
Human Immune Responsiveness to *Lolium Perenne* Pollen Allergen *Lol p* III* (Rye III) Is Associated with HLA-DR3 and DR5

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ABSTRACT: A well-characterized allergen of *Lolium perenne* (perennial rye grass) pollen, *Lol p* III, has been used as a model antigen to study the genetic control of the human immune response. Associations between HLA type and IgE or IgG antibody (Ab) responsiveness to *Lol p* III were studied in two groups of skin-test-positive Caucasoid adults (N = 135 and 67). We found by nonparametric and parametric analyses that immune responsiveness to *Lol p* III was significantly associated with HLA-DR3 and DR5. No association was found between any DQ type and immune responsiveness to *Lol p* III. Geometric mean IgE or IgG Ab levels to *Lol p* III were not different between B8⁺, DR3⁺ subjects and B8⁻, DR3⁺ subjects, showing that HLA-B8 had no influence on the association. *Lol p* III IgG Ab data obtained on subjects after grass antigen immunotherapy showed that 100% of DR3 subjects and 100% of DR5 subjects were Ab⁺. A comparison of all the available protein sequences of DRB gene products showed that the first hypervariable region of DR3 and DR5 (and DRw6), and no other region, contains the sequence Glu₆-Tyr-Ser-Thr-Ser₁₃. Our observations are consistent with the possibility that immune responsiveness to the allergen *Lol p* III is associated with this amino acid sequence in the first hypervariable region of the DRβ₁ polypeptide chain.

ABBREVIATIONS

Ab	antibody	<i>Lol p</i> III	group III allergen from
Ag	antigen		<i>Lolium perenne</i> pollen (Rye
BBS	borate-buffered saline		III)
BSA	bovine serum albumin	RFLP	restriction fragment length
Ig	Immunoglobulin		polymorphism
		RIA	radioimmunoassay

INTRODUCTION

The initiation of an immune response to a T-cell-dependent antigen (Ag) requires that the Ag (or, more probably, a fragment of the Ag) be recognized by helper T cells in the context of an Ia molecule [2,3], which is the product of HLA-D region A and B genes in the human. There are a number of examples showing significant associations between specific HLA-D types and immune responses to

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** *Lol p* III is the designation given under IUIS Allergen Nomenclature System (1).*

various antigens [4–7] or between HLA-D types and various autoimmune disorders [8,9]. The molecular basis of these associations is just beginning to be unravelled. Thus, McDevitt and colleagues [10] demonstrated that susceptibility to insulin-dependent diabetes mellitus was closely related to the type of amino acid at position 57 in the DQ β polypeptide chain. Interestingly, position 57 in DQ β was also shown to be a major determinant of susceptibility to pemphigus vulgaris [11]. Further studies have shown that in addition to the residue 57, more complex factors determine susceptibility or resistance to insulin-dependent diabetes mellitus and to pemphigus vulgaris [12–15]. Unfortunately, in these and most other autoimmune diseases, no clear information exists on the (auto)antigens involved. Our approach is to study the associations between particular HLA-D types and specific immune responsiveness to purified, well-defined antigens in order to be able to relate directly the complementary structures of the Ia molecules (desetopes) to the respective Ia binding sites (agretopes) of the antigens.

In this study, we have used a highly purified *Lolium perenne* (perennial rye) pollen component as a model antigen, namely *Lol p* III (Mr = 11,000). We have previously [16] demonstrated a DR3 association of IgE and IgG Ab responsiveness to two other allergens of *Lolium perenne*, namely *Lol p* I (Mr = 27,000) and *Lol p* II (Mr = 11,000). We will discuss the associations between particular HLA-DR types and Ab responsiveness to *Lol p* III in two groups of Caucasoid adults, from which one may extrapolate that a specific amino acid sequence (Glu₉-Tyr-Ser-Thr-Ser₁₃) in the first hypervariable region of the DR β ₁ chain is positively associated with Ab responsiveness to *Lol p* III.

