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HLA class II restriction specificity of *Dermatophagoides* spp. reactive T lymphocyte clones that support IgE synthesis

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Summary

The results of recent experiments investigating the restriction specificity of cross-reactive, or *Dermatophagoides farinae*-specific, T cell clones isolated from an atopic individual with perennial rhinitis are reviewed. The restriction specificity was examined using serological inhibition, allogeneic presenting cells and murine fibroblasts expressing HLA-D region products. Although serological inhibition studies suggested that DR class II proteins were the major restriction elements used, the patterns of recognition observed with the allogeneic cell panel were complex, generally failing to correlate with the serologically defined MHC class II specificities. Analysis of the restriction patterns indicated that the majority of the T cell clones were restricted by DR β III gene products and this was confirmed using murine fibroblasts expressing DRw52. DR β I gene products functioned as restriction elements in the recognition of house dust mite allergen by the other clones. In an *in-vitro* model of allergen-dependent IgE synthesis, both DR β I and DR β III class II restricted T cells could be shown to provide functional help for IgE synthesized by autologous B cell-enriched populations.

Introduction

Major histocompatibility (MHC) class II gene products form allele-specific molecular interactions with both peptide fragments of foreign antigen and the antigen receptor of CD4+ T lymphocytes [1,2,3]. The formation of this ternary complex is critical for T cell activation and the subsequent induction of immunoglobulin (Ig) synthesis.

With respect to immune recognition of aero-allergens, such as *Dermatophagoides* spp. (house dust mite), it is surprising that investigation of the antigen and restriction specificity of T cell recognition, has in general been neglected, although the requirement for T cells in the induction of IgE is well established [4]. Contrary to the T cell recognition, the specificity and effector function of IgE induced after exposure to *Dermatophagoides* has been extensively investigated [5]. Examination of the reactivity of polyclonal T cells to *Der p* I, defined serologically as a major allergen of *D. pteronyssinus*, proliferative responses were observed in atopic, but not the control (non-atopic),

subjects [6]. An independent investigation, based on inhibition with anti-human MHC class II antibodies alone, suggested that T cell recognition of *D. pteronyssinus*-derived allergens, of molecular weight 15-25 kD, was restricted by HLA-DR gene products [7].

The results of recent experiments investigating the antigen and restriction specificity of *D. farinae*-induced T cell clones, indicate that extensive heterogeneity exists in the T cell repertoire reactive with house dust mite allergen. In this review these findings will be discussed.

Results and discussion

Antigen specificity

T cell clones induced with *D. farinae* were isolated from the peripheral blood of an atopic individual with perennial rhinitis and their antigen specificity analysed [8]. Surprisingly, only two of the T cell clones examined were cross-reactive, proliferating in response to both the inducing antigen, *D. farinae*, and *D. pteronyssinus*, but not to the unrelated allergens (Table 1). More detailed analysis of the fine specificity of these clones using immunoblots of fractionated *Dermatophagoides* and affinity purified components revealed that they recognized

Table 1. Antigen specificity of T cell clones induced with *D. farinae*

T cell clones	Antigen			
	<i>D. farinae</i>	<i>D. pteronyssinus</i>	Grass-mix	<i>Parietaria</i>
DE5	9.8	0.2	0.9	0.5
DE9	28.5	22.6	0.3	0.7
DE12	28.9	0.7	0.4	0.6
DE26	256.0	89.8	1.9	2.9
DE41	42.3	1.2	2.1	2.1
DD11	257.6	3.4	0.7	0.6

