

HUMAN T CELL RESPONSES TO PURIFIED POLLEN ALLERGENS OF THE GRASS, *Lolium perenne*

Analysis of Relationship between Structural Homology and T Cell Recognition¹

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The in vitro proliferative response to purified allergens of the grass, *Lolium perenne* pollens was studied using PBMC from individuals allergic to grass pollens and Ag-specific T cell lines and T cell clones derived from them. The PBMC from all 10 subjects studied showed a strong response to *Lol p* I and most of them (8 of 10) also responded to *Lol p* III. Although *Lol p* II induced a moderate response in 4 of 10 individuals, it did not induce any response in others at all the Ag concentrations tested. However, one of the subjects (JH) responded to, besides *Lol p* I, both *Lol p* II and *Lol p* III equally well. Analysis of Ag-specific T cell lines and clones derived from three individuals showed varied pattern of reactivity to the *Lol p* allergens. Some of the *Lol p* III-specific T cell lines and clones were also stimulated by *Lol p* I and similarly, some of the *Lol p* I-specific T cell clones (derived from four other subjects) were stimulated by *Lol p* III; thus showing a two-way cross-reactivity between those T cells. In both cases, the cross-reactivity to *Lol p* II, when observed, was lower than that seen with *Lol p* I and *Lol p* III. Comparison of amino acid sequences of the three *Lol p* proteins revealed a significant level of structural similarity among them, including several segments of identical sequences. Although one of the synthetic peptides of *Lol p* III sharing appreciable sequence homology with other proteins stimulated PBMC from two subjects, three other peptides did not. Nevertheless, these studies indicated the possible existence of cross-reactive T cell epitope(s) among the grass pollen allergens. Based on these results, the relationship between amino acid se-

quence homology among the *Lol p* proteins and their recognition by T cells is discussed.

One of the major grass species causing allergy in humans is the perennial rye grass, *Lolium perenne* (1-3). Several proteins from this grass pollen have been isolated, characterized, and used in studies on specific serum antibody responses in individuals allergic to grass pollen proteins and mapping of their epitopes by using mAb (4-13). The complete amino acid sequences for three of these proteins, *Lol p* I³ ($M_r = 27,000$, pI 5.7-6.0), *Lol p* II ($M_r = 11,000$, pI 5.0-5.3), and *Lol p* III ($M_r = 11,000$, pI 9.0-9.4) have been recently published (14-16). Humans allergic to grass pollens often show concordant presence of serum antibodies to all of the three *Lol p* Ag (5, 9). About 95% of the individuals allergic to grass pollens possess serum IgE and/or IgG antibodies to *Lol p* I and about 45% of them have serum antibodies to *Lol p* II and III (3, 5). Both *Lol p* II and III are highly cross-reactive with human, goat, and rabbit antibodies (9). However antibodies to *Lol p* I are unique and do not cross-react with *Lol p* II and III (9, 14, 16).

Previous studies have shown an association of HLA-DR haplotypes (e.g., HLA-DR3, -DRw6, -DRw11) with serum antibody responses to *Lol p* proteins have indicated the role of these class II MHC molecules in grass pollen allergy (17-22). However, a functional correlation as to which of these class II MHC molecules are involved as restriction elements for T cell response remains to be shown. Although, there is an obvious requirement for T cell help in the antibody responses to these Ag (17), the extent to which specific T cell responses are responsible for initiating this allergy is much less understood (14, 18). Furthermore, based on structural analysis of the *Lol p* proteins certain segments have been predicted to harbor potential T cell epitopes (16), although experimental verification of such predictions is not yet available. Therefore, it became important to analyze the T cell response to these Ag. In our study, we have examined T cell responses to three purified *Lol p* allergens using PBMC from humans allergic to grass pollens and *Lol p* I- and *Lol p* III-specific T cell lines and T cell clones derived

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³ Abbreviations used in this paper: *Lol p* I, *Lol p* II, and *Lol p* III: group I, II, and III allergens from the pollen of the grass *Lolium perenne*, respectively; pI, isoelectric point.

from them. Although some of these allergen-reactive T cells are specific for a particular *Lol p* protein, other T cells exhibit cross-reactivity with other *Lol p* proteins. To our knowledge, this is the first report demonstrating cross-reactivity among related, but yet different, allergens at the level of T cell recognition and we provide some evidence for a possible structural basis for such a phenomenon.