MATERIALS AND METHODS

Study subjects. Two groups of unrelated Caucasoid adults who were skin-test-positive to one or more inhalant allergens and who were previously studied for Ab positivity to *Lol p* I and II [16] were investigated. Westinghouse subjects: A total of 135 employees [83% male, mean age 39 ± 10 (SD) years; age range 20–61 years] of Westinghouse Electric Corporation, Hunt Valley, Baltimore County, MD, were studied. Thirteen of these subjects (10%) had previously received grass pollen immunotherapy from their private physicians. Clinic patients: The clinic group consisted of 67 subjects [66% male; mean age 39 ± 10 (SD) years; age range 20–64 years] who had high levels of clinical sensitivity and ST positivity to short ragweed and/or rye pollen extracts. Unless otherwise stated, analyses were carried out using sera prior to any grass antigen therapy given in our allergy clinic, except in 13 cases where such therapy had been given in the past by the patient's private physician. Posttreatment sera of 46 of the 67 clinic subjects were available immediately after they had received high-dose immunotherapy in our allergy clinic for 1 to 3 years with grass pollen antigens [17,18].

Antigen. *Lol p* IIIB, the major isoallergenic form of *Lol p* III, was purified using methodology described previously [19]. The preparation was homogeneous by agarose gel electrophoresis at pH 8.6, SDS-PAGE, isoelectric focusing, and by immunoelectrophoresis against hyperimmune goat antisera raised against *Lolium perenne* pollen extract.

Radioimmunoassays (RIAs) of IgE and IgG Abs. A direct-binding double-Ab RIA was used to determine the concentrations of human IgG and IgE Abs to *Lol p* III

[see 19]. The Ag was radiolabeled with ^{125}I by the Chloramine T method to a specific activity of 9×10^4 cpm/ng. For IgG Ab determinations, the human serum was diluted 1:10 in borate-buffered saline (BBS), and for IgE Ab determinations the human serum was diluted 1:2 in a human IgE myeloma serum PS "carrier" (1:200). To 100 μl of the diluted serum was added 100 μl of radiiodinated *Lol p* III (0.5 ng) in BBS containing 5% BSA. After a 4-hr incubation at 23°C, 100 μl of a solution was added containing a slight excess of either goat anti-human IgG (γ -chain specific for IgG Ab determination) or goat anti-human IgE (ϵ -chain specific for IgE Ab determination). After overnight incubation at 4°C, the precipitates were washed four times with BBS and counted in a gamma counter. The Ab contents were computed by comparison against a standard serum of known IgG Ab level. All determinations were performed in triplicate in at least two assays. Further experiments were performed where the coefficient of variation was >10% for the means of the first two assays. Sometimes as many as four assays were necessary to finalize the data for very low Ab levels.

Data analysis. Using the above RIAs, we were unable to discriminate between the cpm values corresponding to Ab levels of <0.3 ng/ml and background. Because 1:10 and 1:2 serum dilutions were the highest concentrations used in the IgG and IgE Ab assays, respectively, we could compute IgG Ab levels of >3 ng/ml and IgE Ab levels of >0.6 ng/ml. Persons having lower than these detectable limits were considered to be negative for nonparametric analyses (Fisher's Exact Test, one-tailed and two-tailed). Such "negative subjects" were characterized as having levels of 3 ng/ml and 0.6 ng/ml of IgG and IgE Ab, respectively, in the case of the parametric statistical analyses. The influence of several independent variables, namely, HLA specificity, log(total IgE), sex, age, and previous immunotherapy on log(IgE Ab) or log(IgG Ab) levels, was then evaluated by stepwise multiple regression analysis [16]. "Previous immunotherapy," which refers to injections of grass pollen extracts by patient's private physician, was ranked for the purpose of analysis as 2 for therapy within the past 5 years, 1 for therapy given >5 years ago, and 0 for no previous immunotherapy.

HLA typings. HLA-A, B, and C typings were performed by standard lymphocytotoxicity technique using 140 well-defined typing sera standardized against International Workshop reagents. HLA-DR and DQ typings were performed by a modification [20] of the method of van Rood et al. [21] using 70 well-defined sera, which permitted the identification of specificities DR1-DR10, DRw52, DRw53, and DQw1-DQw3.

