

Molecular Genetics of Human Immune Responsiveness to *Lolium perenne* (Rye) Allergen, *Lol p* III¹

(Short Communication)

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Abstract. *Lol p* II and III are each about 11-kD protein allergens from the pollen of *Lolium perenne* (rye grass). We have found that human immune responses (IgE and IgG antibodies) to both proteins are significantly associated with HLA-DR3. In addition, the two proteins are cross-reactive with the antibodies in many human sera (about 84% human sera showed the cross-reactivity). We have determined > 90% of the amino acid sequences of the two proteins and found that they are at least 54% homologous. Berzofsky found that 75% of the 23 known T cell sites in various proteins had an amphipathic structure. Our analysis by the same method showed that both *Lol p* II and III have a major region of amphipathicity (at residues 61-67, *Lol p* III numbering) which might contain sites for binding to an Ia molecule and a T cell receptor. This region is identical between *Lol p* II and III, except for an Arg-Lys substitution, and could account, in part, for the DR3 association with responsiveness to both molecules. An interesting difference between the two proteins is that immune response to *Lol p* III is associated with DR5 (in addition to DR3), whereas no DR5 association is found in the case of *Lol p* II. One possibility is that *Lol p* III has an additional site which binds to the DR5 Ia molecule. *Lol p* III indeed has a second highly amphipathic peptide, 24-30 (*Lol p* III 24 R P G D T L A 30), which is different and not amphipathic in *Lol p* II. Therefore, the results of these analyses are consistent with our observations that DR3 is associated with immune responses to both *Lol p* II and III, but DR5 is associated with immune response to only one of them, *Lol p* III. Further experiments are required to examine these postulates.

There are a number of examples showing significant associations between specific human leukocyte antigen D (HLA-D) types and immune responses to various antigens (Ags) [2-5], or between HLA-D types and various autoimmune disorders [6, 7]. These associations result perhaps from the fact that the initiation of an immune response to the specific Ags requires that the Ag (or its fragment) be recognized by helper T cells in the context of an Ia molecule [8, 9] which is a product of *HLA-D* region genes in the human. The molecular basis of these associations is just beginning to be understood. Thus, Todd et al. [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]. In these examples, a key element at the site of the Ia molecule responsible for disease resistance/susceptibility seems to have been identified. Unfortunately, in these and most other autoimmune diseases, no clear information exists on the (auto)Ag involved. Our approach is to study the associations between particular HLA-D types and specific immune responsiveness to purified, well-defined Ags 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 Ags.

We have been using *Lol p* I, II and III as model Ags to study the molecular genetics of human immune responsiveness. *Lol p* III (group III allergen from *Lolium perenne* pollen; rye III) is the designation given under the IUIS Allergen Nomenclature

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System [1]. These are protein allergens from *L. perenne* (perennial rye grass) pollen, with molecular weights of 27,000, 11,000, and 11,000 respectively. In this paper, we will discuss the associations between particular HLA-DR types and antibody (Ab) responsiveness to *Lol p III* in Caucasoid adults. From the data to be presented in this paper, 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*.

Rye-allergic people are often concordantly sensitive to all three of these allergens: *Lol p I*, II, and III. Thus, among 202 subjects, 53 were *Lol p III* IgE Ab⁺, 41 of 53 (77%) were *Lol p II* IgE Ab⁺, and 48 of 53 (91%) were *Lol p I* IgE Ab⁺ (corresponding numbers for IgG Ab positivities: *Lol p II/III* = 58 of 65 or 89%, and *Lol p I/III* = 56 of 65 or 86%). Since earlier studies suggested that these Ags are not cross-reactive, we studied their immunological relatedness by double-antibody radioimmunoassay (DARIA) in order to understand further the immunochemical basis for the concordant recognition of the three allergens. Direct-binding DARIA studies were performed using human sera from 202 allergic subjects. Inhibition DARIA studies were carried out using 17 human sera from grass-allergic patients who were on grass immunotherapy, 1 goat antiserum, and 6 rabbit antisera. None of the sera detected any significant degree of two-way cross-reactivity between *Lol p I* and II or between *Lol p I* and III. From these results [12], we concluded that the concordance of Abs to *Lol p I* on the one hand and to *Lol p II* and/or III on the other was due to specific immune responses to the individual allergens and not merely a reflection of their cross-reactivity. However, the degree of two-way cross-reactivity between *Lol p II* and III exhibited by individual human and animal antisera varied between undetectable and 100%. Therefore, it was not possible to draw a similar conclusion concerning concordance of Abs to *Lol p II* and III. Serum samples, which, by DARIA, showed Abs to both *Lol p II* and III, exhibited a high degree of cross-reactivity between the two Ags (the binding of one Ag could be completely inhibited by the other). But those human sera containing Ab only to *Lol p III* exhibited no cross-reactivity with *Lol p II* and vice versa. As mentioned earlier, out of the 53 *Lol p III* IgE Ab⁺ subjects, 41 were also *Lol p II* IgE Ab⁺; the remainder (12 subjects) produced Abs to *Lol p III* which did not cross-react with *Lol p II* (or

