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MHC class II restriction specificity of cloned human T lymphocytes reactive with *Dermatophagoides farinae* (house dust mite)

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SUMMARY

In this report the antigen and restriction specificity of human T-cell clones induced with *Dermatophagoides farinae* (*D. farinae*) and isolated from an atopic individual with perennial rhinitis has been investigated. Of the six clones analysed, four were species specific and two showed cross-reactivity for the closely related *Dermatophagoides pteronyssinus* ((*D. pteronyssinus*). Inhibition of antigen-dependent proliferation by murine monoclonal antibodies directed against HLA-D-region gene products revealed that all the clones were restricted by HLA-DR molecules. The restriction specificity was investigated further using a panel of histocompatible and allogeneic-presenting cells. Of the clones tested, one appeared to be DR5 restricted while the remainder showed complex patterns suggesting that DRw52 and DRw53 supertypic specificities may be the restriction elements presenting antigen.

INTRODUCTION

The co-recognition of antigen in association with membrane proteins encoded by the major histocompatibility gene complex (MHC) is essential for the activation of T lymphocytes (Shevach & Rosenthal, 1973; Schwartz, 1985). With respect to human CD^{4+} T lymphocytes it has been reported that the HLA-D region loci (DR, DQ and DP) can all function as restriction elements in the presentation of extrinsic antigen (Bergholtz, Thoresen & Thorsby, 1980; Eckels *et al.*, 1982; Qvigstad, Moen & Thorsby, 1984). The evidence for this has been derived from panel studies using allogeneic accessory cells and the serological inhibition of antigen-dependent activation of cloned T cells.

The T-cell dependency of IgE antibody synthesis in response to aero-allergens such as *Dermatophagoides* (house dust mite) is well established (Ishizaka, 1984). In contrast to the detailed serological studies (Heymann, Chapman & Platts-Mills, 1986; Heymann *et al.*, 1987; Stewart & Turner, 1980), the antigen and restriction specificity of the T-cell response in atopic individuals is ill-defined. However, polyclonal T-cell responses to *Der p* I, a major allergen of *D. pteronyssinus*, have been described in atopic but not control non-atopic individuals (Rawle, Mitchell & Platts-Mills, 1984). In addition, human T-cell lines and clones reactive with *D. pteronyssinus* have been isolated, and their restriction specificity has been suggested to be controlled by HLA-DR gene products based on serological inhibition alone using anti-human MHC class II antibodies (Lanzavecchia *et al.*, 1983).

We have described previously T-cell clones induced with *D. farinae* (O'Hehir *et al.*, 1987). In this paper we extend the study by investigating the antigen and restriction specificity of these T cells cloned from the peripheral blood of an atopic individual of DR5(12),7; DRw52 haplotype. Analysis of their antigen specificity revealed both cross-reactive and *D. farinae*-specific T-cell populations. Inhibition with monoclonal antibodies specific for HLA-D-region antigens demonstrated that all six of the clones assayed were restricted by HLA-DR-encoded determinants. However, by panel studies only one clone appeared to be DR5 restricted, with the remaining clones exhibiting a complex pattern, suggesting recognition of antigen may be restricted by elements associated with the DRw52 and DRw53 supertypic specificities.

Abbreviations: *D. farinae*, *Dermatophagoides farinae*; *D. pteronyssinus*, *Dermatophagoides pteronyssinus*; HLA, human MHC; [^{3}H]TdR, tritiated methyl thymidine; mAb, monoclonal antibody; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cells.

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MATERIALS AND METHODS

Antigens

Lyophilized extracts of *D farinae*, *D. pteronyssinus*, grass-pollen mix and parietaria were the generous gifts of Pharmacia (Uppsala, Sweden).

Antibodies

Murine monoclonal antibodies against human MHC class II determinants L243 [anti-HLA-DR (IgG2a), 50 μg immunoglobulin (Ig)/ml], Leu 10 [anti-HLA-DQ (IgG1), 25 μg Ig/ml] and B7/21 [anti-HLA-DP (IgG1), 25 μg Ig/ml] were purchased from Becton-Dickinson (Sunnyvale, CA).