RESULTS

Prevalence Rates of IgE and IgG Abs to *Lol p* III

Among the 135 Westinghouse individuals, 30 produced IgE Ab and 38 produced IgG Ab to *Lol p* III; 8 individuals had IgG Ab without showing any detectable IgE Ab to *Lol p* III. Among the 67 clinic patients, 23 produced IgE Ab and 27 produced IgG Ab to *Lol p* III. In both groups, there was no individual who had IgE Ab in the absence of detectable IgG Ab, and the correlations between log(IgE Ab) and log(IgG Ab) to *Lol p* III were significant ($r = 0.64$, $p < 10^{-5}$ for the Westinghouse group; $r = 0.78$, $p < 10^{-5}$ for the clinic group). These findings are consistent with earlier findings using other inhalant allergens [4,22-26].

Relationship between *Lol p* III Abs and HLA Specificity

The proportions of all the different HLA-A, B, C, and D types between *Lol p* III⁺ and *Lol p* III⁻ Westinghouse subjects were compared using nonparametric statistics (Fisher's Exact Test). The only significant associations observed for both IgE and IgG Ab responsiveness to *Lol p* III were with DR3 and with HLA-A1, B8, and DRw52, all of which are encoded by alleles of genes in linkage disequilibrium (Table 1). In addition, there was a trend for Ab positivity to be

TABLE 1 Associations between HLA-A, B, C, DR, or DQ and Ab positivity to *Lol p* III (nonparametric analysis)

HLA	IgE Ab positivity ^a		IgG Ab positivity ^a	
	Ab ⁺	Ab ⁻	Ab ⁺	Ab ⁻
Westinghouse subjects (N = 135):				
A1	47%	27%	50%	24%
	(0.05)		(0.004)	
	2.4		3.2	
B8	33%	15%	40%	13%
	(0.04)		(0.01)	
	2.8		3.4	
DR3	43%	18%	47%	14%
	(0.007)		(0.0002)	
	3.5		5.3	
DR5	30%	18%	31%	16%
	(0.2)		(0.06)	
	1.9		2.3	
DRw52	83%	50%	82%	48%
	(0.001)		(0.0005)	
	4.9		4.7	
Clinic patients (N = 67):				
A1	35%	16%	33%	15%
	(0.08)		(0.07)	
	2.8		2.8	
B8	48%	16%	41%	17%
	(0.007)		(0.03)	
	4.8		3.2	
DR3	57%	7%	48%	7%
	(0.00001)		(0.0002)	
	17.8		11.5	
DR5	26%	2%	22%	2%
	(0.01)		(0.02)	
	15.1		11.1	
DRw52	91%	25%	78%	28%
	(<0.00001)		(0.00006)	
	31.5		9.2	

^a % of Ab⁺ and Ab⁻ subjects is given in regular type, *p* values are in parentheses, and relative risks are in **bold type**. The *p* values are two-tailed for those associations observed for the first time in the study; this includes all associations in the Westinghouse subjects and only DR5 associations in the clinic group. The *p* values are one-tailed for the associations that are being confirmed in the second study group; this includes all but the DR5 association in the clinic patients.
Note: DR7 and DRw53 were negatively associated with IgE and IgG Ab positivities to *Lol p* III. The *p* values (in parentheses) and the relative risks, in bold face, for DR7 associations were: clinic IgE (0.008) 0.1, IgG (0.04) 0.3. Corresponding values for the DRw53 associations were: Westinghouse IgE (0.003) 0.2, IgG (0.0005) 0.2; clinic IgE (0.01) 0.2. In addition, DRw6 was significantly associated with *Lol p* III IgE Ab responsiveness in the Westinghouse subjects (0.03) 2.7

TABLE 2 Stepwise regression analyses of log(IgE Ab) or log(IgG Ab) levels to *Lol p* III vs. the independent variables: age, sex, log(total IgE), previous grass immunotherapy, and HLA type