Table 1. HLA-DR types of individuals who were *Lol p III* IgE⁺ but *Lol p II* IgE⁻

ID No.	DR type	
116	6,	-
146	5,	-
167	1,	3
454	2,	3
468	4,	5
510	3,	7
611	3,	-
675	3,	5
1042	3,	5
1088	4,	6
1107	3,	5
1112	5,	6

This analysis was done on a total of 202 subjects including 135 Westinghouse subjects who were ST⁺ to LPE and 67 clinic patients who were ST⁻ to LPE but ST⁺ to one or more of other inhalant allergens (extracts of short ragweed and timothy grass pollen, cat dander, house dust, and *Alternaria* spp.).

Lol p I). A look at the HLA-DR types of these 12 individuals (table 1) showed that all, except 2, had DR3 and/or DR5 specificities; the other 2 subjects had DRw6 in common. This observation indicated that immune responsiveness to *Lol p III* might be associated with DR3 and 5 and, possibly, with DRw6.

In order to further assess the HLA-D associations, the proportions of all the different HLA-D types between *Lol p III*⁺ versus *Lol p III*⁻ subjects in a study group of 135 Caucasoids (Westinghouse study group) were compared using non-parametric 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 DRw52 which are encoded by alleles of genes in linkage disequilibrium (table 2). In addition, there was a trend for Ab positivity to be associated with DR5 (p not significant at <0.05). The associations observed in the Westinghouse group were confirmed in another study group of 67 Caucasoids (clinic group, table 2). 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⁻ (skin test) to an unrelated complex allergenic mixture, namely crude short ragweed (23 and 26%, respectively, among Westing-

Table 2. Associations between HLA-DR or DQ and Ab positivity to *Lol p* III (nonparametric analysis)

HLA	IgE Ab positivity ¹		IgG Ab positivity ¹	
	Ab ⁺	Ab ⁻	Ab ⁺	Ab ⁻
<i>Westinghouse subjects (n = 135)</i>				
DR3, %	43 (0.007) 3.5	18	47 (0.0002) 5.3	14
DR5, %	30 (0.2) 1.9	18	31 (0.06) 2.3	16
DRw52, %	83 (0.001) 4.9	50	82 (0.0005) 4.7	48
<i>Clinic patients (n = 67)</i>				
DR3, %	57 (0.00001) 17.8	7	48 (0.0002) 11.5	7
DR5, %	26 (0.01) 15.1	2	22 (0.02) 11.1	2
DRw52, %	91 (<0.00001) 31.5	25	78 (0.00006) 9.2	28

¹ Percent Ab⁺ and Ab⁻ subjects are given in regular type, p values are in parentheses, and relative risks are in italics. 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 italics) for DR7 associations were: clinic IgE (0.008) 0.1, IgG (0.04) 0.3. The 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. *Study subjects:* Two groups of unrelated Caucasoid adults who were ST⁺ to one or more inhalant allergens and who were previously studied for Ab positivity to *Lol p* I and II [12] 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 Corp., Hunt Valley, Baltimore County, Md., USA, were studied. Thirteen of these subjects (10%) had previously received grass pollen immunotherapy from their private physicians. *Clinic patients:* This 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⁺ to short ragweed and/or rye pollen extracts. Thirteen of these subjects

