

Point of view

The cellular and molecular basis of the human T cell response to *Dermatophagoides* spp. (house dust mite)

ROBYN E.O'HEHIR & JONATHAN R.LAMB

Department of Immunology, St Mary's Hospital Medical School, London, U.K., and ImmuLogic Pharmaceutical Corporation, 855 California Avenue, Palo Alto, CA 94304

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Introduction

Population studies have indicated that between 25–30% of individuals are atopic, exhibiting the common feature of producing immunoglobulin E (IgE) in response to environmental allergens. The allergic response is characterized by both specific (IgE; Ishizaka 1984) and non-specific (mast cells and polymorphonuclear granulocytes; Lett-Brown, Alam & Grant 1989) effector arcs. The re-exposure of sensitized skin or airways of atopic subjects to the appropriate allergen induces crosslinking of specific IgE bound to Fcε receptors on the surface of the primary effector cells. The immediate reaction results in degranulation of mast cells and basophils with the subsequent release of primary mediators including histamine. A more delayed, late-phase response follows and is inflammatory in nature. Secondary mediators, including leukotrienes, platelet activating factor and prostaglandins, are released from the cellular infiltrate of eosinophils, neutrophils, macrophages, basophils and lymphocytes during this phase of the allergic reaction (Cromwell 1988, Kay 1988, Frew & Kay 1990).

Despite the dramatic advances in our knowledge of the pathophysiology of atopic disease, the cellular, genetic and molecular mechanisms underlying allergen recognition and the regulation of immune responsiveness remains limited (O'Hehir *et al* 1990c). Similarly, although the importance of T lymphocytes in IgE induction has long been recognized (Okumura & Tada 1971), it is only recently that the specificity and regulatory role of CD4+ T lymphocytes in both arcs of allergic immune responses are being examined in detail (Lett-Brown, Alam & Grant 1989, O'Hehir *et al.* 1990c).

Of the many environmental allergens, the house dust mite (HDM, *Dermatophagoides* spp.) is an important source of allergen associated with the clinical diseases of perennial

Correspondence: Dr Robyn O'Hehir, Department of Immunology, St Mary's Hospital Medical School, Norfolk Place, London W2 1PG, UK.

rhinitis, asthma and atopic dermatitis. In this article recent developments in the analysis of the specificity and regulation of the human cellular immune response to *Dermatophagoides* spp. are discussed.

ACAROLOGY

Dust mites are acarids related to ticks and spiders and closely related to the mites that cause scabies. They are sightless, eight-legged arthropods just invisible to the naked eye ($< 1/3$ mm in length). The main house dust mites are of the family Pyroglyphidae which includes the genus *Dermatophagoides*, with the main species being *D. pteronyssinus*, *D. farinae*, and *D. microceras*. They feed on human skin scales, or fungi growing on such scales, with the major secretory product of the mites being their faecal pellet (10–30 μ diameter) which is enveloped by a peritrophic membrane (Platts-Mills & Chapman 1987). House dust mites are found throughout the world, *D. pteronyssinus* being more prevalent in humid, warm areas and *D. farinae* being more common in dry, cooler regions (van Bronswijk Sinha 1971). They form one of the major sources of allergen and, therefore, play a major aetiological role in symptomatic allergic disease.