Dependent variable	Independent variables	Variable ^a	Regression ^b	
Westinghouse subjects (<i>N</i> = 135):				
anti- <i>Lol p</i> III IgE	DR5	<i>p</i>	R ²	<i>p</i>
	DRw6	0.01	0.05	0.01
	DRw8	0.01	0.09	0.001
	DR3	0.02	0.13	0.0004
	B14 (-'ve) ^c	0.03	0.16	0.0001
	Previous Rx ^d	0.03	0.19	0.00004
	Total IgE	0.03	0.22	0.00001
anti- <i>Lol p</i> III IgG	DR3	0.03	0.25	0.00001
	DR3	0.0007	0.08	0.0007
	Previous Rx	0.003	0.14	0.0004
	DRw6	0.003	0.20	<1 × 10 ⁻⁵
	DR5	0.01	0.24	<1 × 10 ⁻⁵
Clinic patients (<i>N</i> = 67):				
anti- <i>Lol p</i> III IgE	B14 (-'ve)	0.02	0.27	<1 × 10 ⁻⁵
	DR3	0.0003	0.18	0.0003
anti- <i>Lol p</i> III IgG	DR5	0.007	0.27	0.0004
	DR3	0.0009	0.16	0.0009
	DR5	0.004	0.26	0.0007

^a This set of *p* values indicates the significance of the partial F statistic for each of the independently cited variables.^b R² indicates how well the combination of independent variables predicts the dependent variable, 0 < R² ≤ 1. (R² = 0.05 for the first regression equation which includes only DR5; R² = 0.09 for the regression equation which includes both DR5 and DRw6, etc.) The *p* values indicate the significance of the overall F statistic in each case; i.e., where the cited variable and all preceding variables are included in the regression equation.^c Significant negative associations (in bold print).^d Immunotherapy (Rx) with grass antigens.

associated with DR5 (*p* values were not significant at <0.05). Except for A1, all the associations observed in the Westinghouse group were confirmed in the clinic group (Table 1). In addition, the clinic group showed strong and significant associations of DR5 with both IgE and IgG Ab responsiveness to *Lol p* III. No DQ association was found in either study group.

The proportions of DR3⁺ subjects were not different between subjects ST⁺ or ST⁻ to an unrelated complex allergenic mixture, namely crude short ragweed (23% and 26%, respectively, among Westinghouse subjects). Thus, the observed DR3 association with immune responsiveness to *Lol p* III does appear to be antigen-selective.

A series of stepwise multiple regression analyses were carried out in order to obtain further information on the HLA associations and to investigate the possible associations of responsiveness to *Lol p* III with age, sex, etc. (Table 2). Log(IgE Ab) and log(IgG Ab) to *Lol p* III were the dependent variables; the different HLA types, age, sex, previous grass immunotherapy, and log(total serum IgE) were the independent variables. Unlike the nonparametric analyses, which are based on classification of the subjects as responders and nonresponders, the multiple regression analyses take into account the actual specific Ab levels in the patients' sera. The results of parametric analyses confirmed the HLA-DR3 and DR5 associations with responsiveness to *Lol p* III in both the

TABLE 3 Responsiveness to *Lol p* III before and after grass antigen immunotherapy^a of the 46 clinic subjects who received immunotherapy

	Total subjects	<i>Lol p</i> III IgG Ab+ subjects	
		Pre-Rx	Post-Rx
DR3:			
All DR3 ⁺ subjects	14 ^b	11 (79%)	14 (100%)
DR3 ⁺ ,DR5 ⁻	12	11 (92%)	12 (100%)
DR5:			
All DR5 ⁺ subjects	7 ^b	6 (86%)	7 (100%)
DR5 ⁺ ,DR3 ⁻	5	4 (80%)	5 (100%)
Non-DR3,5:			
All DR3 ⁻ ,DR5 ⁻ subjects	27	8 (30%)	10 (37%)

^a Rx = immunotherapy with grass pollen extract.

^b Includes two subjects who were DR3⁺ and DR5⁺. Thus, the total number of DR3 and/or DR5⁺ subjects was 19 (14 + 7 - 2).

study groups. In addition, in the Westinghouse group, DRw6, DRw8, previous immunotherapy, and total serum IgE level were also found to be predictors of anti-*Lol p* III IgE Ab positivity; and previous immunotherapy and DRw6 were found to be predictors of anti-*Lol p* III IgG Ab positivity.

