

Use of altered peptide ligands to modulate immune responses as a possible immunotherapy for allergies

Allergies are dramatically increasing in prevalence, and the management of these diseases is a heavy burden on the health-care systems of developed countries. In recent years, many efforts have been made to improve the therapy of allergies and to develop new approaches for immunotherapy. Here we briefly review the use of peptides to modulate T-cell responses to allergens. We focus mainly on the possibility of using altered peptide ligands (APLs), i.e., peptides tailored on immunodominant T epitopes and bearing a single amino-acid substitution, as a tool to modulate immune responses to allergens. These peptides may be recognized by the specific T cells triggered by the agonist peptides, but they are unable to elicit T-cell responses; thus, they could be ideal candidates to modulate immune responses to allergens. The availability of these peptides could allow new approaches for immunotherapies.

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The control of the immune responses by induction of long-term, antigen-specific immunologic unresponsiveness is a crucial point in curing immune-mediated diseases, including allergies. In fact, unlike current treatments, therapies able to induce specific tolerance would affect only undesired immune responses without affecting the protective immunity. In the last few years, several approaches to immunologic tolerance induction have been explored, and researchers have tried to modulate the immune responses acting on the different steps of the activation of the immune system (reviewed in ref. 1). In this report, we focus on the possible use of altered peptide ligands (APLs) as a therapy to modulate allergic responses.

APLs, antagonists, and partial agonists

Antigens are specifically recognized as peptides bound in an autologous MHC context by specific receptors expressed on T cells, T-cell receptors (TCRs). The interaction MHC/peptide/specific TCR starts a cascade of biochemical events that leads to T-cell activation (reviewed in ref. 2). Upon activation, T cells display

their functions, such as helper T-cell proliferation and cytokine production aiming to promote secretion of antibodies and cytotoxicity (2). Recently, many data have shown that it is possible to block selectively T cells specific for a given antigen by using APLs, also called analog peptides (3, 4). Usually, APLs bear a single substitution of a putative T-cell contact residue of a characterized antigenic peptide, and they retain similar properties of MHC binding (3, 4). Therefore, APLs bind MHC with similar affinity to the unmodified peptide and can be recognized by the specific TCRs; but they do not elicit a T-cell response (3, 4). Many groups have studied in detail the effect of a single amino-acid substitution of antigenic peptides on T-cell activation (reviewed in ref. 5), but, despite our increasing knowledge, the way APLs affect T-cell activation is not yet completely understood.

APLs may indeed affect T-cell function in different ways. It is likely that, at least in some cases, the TCR–APL interaction is unable to originate T-cell signaling, thus resulting in an impairment of the specific T-cell activation. This phenomenon is referred to as TCR antagonism, and the peptides are defined as antagonists (4, 5).

Antagonist peptides are able to block T-cell proliferation, and antagonist peptides have been described by different groups in murine and human systems (4–7). Moreover, TCR antagonism has been observed in several MHC class I and class II restricted responses and in several different antigenic systems (4–7), including superantigen stimulation (8). It is likely that antagonism is a rather general phenomenon that can be observed in many different experimental systems.

While the antagonist peptides do not initiate T-cell signaling, some peptides are able to elicit some but not all of the signals required for full T-cell activation. In this case, the peptides are defined as partial agonists (4, 5).

Partial agonists have been described that uncouple different T-cell responses by given TCR ligands. For example, on the same T-cell clones, an analog may trigger cytotoxicity alone, whereas a different one may induce only cytokine production (9–11).

The mechanisms underlying the effects of APLs have been extensively investigated in the last few years. According to current models of T-cell recognition, the effects of APLs could be due to altered kinetics of TCR–MHC association or to a conformational modification of the MHC/peptide complex that triggers the TCR in a different way (reviewed in ref. 5).

Other molecules, such as CD4, CD8, and CD45, are involved in T-cell recognition and may affect the efficiency of antagonism (12, 13), thus suggesting that the low avidity of the whole interaction could be the critical point.

The affinity of the TCR for antagonists seems to be much lower than the one for agonist peptides, according to biochemical measurements. Several experiments performed by surface plasmon resonance have shown that the TCR/agonist/MHC complex has a K_d of 21 $\mu\text{mol/l}$, whereas, in the presence of an antagonist, a K_d 10^{-4} times lower has been measured (5, 14, 15).