T CELL ANTIGEN RECOGNITION OF *DERMATOPHAGOIDES* SPP

(i) Epitope mapping

T lymphocytes capable of responding specifically to components of *Dermatophagoides* spp. are present in the T cell repertoires of both atopic and non-atopic individuals (Halvorsen *et al.* 1986, O'Hehir *et al.* 1988). This has been confirmed by the isolation of HDM specific T cell clones from both groups of subjects (O'Hehir *et al.* 1988). At the polyclonal level, the T cell proliferative responses to the major species of HDM, *D. pteronyssinus* and *D. farinae*, were comparable (O'Hehir *et al.* 1987). However, from analysis of the specificity of long term lines and monoclonal populations of T cells it was revealed that species-specific determinants were predominantly recognized, contrasting with the humoral response to HDM (Platts-Mills & Chapman 1987). Analysis with unfractionated preparations of dust mites and their culture medium provides no information on the individual antigenic determinants that are being recognized in the specific immune response. In an attempt to identify the stimulatory proteins, without the bias of using only preparations of major allergens selected by serology, allergen fractionated on SDS-PAGE and immobilized on nitrocellulose has been used to isolate immunogenic components (O'Hehir *et al.* 1987, Lamb, O'Hehir & Young 1988). The antigen specificities of a panel of T cell clones and a long term T cell line induced with *D. farinae* were probed using this approach. T cells reactive with either the group I or group II allergens (26 kD and 12 kD, respectively) were identified, but the dominant component of the repertoire appeared to be directed towards the group II allergens (Figure 1, O'Hehir *et al.* 1987). Proliferation in response to affinity-purified preparations of these proteins (generous gifts of M. Chapman and P. Heymann, Figure 2) confirmed the T cell specificity. Additional antigenic determinants were located using polyclonal T cell populations in which four distinct peaks of activity were observed comigrating with proteins of molecular weights 12, 26, 39 and 58 kD (O'Hehir *et al.* 1990c). Serological examination has recently defined a group IV allergen of *Dermatophagoides* spp, α amylase

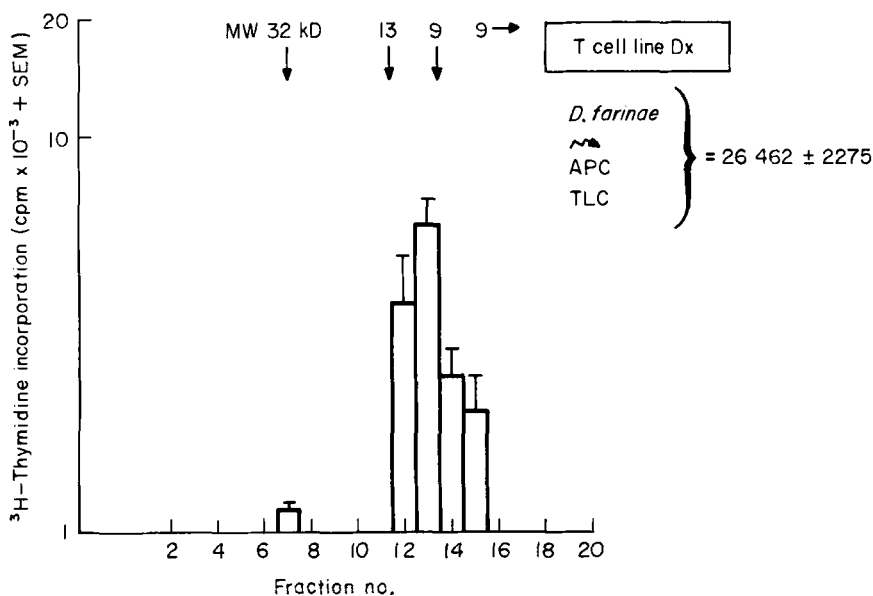


Figure 1. Pattern of reactivity of *D. farinae* specific T cell line to fractionated house dust mite. *D. farinae* mite body extract was fractionated by separation on SDS-PAGE under denaturing conditions and transferred to nitrocellulose. The fractions were then added to proliferation assays containing cells of the long-term line (DX) and autologous irradiated peripheral blood mononuclear leucocytes as a source of antigen presenting cells. Proliferation of the T cells was correlated with the incorporation of tritiated thymidine. The figure is reproduced from O'Hehir *et al* (1987) by kind permission of Blackwell Scientific Publications.

