

EDITORIAL

Clinical and Experimental Allergy

Of cats and men: immunodominance and the role of HLA-DP/DQ

This editorial discusses the findings of the paper in this issue by Bateman et al. [24] pp. 1760–8

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Among chronic immunological diseases, allergic disorders provide a unique opportunity to both diagnose and treat in an allergen/antigen-specific fashion. Concepts such as component-resolved diagnosis (CRD) [1], combined with technology such as allergen arrays [2], allow precise definition of allergen sensitization within an individual. Accurately identifying the allergens-driving disease is key to extending the concept of CRD to treatment. Current treatment for allergic disease is largely symptomatic and fails to address the increasing prevalence of disease. Specific allergen immunotherapy (SIT) is disease-modifying and has been shown to both reduce the progression of mild to severe disease [3, 4], and also to prevent development of sensitization [5]. Despite the efficacy of SIT, when properly administered, its use is limited by the frequent occurrence of adverse events related to the allergenicity of the preparation employed. Many strategies have been employed in an attempt to reduce the IgE reactivity of allergen extracts, and more recently recombinant allergens (reviewed in reference [6]). Initially, aldehyde modification of allergen extracts was used to generate 'allergoids' with reduced IgE reactivity. These products are still used for treatment but offer only modest protection from allergic adverse events. Recombinant allergens offer many advantages over extracts although providing full coverage of all allergens present in a natural allergen extract may not be logistically or

financially viable. However, a recent study with five major recombinant grass pollen allergens demonstrated efficacy equivalent to extract therapy [7], implying that inclusion of only the most dominant allergens will be sufficient to achieve therapeutic success in most cases. Identification of naturally occurring low-allergenicity isoforms of natural allergens [8], or modification of allergen molecules using recombinant DNA technology, has provided molecules for therapy that have reduced IgE reactivity [9]. To date, only two examples of such molecules have been evaluated therapeutically [10–12]. Other approaches that have been evaluated in human trials include covalent linkage of allergen to pathogen-associated molecular patterns such as unmethylated oligonucleotides (immunostimulatory sequences) [13, 14], delivery of allergen together with virus-like particles [15] and synthetic peptides [16–19]. In all cases, few clinical studies have been reported and no meaningful direct comparisons have been made with conventional therapy.

The use of synthetic peptides offers potential advantages over other approaches including ease, precision and economy of manufacture. Furthermore, if the sequences employed are sufficiently short, cross-linking of neighbouring IgE molecules on effector cells such as mast cells and basophils is unlikely [20]. Peptide strategies targeting B and T cells are under development but at present, only T cell-targeted therapy has been taken into the clinic. For this form of therapy to be successful, it is necessary to treat with peptides that are capable of binding to a diverse array of commonly expressed major histocompatibility complex (MHC) molecules which display peptides for T cell recognition.

Just as there are more allergen molecules in many extracts than it is feasible to include in a therapeutic preparation of recombinant allergens, the potential number of individual MHC T cell epitope combinations in any given allergen exceeds what can be reasonably combined

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in a product for treatment. However, this *embarass de riches* can be rationalized by selecting peptides sequences that are promiscuous in two senses: (i) they contain overlapping/overlaid T cell epitopes that are capable of binding to many MHC molecules and (ii) the epitopes themselves are capable of binding to more than one MHC molecule. For example, a single peptide containing only 13 residues may be capable of binding to multiple MHC molecules and thus being recognized by a large percentage of the population [21, 22].

The dominant role of CD4⁺ T cells in allergic disease (with the exception of atopic dermatitis where both CD4⁺ and CD8⁺ T cells are implicated) dictates that peptides for T cell-targeted therapy be selected from those that bind to MHC Class II molecules. The latter exist in a number of subtly different loci including human leucocyte antigen (HLA)-DR, DP and DQ. HLA-DR molecules form the most diverse of these groups and have generated the most examples of peptide-MHC interaction in the immunological literature. However, each of the many families of HLA-DR molecules is relatively restricted in their distribution at the population level. For example, the HLA-DR1 family of molecules occurs at an approximate frequency of 10% in a Caucasian population, with the archetypal HLA-DRB1*0101 member of the family being the most common. Many other HLA-DR allele families have similar prevalence of distribution. Thus, epitopes identified on the basis of HLA-DR binding may contribute in a restricted manner to population coverage of a peptide vaccine. Of course, such restriction at the epitope level can be offset, to some extent, by the fact that the epitope in question may also bind to other HLA-DR families, and may also form part of a peptide sequence that encompasses several other overlaid epitopes of different specificity. Other loci such as HLA-DP and DQ encode fewer polymorphisms in their alleles, giving rise to smaller numbers of DP and DQ molecules when compared with HLA-DR. However, these molecules are more widely distributed in the population. As a result, epitopes, which can be identified as DP or DQ binders, may offer broader population coverage in a vaccine and are therefore desirable. Two molecules in particular stand out as being expressed widely across the population, HLA-DP*0401 and 0402 (collectively referred to as DP4). One or other of these alleles is present in approximately 50% of the population [23].

