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Modulation of autoimmunity with artificial peptides

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Abstract

The loss of immune tolerance to self antigens leads to the development of autoimmune responses. Since self antigens are often multiple and/or their sequences may not be known, one approach to restore immune tolerance uses synthetic artificial peptides that interfere or compete with self peptides in the networks of cellular interactions that drive the autoimmune process. This review describes the rationale behind the use of artificial peptides in autoimmunity and their mechanisms of action. Examples of use of artificial peptides in preclinical studies and in the management of human autoimmune diseases are provided.

Keywords

Artificial peptides; Immune tolerance; Multiple Sclerosis; Systemic Lupus Erythematosus; Glatiramer Acetate

1. Introduction

T cells recognize peptides bound to the major histocompatibility complex molecules (MHC) on antigen presenting cells (APC). The same peptides that cause immunogenic T cell responses in autoimmunity may be used to induce tolerance through an immune deviation towards non-pathogenic responses (1). This can be achieved by peptide administration through the oral, intravenous, intraperitoneal, or intranasal routes. In general, high dose regimens lead to the deletion of antigen-specific cells and/or T cell anergy, while both high- and lowdoses can induce T cells with immunoregulatory activities (2).

Although the administration of peptides for the induction of peripheral immune tolerance can be an approach to therapy of autoimmune diseases, it is often hampered by multiple T cell reactivities associated with a disease and by the fact that the functional blockade of T cells specific for a single epitope is unlikely to be therapeutically effective. Additionally, a specific peptide can bind to one or more MHC alleles, and not to others (3).

For certain autoimmune diseases, target autoantigens are relatively well defined. For other autoimmune conditions, the lack of knowledge of the T-cell epitopes that are critical to the disease pathogenesis may not allow the targeting of selected self antigens. This aspect may be

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the most significant obstacle in using peptides as surrogate antigens for the induction of immune tolerance. MHC polymorphisms further complicate the possibility to use peptides in large numbers of individuals. Lastly, the antigens that associate with autoimmunity may often include multiple epitopes that can be temporally, quantitatively and qualitatively involved in different ways and/or in diverse phases of the disease pathogenesis. By the time of peptide therapy, an initiating antigen can be less relevant in a process that is self-sustaining through other mechanisms driven by other epitopes.

2. Artificial peptides

Several artificial peptides have been tested as tolerogenic modulators of pathogenic autoimmunity. To design artificial peptides, investigators first study the binding of synthetic overlapping peptides that encompass the target antigen to purified MHC molecules. Subsequently, binding data are analyzed by alignment using specific algorithms, to predict the affinity of binding. This bioinformatic prediction is then validated experimentally. The combination of bioinformatic methods with experimental data results in general in the construction of artificial peptides that can contribute significantly to the reduction in the number of peptides required to define T-cell epitopes in a specific autoimmune disease.

At a functional level, the binding of an artificial peptide to the MHC has to mimic the natural self antigen, and it has to lack the inflammatory response elicited by the native peptide. Although the primary structure of the artificial peptide must structurally resemble that of the self peptide that drives and/or sustains the autoimmune response, the conformational change of the artificial peptide - that is not identical three-dimensionally - makes the ligand/receptor binding that would lead to activation of APC or T cells either unproductive or partly ineffective. As a consequence, the artificial peptide will inhibit immune cell activation (4).

We discuss here the use of artificial peptides in organ-specific and systemic autoimmune disease (multiple sclerosis and systemic lupus erythematosus, respectively). We also include a brief description of artificial carriers that anchor multiple copies of B and/or T cell epitopes, including dendrimers that bear branching segments and multimeric core matrices.

