

MHC Class II and allergen-specific T cell clones

Robyn E. O'Hehir and Jonathan R. Lamb

ImmuLogic Pharmaceutical Corporation, 855 California Avenue, Palo Alto, CA 94304, U.S.A. and Department of Immunology, Wright-Fleming Institute, St. Mary's Hospital Medical School, London W2 1PG, U.K.

Introduction

Although elevated levels of specific immunoglobulin E (IgE) are common in all individuals affected by atopic disease, the genetic basis of atopy remains ill-defined. This is attributable, in part, to an incomplete understanding of the mechanisms regulating IgE production [1] and the apparent polygenic nature of inherited susceptibility to allergies [2]. Nevertheless, the major histocompatibility gene complex (MHC) clearly influences susceptibility in certain allergic diseases. Population genetics and the molecular analysis of specific responsiveness to defined protein pollen allergens have provided much of the information establishing linkage between the HLA class II molecules and allergic disease [3-6]. However, where population analysis has failed to establish HLA associations, the isolation of allergen-reactive T cell clones have allowed the contribution of individual MHC class II molecules to be explored at the molecular level [7]. Having identified dominant use of particular MHC class II gene products in the response to specific allergens it is possible to develop experimental models for investigating novel strategies of immunotherapy. One approach is to exploit the ability of different peptides to compete with each other for occupancy of the antigen combining site of MHC class II molecules [8]. This theoretically provides an opportunity to regulate allergic responses by disrupting the formation of molecular interactions between peptides and MHC molecules.

Results and Discussion

Analysis of MHC class II restriction specificity at the clonal level, using both allogeneic presenting cell panels [9] and murine fibroblasts transfected with the appropriate HLA-D regions genes [10], has indicated that the HLA-DRAB3 gene products (DRw52) may be the major restriction elements in T cell recognition of *Dermatophagoides* spp. In this report we describe the results of *in vitro* experiments

investigating the ability of non-allergen derived peptide analogues from the carboxyl terminal region of the HA-1 molecule of influenza haemagglutinin (HA; residues 307-319) to regulate DRAB3 and DRAB1-restricted T cell recognition of house dust mite.

In preliminary studies non-allergen derived peptides were screened for their ability to modulate the response of polyclonal T cells, from atopic individuals, activated with house dust mite under optimal conditions. The addition of an analogue of HA 307, monosubstituted with serine for tyrosine at position 309 (S-309), inhibited the proliferative response in a dose dependent manner, whereas as a control keratin peptide of similar molecular weight had no inhibitory effects [11]. In order to examine the antigen specificity of peptide-mediated inhibition the HA S-309 analogue was added to polyclonal T cells stimulated with *M. tuberculosis*. Modulation of the response to the recall antigen was minimal, suggesting that only a minor component of the T cell repertoires reactive with mycobacterial and house dust mite have overlapping restriction specificities. Alternatively, if the same MHC class II molecules are restricting T cell recognition of these antigens, the HA S-309 peptide may bind with lower affinity than the majority of mycobacterial peptides, and thus fail to inhibit proliferation.

Examining down-regulation of the response of heterogenous T cell populations to intact house dust mite is valuable in determining the effectiveness of peptide competition on the overall response. Nevertheless, it provides no information on the MHC class II molecules involved or the specificity of their interaction with peptides. However, a major requirement of peptide competition in the regulation of allergic immune responses is that the blocking peptide has specificity for the disease associated MHC class II molecules. To address this question the S-309, HA analogue was assayed for its ability both to bind to different MHC class II molecules and, to inhibit the proliferative response of house dust mite specific T cell clones of different restriction specificities. The S-309 peptide blocked the antigen dependent response of cloned T cells reactive with the group I (*Der p* I and *Der f* I) allergens, and restricted by DRw12B1, DR2Dw2B5 and DR2Dw21B1 class II molecules [9,10]. However, the response of DRw11 restricted ragweed reactive T cell clones was unaffected. The activation of T cell clones restricted by the DRAB3 gene products, a dominant restriction element in the recognition of house dust mite antigens, was blocked by HA S-309, as illustrated for the T cell clone DE47 (Fig. 1A).