Isolation of antigen-reactive T-cell clones

T-cell clones were isolated as described previously (Lamb *et al.*, 1982; O'Hehir *et al.*, 1987). Briefly, peripheral blood mononuclear cells (PBMC; $2{\cdot}5 \times 10^5$/ml) were primed with optimal concentrations of *D. farinae* antigen [10^3 biological units (BU)/ml] for 7 days in RPMI-1640 (Flow Labs, Irvine, Ayrshire) medium supplemented with 2 mM L-glutamine, 100 IU/ml penicillin/streptomycin and 5% screened, inactivated human A^+ serum (National Blood Transfusion Service, Edinburgh). Lymphoblasts enriched on Ficoll–Paque (Pharmacia) were established as a long-term line in the presence of irradiated (2500 rads) autologous PBMC, *D. farinae* and interleukin-2 (IL-2; Lymphocult T, Biotest Folex, Frankfurt, FRG; 10% v/v) and subsequently cloned by limiting dilution from the line. For cloning, viable cells (0·3 cells/well) were plated in Microtest II trays together with irradiated autologous PBMC (5×10^5/ml), *D. farinae* and IL-2 (10% v/v). After 7 days growing clones were transferred to flat-bottomed 96-well microtitre trays and subsequently to 24-well trays. At each transfer the clones received fresh IL-2 and filler cells together with specific antigen. The clones were maintained with further IL-2 every 3–4 days and filler cells together with antigen were added every 7 days. Before use in proliferation assays, the clones were rested for 6–8 days after the last addition of filler cells and antigen.

T-lymphocyte proliferation assays

T-cell clones (1×10^4 cells/well) were cultured with *D. farinae* in the presence of a panel of HLA-typed histocompatible and histo-incompatible irradiated PBMC ($2{\cdot}5 \times 10^4$/well) as a source of accessory cells. In serological inhibition assays, antibodies were added over a concentration range at the initiation of the cultures. Proliferation, as correlated with tritiated methyl thymidine ([^{3}H]TdR; Radiochemicals Inc. Amersham, Bucks) incorporation, was measured by liquid scintillation spectroscopy. The results are expressed as the mean counts per minute (c.p.m. + %SEM).

Statistical methods

Negative and positive responses were classified objectively by employing the T-max analysis suggested by Mendell (Mendell *et al.*, 1977). For each clone in each experiment c.p.m. data were first normalized by $\log_{10}$ transformation, ranked and partitioned into high (positive) and low (negative) groups. This approach has been used successfully to analyse the response patterns of alloreactive T cells (Rosen-Bronson *et al.*, 1986).

RESULTS

Antigen specificities of cloned T lymphocytes

The cloned T cells proliferated in response to the inducing antigen *D. farinae* but failed to recognize the allergens grass-pollen mix and parietaria over a range of concentrations (Table 1). Clones DE9 and DE26 gave strong proliferative responses to the closely related member of the same genus, *D. pteronyssinus*. The other clones (DE5, DE12, DD11 and DE41) failed to recognize this allergen, being highly species-specific.

Table 1. Antigen specificities of cloned T lymphocytes

Clones	*D. farinae*	*D. pteronyssinus*	Grass-mix	Parietaria	No Ag
DE41	6435 (7)	197 (40)	324 (8)	325 (9)	152 (44)
DE9	24,212 (6)	19,255 (2)	245 (39)	561 (41)	849 (38)
DD11	24,476 (5)	319 (81)	65 (49)	58 (16)	95 (32)
DE5	16,279 (9)	401 (28)	1441 (21)	835 (12)	1659 (10)
DE12	12,845 (6)	290 (19)	169 (27)	247 (6)	444 (24)
DE26	12,801 (3)	4492 (6)	95 (13)	147 (47)	50 (13)

T lymphocytes (5×10^4/ml) were cultured with autologous irradiated PBMC (1.25×10^5/ml) in the presence of the inducing antigen *D. farinae*, *D. pteronyssinus*, five-grass-mix or parietaria. Proliferation as correlated with [^{3}H]TdR incorporation was measured at 72 hr. The results are expressed as mean c.p.m. (%SEM) for triplicate cultures. The data shown is count of maximum proliferation over a dose–response curve for each antigen. Underlining indicates positive response.