3. Artificial peptides in organ-specific autoimmunity

3a. Glatiramer acetate (GA, Copaxone® or Copolymer-1) and multiple sclerosis (MS)

GA was designed over thirty years ago as a synthetic analogue of myelin basic protein (MBP) by a group of scientists at the Weizmann Institute in Israel. It is a standardized mixture of polypeptides (range: 4.7–11 kDa) that are randomly polymerized from the L-amino acids glutamate, lysine, alanine and tyrosine in a molar ratio of 0.14:0.34:0.43:0.09 - which is the same ratio found in the amino acid sequence of MBP (5). The original idea was to create a synthetic molecular mimic of MBP that could induce experimental autoimmune encephalomyelitis (EAE) - a mouse model of human MS - because at that time MBP was not available and its primary structure was not known. GA, which was designed as a MBP analogue on the basis of the MBP amino acid composition, surprisingly prevented EAE, instead of inducing it (6). The confirmation of these studies eventually defined the beneficial effects of GA not only in EAE but also in human MS (7), where the clinical efficacy of GA in a randomized clinical trial (8) led to the approval of GA in relapsing remitting (RR)MS, a subset of MS in which GA currently represents one of the first-line treatments, together with b-interferons (9).

Mechanistically, the high affinity binding of GA to the MHC on APC prevents MBP binding and provides a competitive antagonism for MBP-derived peptides that fuel the autoreactive T-cell responses to MBP in the disease (10). GA also induces a shift in the phenotype of Th1 to

Th2 cells (11) through the inhibition of the production of pro-inflammatory cytokines such as IL-12p70 from dendritic cells (12). Finally, GA has neuroprotective effects that involve the stimulation of neurotrophin secretion, which promotes axonal protection and myelin repair in the central nervous system (13).

3b. hmTAP and EAE

To inhibit multiple T cell autoreactivities in EAE, artificial multiantigen/multiepitope molecules have been constructed to encode simultaneously several disease-relevant epitopes of MBP, proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG) (14–15). When systemically administered in a tolerogenic fashion, a synthetic human multitarget autoantigen protein (hmTAP) efficiently inhibited EAE induced by immunization to PLP or by the transfer of encephalitogenic T cells reactive to MBP, PLP and MOG (14).

4. Artificial peptides in systemic autoimmunity

4a. pConsensus (pCons) and systemic lupus erythematosus (SLE)

Several autoantibodies to DNA – the hallmark antibodies (Ab) of systemic lupus erythematosus (SLE) – contain amino acid sequences in their VH regions that induce *in vitro* T cell proliferation and cytokine secretion (16). The identification of T-cell epitopes in many murine anti-DNA Ab indicated that immunogenic sequences had similar location and amino acid content in several murine anti-DNA Ab (17–19). An algorithm was derived to design a 15-mer synthetic consensus peptide (pCons) that described most of the stimulatory sequences in the CDR1/FR2 region of VH regions of several murine anti-DNA Ab (19). pCons contained MHC class II binding determinants that could be presented by I-Ed but also by Kd of MHC class I, and consisted of 3 basic amino acids separated by one or two uncharged amino acids. The surrounding amino acids were chosen from over 400 peptides from the VH regions of anti-DNA Ab from (NZB x NZW)F1 (BWF1) mice that could stimulate proliferation of syngeneic T cells (19). A non-stimulatory peptide with analogous I-Ed binding motifs, yet violating the consensus motif, was synthesized as a negative control and was called pNeg (19).

Monthly intravenous injections of pCons delayed the appearance of multiple autoantibodies and nephritis in lupus-prone BWF1 mice treated before the onset of disease, and prolonged the survival of BWF1 mice treated after the appearance of anti-DNA Ab in serum (19). Tolerance was induced by pCons but not by pNeg, in spite of their strikingly similar molecular structure (16). The immunization of tolerized mice with another antigen, hen egg lysozyme (HEL), induced a good Ab response which was equivalent to the level induced in naïve BWF1 mice, suggesting that the suppression of multiple autoantibodies (IgG antidsDNA, anti-nucleosome and anti-cardiolipin Ab) induced by pCons did not interfere with a normal immune response to other antigens (19). Since pCons contained amino acid sequences that bound MHC class II molecule and MHC class I molecules, tolerization by pCons affected both the CD4+ and CD8+ T cell compartments (20–21). T inhibitory (Ti) CD8+ cells and pCons-reactive CD4+CD25+Foxp3+ regulatory T cells (Tregs) expanded *in vivo* in pCons-treated mice, and both cell subsets were capable to suppress anti-DNA Ab production in B cells (20–21). CD8+ Ti cells were detectable up to 8 weeks after a single injection of pCons (20). These cells suppressed the proliferation of CD4+ T cells and the production of anti-DNA Ab from B cells through soluble factors, as indicated by transwell experiments (20). The suppressive effects were abrogated by the addition of anti-TGF- β to cultures, but not by anti-IFN- γ or anti-IL10 Ab (20) – an interesting finding considering that TGF- β has an important role in the differentiation and development of peripheral regulatory T cells (22). Moreover, a single transfer of Ti cells to irradiated BWF1 recipients significantly reduced the appearance of anti-DNA Ab and proteinuria in recipient mice, and prolonged their survival (20). The Ti cells were resistant to apoptosis, had increased expression of *Foxp3*, elevated production of TGF- β , and