Cloned T cells were stimulated with antigen in the presence of irradiated autologous PBMC and proliferation was determined by the incorporation of [³H]TdR as described in detail elsewhere [9]. The results are expressed as stimulation index (SI):

$$SI = \frac{\text{T cells + PBMC + antigen}}{\text{T cells + PBMC}}$$

Der p I and *Der f* I [9, 10]. This parallels the specificity of the human IgE antibody response to the major allergens, *Der p* I and *Der f* I of which 80–95% is cross-reactive [11]. The remaining clones were species-specific, recognizing only *D. farinae*, and in the case of DD11 the determinant was located in *Der f* II [10]. Probing the specificity of a polyclonal T cell line with immobilized and fractionated *Dermatophagoides* demonstrated that a species-specific epitope of *Der f* II was predominantly recognized, indicating that clone DD11 was not of unique specificity. In contrast, cross-reactive epitopes in *Der p* II and *Der f* II are predominantly recognized by human IgE antibody and this was reflected in the serum of the individual from whom the T cell clones were derived. These observations suggest that T cells reactive with species-specific epitopes are able to provide B cell help for the induction of cross-reactive antibodies.

HLA class II restriction specificity

Although HLA-DR, -DQ and -DP class II proteins are all able to function in the presentation of foreign antigen to CD4⁺ T cells, HLA-DR appears to be the most frequently used of the restriction elements [12–14]. This may reflect the quantitative expression, or time of appearance, of MHC class II proteins on the cell membrane. Similarly, serological inhibition studies indicated that T cell recognition of house dust mite allergens was predominantly restricted by HLA-DR proteins, as antigen dependent proliferation was inhibited by murine monoclonal anti-HLA-DR, but not by anti-HLA-DQ or -DP antibodies reactive with invariant determinants of HLA-D region

Table 2. HLA class II restriction pattern of T lymphocyte clones

Cloned T cells	HLA class II specificity of accessory cells
DE5	1/3 DR4+; 5/15 DR5+; 6/8 DR7+
DE9	1/3 DR2+; 9/15 DR5+; 1/2 DR8+
DE12	1/3 DR+; 13/15 DR5+; 5/8 DR7+
DE26	12/15 DR5+; 6/8 DR7+
DE41	12/15 DR5+
DD11	13/15 DR5+; 4/8 DR7+

Cloned T cells were tested for their ability to recognize *D. farinae* presented by an accessory cell panel of DR-matched and DR-mismatched PBMCs from 30 individuals including an autologous control. Positive and negative responses were objectively assigned using the T max programme [8].

molecules [8]. From the results of these inhibition experiments the restriction specificity of the T cell clones was determined using an allogeneic accessory cell panel of HLA-DR matched and mismatched cells including the autologous control (DR 5(12),7; DRw52, —) to present allergen. The restriction specificities observed were complex and revealed no consistent patterns which corresponded with the serologically defined HLA-DR specificities (Table 2). The MHC class II proteins, restricting antigen recognition by the T cell clone DE41 showed the closest association with a serological class II specificity. Nevertheless, the ability of only 12/15 DR5+ accessory cells to present *D. farinae* implied that this was a subtype of DR5+ [15, 16]. Analysis at the population level of the presence of specific serum IgE antibodies following natural exposure to the 11.5 kD allergen (*Amb a* VI) of short ragweed appeared to be associated with HLA-DR5 when defined serologically [17]. The epitopes recognized by anti-MHC class II antibodies are predominantly located on the exposed surfaces of class II molecules, and are distinct from those involved in either peptide binding or the formation of contacts with the T cell receptor, therefore it is not surprising that T cell restriction and serologically defined class II specificities do not always overlap. This is illustrated by the pattern of restriction of T cell clone DE5 that responded to allergen presented by some DR4+ and DR7+ accessory cells. This suggested that the supertypic class II specificity DRw53 ($\alpha\beta$ III gene product) was the restriction element. The recent identification of a second allelic form of DRw53 may explain the inability of all the DR4+ and

	10	20	30	40	50
DR conserved	GDTRPRFL	---ECHFFNGT	ERV--L-R--NQEE	--RFDS	SDVGE-RAV
DR2Dw2 β I		WQDKY.....	RF.H.DFY.....	DL.....	Y.....
DR5Dw5 β I		EYSTS.....	RF.D.YFY.....	YV.....	F.....
DR8Dw8 β I		EYSTG..Y.....	RF.D.YFY.....	YV.....	Y.....