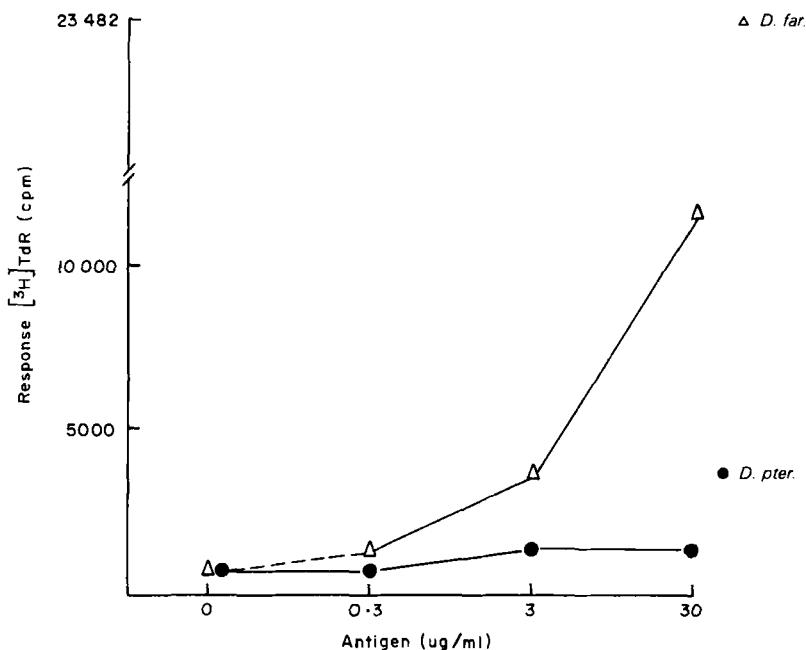


Figure 2. T cell recognition of species-specific epitopes in the group II allergen of *D. farinae*. T cells of a long-term IL-2 dependent line induced with *D. farinae* were stimulated with affinity purified group II allergen isolated from *D. farinae* (Δ) or *D. pteronyssinus* ($\bullet$) in the presence of antigen presenting cells. The response to unfractionated house dust mite is shown by the data points on the right of the figure.

(60 kD; Stewart 1990), potentially corresponding to the 58 kD fraction which was stimulatory to T cells.

The further identification of T cell epitopes within these determinants has been hampered by the lack of amino acid sequence data of the major allergens. Recently, however, the group I and II HDM proteins have been cloned and sequenced (Chua *et al.* 1988, Thomas, personal communication). This has allowed overlapping peptides to be synthesized and used to probe the HDM reactive T cell repertoire at both the clonal and polyclonal levels. Sublibraries of recombinant antigens have, therefore, also become available, providing an additional approach with which to localize T cell epitopes (Lamb *et al.* 1988). Results to date using both methods have been disappointing, raising a question as to the possible importance of conformational determinants in allergen epitope recognition, as has been suggested for PLA₂ (Kuo *et al.* 1989). The group I allergen of *D. pteronyssinus*, *Der p* I, has been the subject of most study with a number of possible epitopes being identified, including residues 56-65, 81-102, 85-109 and 107-117 (O'Hehir *et al.* 1990c, O'Hehir, Kuo, Thomas & Lamb, unpublished results). Polyclonal analysis using truncated recombinant *Der p* I suggested additional epitopes at the amino terminus (residues 1-56; Thomas, unpublished results). However, none of these sites has been consistently recognized on screening panels of clones and polyclonal T cell populations.

(ii) *HLA class II restriction specificity of T cell recognition*

The activation of CD4+ T lymphocytes and the subsequent induction of immune responses requires the formation of a ternary complex in which products of the class II major histocompatibility gene complex (MHC) form allele-specific molecular interactions between the specific antigen receptor of T cells (TCR) and peptidic fragments of foreign antigen (Schwartz 1985, Moller 1987, Davis & Bjorkman 1988). The HLA class II restriction specificity of cross-reactive and *D. farinae* specific T cell clones was examined using serological inhibition, allogeneic presenting cell panels and murine fibroblasts transfected with HLA-D region genes. Initial studies using antibody inhibition (Lanza-vecchia *et al.* 1983) demonstrated that antigen recognition was regulated by immune response genes of the HLA-DR subregion, as monoclonal antibodies directed against HLA-DR, but not HLA-DP or -DQ, were able to markedly inhibit antigen-dependent proliferation (O'Hehir *et al.*, 1988).