MATERIALS AND METHODS

Subjects. Fourteen subjects allergic to grass pollens showing significant levels of serum IgG antibodies to the major allergen, *Lol p* I, were included in the study. Eleven of these volunteers were Caucasians and three were Asian Indians. Twelve of 14 subjects did not receive any immunotherapy and the two who did (JH and KM), had discontinued immunotherapy at least 2 yr before this study. The study group contained nine males and five females of ages between 25 and 45 yr.

Ag. The *Lol p* I, *Lol p* II, and *Lol p* III proteins were purified from the pollen extract by methods described previously (1, 5, 9) and each of these proteins was homogeneous by agarose gel electrophoresis, SDS-PAGE, IEF, and immunoelectrophoresis against hyper immune goat antisera to *Lol p* pollen extract (9). The 15mer peptides of *Lol p* II (p72-86, SEKGMRNVFDDVPA-amide) and *Lol p* III (p20-34, IKYTRPGDTLAEVEL-amide; p70-84, SKGGMKNVFDEVIPT-amide) were purchased from Multiple Peptide Systems, San Diego, CA. These peptides were synthesized using a variation of Merrifield's (23) original solidphase procedures in conjunction with the method of simultaneous multiple peptide synthesis (24) using t-Boc chemistry. The molecular identity of these peptides were reconfirmed by reverse phase HPLC analysis and mass spectral analysis using ABI Bio-Ion 20 Mass Spectrometer Applied Biosystems, Foster City, CA. The 20mer peptide of *Lol p* III (p1-20, TKVDLTVEKGSADKTLVLNI) was synthesized on a Applied Biosystem 430A Peptide Synthesizer (Applied Biosystems, Foster City, CA) using t-BOC method according to manufacturers' directions at the Biopolymer Laboratory of the Howard Hughes Medical Institute, Baltimore, MD. The peptide was cleaved with anhydrous hydrogen fluoride and purified using reverse phase HPLC on a Vydac C-18 column. The sequence of the peptide was confirmed by amino acid analysis using the Applied Biosystem 420A Derivatizer/Analyzer.

Assay for in vitro proliferation. PBMC were isolated from heparinized blood by gradient centrifugation on Histopaque-1077 (Sigma Chemical Co., St. Louis, MO) by the method described elsewhere (25). After three washes in Dulbecco's PBS, pH 7.2, the PBMC were resuspended to a concentration of 5×10^6 /ml in the culture medium RPMI 1640 supplemented with 5% human AB⁺ serum, 2 mM L-glutamine, 25 mM HEPES, 100 U/ml penicillin, 100 µg/ml streptomycin, 10 µg/ml gentamycin (all the reagents mentioned above were from GIBCO, Grand Island, NY) and 5×10^{-5} M 2-ME (Sigma). PBMC (5×10^5) were cultured in triplicate at 200 µl/well in 96-well microtiter plates in the absence or presence of different concentrations of *Lol p* Ag at 37°C and 5% CO₂ in air. Five days later 1 µCi/well [methyl-³H]TdR (Du Pont/New England Nuclear, Boston, MA) was added and its incorporation into DNA was measured after 16 to 18 h by liquid scintillation counter (Beckman, model LS 5000TD, Beckman Instruments Inc., Fullerton, CA). This was taken as an index of PBMC proliferative response to the purified *Lol p* Ag. When T cell lines and clones were used, 1 to 2×10^4 T cells were cultured with 2 to 3×10^5 irradiated (5000 rad) autologous PBMC (as a source of APC) in the presence or absence of appropriate Ag for 3 days and [methyl-³H]TdR was present during the last 16 to 18 h of culture. In each case the data from a representative experiment is shown. The values given are the arithmetic mean cpm of triplicate cultures and the SD was always <10% of the mean.

Derivation of T cell clones. Inasmuch as the frequency of T cells specific for a particular allergenic protein could be very low in peripheral blood, the procedure described elsewhere (14) was adopted to derive Ag-specific T cell clones. Briefly, 10^5 PBMC were stimulated with 5 µg/ml *Lol p* III or *Lol p* I in microtiter plates. Seven days later human rIL-2 was added at a concentration of 10 U/ml. Thereafter, rIL-2 was added every 3 to 5 days to expand the proliferating cells. When sufficient cells were obtained (approximately 4 wk), they were screened for Ag-specific reactivity by culturing 10^4 T cells and 10^5 irradiated (5000 rad) autologous PBMC in the absence or presence of appropriate Ag. The Ag-specific T cell lines thus obtained were cloned by limiting dilution and the growing T cells were propagated by stimulation with PHA, rIL-2, and irradiated autologous or allogenic PBMC. The cloned T cells were used in further experiments.