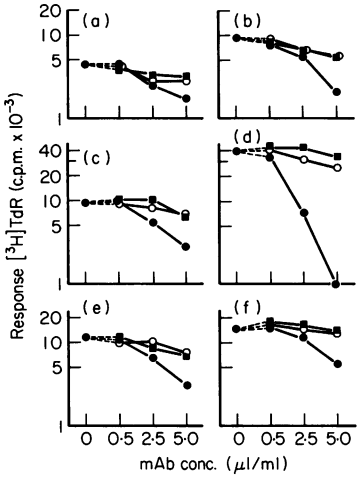

Figure 1. Six clones showing dose–response of blocking effect of murine monoclonal antibodies against human MHC class II determinants. Closed circles, anti-HLA-DR (L243); open circles, anti-HLA-DP (B7/21); closed squares, anti-HLA-DQ (Leu 10). (a) DE41, (b) DE26, (c) DD11, (d) DE5, (e) DE9, (f) DE12.

Serological inhibition of the proliferative response of the T-cell clones

In order to initially define the subsets of MHC class II molecules that might be acting as restriction elements in this system, monoclonal antibodies directed against framework antigens of HLA-D-region molecules were used to block antigen-dependent proliferation of the T-cell clones (Fig. 1). Although a small degree of inhibition was observed with the Leu 10 (HLA-DQw1, w3) and B7/21 (HLA-DP) antibodies, maximal blocking was obtained with L243, which recognizes a determinant common to

Table 2. Genetic restriction of T-lymphocyte clones

Panel	DR	DRw	DE41	DE9	DD11	DE5	DE12	DE26
RM*	7, 5 (12)	52, —	7732	28,535	13,130	15,815	47,541	16,489
030G	7, 5 (11)	52, 53	1178	9548	3864	4195	26,873	101
857D	5, 7	52, 53	2040	11,440	7447	3703	32,439	8901
305G	2, 7	—, —	337	619	284	2547	3638	1875
903E	4, 7	53, —	105	124	123	1989	2657	1464
239E	7, 9	53, —	205	287	792	4111	8632	2844
189P	3, 7	52, 53	541	251	2367	902	14,463	3551
005G	1, 7	53, —	182	201	288	128	166	407
814D	4, 5 (12)	52, 53	573	103	1895	1479	16,401	3715
150G	4, 5 (12)	52, 53	1606	15,437	6300	3601	34,711	9397
056G	5, —	52, 53	1243	7721	4302	279	26,071	5453
129E	3, 5	52, —	1875	11,084	4896	207	26,719	7885
192E	1, 5	52, —	809	5208	3229	225	28,740	6597
926D	5 (11), —	52, —	5529	17,683	10,045	317	45,779	509
023C	8, 5 (11)	52, —	1797	339	5237	654	38,538	6200
826E	5, 6	52, —	1335	275	4223	245	23,150	7049
088G	1, 5	52, —	853	220	3309	203	16,527	4561
572G	10, 5 (12)	52, —	757	341	1802	96	13,779	3809
557G	10, 5	52, —	461	9898	451	401	1853	2185
589G	1, 5 (11)	52, —	311	268	381	102	1599	387
224G	1, —	—, —	206	277	470	405	845	933
161E	2, 9	53, —	143	133	273	433	889	289
855E	2, 4	53, —	122	453	159	772	1616	166
001C	2, —	—, —	315	5439	466	489	1295	1163
1009	3, 8	—, —	124	2000	571	234	1254	772
004C	3, —	52, —	201	289	335	268	697	421
804E	3, 6	52, —	225	1166	846	537	2902	1061
847E	4, —	53, —	215	344	279	7189	9617	349
727E	4, 6	52, 53	213	142	455	120	339	206
013E	8, —	52, —	189	195	137	107	204	135

* Autologous cells.
T-max analysis.
Underlining indicates positive response.

all DR ($\alpha\beta$I and $\alpha\beta$III) molecules. Thus, the clones presented here would seem to be restricted by epitopes of the HLA-DR class II molecules.