The restriction specificity was further investigated using a presenting cell panel of 30 allogeneic accessory cells of known haplotype. The patterns of responsiveness observed were complex, in general failing to correlate with the serologically-defined classical HLA-DR specificities encoded by the DRB1 genes (O'Hehir *et al.* 1988). Responsiveness due to alloreactivity was excluded by control cultures in which no antigen was present. Surprisingly, the majority of the T cell clones appeared to be restricted by products of the DRB3 gene locus (DRw52, DRw53) with a minority restricted by DRB1 gene products. Quantitation of mRNA for class II proteins suggests that the expression of B3 gene products is two to five fold less than that of B1 gene products (Berdoz *et al.* 1987). Moreover, in the majority of antigen systems investigated, DRB1 gene products have been demonstrated as the restriction specificity (Ottenhoff *et al.* 1985). The specificities suggested by the panel of allogeneic presenting cells were then confirmed using murine fibroblast cell lines transfected with the appropriate HLA-DR gene products and panels of EBV-transformed B cell lines (O'Hehir *et al.* 1990d). In a panel of seven *D. farinae*

reactive T cell clones, five were restricted by DRAB3 gene products. The apparent dominance of the supertypic specificity in HDM recognition in this individual was confirmed when it was demonstrated that a *D. farinae* reactive, long-term T cell line proliferated strongly in response to HDM presented by the DRw52b+ murine fibroblasts.

The supertypic specificity DRw52 is in linkage disequilibrium with the alleles HLA-DR3, -5, -w13, -w14, with DRw53 being coexpressed with HLA-DR4, -7, and -9 alleles. A large percentage of the population, therefore, expresses a B3 gene product which may account for the observed high frequency of HDM allergy. However, population studies have not suggested that the inheritance of HDM allergy is regulated solely by HLA gene products (Blumenthal *et al.* 1986, Luoma & Koivikko 1982). Instead, the MHC may determine the specificity of the allergic response with non-MHC linked genes, such as the putative 'atopy gene' located on chromosome 11q, determining the overall propensity to develop allergic diseases (Cookson *et al.* 1989).

Functional activity of HDM-specific T cells isolated from atopic and non-atopic individuals

The importance of IgE in determining allergic sensitivity in atopic subjects is well-recognized. However, the immunological basis underlying the reason why atopic individuals support allergen-specific IgE synthesis and non-atopic subjects fail to do so is not understood. Many of the models investigating IgE regulation have been derived from studies of pathological variants resulting in hyper-IgE production or from autoreactive and alloreactive T cell clones (Leung & Geha 1987). Therefore, an allergen-specific *in vitro* model for total IgE production by autologous B cells was developed, using HDM specific T cells isolated from both groups of individuals, to allow the identification of possible differences in regulation (O'Hehir *et al.* 1989).

Two HDM specific T cell clones were selected from an atopic HDM allergic subject, one restricted by DRAB3 gene products (DRw52b) and the other restricted by DRAB1 gene products (DRw12 and degenerate on DR2Dw2B5 and DR2Dw21B1). Investigation of the IL-2 and IL-4 lymphokine profiles of the two clones by dot blot analysis demonstrated that both clones had transcripts for IL-2, whereas, only one of the clones had marked levels of IL-4 mRNA. The presence of both IL-2 and IL-4 mRNA indicates that these T cell clones do not readily segregate into the T_H1 and T_H2 subsets based on their lymphokine profiles (Mossman & Coffman 1987). In contrast, cloned HDM-specific T cells, isolated from the non-atopic subject, had mRNA for IL-2 and not IL-4. The functional responses to HDM and phenotypic expression of all three T cell clones were comparable (O'Hehir *et al.* 1989).

It was demonstrated that T cell clones from an atopic subject were able to support IgE synthesis which was IL-4 dependent, either from endogenously-produced IL-4 or exogenous IL-4. In contrast, the HDM-specific T cell clone from a non-atopic subject failed to support IgE synthesis either in the presence or absence of exogenous IL-4 (O'Hehir *et al.* 1989). These results have been confirmed and extended (Wierenga *et al.* 1990). Furthermore, the IL-4 dependence of IgE synthesis in the atopic system was supported by the ability of a monoclonal antibody putatively directed against the IL-4 receptor to abrogate the production of antigen-induced IgE from the B-cell populations (Larche *et al.* 1988).