T-cell antagonism is also a “natural” phenomenon. Some viruses use T-cell antagonism to escape the immune response, and it has been reported that the HBV and HIV may give rise to viral epitopes bearing a substitution in TCR contact residues and no longer recognized by specific cytotoxic T cells (16, 17). Furthermore, T-cell antagonism seems to play a role in positive thymic selection and in the establishing of the T-cell repertoire (18, 19).

Immunomodulation of responses to allergens

Recently, several groups reported the inhibition of T-cell responses to allergens by different approaches (reviewed in ref. 1). Several authors were able to block T-cell responses to allergens with isoforms of major allergens (20–22), and other groups reported the ability to induce anergy with T-cell epitopes (22–24). In some studies, the vaccination of allergen-sensitized animals, performed by

using plasmids containing the allergen T-cell epitope, abolished the response (1, 25). In all these cases, T-cell production of cytokines was impaired so that T cells were no longer producing Th2-like cytokines. The mechanisms of these effects are not yet elucidated. However, since these reports, clinical trials to evaluate the effects of vaccination using the major T-cell epitopes from Fel d 1 have been undertaken (20–29).

To date, there are no definitive data on the efficacy of this new immunotherapy.

APLs and their use to inhibit responses to allergens

It is clear that the antagonism phenomenon, as well as the availability of antagonist and partial agonist peptides for specific antigens, may represent an important tool for the modulation of immune responses. As mentioned above, the use of such an approach could be able to inhibit only T cells responsible for dangerous immune responses, as in autoimmune diseases, transplant rejection, and allergies.

In a large majority of cases, allergies are due to an ongoing production of antigen-specific IgE, which is sustained by a persistent recognition of allergens by Th2 cells. After the specific recognition, Th2 cells start to produce cytokines, IL-4, IL-5, and others, so promoting the production of antibodies by B cells and the IgG–IgE switch (30).

On this basis, the ability to impair the activation of allergen-specific T cells should permit us to avoid, or at least to diminish, the persistence of IgE production, with a consequent lowering of allergic responses, but no effect on the overall protective functions of the immune system.

Moreover, the availability of partial agonists could allow us to block only some functions of T cells, such as cytokine production by Th2-like cells.

Over the years, numerous attempts have been made to use APLs to affect allergen stimulation *in vitro*. APLs have been used to inhibit the stimulation of T-cell clones specific for a major allergen of house-dust mites (HDM) Der p 2 (23, 32) and the major allergens of Japanese cedar pollen, Cry j 1 and Cry j 2 (33). In the first case, the APLs worked as partial agonists, while antagonists and partial agonists were identified by Fasler et al. (31). These authors also showed that APLs downregulate the expression of CD40L on T cells, thus affecting the T–B interactions (32).

Some members of our group have shown that it is possible to inhibit the proliferation of T-cell lines specific for Par j 1, a major allergen of *Parietaria judaica* pollen, with APLs (34). One immunodominant epitope was found along the sequence of Par j 1; interestingly, this epitope was found to be a promiscuous MHC class II binder. APLs derived from this Par j 1 epitope were able to block the proliferation of Par j 1-specific T-cell clones

and T-cell lines, even when the cell stimulation was done with the whole-pollen extract.

These results hold good promise for an eventual immunotherapy of allergy, at least when the sensitizing allergen is Par j 1. Indeed, because it is a promiscuous MHC class II binder, it is likely that this epitope is recognized by a large number of persons; thus, APLs would also be recognized and could be used for a large part of the population.

Conclusion

The prevalence of allergies has been dramatically increasing in recent years at a high cost to the health-care systems in the developed countries. Therefore, there is great interest in improving the therapy for these

diseases. The availability of peptides able to down-regulate the allergen-specific T-cell response could lead to a lower synthesis of IgE, which is responsible for allergies. Furthermore, this approach could avoid the risks of conventional immunotherapy, in which the whole allergen is used. Being T-cell epitope-derived, the APLs should not bind IgE, thus eliminating the side-effects of conventional immunotherapy. The finding of APLs that bind to several MHC class II complexes suggests that they may be of general use.

Further studies need to clarify the mechanisms of APLs effects, and animal models need to be prepared to test the validity of this approach; however, the ability to regulate T-cell functions could represent a key approach to a new therapy for allergies, as well as for other immune-mediated diseases.

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