Table 3. Stepwise regression analyses of log [IgE Ab] or log [IgG Ab] levels to *Lol p* III versus the independent variables age, sex, log [total IgE], previous grass immunotherapy (Rx), and HLA-D type

Dependent variable	Independent variables	Variable p ^a	Regression ^b	
			R ²	p
<i>Westinghouse subjects (n = 135)</i>				
Anti- <i>Lol p</i> III IgE	DR5	0.01	0.05	0.01
	DR6	0.01	0.09	0.001
	DR8	0.02	0.13	0.0004
	DR3	0.03	0.16	0.0001
	previous Rx ^c	0.03	0.22	0.00001
	Total IgE	0.03	0.25	0.00001
Anti- <i>Lol p</i> III IgG	DR3	0.0007	0.08	0.0007
	previous Rx	0.003	0.14	0.00004
	DR6	0.003	0.20	< 1 × 10 ⁻⁵
	DR5	0.01	0.24	< 1 × 10 ⁻⁵
<i>Clinic patients (n = 67)</i>				
Anti- <i>Lol p</i> III IgE	DR3	0.0003	0.18	0.0003
	DR5	0.007	0.27	0.0004
Anti- <i>Lol p</i> III IgG	DR3	0.0009	0.16	0.0009
	DR5	0.004	0.26	0.00007

^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, $0 < R^2 \leq 1$. (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 the preceding variables are included in the regression equation.

^c Immunotherapy with grass Ags.

(19%) had previously received grass pollen immunotherapy. Additional posttreatment sera on 46 subjects were available immediately after they had received high-dose immunotherapy in our allergy clinic for 1–3 years with grass pollen Ags [13, 14]. Unless otherwise stated, analyses were carried out using sera prior to any grass Ag therapy, except in those cases where such therapy had been given in the past by the patient's private physician.

house subjects). Thus, the observed DR3 association with immune responsiveness to *Lol p* III does appear to be Ag 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 3). log[IgE Ab] and log[IgG Ab] to *Lol p* III were the dependent variables; the different HLA-D types, age, sex, previous grass immunotherapy, and log[total serum IgE] were the independent variables. The results of parametric analyses confirmed the HLA-DR3 and DR5 associations with responsiveness to *Lol p* III in both 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

Table 4. Responsiveness to *Lol p* III before and after grass Ag immunotherapy of the 46 clinic subjects who received immunotherapy

	Total subjects	<i>Lol p</i> III IgG Ab ⁺ subjects			
		before Rx		after Rx	
		n	%	n	%
<i>DR3</i>					
All DR3 ⁺ subjects	14 ^a	11	79	14	100
DR3 ⁺ , DR5 ⁻	12	11	92	12	100
<i>DR5</i>					
All DR5 ⁺ subjects	7 ^a	6	86	7	100
DR5 ⁺ , DR3 ⁻	5	4	80	5	100
<i>Non-DR3, 5</i>					
All DR3 ⁻ , DR5 ⁻ subjects	27	8	30	10	37

^a Includes 2 subjects who were DR3⁺ and DR5⁺. Thus, the total number of DR3⁺ and/or DR5⁺ subjects was 19 (14 + 7-2). Rx = Immunotherapy with grass Ags.

^a Includes 2 subjects who were DR3⁺ and DR5⁺. Thus, the total number of DR3⁺ and/or DR5⁺ subjects was 19 (14 + 7 - 2). Rx = Immunotherapy with grass Ags.

Table 5. Amino acid sequence of various DRβ polypeptide chains at residue position 9-13