Although able to inhibit recognition of house dust mite allergens by T cell clones, the native HA sequence, unlike the S-309 analogue, induced proliferation at

the polyclonal level in some individuals. Clearly a non-stimulatory peptide is preferable as a competitive inhibitor. Furthermore, the binding of the native HA peptide to HLA-D region molecules expressed on the membrane of EBV transformed B cells was reported to be extremely permissive [12]. In contrast, the HA S-309 analogue bound to only selected B cell lines, the majority of which expressed both DRB1 and DRB3 gene products. Additional studies using murine fibroblasts expressing the appropriate HLA-D region genes demonstrated that HA S-309 was able to bind to DR2Dw2B5 and DRw52b molecules, consistent with the ability of this peptide to inhibit the proliferative response of the cloned T cells.

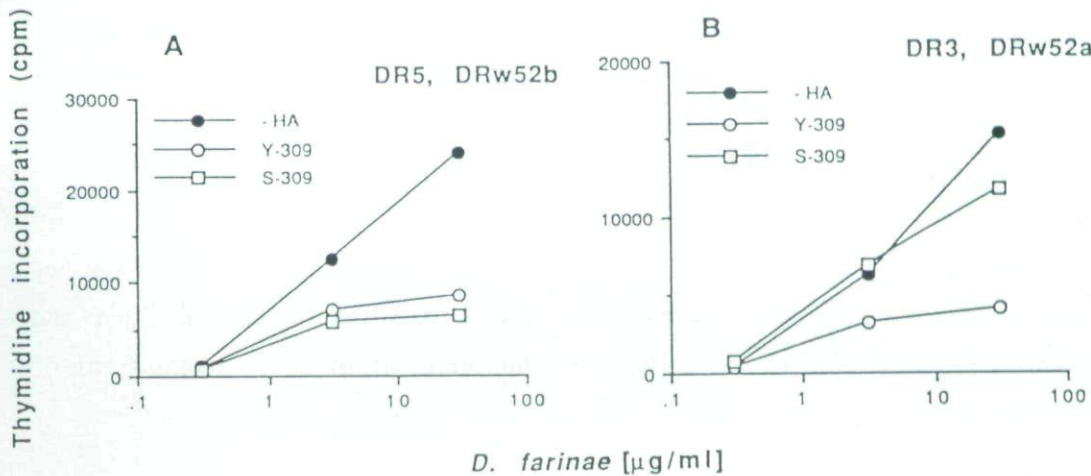


Fig. 1 Cloned T cells (DE47) were stimulated with *D. farinae* at different concentrations (-●-) in the presence of (B) DR3, DRw52a or (A) DR5, DRw52b expressing EBV transformed B cells. Peptides (HA Y-309, -O- or HA S-309, -□-, 100μg/ml) were added at the initiation of the cultures and proliferation as correlated with thymidine incorporation was determined after 60 hours. Background responses of the T cells in the absence of *D. farinae* or to the competing peptides was <1000 cpm.

The presentation of allergen by DR5, DRw52b and DR3, DRw52a expressing EBV-B cells (Fig. 1A and B), together with the hybridisation patterns of oligonucleotide probes specific for the DRw52a, b and c alleles in allogeneic presenting cell panel studies, indicated that recognition of *D. farinae* by clone DE47 was degenerate on DRw52a and b. Interestingly, while the native HA peptide blocked allergen recognition restricted by both DRAB3 gene products, the S-309 analogue only inhibited house dust mite presented in the context of DRw52b (Fig. 2B). Binding studies using transfectants expressing the allelic forms of DRw52 confirmed that the HA S-309 analogue failed to bind to DRw52a.