RESULTS

Comparison of PBMC responses to *Lol p* Ag. We first investigated the PBMC responses to different concentrations of *Lol p* I, *Lol p* II, and *Lol p* III in 10 individuals allergic to grass pollens. For the sake of comparison, only the maximum response obtained with each Ag is given; for *Lol p* I and *Lol p* II it was at 25 µg/ml and for *Lol p* III it was at 5 µg/ml (Table I). That this selective representation was not misleading is described below. All the individuals showed significant but varied levels of response to *Lol p* I. Except for two individuals (SS, DS), the rest of the group responded to *Lol p* III as well. Although four out of ten subjects (JH, BMc, TS, JDa) showed moderate response to *Lol p* II, the rest showed very poor or no response to *Lol p* II. The magnitude of the response to these three Ag was in the following order: *Lol p* I > *Lol p* III > *Lol p* II; and those subjects who showed comparatively higher response to *Lol p* I also showed appreciable response to *Lol p* II and III.

To confirm that the response to *Lol p* II is relatively poor, the dose response curves to all the three Ag have been compared between three representative subjects (Fig. 1). The PBMC from these subjects gave moderate (TS), poor (LGe), or no (KM) response to *Lol p* II. They all showed dose dependent increase in response to both *Lol p* I and *Lol p* III. Uniformly, the response to *Lol p* II was low (or none) in each of them at all the doses tested. The same was also true with the other subjects (data not shown).

Cross-reactivity at T cell level. It was of interest to study whether the concordant PBMC response to all the three *Lol p* Ag was due to specific T cell reactivity to individual Ag or cross-recognition of these antigens. Initial experiments were performed with *Lol p* III- and *Lol p* I-induced T cell lines derived from atopic donors (Fig. 2). The JD-12 T cell line derived by initial stimulation with *Lol p* III gave significant response to *Lol p* III. In addition, it weakly responded to *Lol p* I, but did not respond to *Lol p* II at all the concentrations tested. Another T cell line (JH-1) derived the same way but from a different individual, gave significant dose dependent response to all of the three *Lol p* Ag. Although the sensitivity of these T cells (JH-1) to the different *Lol p* Ag varied, at 1 µg/ml, all the three Ag induced more or less similar levels of response. By contrast, the BM-7 T cell line, generated by

TABLE I
PBMC responses to purified *Lol p* Ag in humans allergic to grass pollens^a

Subjects	No Ag	Proliferative Response In Presence of		
		<i>Lol p</i> I	<i>Lol p</i> II	<i>Lol p</i> III
JH	980	49,846	16,132	16,489
KM	4,304	23,145	0	9,789
TS	1,502	39,975	8,491	15,563
LGe	609	29,144	883	7,786
SS	2,192	17,104	114	1,468
AA	1,786	16,589	123	7,370
GK	3,802	18,676	0	19,621
BMc	7,300	62,517	9,044	21,567
DS	727	7,449	244	675
JDa	2,679	21,730	3,420	13,106

^a A total of 5×10^5 PBMC was cultured in the absence or presence of different amounts of *Lol p* I, *Lol p* II, or *Lol p* III and the maximum response obtained with *Lol p* I (25 µg/ml), *Lol p* II (25 µg/ml), and *Lol p* III (5 µg/ml) alone is depicted above. The values given are the arithmetic mean cpm in triplicate cultures and the responses in the presence of Ag are Δ cpm (response in the absence of Ag was subtracted).