Restriction pattern of T-cell clones

Based on the serological inhibition analysis, T-cell clones were tested further for responsiveness to an antigen-presenting panel consisting of DR-matched and DR-mismatched PBMC from 30 individuals, including RM as the DR5(12), 7,(52, −) autologous control (Table 2). The results of control cultures (data not shown), when the T-cell clones and filler cells were incubated together in the absence of antigen, ranged from 47 to 540 c.p.m. (mean = 144 c.p.m.). In all cases, the percentage SEM for triplicate cultures was less than 25%. Analysis of restriction specificities was performed after objectively assigning positive and negative responses using the T-max programme.

Alone of the six clones studied, DE41 showed the closest association with a serologically defined HLA-DR specificity in that it recognized antigen presented by 12/15 DR5$^+$ accessory cells, suggesting it was DR5 restricted. The failure of the remaining three DR5$^+$ accessory cells (557G, 589G and 814D) to present antigen to DE41 may reflect that they express a subtype of DR5.

Clone DE5 recognized 6/8 DR7$^+$ accessory cells and 4/6 DR4$^+$ accessory cells. From the known Dw-subtype heterogeneity of both DR4 and DR7, DE5 may recognize a DRw53-associated restriction element.

T-cell clone DE9 recognized 9/15 DR5$^+$ accessory cells, 1/3 DR2$^+$ accessory cells and the DR8$^+$ accessory cell 1009.

The patterns of restriction observed with the remaining clones DD11, DE12 and DE26 suggest that they may recognize antigen in association with subtypes of DRw52. DD11 recognized 13/15 DR5$^+$ accessory cells and 1/4 informative DR3$^+$ accessory cells. It is noteworthy that 189P is a DR3 variant associated with HLA-B18 rather than the usual HLA-B8.

Clone DE12 appears similar to DD11; however, the response pattern is slightly different, primarily with respect to the recognition of accessory cells 239E (DR7, w9), 305G (DR2,7) and 847E (DR4, −).

Clone DE26 shows a restriction pattern that is not dissimilar from DD11 and DE12, with primary differences being in the recognition of accessory cells 239E (DR7,w9), and 305G (DR2,7).

It is possible that DE12 and 26 are doublets; however, we consider this unlikely since by plating 0·3 viable cells/well, by Poisson distribution the probability of monoclonality is greater than 95%.

DISCUSSION

In this paper we confirm our previous observation that T cells responsive to antigens of the house dust mite, one of the commonest clinically relevant aero-allergens, can be cloned from the peripheral blood of an atopic individual (O'Hehir *et al.*, 1987). Investigation of the antigen specificity of these clones revealed that the majority are species-specific for the inducing allergen *D. farinae* with a minority recognizing cross-reactive determinants also present in the closely related species *D. pteronyssinus*. Although both polyclonal and monoclonal T-cell responses to *D. pteronyssinus* have been described, their cross-reactivity with *D. farinae* was not determined (Lanzavecchia *et al.*, 1983; Rawle *et al.*, 1984).