In the current issue of *Clinical and Experimental Allergy*, Bateman et al. [24] describe a single peptide sequence, containing two epitopes, from the major cat allergen Fel d 1, which binds to broadly expressed MHC Class II molecules. Such epitopes would be expected to provide broad population coverage within the context of a peptide vaccine.

To define the epitopes, the authors evaluated the T cell response to overlapping 20mer (20 amino acid residue) peptides from the primary sequence of the Fel d 1

molecule. Responses were assessed by ELISpot assay which quantified the frequency of IL-4-, IL-10- and IFN- γ -producing T cells in peripheral blood mononuclear cells (PBMCs) from 26 subjects with moderate to severe atopic dermatitis (all with positive IgE antibodies to cat dander and the majority exhibiting disease exacerbation upon exposure to cats) and 42 laboratory staff, without atopic dermatitis, as controls.

The authors observed that many of the individuals (approximately 50%) that mounted a cytokine response to the immunodominant B1-20 peptide expressed the HLA-DQB1*06, which is a commonly expressed allele and was found in approximately 50% of the individuals in this study, including those with and without atopic dermatitis. This recognition of the peptide was facilitated through presentation via HLA-DQB1*06 (established using blocking antibodies directed against HLA-DP, HLA-DR and HLA-DQ). Importantly, T cell lines specific for the B1-20 epitope restricted by HLA-DQB1*06 were also capable of recognizing native Fel d 1, demonstrating that the B1-20 epitope is a naturally processed peptide from the allergen.

The fact that not all B1-20 responses could be explained by restriction via DQB1*06 prompted the authors to look for alternative restriction elements for this epitope. They found that the majority of individuals recognizing the immunodominant peptide in the absence of DQB1*06 did share expression of the common HLA-DP molecule DPB1*0401 (13 of 16 individuals). Using T cell lines and transformed B cell lines (as antigen-presenting cells) from individuals expressing DPB1*0401 (but negative for DQB1*06), the authors were able to demonstrate that the former was indeed capable of binding and presenting this epitope and that antibodies specific for DP alleles blocked the responses.

Fine epitope mapping using truncated peptides established that the minimal epitopes restricted by DPB1*0401 and DQB1*06 were overlapping, but offset in register by one amino acid (alanine) at the amino terminus. Taken together, these results demonstrate that a single 20mer peptide can be restricted by two very commonly expressed MHC Class II molecules and identifies this peptide sequence as one of potential importance for peptide immunotherapy studies.

To address issues around the quality of the immune response and pathogenesis of atopic dermatitis, comparisons were made between subjects with and without atopic dermatitis. In general, IL-4 and IFN- γ responses were found in both groups, although IL-4 responses tended to be higher in subjects with disease, while IFN- γ responses were lower (i.e. higher Th2:Th1 ratio in disease). Interestingly, there also appeared to be a trend towards decreased IL-10 responses in those individuals with atopic dermatitis. These results are in accordance with earlier studies evaluating ELISpot and cytokine capture responses to aeroallergens and food allergens which demonstrated a

relative deficit of IL-10 responses in subjects with allergic disease compared with healthy controls [25]. Similar patterns of cytokine response have been observed in autoimmune disease [26].

In conclusion, Bateman et al. have identified an immunodominant epitope in chain 2 of the Fel d 1 molecule, which is restricted by very commonly expressed by HLA-DQ and HLA-DP alleles. The broad pattern of expression of these molecules within the population identifies this peptide sequence as one that may be useful in future peptide immunotherapy strategies. Furthermore, detailed analysis of epitope-specific cytokine responses has revealed qualitative differences between individuals allergic to Fel d 1 with and without atopic dermatitis, suggesting that the latter is associated with a more Th2-skewed response in association with reduced production of IL-10. This study makes an important contribution to our understanding of the immunopathogenesis of allergen-driven atopic dermatitis and identifies an important epitope for peptide-based treatment strategies.

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