WHAT CAUSES ALLERGIC AND AUTOIMMUNE DISEASES?

The immune response is designed to protect the body from foreign substances that can cause disease, especially infectious agents. In certain individuals, however, the normal immune response to substances recognised by the body is exaggerated and an excessive response is triggered. This response can be directed to an externally encountered substance, such as pollen or a foodstuff, or to a substance that is a component of the body, such as the myelin sheath surrounding nerve axons. Conditions arising from externally delivered antigens are known as allergies, and those arising from a response to an antigen that occurs naturally in the body as autoimmune disorders. Autoimmune disorders may be generalised (for example, systemic lupus erythematosus) or specific to a single organ (for example, multiple sclerosis, diabetes).

Allergic and autoimmune diseases generally involve different arms of the immune system. Allergies are caused by the triggering of mediator release from mast cells via their surface-bound IgE antibody. Production of IgE antibodies requires an association between antibody producing B-cells and cytokine secreting T-cells; in this case, the T-cells must be of the Th2 type secreting cytokines such as interleukins 4 and 5 (IL-4 and IL-5). Most of the cells involved in inflammatory autoimmune diseases, on the other hand, require help from T-cells of the Th1 or Th17 type, secreting cytokines such as interferon gamma and IL-17 respectively. Currently non-specific, immune-suppressive drugs are used to control these diseases.

Antibodies are produced by B lymphocytes, and these require help from T helper cells for their growth and differentiation. Both sets of lymphocytes are dependent on antigen for their activation. Even though the receptors on these two distinct types of lymphocyte bear structural homology, the nature of antigen recognition is completely different. B cells express a surface immunoglobulin molecule that recognises intact proteins or carbohydrate structures. T-cells, on the other hand, bear a receptor that recognises short fragments of proteins when these have been degraded inside cells. The resulting peptides must first bind to MHC (major histocompatibility complex) molecules, and the MHC-peptide complexes must then traffic to the cell surface. Only certain types of cell have the capacity to present antigen to T-helper (CD4+) cells and these antigen-presenting cells (APCs) include dendritic cells, macrophages and B cells.

ALLERGEN DESSENSITISATION AND THE CONCEPT OF PEPTIDE THERAPY

In 1911, Noon and colleagues first described how injections of allergen could be used to suppress disease in allergy sufferers. It has taken almost a century to understand how this therapeutic approach works. Antigen-specific immunotherapy (SIT) works by immune deviation and the induction of regulatory
T-cells (1). Treatment with intact allergens is, however, potentially dangerous and can lead to fatal anaphylactic reactions. Fortunately, this danger can be avoided by first breaking down the antigen and administering peptides known to stimulate T-cells (T-cell epitopes) as opposed to the intact antigen. Peptide therapy of allergic disorders has been tested in both experimental models and in clinical trials in man. Clinical trials have revealed that repeated administration of peptide antigen leads to down-regulation of pro-allergic T-helper cells, with concomitant expansion of regulatory T-cells (Treg) secreting IL-10. This then opens up tremendous opportunities for the development of peptide therapies for the many allergic disorders that are increasingly prevalent in developed countries. Furthermore, similar approaches have been highly successful in the prevention and treatment of autoimmune disorders in experimental models (2). As yet, clinical trials of peptide therapy in human autoimmune diseases have lagged behind those in allergy.

**DESIGNING PEPTIDES FOR THERAPY OF AUTOIMMUNE AND ALLERGIC DISORDERS**

T-helper cells control immune responses to infectious agents, allergens and self-antigens. These cells only respond to antigen when this is presented by MHC class II molecules in the form of a fragment of antigen. Generally speaking, these fragments or peptides are 13 to 17 amino acids in length – a size that allows the peptide to fill the peptide-binding cleft in the class II molecule. The first step in designing peptides for immunotherapy is to identify the nature of the MHC-bound peptide. Recently, various algorithms have been developed to predict the most likely peptides in a protein sequence to bind class II molecules. Furthermore, sophisticated fast atom bombardment, tandem mass spectrometry techniques have been used to reveal the sequence of peptides eluted from particular MHC molecules. This technique can be adapted for specific antigens; thus, APCs are incubated with specific antigen, the MHC molecules purified and peptides eluted from the molecule. The spectrum of eluted peptides is then compared with that of cells incubated without specific antigen. Differential peaks are then sequenced and the origin of the peptide identified by bioinformatics. Alternatively, T-cell clones specific for a given antigen can be used to map epitopes by using overlapping synthetic peptides (3).

Unanue and colleagues have shown that peptides added directly to MHC molecules may not bind to the molecule in an appropriate conformation (4). This group identified the nature of a naturally processed epitope using the mass spectrometry technique described above. They then prepared the synthetic peptide based on this sequence. Previously, they had isolated T-cell clones specific for the naturally processed epitope; they referred to these as type A T-cells. When T-cells were raised against the identified T-cell epitope as a synthetic peptide, few of the resulting T-cells responded to native antigen even though they responded to the synthetic peptide. These cells were referred to as type B T-cells. The reason that type B T-cells do not recognise the naturally processed antigen is because their antigen – the synthetic peptide – binds to the MHC in a different conformation to that generated from the intact antigen. The epitopes recognised by type B T-cells are therefore referred to as ‘cryptic’ epitopes.