Lol p III IgG Ab data on the clinic subjects after grass antigen immunotherapy provided further evidence for DR3 and DR5 associations with *Lol p* III responsiveness (Table 3). Thus, among the 46 subjects who received immunotherapy, 14 were DR3⁺, 11 of whom were *Lol p* III IgG Ab⁺ before immunotherapy, and the remaining 3 became IgG Ab⁺ after immunotherapy. In order to eliminate any contribution of DR5 toward the responsiveness, we also reviewed the 12 subjects within this group who were DR3⁺,DR5⁻; 11 of these 12 subjects were *Lol p* III IgG Ab⁺ before immunotherapy, and the remaining one patient became Ab⁺ after immunotherapy. Similar analysis for DR5 showed that in the clinic group of 46 subjects who received immunotherapy, 7 were DR5⁺, 6 of whom were *Lol p* III IgG Ab⁺ before immunotherapy, and the remaining subject became Ab⁺ after immunotherapy. Thus, 100% of the 19 DR3⁺ and DR5⁺ subjects were responders to *Lol p* III after grass antigen immunotherapy. On the contrary, only 37% of the 27 DR3⁻,DR5⁻ subjects were *Lol p* III IgG Ab⁺ after immunotherapy ($p = 0.00001$).

Finer Definition of the HLA-DR3 Association

Recent studies using restriction fragment length polymorphism (RFLP) mapping [27] have shown the DR3 type may be divided into two distinct major subtypes in Caucasoids: B8⁺,DR3⁺ haplotypes and B8⁻,DR3⁺ haplotypes. DR3 expresses only one α chain, which is invariant among the subtypes, and two β chains, β_1 and β_{III} . Nucleotide sequence studies have shown that the DR β_1 allele is identical but the DR β_{III} allele differs in these two RFLP-defined subtypes [28,29]. Consequently, we investigated whether the immune responsiveness to *Lol p* III was associated with the B8⁺,DR3⁺ phenotype or with the B8⁻,DR3⁺ phenotype, making the assumption that in most cases the B8⁺,DR3⁺ phenotype would correspond to the respective haplotype because of the strong linkage disequilib-