There is precedent for this approach in the regulation of immune responsiveness [13,14]. It has been demonstrated that competitive inhibition with non "disease-related" peptides may prevent the development of experimental allergic encephalitis [15]. The ability to downregulate allergen specific immune responses in atopic individuals with non-stimulatory, non-allergen derived peptides offers a novel approach to immunotherapy.

Acknowledgements

This research was supported by in part by grants from the Wellcome Trust and the Medical Research Council. Dr. R. E. O'Hehir is the recipient of a Wellcome Senior Research Fellowship in Clinical Science.

References

- 1 Leung DYM, Geha RS. Regulation of the human IgE antibody response. *Int Rev Immunol* 1987; 2:75-91.
- 2 Blumenthal MN, Yunis E, Mendell N, Elston RC. Preventive allergy: genetics of IgE-mediated diseases. *J Allergy Clin Immunol* 1986; 78:962-968.
- 3 Marsh DG, Hsu SH, Roebber M, Erlich-Kautzky E, Freidhoff LR, Meyers DA, Pollard MK, Bias WB. 1982. HLA-Dw2: A genetic marker for human immune response to short ragweed pollen allergen Ra5. I. Response resulting primarily from natural antigenic exposure. *J Exp Med* 1982; 155:1439-1463.
- 4 Marsh DG, Freidhoff LR, Ehrlich-Kautzky E, Bias WB, Roebber M. Immune responsiveness to *Ambrosia artemisiifolia* (short ragweed) pollen allergen Amb a VI (Ra6) is associated with HLA-DR5 in allergic humans. *Immunogenetics* 1987; 26:230-236.

- 5 Ansari AA, Friedhoff LR, Meyers DA, Bias WB, Marsh DG. Human immune responsiveness to *Lolium perenne* pollen allergen *Lol p* III (Rye III) is associated with HLA-DR3 and DR5. *Human Immunol* 1989; 25:59-71.
- 6 Marsh DG, Zwollo P, Huang SK, Ghosh B, Ansari AA. Molecular studies of human immune response to allergens. *Cold Spring Harbour Symp Quant Biol* 1989; LIV: 459-470.
- 7 O'Hehir RE, Garman R, Greenstein J, Lamb JR. The specificity and regulation of T cell responsiveness to allergens. *Ann Rev Immunol* 1991; in press.
- 8 Werdelin O. Chemically related antigens compete for presentation by accessory cells to T cells. *J Immunol* 1982; 129:1883-1891.
- 9 O'Hehir RE, Eckels DD, Frew AJ, Kay AB, Lamb JR. MHC-class II restriction specificity of cloned human T lymphocytes reactive with *Dermatophagoides farinae* (house dust mite). *Immunology* 1988; 64:627-631.
- 10 O'Hehir RE, Mach B, Berte C, Greenlaw R, Tiercy J-M, Bal V, Lechler R, Trowsdale J, Lamb JR. Direct evidence for a functional role of HLA-DRB3 gene products in the recognition of *Dermatophagoides* spp. by helper T cell clones. *Int Immunol* 1990; in press.
- 11 O'Hehir RE, Lamb JR. Immunomodulation of cellular responses to house dust mite using non-stimulatory peptides. *J Allergy Clin Immunol* 1990; 58:200 (Abstr).
- 12 Busch R, Strang G, Howland K, Rothbard JR. Degenerate binding of immunogenic peptides to HLA-DR proteins on B cell surfaces. *Int Immunol* 1990; 2:443-451.
- 13 Adorini L, Muller S, Cardineaux F, Lehmann PV, Falcioni F, Nagy ZA. *In vivo* competition between self peptides and foreign antigens in T cell activation. *Nature* 1988; 334:623-625.
- 14 Urban JL, Horvath SJ, Hood L. Autoimmune T cells: Immune recognition of normal and variant peptide epitopes and peptide-based therapy. *Cell* 1989; 59:257-271.
- 15 Zamvil SS, Steinman L. The T lymphocyte in experimental allergic encephalomyelitis. *Ann Rev Immunol* 1990; 8:579-621.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.