	Amino acid sequence (positions 9-13)					Sources	Reference
DR3β ₁	Glu	Tyr	Ser	Thr	Ser	AVL (HTC)/WT 49 (HTC)	16/17
DR5 Dw5β ₁	-	-	-	-	-	Swei (HTC)	18
DRw6aβ ₁	-	-	-	-	-	HHK (HTC)/APD (HTC)	16/17
DRw6bβ ₁	-	-	-	-	-	DR4/w6 cell line	16
DRw8β ₁	-	-	-	-	Gly	Madura (HTC)	17
DR1 Dw1β ₁	Trp	Gln	Leu	Lys	Phe	LG2 (HTC)/B cell line 45.1	17/19
DR1 Dwβ ₁	Trp	Gln	Leu	Lys	Phe	1568 clone 69	20
DR2 Dw2β ₁	Gln	Gln	Asp	Lys	Tyr	PGF (HTC)	17
DR2 Dw2β _{III}	Gln	Gln	Pro	Lys	Arg	PGF (HTC)	17
DR2 Dw12β ₁	Gln	Gln	Asp	Lys	Tyr	BGE (HTC)/DHO (HTC)	21/22
DR2 Dw12β _{III}	Gln	Gln	Pro	Lys	Arg	BGE (HTC)	21
DR2 Dw21β ₁	Gln	Gln	Asp	Lys	Tyr	AZH (HTC)/DR2-MN2	21/22
DR2 Dw21β _{III}	Trp	Gln	Pro	Lys	Arg	AZH (HTC)/DR2-MN2	21/22
DR2 Dw22β ₁	Gln	Gln	Asp	Lys	Tyr	REM	23
DR2 Dw22β _{III}	Trp	Gln	Pro	Lys	Arg	REM	23
DR3 DRw52a	-	Leu	Arg	Lys	-	AVL (HTC)	16
DR3 DRw52b	-	Leu	Leu	Lys	-	DR4/w6 cell line	19
DR4 Dw4β ₁	-	Gln	Val	Lys	His	Priess/GM 2103/WT 51 (HTC)	24/25/17
DR4 Dw10β ₁	-	Gln	Val	Lys	His	FS (HTC)	25
DR4 DDw14β ₁	-	Gln	Val	Lys	His	BIN-40 (HTC)	25
DR4 DRw53	-	Gln	Ala	Lys	Cys	Priess (HTC)	24, 17
DR7β ₁	Trp	Gln	Gly	Lys	Tyr	LBF (HTC)	17
DRw9β ₁	Lys	Gln	Asp	Lys	Phe	DKB (HTC)	17
DRw10β ₁	-	Glu	Val	Lys	Phe	Raji cell line	26

HTC = Homozygous typing cell.

grass Ag immunotherapy provided further evidence for DR3 and DR5 associations with *Lol p* III responsiveness (table 4). 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 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 ($p = 0.00001$).

The two DR types, DR3 and 5, belong to the 'DRw52 family', other members being DRw6 and DRw8. We investigated whether there was any specific sequence common in the hypervariable regions of the β -polypeptides of DR3 and DR5. 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 (residues 9-13) in DR3 and DR5 (table 5). The only other DR β polypeptides that are known to contain this sequence are two subtypes of DRw6 (table 5).

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 _I polypeptide chain. It is interesting to note that this region has also been implicated in the responsiveness to tetanus toxoid [26]. 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 exposure or immunotherapy. (2) Immune responsiveness to *Lol p* III also shows a weak association with DRw6 (see table 3), two subtypes of which contain the sequence EYSTS in DR β _I polypeptide chain. The lack of a strong association between DRw6 and responsiveness to *Lol p* III may be a result of the heterogeneity in the DRw6 type. (3) Little or no association is found between DRw8 and responsiveness to *Lol p* III

(see tables 2 and 3). It should be noted that in our total study 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 _I-chain differs between DR3 and DR5 β _I-chains only by one amino acid (position 13) in the first hypervariable region (table 5), 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 Ag may be involved.

Our results on *Lol p* III bear some striking similarities with those we reported earlier for *Lol p* I and II [13]. 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 Ag fragment (allowing for some degeneracy in the specificity of Ia-Ag interaction) has not only been documented [28, 29], 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 Ags are important in understanding the molecular basis of the responsiveness. It will be interesting to investigate whether *Lol p* I, II, and III have a common structural feature (agretope) which 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. Such studies have become even more interesting now as the X-ray crystallographic structure of a class I molecule [30, 31] has been used to derive the structure of the Ag-binding site of class II molecules [32].