It has been suggested that the negative regulation of IgE synthesis is controlled by differential IFN- γ and IL-4 production (Vercelli *et al.* 1990). Therefore, antibodies directed against IFN- γ were also added in the presence of exogenous IL-4 and resulted in the enhancement of IgE production by the atopic cloned T lymphocytes but again failed to induce IgE synthesis by the non-atopic clone (O'Hehir & Lamb, unpublished observations). To delineate further whether the regulatory defect lies with the T cell or B cell, mixing experiments using histocompatible atopic and non-atopic donors are underway. The ability of the cloned HDM reactive T cells to support IgE synthesis *in vitro* in a physiological system such as this supports the validity of study of the antigen recognition and the functional activity of allergen reactive T cell clones as models for allergic diseases.

IMMUNOMODULATION OF HDM RECOGNITION BY T CELLS

Despite the widespread popularity of allergen hyposensitization as a therapeutic regimen, the information available as to the immunological basis for this approach is limited. Knowledge of antigen specificity, restriction specificity and the functional activity of allergen-specific T cell populations allows more detailed evaluation of this question. The importance of the ternary complex formed between TCR, MHC and antigen in the initiation of immune responsiveness has been discussed. It follows, therefore, that interference with any of the components of this complex may downregulate responsiveness. The induction of non-responsiveness in the periphery may be established by direct regulation of the 'disease-related' T cells through their TCRs (Lamb *et al.* 1983, Lamb & O'Hehir 1989) or, conversely, by non-stimulatory peptides competing at the level of the class II MHC molecules (Adorini *et al.* 1988, O'Hehir *et al.* 1990b, O'Hehir & Lamb 1990a, Zamvil & Steinman 1990).

(i) *Functional inactivation of HDM-reactive T cells*

Deletion or functional inactivation of T cells through their antigen specific receptors allows exquisite specificity of anergy to be achieved due to the clonal distribution of TCRs. If a limited number of 'disease-related' T cell epitopes were identified in the allergic response to HDM, then such an approach might be feasible to allow the design of desensitizing agents based on allergen structure. The observation that components of the HDM reactive T cell repertoire are able to recognize cross-reactive and species-specific determinants within the different allergens (O'Hehir *et al.* 1987, 1988) renders this approach less favourable. However, preliminary studies *in vitro* demonstrated that a low dose hyposensitization protocol was able to decrease the HDM reactivity of one T cell clone that was known to be capable of supporting allergen-dependent IgE synthesis (O'Hehir *et al.* 1989). The T cells were initially activated by *D. farinae* presented by autologous accessory cells and then the subsequent exposure to escalating concentrations of the the complex *D. farinae* antigen mixture resulted in a 42% decrease in the responsiveness of the T cells to challenge by their native ligand. The decreased antigen reactivity was not due to cytolysis for IL-2 responsiveness was enhanced, as has been observed in other models of peripheral T cell anergy.

If oligoclonality of HDM reactive T cells were demonstrable in their TCR V β gene usage, this would facilitate an alternative approach of inducing anergy by modulation of

TCRs through the shared features of their V β gene products. A family of antigens which includes the *Staphylococcus aureus* (*S. aureus*) enterotoxins, are termed 'superantigens' based on their ability to stimulate powerful polyclonal proliferative responses of T cells bearing particular V β gene products (Choi *et al.* 1989, Marrack & Kappler 1990). The staphylococcal enterotoxins are also capable of functionally inactivating T cells (O'Hehir & Lamb, 1990b). Examination of the V β gene usage of a number of HDM reactive T cell clones revealed that a proportion of these expressed TCR V β 6+ or V β 3+ gene products. The V β 6+ T cell clones were able to be modulated by pretreatment with the staphylococcal toxin E (SEE) and those expressing V β 3 were inactivated by exposure to SEB (O'Hehir *et al.* 1990a). The anergic T cells failed to respond to an immunogenic challenge with their natural ligand (HDM), but retained the ability to proliferate to exogenous IL-2, indicating that the mechanism operating was that of nondeletional tolerance. The loss of the ability of the cloned T cells to proliferate to specific antigen was associated with downregulation of membrane Ti-CD3 and enhanced expression of CD25 (IL-2 receptor, O'Hehir & Lamb 1990b, O'Hehir *et al.* 1990a). Recent reports have demonstrated that the enterotoxicity of SEA and SEB may be removed by carboxymethylation without the loss of their immunogenic properties (Purdie, Hudson & Fraser 1990, Alber, Hammer & Fleischer 1990). Further refinement of the tolerogenicity of these reagents may allow the preparation of alternative vaccines.