**THE NEED TO OPTIMISE PEPTIDE DESIGN**

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CRYPTIC EPITOPES ARE INEFFECTIVE FOR PEPTIDE THERAPY

Our laboratory has investigated the requirements for effective peptide therapy of autoimmune diseases. Myelin basic protein (MBP) is a major target antigen in the human autoimmune, demyelinating disease multiple sclerosis (MS). The dominant epitope for induction of experimental MS in a mouse model was found within amino acids 89-101 of MBP. This peptide failed, however, to suppress disease when administered as a peptide therapy. The reason for this was subsequently revealed when it was found that 89-101 contains a dominant cryptic epitope (5). As a result, the synthetic peptide fails to engage with disease-relevant, encephalitogenic T-cells when administered in soluble form. Taken together, these results prove that synthetic peptides for peptide therapy must be designed to mimic the naturally processed epitope. If they do not, they will simply be ignored by disease-relevant T-cells.

HOW TO DESIGN PEPTIDES FOR IMMUNOTHERAPY

As pointed out above, it is not sufficient to simply identify T-cell epitopes to develop peptides for immune therapy. How then can we ensure that peptides mimic the naturally processed epitope? T-cell clones may be selected by stimulating with the protein antigen associated with disease. These T-cell clones are then used to screen for peptides that mimic the naturally processed epitope when bound to isolated MHC molecules. For this purpose, purified MHC protein, either bound to a solid matrix or dispersed in planar lipid membranes, can be treated with isolated peptides. The MHC-peptide complexes are washed to remove free peptide and the T-cell clone is added. The T-cell clone will only respond if the peptide mimics the naturally processed epitope.

APCs fixed with a reagent such as paraformaldehyde, to inhibit antigen-processing, serve as an alternative antigen processing independent presentation system. The peptide epitopes capable of stimulating disease-related T-cells without antigen processing are referred to as antigen processing independent epitopes or apitopes.

HOW DO APITOPES SUPPRESS ALLERGIC AND AUTOIMMUNE CONDITIONS?

An essential feature of peptide therapy is that the peptide antigen should be soluble. The soluble peptide is injected into the individual by one of a variety of routes. Studies in experimental animals have shown that mucosal routes (oral or intranasal) or systemic routes (intravenous, subcutaneous or intradermal) are effective, although this may vary from peptide to peptide with some peptides being destroyed in the gut, for example. Allergic desensitisation with synthetic peptides has shown that small doses of peptide are sufficient to cause dramatic effects (1). The administered peptide associates directly with dendritic cells in the body. In their resting/immature state, dendritic cells are tolerogenic; in other words, presentation of antigen by the cells ‘switches off’ potentially harmful T cells. Extensive studies in experimental models have shown that the delivery of T-cell epitopes by immature dendritic cells leads to deletion, functional anergy or the induction of T reg cells in vivo. The resulting T reg cells then maintain neighbouring dendritic cells in the immature state and produce local immune suppression. It is believed that this explains why delivery of soluble epitopes derived from antigen A are able to suppress responses to antigen B of the same tissue.

PEPTIDE THERAPY IN HUMANS, THE STORY SO FAR

The administration of soluble peptide based on known T-cell epitopes leads to suppression of the specific immune response, induction of bystander suppression, and the prevention and treatment of hypersensitivity conditions. This has been extensively studied in experimental models of autoimmune disease and allergy.
A number of studies have correlated efficacy of treatment with the induction of T reg cells in vivo. Our laboratory has described the properties of a novel, IL-10 secreting T reg cell arising from the administration of soluble peptide (7).

Two recent studies in man have shown how synthetic peptides can be used for immunotherapy of allergic diseases. Antigen-specific immunotherapy of bee venom allergy was achieved by repetitive injections of peptides from the bee venom allergen (8); this resulted in the appearance of IL-10 secreting T reg cells. Similarly, repeat injections of peptides from cat dander allergen resulted in reduction of the clinical response with a concomitant increase in IL-10 secretion (9). Furthermore, a recent trial of heat shock protein peptide treatment in rheumatoid arthritis has shown a similar generation of IL-10 secreting cells as a consequence of treatment (10).

Taken together, these clinical studies imply that humans have the same type of IL-10 secreting T reg cell as that defined in experimental animal models. This provides a clear rationale for the application of peptide therapy to a broad range of autoimmune and allergic conditions in man. At Apitope Ltd (Bristol, UK) we are designing apitopes for the treatment of multiple sclerosis and will shortly initiate clinical trials. Apitopes for the treatment of type I diabetes, inhibitor antibody formation in haemophilia and house dust mite allergy are under development. Peptide therapy of autoimmune and allergic diseases will greatly improve the safety of specific immunotherapy, while reducing the reliance of patients on non-specific immune suppressive drugs.

References