	60	70	80	90
DR conserved	-ELGRP-AE-WNSQKD	LE--R--VD	YCRHNYGV	-ESFTVQRR
DR2Dw2 β I	T.....D..Y.....	F..DR..AA..T.....	G.....	
DR5Dw5 β I	T.....DE.Y.....	F..DR..AA..T.....	G.....	
DR8Dw8 β I	T.....S..Y.....	F..DR..AL..T.....	G.....	

Fig. 1. Amino acid sequences of the β 1 domains of the HLA-DR class II proteins, DR2Dw2, DR5Dw5 and DR8Dw8. Amino acids are represented by the single letter code. The box area shows a region of homology in the β 1 helix.

DR7+ accessory cells to function as presenting cells [Gorski, personal communication].

Interestingly, the T cell clone DE9 responded to *D. farinae* presented by some DR2+, 5+ and 8+ accessory cells (Table 2). Comparison of the primary amino acid sequences of these alleles indicated that the residues forming the third variable region (residues 67–71) of the β 1 domain are conserved FLEDR (Fig. 1). From the structural similarities and sequence homologies between class I and II MHC proteins, a predictive structure of the antigen combining site of class II can be modelled, based on the crystal structure of HLA-A2 [18–20]. In this model, residues 67–71 would be located in a central position on the helix, forming a wall of the antigen combining site, residue 70 being predicted to point up towards the T cell receptor, and 71 pointing in towards the combining site. The residue at position 67 points both inwards and upwards, with the potential to form contacts with both antigen and T cell receptor (Fig. 2). The functional importance of the polymorphic residues in the β 1 helix was demonstrated from the comparison of T cell responses restricted by the I-A^b wild type and the I-A^b β ^{bm12} mutant. These alleles differ by only the three amino acids at 67, 70 and 71 of the β 1 helix, but the effects on the antigen and restriction specificities are profound [21,22]. The conservation of amino acids at positions 67, 70 and 71 in the DR2, 5 and 8 proteins could allow the binding of the peptide and the formation of favourable contacts with T cell receptors. This may occur despite extensive polymorphism in the β sheets that form the floor of the combining site and thus contribute to antigen binding [23]. Specific IgE antibody responses to the low molecular allergens *Amb a* V, *Amb t* V and *Amb p* V of ragweed are all associated with the DR2Dw2 haplotype [24,25]. Although the B cell response is reported to be specific for the three individual allergens, it is possible that the helper T cells are cross-reactive, or that DR2Dw2 class II molecules can bind peptides derived from each.

The pattern of reactivity of the remaining T cell clones on the allogeneic presenting cell panel suggested that the

restriction specificity was related to the supertypic class II specificity, DRw52, encoded by the $\alpha\beta$ III genes. DNA sequencing has revealed that there are at least three allelic forms of the β III gene encoding DRw52. Analysis of the restriction specificity of tetanus toxoid reactive T cell clones, using oligonucleotide typing, demonstrated that both DRw52a and b were able to present antigen [26,27]. To confirm that DRw52 class II proteins were able to restrict the presentation of *D. farinae*, murine fibroblasts expressing DRw52b or DRw52c were used as accessory cells. Murine fibroblasts have no endogenous class II proteins and therefore only express the transfected gene. The T cell clone DE26 proliferated when antigen was presented by DRw52b, but not by DRw52c or the control DR1+ and Ltk- transfectants. The efficiency of antigen presentation by the L cells expressing DRw52b was reduced compared to that obtained using peripheral blood mononuclear cells (PBMC). This may reflect a lower level of class II expression on the transfectants. Alternatively, in the absence of the appropriate accessory molecules the threshold of T cell activation may be altered. To determine that T cells, restricted by DR β III gene products, were not unique characteristics of the clones examined, but representative of the polyclonal response, the restriction specificity of the T cell line to *D. farinae* presented by the DRw52b transfectant was examined. A proliferative response of >60% of that achieved when PBMC were used as accessory cells was obtained, confirming that a large proportion of the house dust mite-reactive T cell repertoire of this subject use this restriction element. This result is in contrast to the findings with other antigens, where the major restriction specificities are associated with the β I gene products [28].