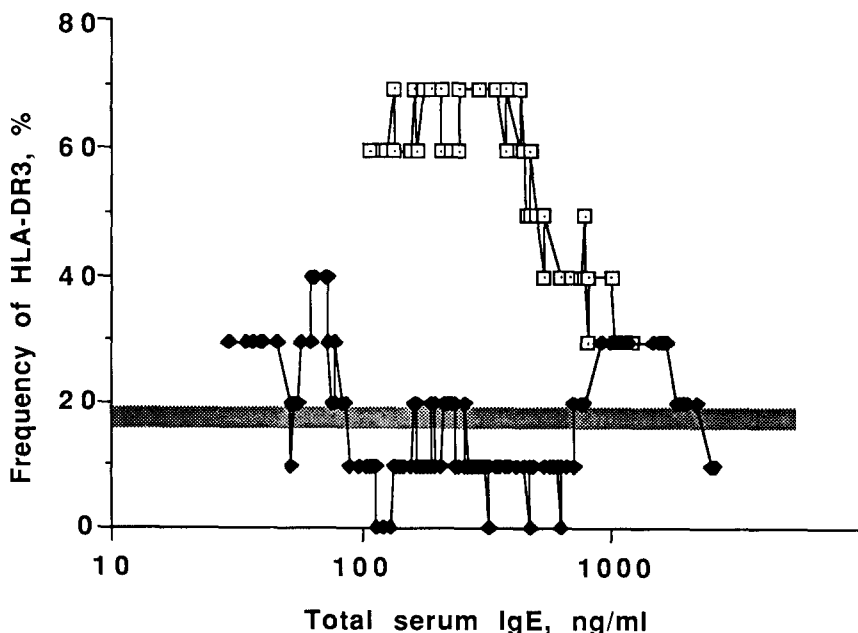


FIGURE 1 Frequency of DR3⁺ subjects in consecutive, overlapping groups of 11 *Lol p* III IgE Ab⁺ subjects (open squares) and *Lol p* III IgE Ab⁻ subjects (filled symbols) (e.g., for the *Lol p* III IgE Ab⁺ plot, the 11 subjects with the lowest total serum IgE levels constituted the first group. For the second group, the subject with the lowest total serum IgE level was deleted from the first group and replaced by the subject with the next highest total serum IgE level). The percent of DR3⁺ subjects in each group was plotted at the median total serum IgE level of that group. The Caucasian population frequency of HLA-DR3 is 19% (hatched bar).

rium between B8 and DR3. For both IgE Ab and IgG Ab, the associations with the B8⁺,DR3⁺ phenotypic combination were not better than with DR3 alone. Also, the geometric means of Ab levels to *Lol p* III in B8⁺,DR3⁺ subjects were not significantly different from those in B8⁻,DR3⁺ subjects (respectively, IgE Ab, 3.4 vs. 2.7 ng/ml; IgG Ab, 1.1 vs. 1.2 ng/ml). Furthermore, the mean log(Ab) levels for B8⁺,DR3⁻ subjects and B8⁻,DR3⁻ subjects were not different, precluding any independent influence of B8.

Relationship between HLA-DR3 Frequency and Total Serum IgE Levels among Subjects IgE Ab⁺ or IgE Ab⁻ to *Lol p* III

We wish to investigate further the influence of *total* serum IgE leveled on *Lol p* III IgE Ab positivity in view of the relationship noted in Table 2 and previous findings that suggested interrelationships between total IgE and HLA type among subjects responding to other allergens [30]. First, we found that the geometric mean total serum IgE levels in *Lol p* III IgE Ab⁺/DR3⁺ subjects (262 ng/ml) vs. *Lol p* III IgE Ab⁻/DR3⁻ subjects (458 ng/ml) were significantly different ($p = 0.05$, two-tailed). Secondly, we plotted the frequencies of DR3 against the total serum IgE level in consecutive, overlapping groups of 11 subjects for both *Lol p* III IgE Ab⁺ and IgE Ab⁻ subgroups (Figure 1). Among the *Lol p* III IgE Ab⁻ subjects, the frequency of DR3 varied randomly about its normal Caucasoid

population frequency (19%). However, for *Lol p* III IgE Ab⁺ subjects, the frequency of DR3 was nonrandomly distributed with respect to total serum IgE level; subjects having low total IgE levels had a high DR3 frequency (~65%), which decreased to about 30% as the total IgE level increased. These observations suggest that subjects who produce high levels of IgE of any specificity tend to produce specific IgE Ab to *Lol p* III as well, irrespective of their HLA-D specificity; whereas low IgE producers may require an *Ir* gene associated with DR3 in order to respond to *Lol p* III. Among all the *Lol p* III IgE Ab⁺ subjects, mean total serum IgE levels in DR5⁺ subjects (468 ng/ml) vs. DR5⁻ subjects (309 ng/ml) were not significantly different.

DISCUSSION

We found, by both nonparametric and parametric analyses, that immune responsiveness to *Lol p* III was significantly associated with HLA-DR3 as well as with B8. Mean IgE or IgG Ab levels to *Lol p* III were not different between B8⁺,DR3⁺ subjects vs. B8⁻,DR3⁺ subjects, showing that HLA-B8 had no influence on the DR3 association. Therefore, the association of *Lol p* III responsiveness with B8 (and a weaker association with A1) presumably results from the strong linkage disequilibrium between the respective alleles. As already noted, the DR3B_I gene is identical, but the DR3B_{III} gene differs between the B8⁺ and B8⁻ subtypes. While the involvement of the DR3B_{III} gene cannot be completely ruled out, observation that the presence of B8 had no influence on the level of *Lol p* III-specific Abs in DR3⁺ subjects indicates that DR3B_I gene, and not DR3B_{III} gene, is more likely to be involved in the control of response to *Lol p* III.