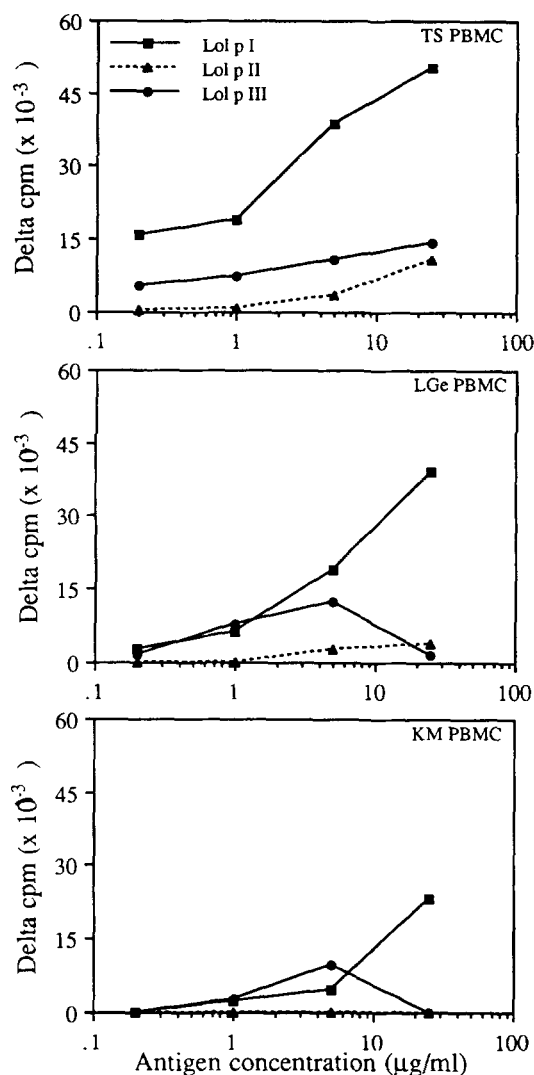


Figure 1. PBMC response to the purified *Lol p* Ag. The PBMC (5×10^5) from three representative subjects, TS, LGe, and KM were cultured in the absence or presence of different doses of *Lol p* I (■), *Lol p* II (▲), and *Lol p* III (●). The background response in the absence of Ag (2160, 5121, and 4304 cpm for the three subjects, respectively) was subtracted and the values shown are the arithmetic mean Δ cpm in triplicate cultures.

initial stimulation with *Lol p* I was highly specific, and did not respond to *Lol p* II or III at all the concentrations tested. These results indicated that in certain individuals T cells exist that can recognize more than one *Lol p* proteins.

However, at this point it was not clear whether the cross-reactivity observed between *Lol p* I and III was exhibited by individual T cells or separate populations of T cells present in the Ag-specific T cell lines. Therefore, cloned Ag-specific T cells were used in further analysis. Three of five *Lol p* III-specific T cell clones (all from the same donor, JDa) were able to recognize *Lol p* I as well, but not *Lol p* II (Table II). Two additional clones from another donor (JH) responded to *Lol p* III and in addition, gave relatively poor response to both *Lol p* I and II (Table II). A comparison of the dose response curves generated by *Lol p* III and *Lol p* I revealed no significant difference in all the three cross-reacting T cell clones studied (Fig. 3). Equimolar concentrations (except very low) of both *Lol p* III and I induced similar levels of proliferative response in these *Lol p* III-specific T cells (Fig. 3). Once again, *Lol p* II was unable to induce any detectable re-

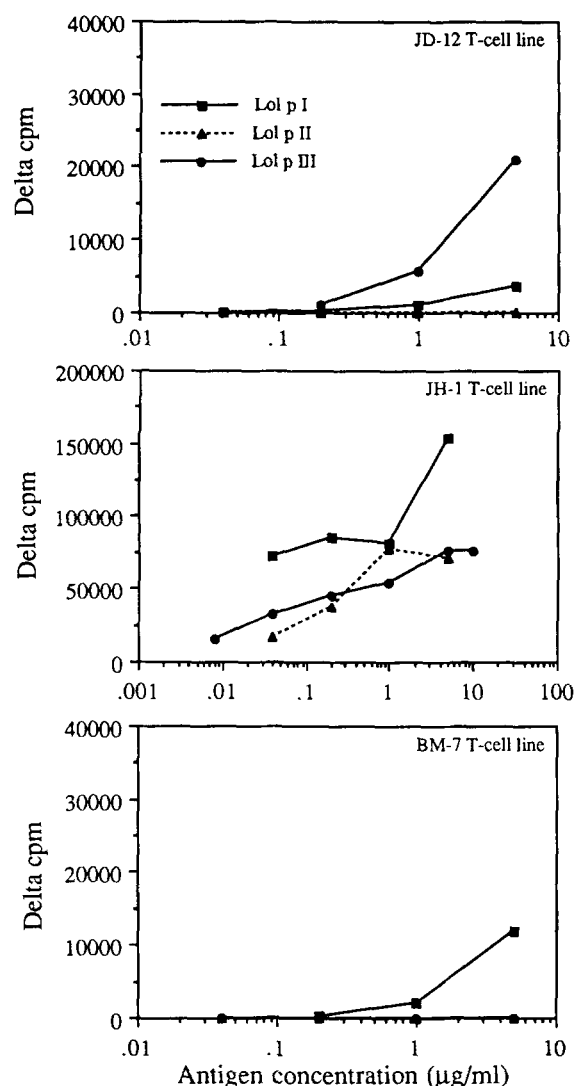


Figure 2. Cross-recognition of *Lol p* Ag by T cell lines. A total of 2×10^4 T cells from three different T cell lines (JD-12, JH-1, and BM-7) was cultured with 3×10^5 irradiated autologous PBMC and different concentrations of *Lol p* I (■), *Lol p* II (▲), and *Lol p* III (●). The response in the absence of Ag (121 cpm in JD-12; 35714 cpm in JH-1; and 305 cpm in BM-7) was subtracted and the values shown are the arithmetic mean Δ cpm in triplicate cultures.