The initial analysis of the restriction specificity of the T-cell clones suggested that antigen recognition was regulated by immune-response genes of the HLA-DR subregion, since only anti-HLA-DR- and not HLA-DQ- or HLA-DP-specific antibodies markedly inhibited antigen-dependent proliferation. Others have demonstrated the ability of murine monoclonal anti-HLA-DR antibodies and a rabbit anti-human 'Ia' antiserum to inhibit antigen-dependent proliferation of a *D. pteronyssinus*-reactive T-cell line and suggested that HLA-DR molecules are able to function as restriction elements for the presentation of *Dermatophagoides* antigens (Lanzavecchia *et al.*, 1983). To determine the MHC class II alleles restricting T-cell recognition of *D. farinae*, a panel consisting of DR-matched and mismatched PBMC were used as accessory cells. From the results obtained with large panels of presenting cells, definition of the restriction elements appears to be more complex than suggested by the serological analysis. In this context it should be remembered that the use of a small panel could falsely suggest simple restriction patterns and thereby lead to underestimation of the usage of MHC class II molecules in

the presentation of antigen and the diversity of the T-cell repertoire. Thus, in our experiments, if only three or four DR5$^+$-presenting cells had been used it is possible that the differences between DE41 and other clones, such as DE9, which also respond to antigen in association with some of the DR5$^+$-presenting cells, would have been overlooked. Interestingly, only one clone (DE41) appeared to be restricted by MHC class II molecules with parallel serological definition; even then this appeared to be a subtype of DR5 (Bell *et al.*, 1987; Todd, Bell & McDevitt, 1987). Studies at the population level have demonstrated that specific immune responsiveness to the purified pollen antigen *Amb a VI* in atopic individuals, as determined by the presence of serum IgE, is HLA-DR5-associated (Marsh *et al.*, 1987). In contrast, responsiveness to *Amb a V* in the allergic population appeared to be restricted to the DR2/Dw2 haplotype (Marsh *et al.*, 1982). To identify MHC class II alleles determining high responder status to *Dermatophagoides*, T-cell epitopes within the major allergens (*Der f* I and II) need to be identified in atopic individuals and distinguished from those recognized by non-atopics (Buckley *et al.*, 1977; R. E. O'Hehir, unpublished results).

Although the remaining T-cell clones reported here are restricted they showed no consistent patterns that corresponded to serologically defined HLA-DR specificities. However, this is not entirely unexpected since the regions of MHC class II determinants recognized by antibodies do not necessarily overlap with those involved in antigen binding or those that form contacts with T-cell receptor molecules. The complexity in the restriction patterns we observed for these clones may be related to DRw52 and DRw53 specificities encoded on the DRβIII chain. Little is known about antigen-specific responses that are restricted by these molecules, perhaps because of the complexity of the system, which has now been examined at the molecular level (Gorski & Mach, 1986; Owerbach, Rich & Taneja, 1986). Although some human T cells are known to be restricted by HLA-DQ (Qvigstad *et al.*, 1984) and HLA-DP (Eckels *et al.*, 1983), the predominant restriction elements appear to be those encoded by HLA-DR (Eckels *et al.*, 1984). Of particular interest is the observation that some DR2$^+$-, DR5$^+$- and DR8$^+$-presenting cells can activate the T-cell clone DE9, suggesting antigen may bind to conserved residues in the beta-chain of these alleles, such as the sequence (FLED) corresponding to residues 67–70. This is speculative, although such conservation of residues in the third hypervariable domain may allow for the appropriate contact between MHC class II, antigen and the T-cell receptor (Ronchese, Brown & Germain, 1987).

Furthermore, no correlation was observed between the antigen and restriction specificities of the T-cell clones, although phenotypically they were identical (CD4$^+$, CD3$^+$, UCHL1$^+$, CD8$^-$), implying they are of the helper/inducer subset of T cells. Thus neither the cross-reactive nor species-specific determinants of *D. farinae* appeared to be preferentially restricted by either of the haplotypes for this individual, such as has been reported for the cytotoxic response to the internal components of influenza virus (Gotch *et al.*, 1987). Since allergic responses represent aberrant immunological recognition, an intriguing hypothesis would be that some T cells that recognize 'allergenic epitopes' may interact with restriction elements encoded on the DRβIII chain. Systematic study in allergic and non-allergic populations could elucidate this possibility and may prove of practical value in the design of desensitization agents based on synthetic peptides.

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