(ii) *Inhibition of responsiveness by antigen competition*

Direct binding assays have demonstrated that gene products of specific MHC class II alleles are degenerate in that they are capable of binding a large number of different peptides. Similarly, individual peptides are able to bind to more than one MHC class II protein (Busch *et al.* 1990, Panina-Bordignon *et al.* 1989). Despite the apparent permissiveness, certain patterns of binding are observed to occur preferentially. The preferential binding and ability for peptides to compete for occupancy of the antigen combining site raises the question of whether non-stimulatory peptides may provide an alternative approach for modification of the aberrant allergic immune response.

Binding studies have demonstrated that a peptide analogue derived from the carboxyl terminus of the HA-1 molecule of influenza haemagglutinin (HA; residues 307-319), with the tyrosine at position 309 substituted with serine, bound with high affinity to the DRAB3 gene product DRw52b expressed in isolation from DRAB1 class II molecules on murine fibroblasts. The DRAB3 gene products appear to be major restriction elements in HDM recognition (O'Hehir *et al.* 1988, 1990d). Extension of the binding studies using homozygous EBV transformed B cells expressing MHC class II molecules representative of a large number of different DR alleles indicated that the binding of the Ser-309 HA analogue was restricted to selected class II specificities including subtypes of DR2, DR5 and the B3 gene products (O'Hehir *et al.* 1990b). Interestingly, the same HLA-DRAB1 class II molecules that bind the Ser-309 HA analogue have been observed to restrict T cell recognition of HDM (O'Hehir *et al.* 1990d). Functional studies demonstrated that this analogue was able to inhibit T cell proliferative responses to HDM from a number of individuals with symptomatic HDM allergy, at both the polyclonal and monoclonal levels. The peptide itself was non-stimulatory to the polyclonal T cells, suggesting that the repertoires of the individuals examined did not possess T cells naturally primed to Ser-309. Neither was the HA analogue toxic to the T cells at concentrations capable of

inhibiting HDM-dependent proliferation. A certain degree of antigen specificity was evident in that polyclonal antimycobacterial and anti-ragweed T cell responses were not inhibited. This may result from the antigenic determinants in ragweed and mycobacteria binding to different MHC class II molecules to the inhibitory peptide, or that they bind with higher affinity than the Ser-309 HA analogue to the same alleles. Moreover, an inability to block either PHA or anti-CD3 antibody induced proliferation, suggested that antigenic competition for binding to MHC class II was occurring rather than an effect mediated directly at the level of the T cells. As a non-allergen derived peptide, and an analogue of the naturally occurring HA influenzae, the likelihood of such reagents as these being capable of inducing anaphylaxis is decreased.

Precedent exists for the use of non-stimulatory peptides to regulate immune responsiveness *in vivo* (Adorini *et al.* 1988, Zamvil & Steinman 1990). Adorini *et al.* (1988) demonstrated the application of such an approach using murine lysozyme to compete for hen egg lysozyme recognition in high responder mouse strains. Preliminary studies in the mouse, coimmunizing with HDM peptide have been encouraging, suggesting that the HA peptide is able to downregulate *Der p 1* specific IgE responses (Chapman, O'Hehir & Lamb, unpublished observations). A competitive approach such as this may be particularly effective for localized disease, such as observed in perennial rhinitis.

Conclusions

Allergic diseases are the clinical manifestations of the aberrant immune responses invoked in certain individuals when exposed to common environmental allergens. Knowledge of the cellular and molecular interactions which occur between specific allergen, the MHC class II molecules expressed on the membrane of antigen presenting cells and CD4+ T lymphocytes is central to an understanding of the complex cascades which form the specific and non-specific effector mechanisms of the allergic immune response. Being the source of commonly-encountered ubiquitous allergens, the HDM (*Dermatophagoides* spp) provides a suitable model for the dissection of such interactions. The clarification of the antigen and restriction specificity and functional properties of HDM-reactive T cells has now allowed experimental systems for the examination of protocols of hyposensitization to be developed.

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