TABLE II
Cross-reactivity of *Lol p* III-specific T cell clones^a

Clone	No Ag	Proliferative Response in Presence of		
		<i>Lol p</i> III	<i>Lol p</i> I	<i>Lol p</i> II
JDTC-3	291	44,566	31,624	322
JDTC-4	269	36,857	22,715	465
JDTC-6	493	33,991	17,372	423
JDTC-2	350	3,599	463	353
JDTC-5	488	3,547	735	601
JHTC-2	669	5,044	1,755	1,865
JHTC-3	699	6,186	1,743	2,268

^a A total of 2×10^4 *Lol p* III-specific cloned T cells from two allergic subjects (JD and JH) was cultured with 3×10^5 irradiated autologous PBMC in the absence or presence of different amounts of *Lol p* Ag. The response to 25 $\mu\text{g/ml}$ *Lol p* Ag alone is depicted. The values given are the arithmetic mean cpm in triplicate cultures.

sponse at all the concentrations tested, as observed with PBMC (Fig. 2).

In a separate study, a total of 24 *Lol p* I-specific T cell clones were derived from four donors (AN, VI, AL, and BAL). Although some of these clones responded to only *Lol p* I, the others (about 37%) were stimulated, in addi-

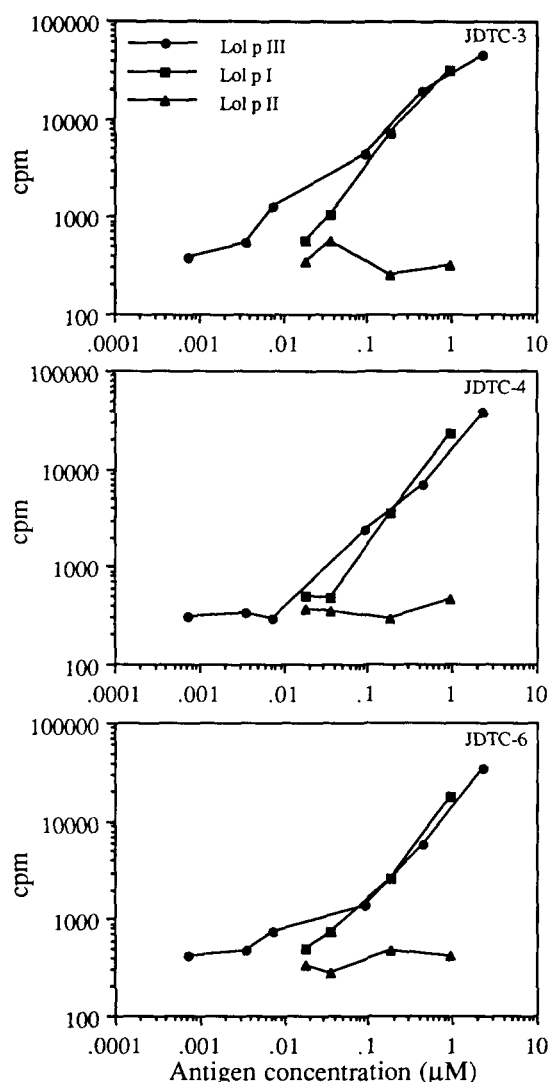


Figure 3. Dose response curves for *Lol p* Ag in *Lol p* III-specific T cell clones. Cloned T cells (2×10^4) were cultured with 3×10^5 irradiated autologous PBMC and different concentrations of *Lol p* I (■), *Lol p* II (▲), and *Lol p* III (●). The response in the absence of Ag was 291 cpm in JDTC-3, 269 cpm in JDTC-4, and 493 cpm in JDTC-6. The values shown are the arithmetic mean cpm in triplicate cultures.

tion, by either *Lol p* III, *Lol p* II, or both (Table III). In some of the latter clones the response to *Lol p* III was relatively higher than that to *Lol p* II. Together, the above results clearly demonstrate the cross-reactivity among the *Lol p* proteins at the level of T cell recognition although, in general, the cross-reactivity to *Lol p* II was relatively less pronounced. And the proportion of such cross-reacting T cells seems to vary among the allergic subjects studied.