References

- 1 Marsh, D.G.; Goodfriend, L.; King, T.P.; Löwenstein, H.; Platts-Mills, T.A.E.: Allergen nomenclature. Bull. Wld Hlth Org. 64: 767 (1986).
- 2 Marsh, D.G.; Hsu, S.H.; Roebber, M.; Kautzky, E.E.; Freidhoff, L.R.; Meyers, D.A.; Pollard, M.K.; Bias, W.B.: 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.* 155: 1439 (1982).
- 3 Marsh, D.G.; Freidhoff, L.R.; Meyers, D.A.; Roebber, M.; Norman, P.S.; Kautzky, E.E.; Hsu, S.H.; Bias, W.B.: HLA-Dw2: a genetic marker for human immune response to short ragweed pollen allergen Ra5. II. Response following ragweed immunotherapy. *J. exp. Med.* 155: 1452 (1982).
 - 4 Roebber, M.; Klapper, D.G.; Goodfriend, L.; Bias, W.B.; Hsu, S.H.; Marsh, D.G.: Immunochemical and genetic studies of *Amb.i.* V (Ra5G), an Ra5 homologue from giant ragweed pollen. *J. Immun.* 134: 3062 (1985).
 - 5 Goodfriend, L.; Choudhury, A.M.; Klapper, D.G.; Coulter, K.M.; Dorval, G.; Delcarpio, J.; Osterland, C.K.: Ra5G, a homologue of Ra5 in giant ragweed pollen: isolation, HLA-DR-associated activity and amino acid sequence. *Mol. Immunol.* 22: 899 (1985).
 - 6 Tiwari, J.L.; Terasaki, P.I.: HLA and disease associations, p. 33 (Springer, New York 1985).
 - 7 Ryder, L.P.; Andersen, E.; Svejgaard, A.: HLA and disease registry: third report (Munksgaard, Copenhagen, 1979).
 - 8 Rosenthal, A.S.; Shevach, E.M.: Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. *J. exp. Med.* 138: 1194 (1973).
 - 9 Schwartz, R.H.: T-lymphocyte recognition of antigen in association with gene products of the major histocompatibility complex. *Annu. Rev. Immunol.* 3: 237 (1985).
 - 10 Todd, J.A.; Bell, J.I.; McDevitt, H.O.: HLA-DQ β gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature* 329: 599 (1987).
 - 11 Sinha, A.A.; Brautbar, C.; Szafer, F.; Friedmann, A.; Tzfoni, E.; Todd, J.A.; Steinmann, L.; McDevitt, H.O.: A newly characterized HLA-DO β allele associated with pemphigus vulgaris. *Science* 239: 1026 (1988).
 - 12 Ansari, A.A.; Kihara, T.K.; Marsh, D.G.: Immunological studies of *Lolium perenne* (rye grass) pollen allergens, *Lol p* I, II and III. *J. Immun.* 139: 4034 (1987).
 - 13 Freidhoff, L.R.; Kautzky, E.E.; Meyers, D.A.; Ansari, A.A.; Bias, W.B.; Marsh, D.G.: Association of HLA-DR3 with human immune response to *Lol p* I and II allergens in allergic subjects. *Tissue Antigens* (in press, 1988).
 - 14 Norman, P.S.; Lichtenstein, L.M.: The clinical and immunologic specificity of immunotherapy. *J. Allergy clin. Immunol.* 61: 370 (1978).
 - 15 Creticos, P.S.; Marsh, D.G.; Norman, P.S.: Mixed allergoid booster immunotherapy (IT) in patients (pts.) allergic to ragweed, grass, oak and plantain. *J. Allergy clin. Immunol.* 75: 164 (1985).
 - 16 Gorski, J.; Mach, B.: Polymorphism of human Ia antigens: gene conversion between two DR beta loci results in a new HLA-D/DR specificity. *Nature* 322: 67 (1986).
 - 17 Bell, J.I.; Denney, D., Jr.; Foster, L.; Belt, T.; Todd, J.A.; McDevitt, H.O.: Allelic variation in the DR subregion of the human major histocompatibility complex. *Proc. natn. Acad. Sci. USA* 84: 6234 (1987).