In addition to DR3, DR5 was also significantly, but more weakly, associated with IgE and IgG Ab responsiveness to *Lol p* III. *Lol p* III IgG Ab data obtained on the clinic subjects after grass antigen immunotherapy (Table 3) showed that 100% of DR3⁺ subjects and 100% of DR5⁺ subjects were Ab⁺, whereas only 23% of DR3⁻,DR5⁻ subjects were Ab⁺.

The two DR types, DR3 and 5, belong to the "DRw52 family," other members being DRw6 and w8. That immune responsiveness to *Lol p* III is associated with the DR types belonging to DRw52 family, evident from the analyses in Tables 1 to 3, is further supported by the following observation: We reported earlier [19] that while *Lol p* I did not cross-react with *Lol p* III, a high degree of cross-reactivity was exhibited between *Lol p* II and III using some, but not all, human sera. In our total combined study group of 202 subjects, there were 12 exceptional subjects who produced IgE Ab to *Lol p* III without producing any detectable IgE Ab to *Lol p* II. Thus, analyses of Ab data in these subjects would be free of any complications that may be caused by the cross-reactivity between *Lol p* II and III. We found that 10 of these 12 subjects (83%) had the DR3 and/or DR5 specificities. The remaining two subjects had DRw6, which also showed some evidence of association with immune responsiveness to *Lol p* III (Table 2). Similar analysis for IgG Ab showed that in our study group of 202 subjects there were 7 exceptional subjects who produced IgG Ab to *Lol p* III in absence of any detectable IgG Ab to *Lol p* II; 5 of these subjects (71%) were DR3⁺ and/or DR5⁺, one was DRw6⁺, and only one patient did not have any of these three DR specificities.

We investigated whether there was any specific sequence common in the hypervariable regions of the β polypeptides of DR3 and 5. A comparison of all the available DR β _I and DR β _{III} sequences showed that the first hypervariable region, and no other region, contains such a sequence, Glu-Tyr-Ser-Thr-Ser

TABLE 4 Amino acid sequence of various DR β polypeptide chains at residue positions 9 through 13

	Amino acid sequence (pos. 9–13)					Source	Reference
DR3 β_1	Glu	Tyr	Ser	Thr	Ser	AVL(HTC/WT49(HTC) ^a	31/32
DR5 Dw5 β_1	—	—	—	—	—	Swei(HTC)	39
DRw6 β_1	—	—	—	—	—	HHK(HTC)/APD(HTC)	31/32
DRw8 β_1	—	—	—	—	Gly	Madura(HTC)	32
DR1 β_1	Trp	Gln	Leu	Lys	Phe	LG2(HTC)/B-cell line 45.1	32/35
DR2 Dw2 β_1	Gln	Gln	Asp	Lys	Tyr	PGF(HTC)	32
DR2 Dw2 β_{III}	Gln	Gln	Pro	Lys	Arg	PGF(HTC)	32
DR2 Dw12 β_1	Gln	Gln	Asp	Lys	Tyr	BGE(HTC)/DHO(HTC)	36/37
DR2 Dw12 β_{III}	Gln	Gln	Pro	Lys	Arg	BGE(HTC)	36
DR2 AZH β_1	Gln	Gln	Asp	Lys	Tyr	AZH(HTC)/DR2-MN2	36/37
DR2 AZH β_{III}	Trp	Gln	Pro	Lys	Arg	AZH(HTC)/DR2-MN2	36/37
DR3 DRw52a	—	Leu	Arg	Lys	—	AVL(HTC)	31
DR3 DRw52b	—	Leu	Leu	Lys	—	DR4/w6 cell line	35
DR4 Dw4 β_1	—	Gln	Val	Lys	His	Priess/GM2103/WT51(HTC)	38/39/32
DR4 Dw10 β_1	—	Gln	Val	Lys	His	FS(HTC)	39
DR4 Dw14 β_1	—	Gln	Val	Lys	His	BIN-40(HTC)	39
DR4 DRw53	—	Gln	Ala	Lys	Cys	Priess(HTC)	38,32
DR7 β_1	Trp	Gln	Gly	Lys	Tyr	LBF(HTC)	32
DRw9 β_1	Lys	Gln	Asp	Lys	Phe	DKB(HTC)	32
DRw10 β_1	—	Glu	Val	Lys	Phe	Raji cell line	34

^a HTC = homozygous typing cell.

