PLoS One* 2015, **10**:e0143366.
35. Bar-Or A, Vollmer T, Antel J, Arnold DL, Bodner CA, Campagnolo D, Gianettoni J, Jalili F, Kachuck N, Lapiere Y *et al.*: **Induction of antigen-specific tolerance in multiple sclerosis after immunization with DNA encoding myelin basic protein in a randomized, placebo-controlled phase 1/2 trial.** *Arch Neurol* 2007, **64**:1407-1415.
36. Garren H, Robinson WH, Krasulova E, Havrdova E, Nadj C, Selmaj K, Losy J, Nadj I, Radue EW, Kidd BA *et al.*: **Phase 2 trial of a DNA vaccine encoding myelin basic protein for multiple sclerosis.** *Ann Neurol* 2008, **63**:611-620.
37. Serra P, Santamaria P: **Antigen-specific therapeutic approaches for autoimmunity.** *Nat Biotechnol* 2019, **37**:238-251.
38. Writing Committee for the Type 1 Diabetes TrialNet Oral Insulin Study G, Krischer JP, Schatz DA, Bundy B, Skyler JS, Greenbaum CJ: **Effect of oral insulin on prevention of diabetes**

- in relatives of patients with Type 1 diabetes: a randomized clinical trial.** *JAMA* 2017, **318**:1891-1902.
39. Herold KC, Bundy BN, Long SA, Bluestone JA, DiMeglio LA, Dufort MJ, Gitelman SE, Gottlieb PA, Krischer JP, Linsley PS *et al.*: **An anti-CD3 antibody, Teplizumab, in relatives at risk for Type 1 diabetes.** *N Engl J Med* 2019, **381**:603-613.
- A positive trial of anti-CD3 to prevent the onset of type 1 diabetes in relatives at risk for type 1 diabetes.
40. Sherry N, Hagopian W, Ludvigsson J, Jain SM, Wahlen J, Ferry RJ Jr, Bode B, Aronoff S, Holland C, Carlin D *et al.*: **Teplizumab for treatment of type 1 diabetes (Protege study): 1-year results from a randomised, placebo-controlled trial.** *Lancet* 2011, **378**:487-497.
 41. Hagopian W, Ferry RJ Jr, Sherry N, Carlin D, Bonvini E, Johnson S, Stein KE, Koenig S, Daifotis AG, Herold KC *et al.*: **Teplizumab preserves C-peptide in recent-onset type 1 diabetes: two-year results from the randomized, placebo-controlled Protege trial.** *Diabetes* 2013, **62**:3901-3908.
 42. Woodle ES, Bluestone JA, Zivin RA, Jolliffe LK, Auger J, Xu D, Thistlethwaite JR: **Humanized, nonmitogenic OKT3 antibody, huOKT3 gamma(Ala-Ala): initial clinical experience.** *Transplant Proc* 1998, **30**:1369-1370.
 43. Ho PP, Fontoura P, Ruiz PJ, Steinman L, Garren H: **An immunomodulatory GpG oligonucleotide for the treatment of autoimmunity via the innate and adaptive immune systems.** *J Immunol* 2003, **171**:4920-4926.
 44. Tavora B, Barcenilla H, Wahlberg J, Achenbach P, Ludvigsson J, Casas R: **Intralymphatic glutamic acid decarboxylase-alum administration induced Th2-like-specific immunomodulation in responder patients: a pilot clinical trial in Type 1 diabetes.** *J Diabetes Res* 2018, **2018**:9391845.
 45. Pedotti R, Sanna M, Tsai M, DeVoss J, Steinman L, McDevitt H, Galli SJ: **Severe anaphylactic reactions to glutamic acid decarboxylase (GAD) self peptides in NOD mice that spontaneously develop autoimmune type 1 diabetes mellitus.** *BMC Immunol* 2003, **4**:2.
 46. Ruiz PJ, Garren H, Ruiz IU, Hirschberg DL, Nguyen LV, Karpuz MV, Cooper MT, Mitchell DJ, Fathman CG, Steinman L: **Suppressive immunization with DNA encoding a self-peptide prevents autoimmune disease: modulation of T cell costimulation.** *J Immunol* 1999, **162**:3336-3341.
 47. Bakdash G, Sittig SP, van Dijk T, Figdor CG, de Vries IJ: **The nature of activatory and tolerogenic dendritic cell-derived signal II.** *Front Immunol* 2013, **4**:53.
 48. Feldmann M, Steinman L: **Design of effective immunotherapy for human autoimmunity.** *Nature* 2005, **435**:612-619.
 49. Pishesha N, Bilate AM, Wibowo MC, Huang NJ, Li Z, Deshycka R, Bousbaine D, Li H, Patterson HC, Dougan SK *et al.*: **Engineered erythrocytes covalently linked to antigenic peptides can protect against autoimmune disease.** *Proc Natl Acad Sci U S A* 2017, **114**:3157-3162.
 50. Kontos S, Kourtis IC, Dane KY, Hubbell JA: **Engineering antigens for in situ erythrocyte binding induces T-cell deletion.** *Proc Natl Acad Sci U S A* 2013, **110**:E60-68.
 51. Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D: **Identification of tissue transglutaminase as the autoantigen of celiac disease.** *Nat Med* 1997, **3**:797-801.
 52. Goel G, King T, Daveson AJ, Andrews JM, Krishnarajah J, Krause R, Brown GJE, Fogel R, Barish CF, Epstein R *et al.*: **Epitope-specific immunotherapy targeting CD4-positive T cells in coeliac disease: two randomised, double-blind, placebo-controlled phase 1 studies.** *Lancet Gastroenterol Hepatol* 2017, **2**:479-493.
 53. Daveson AJM, Ee HC, Andrews JM, King T, Goldstein KE, Dzuris JL, MacDougall JA, Williams LJ, Treohan A, Cooreman MP *et al.*: **Epitope-specific immunotherapy targeting CD4-positive T cells in celiac disease: safety, pharmacokinetics, and effects on intestinal histology and plasma cytokines with escalating dose regimens of Nexvax2 in a randomized, double-blind, placebo-controlled Phase 1 study.** *EBioMedicine* 2017, **26**:78-90.
- Peptide specific approach to tolerance to gluten peptides in celiac disease.
54. Immusan TI: **ImmusanT Discontinues Phase 2 Clinical Trial for Nexvax2 in Patients with Celiac Disease.** 2019 <https://www.globenewswire.com/news-release/2019/2006/2025/1874108/1874100/en/ImmusanT-Discontinues-Phase-1874102-Clinical-Trial-for-Nexvax2-in-Patients-With-Celiac-Disease.html>.
 55. Steinman L: **The road not taken: antigen-specific therapy and neuroinflammatory disease.** *JAMA Neurol* 2013, **70**:1100-1101.