downregulated the surface expression of programmed death (PD)-1 (20,23–24). T_H1 cells that suppressed disease and improved mice survival could also be induced by gene vaccination of BWF1 mice with a plasmid that encoded pCons (25).

Complementarily, the Tregs induced by pCons suppressed the production of autoantibodies through cell contact-mediated mechanisms that involved membrane-bound TGF- β and glucocorticoid-induced TNF-receptor family member 18 (GITR) expression (21).

These findings in the mouse have led to *in vitro* testing of a possible tolerogenic capacity of pCons on immune cells of patients with SLE. It has been found that pCons can elicit a numerical expansion of functional Tregs in those SLE patients who are seropositive for anti-DNA Ab (26), due to an “immunological memory” of the Tregs reactive to the anti-DNA sequences encoded by pCons (27).

4b. DNA-mimicking peptide/anti-mouse Fc γ RIIb Ab and SLE

To selectively suppress the activity of anti-DNA-producing B cells, an artificial molecule cross-linking the B cell receptor with the inhibitory Fc γ RIIb receptors was designed for administration to lupus mice (28). The hybrid molecule was constructed by coupling a DNA-mimicking peptide to a monoclonal anti-mouse Fc γ RIIb Ab. This DNA-peptide chimeric Ab inhibited anti-DNA Ab production *in vitro* and *in vivo*, and delayed the appearance and progression of disease in MRL/lpr lupus mice (28).

4c. Abetimus (LJP 394) and SLE

LJP394 consists of four double-stranded (ds)DNA oligonucleotide epitopes coupled to an inert platform (29). It binds Ab to dsDNA in the blood and on B cells, and induces B cell tolerance (29). The relative affinity of Ab from SLE patients for dsDNA on LJP 394 may influence a patient's pharmacodynamic response to the drug, and patients with high-affinity Ab to LJP 394 have a reduction in affinity during four month weekly exposure to LJP 394 (30). Abetimus has been evaluated so far in 13 clinical trials on more than 800 SLE patients, over a 10-year time span (31). At 100 mg/week dose, LJP 394 has shown multiple positive trends in renal end points and significantly reduced anti-dsDNA Ab levels, although it did not significantly prolong time to renal flare when compared with placebo (32).

5. Conclusion

The results in several experimental models and the clinical efficacy of GA in RRMS suggest the validity of artificial peptides in the immune modulation of those autoimmune diseases where more than a single self-antigen drive and/or sustain the disease process. Non-stimulatory antagonistic peptide analogues of disease-related T cell epitopes hold highest promise for possible therapeutic applications. While it is apparent that mechanisms of protection induced by artificial peptides involve regulatory T cells and cytokine switch (33–35), more investigations are needed not only to better define the mechanistic basis of protection but also to optimize peptide sequences and routes of administration, timing and dosage, to ultimately employ more artificial peptides in the therapeutic management of autoimmune disease, complementarily with standard therapies.

Take-home messages

- Artificial peptides can effectively downregulate autoreactive immune responses in mice and in humans.

- The biochemical mechanisms that are induced by artificial peptides include antagonism, partial agonism, competitive inhibition, and the displacement of pathogenic peptides driving autoimmune responses.
- Some of the mechanisms that are induced by artificial peptides involve a desensitization of the target cell, a cytokine switch from the Th1/Th17 to the Th2 profile, and the activation of immunoregulatory pathways.

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