The dominant use of the DR β III gene products as restriction elements in T cell recognition may contribute to the high incidence of allergy. Clearly, a systematic analysis of T cell recognition at the population level is necessary to substantiate this hypothesis. Additionally, the characteristics of the allergic immune response, unique to atopic individuals, may depend in part on the functional activity of the T cell help induced, and this may be regulated by antigen recognition associated with DR β III gene products. To investigate this possibility, both $\alpha\beta$ I and $\alpha\beta$ III class II restricted T cell clones were examined for their ability to provide B cell help in an allergen-dependent *in-vitro* model of IgE synthesis [29]. The T cell clones DE9 and DE26, when cultured with autologous B cells and macrophages in the presence of house dust mite were able to support the production of both IgG and IgE antibodies (Table 3), confirming that both DR $\alpha\beta$ I and DR $\alpha\beta$ III encoded class II proteins are able to restrict T cells capable of providing B cell help. Furthermore, in agreement with observations in both the

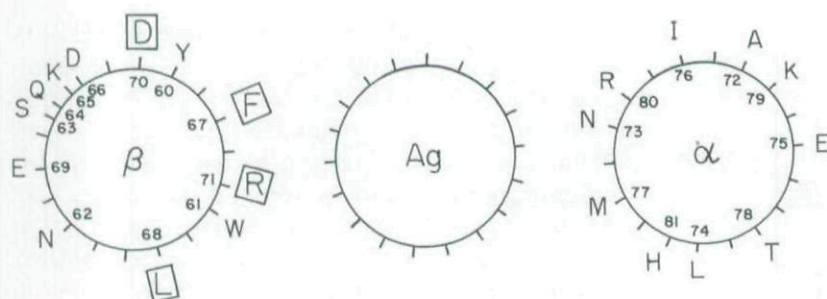


Fig. 2. Schematic cross-sectional diagram illustrating antigen positioned between the α and β chains that form the helical walls of the putative antigen combining site of MHC class II proteins. The β sheet floor of the combining site is not shown. The boxed residues are those in the β 1 helix conserved between DR2, DR5 and DR8.

Table 3. *In-vitro* immunoglobulin synthesis induced by cloned HDM specific T cells isolated from an atopic individual

T cells	E- cells	Antigen	IL-4	IgE (pg/ml)	IgG (ng/ml)
DE26					
+	+	—	—	<98	63
+	+	<i>D. farinae</i>	—	350	580
+	+	Grass pollen	—	<98	45
+	+	<i>D. farinae</i>	+	780	460
—	+	—	—	<98	<15
—	+	Grass pollen	—	<98	<15
DE9					
+	+	—	—	<90	<15
+	+	<i>D. farinae</i>	—	<90	88
+	+	Grass pollen	—	<90	<15
+	+	<i>D. farinae</i>	+	685	2858
—	+	<i>D. farinae</i>	—	<90	27
—	+	Grass pollen	—	<90	<15

T cell clones DE26 and DE9 reactive with *D. farinae* and isolated from an atopic donor were co-cultured with antigen and autologous E- cells in the presence (+) or absence (—) of recombinant IL-4. Supernatant total IgE and IgG were determined by ELISA after 10 days. Backgrounds of the assays were: DE26, IgE <98 pg/ml, IgG <15 ng/ml; DE9, IgE <90 pg/ml, IgG <15 ng/ml.

mouse [30] and human [31,32] the induction of IgE was IL-4 dependent. Interestingly, cloned and polyclonal T cells specific for *D. farinae*, isolated from a non-atopic individual, although capable of inducing IgG, failed to support allergen-dependent IgE synthesis even in the presence of exogenous IL-4 [29]. This result suggests that qualitative aspects of the T cell helper activity, induced in atopic and non-atopic individuals, may be as important as the epitopes recognized in the outcome of immune responsiveness to *Dermatophagoides*. The practical implications of this are in the design of novel protocols for immunotherapy.

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