 - 18 Tieber, V.L.; Abruzzini, L.F.; Didier, D.K.; Swartz, B.D.; Rotwein, P.: Complete characterization of sequence of an HLA class II DR beta chain cDNA from the DR5 haplotype. *J. biol. Chem.* 261: 2738 (1986).
 - 19 Long, E.O.; Wake, C.T.; Gorski, J.; Mach, B.: Complete sequence of an HLA-DR β chain deduced from a cDNA clone and identification of multiple non-allelic DR β chain genes. *Eur. molec. Biol. Org. J.* 2: 389 (1983).
 - 20 Hurley, C.K.; Ziff, B.L.; Silver, J.; Gregersen, P.K.; Hartzman, R.; Johnson, A.H.: Polymorphism of the HLA-DR1 haplotype in blacks. Identification of DR1 β -chain determinant recognized in the mixed lymphocyte reaction. *J. Immun.* 140: 4019 (1988).
 - 21 Lee, B.S.M.; Ruse, N.A.; McMichael, A.J.; McDevitt, H.O.: HLA-DR2 subtypes from an additional supertypic family of DR beta alleles. *Proc. natn. Acad. Sci. USA* 84: 4591 (1987).
 - 22 Wu, S.; Saunders, T.L.; Bach, F.H.: Polymorphism of human Ia antigens generated by reciprocal intergenic exchange between fine DR beta loci. *Nature* 324: 676 (1986).
 - 23 Liu, C.P.; Bach, F.H.; Wu, S.: Molecular studies of a rare DR2/LD-5a/DQw3 HLA class II haplotype. Multiple genetic mechanisms in the generation of polymorphic HLA class II genes. *J. Immun.* 140: 3631 (1988).
 - 24 Spies, T.; Sorrentino, R.; Boss, J.M.; Okada, K.; Strominger, J.L.: Structural organization of the DR subregion of the human major histocompatibility complex. *Proc. natn. Acad. Sci. USA* 82: 5165 (1985).
 - 25 Gregersen, P.K.; Shen, M.; Song, Q.L.; Merryman, P.; Degar, S.; Seki, T.; Maccari, J.; Goldberg, D.; Murphy, H.; Schwenzer, J.; Wang, C.Y.; Winchester, R.J.; Nepom G.T.; Silver, J.: Molecular diversity of HLA-DR4 haplotypes. *Proc. natn. Acad. Sci. USA* 83: 2642 (1986).
 - 26 Gustafsson, K.; Wiman, K.; Emmoth, E.; Larhammar, D.; Bohme, J.; Hyldig-Nielsen, J.J.; Rone, M.; Peterson, P.A.; Rask, L.: Mutations and selection in the generation of class II histocompatibility antigen polymorphism. *Eur. molec. Biol. Org. J.* 3: 1655 (1984).
 - 27 Mellins, E.; Ochs, B.; Arp, B.; Pious, D.: HLA-DR3: structure/function relationships (Abstract No. 417). 10th Int. Histocompatibility Conf. and 13th Annu. Meet. Am. Soc. of Histocompatibility and Immunogenetics, 1987, p. A53.
 - 28 Jean-Gerrard, G.; Ming-Zong, L.; Briner, T.J.; Smith, J.A.; Geffer, M.L.: Interaction of peptide antigens and class II major histocompatibility complex antigens. *Nature* 324: 260 (1986).
 - 29 Jean-Gerrard, G.; Ming-Zong, L.; Briner, T.J.; Buus, S.; Sette, A.; Grey, H.M.; Smith, J.A.; Geffer, M.L.: Immunological self, nonself discrimination. *Science* 235: 865 (1987).
 - 30 Bjorkman, P.J.; Saper, M.A.; Samaroui, B.; Bennett, W.S.; Strominger, J.L.; Wiley, D.C.: Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* 329: 506 (1987).
 - 31 Bjorkman, P.J.; Saper, M.A.; Samraoui, B.; Bennett, W.S.; Strominger, J.L.; Wiley, D.C.: The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature* 329: 512 (1987).
 - 32 Brown, J.H.; Jardetzky, T.; Saper, M.A.; Samraoui, B.; Bjorkman, P.J.; Wiley, D.C.: A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. *Nature* 332: 845 (1988).

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