(residues 9–13) in DR3 and 5 (Table 4). The only other DR β polypeptides that are known to contain this sequence are DRw6 (Table 4).

We also considered the possibility that the primary association of the immune responsiveness to *Lol p* III might rather be with a sequence in the DRw52 β chain. In this case, one or more of the hypervariable regions of DRw52 β chain could possibly be involved in the recognition of the relevant *Lol p* III peptide. This would be particularly true for the third hypervariable region which is similar in DR3 and DRw52. However, dot-blot analysis of the polymerase chain reaction (PCR)–amplified DNA from 27 *Lol p* III responders and 25 nonresponders using an oligonucleotide probe that bound to the third hypervariable region of DR3/DRw52 sequence (AGCAGAAGCGGGGCCG) showed no relationship between the presence of this sequence and responsiveness to *Lol p* III (unpublished observation).

Thus, we hypothesize that immune responsiveness to *Lol p* III is associated with the specific amino acid sequence (EYSTS) in the first hypervariable region of the DR β_1 polypeptide chain. it is interesting to note that this region has also been implicated in responsiveness to tetanus toxoid [40]. Our hypothesis is supported by the following observations: (1) In the clinic group, 100% of the subjects who are DR3⁺ or DR5⁺ respond to *Lol p* III as a result of natural grass pollen exposure or grass immunotherapy. (2) In one of the two study groups, immune responsiveness to *Lol p* III also shows a weak association with DRw6 (Table 2), which also contains the sequence EYSTS in DR β_1 polypeptide chain. The reason for the lack of a stronger association between DRw6 and responsiveness to *Lol p* III is not clear to us, but may reflect the involvement of other DR β polymorphic

residues, in addition to the EYSTS sequence, in antigen binding. (3) Little association is found between DRw8 and responsiveness to *Lol p* III (Tables 1 and 2). It should be noted that in our total group of 202 subjects only 6 of 15 DRw8⁺ subjects were *Lol p* III IgE or IgG Ab⁺. This observation, together with the fact that the DRw8 β_1 chain differs from DR3 and 5 β_1 chains only by one amino acid (position 13) in the first hypervariable region (Table 4), points to the possible importance of position 13 in immune responsiveness to *Lol p* III.

One question is why some DR3⁻, DR5⁻, and DRw6⁻ subjects respond to *Lol p* III. We speculate that in such cases, the gene sequence critical for the Ia recognition of the antigen fragment (such as the gene sequence coding for EYSTS), may have been transposed to another gene by a process such as gene conversion. Alternatively, a different Ia recognition site (agretope) of the antigen may be involved.

Our results on *Lol p* III bear some striking similarities with those we reported earlier for *Lol p* I and II [16]. Thus, like *Lol p* III, immune responsiveness to *Lol p* I and II is associated with HLA-DR3, and B8 has no influence on the level of *Lol p* I and II-specific Abs in DR3⁺ subjects. However, unlike *Lol p* III, no DR5 association was found for responsiveness to *Lol p* I and II. That the same Ia molecule can present more than one, similar antigen fragment (allowing for some degeneracy in the specificity of Ia-antigen interaction) has not only been documented [41,42], but also appears to be an essential feature of the immune system. Identification and sequencing of the Ir gene(s) that control human immune responses to these antigens are important in understanding the molecular basis of the responsiveness. Such studies have become even more interesting now that the x-ray crystallographic structure of a class I molecule has been determined [43,44], in view of its homology with class II. It will be interesting to investigate whether *Lol p* I, II, and III have a common structural feature (agretope) that is recognized by DR3 molecules, and whether any antigens implicated in certain DR3-associated autoimmune diseases have agretopes resembling those of *Lol p* I, II, and III.

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