Analysis of structural basis for T cell cross-reactivity. In an attempt to obtain a structural basis for the observed T cell cross-reactivity among the *Lol p* proteins, we compared the amino acid sequences of *Lol p* II and *Lol p* III (15, 16) with the carboxyl-terminal part of *Lol p* I (14). With an allowance for the conservative residue changes, it is evident that several segments of these proteins exhibit sequence homology with each other and some of these could serve as cross-reactive T cell epitope(s) (Fig. 4).

To study this more closely, we used synthetic peptides of the *Lol p* proteins containing a high degree of sequence homology. Based on the amphipathic nature, previous

TABLE III
Cross-reactivity of *Lol p* I-specific T cell clones^a

Clone	No Ag	Proliferative Response in Presence of		
		<i>Lol p</i> I	<i>Lol p</i> II	<i>Lol p</i> III
AN40	287	5,217	472	946
AN62	276	3,286	817	560
V17	890	13,896	2,606	8,841
V146	467	10,291	379	4,816
V165	797	4,351	817	2,579
V123	1,190	3,784	912	951
V133	540	10,560	1,832	447
V124	324	7,791	804	948
V120	334	3,050	643	451
V119	329	10,180	746	531
V132	273	10,544	509	654
V19	866	9,918	3,359	4,360
V126	346	4,374	1,639	2,740
V153	190	3,887	862	1,640
AL57	386	4,673	808	681
AL41	292	2,724	836	647
AL15	379	3,390	662	660
BAL83	329	9,371	4,559	271
BAL87	468	8,204	3,603	3,806
BAL46	154	9,034	787	317
BAL43	276	15,219	1,403	359
BAL51	253	2,816	1,500	1,833
BAL25	243	5,615	493	325
BAL17	414	5,529	1,514	396

^a A total of 2×10^4 *Lol p* I-specific cloned T cells from four allergic subjects (AN, VI, AL, and BAL) were cultured with 5×10^4 irradiated autologous PBMC in the absence or presence of 25 μ g/ml *Lol p* Ag. The values given are the arithmetic mean cpm in triplicate cultures. The responses to Ag are considered significant when they are at least three times higher than in the absence of Ag.

studies have predicted that the 15-mer peptides, residues p215–229 (*Lol p* I), p72–86 (*Lol p* II), and p20–34 and p70–84 (*Lol p* III), might contain T cell epitope(s) of the respective proteins (16). In addition, these peptides also shared fairly good amount of sequence homology (12/15 homologous residues between *Lol p* II and III; 11/15 residues between *Lol p* I and III). Therefore, preliminary experiments were carried out to see if these peptides can stimulate PBMC from “responder” subjects. The results are shown in Figure 5. Although *Lol p* I induced a similar dose dependent response in both subjects (JH and JD), the responses to *Lol p* II and *Lol p* III differed among them. In particular, *Lol p* II and *Lol p* III generated comparable dose response curves in JH and only *Lol p* III induced significant response in JD. However, the 20mer peptide (p1–20 of *Lol p* III) induced appreciable response in PBMC from both the subjects none of the 15mer peptides mentioned above (p72–86 (*Lol p* II) and p20–34 and p70–84 (*Lol p* III)) induced a detectable response. These results indicated that further experiments with a series of overlapping peptides are required to delineate all the potentially cross-reacting T cell epitope(s).

DISCUSSION

To understand the molecular basis of T cell responses to *Lol p* allergens, the present study addressed two questions: 1) are there specific T cell responses against the individual *Lol p* allergens in humans allergic to grass pollens, and 2) is there any cross-reactivity (as seen in serum antibody responses) exhibited by the T cells recognizing the *Lol p* Ag. Although PBMC from some of the individuals allergic to grass pollens responded to all of the three *Lol p* proteins, PBMC from others responded to *Lol p* I and/or *Lol p* III (Table I). Although the magnitude

Figure 4. Comparison of amino acid sequences of *Lol p* proteins. The carboxyl-terminus part of *Lol p* I (residues 139–240) was aligned with the complete sequences of *Lol p* II and *Lol p* III. Single letter code for the amino acids is used. Boxes enclose identical residues and conservative changes of residues: A=S=T, D=E, F=Y=W, I=L=M=V, N=Q, and R=K. Dashes are inserted to achieve the maximum possible sequence homology.

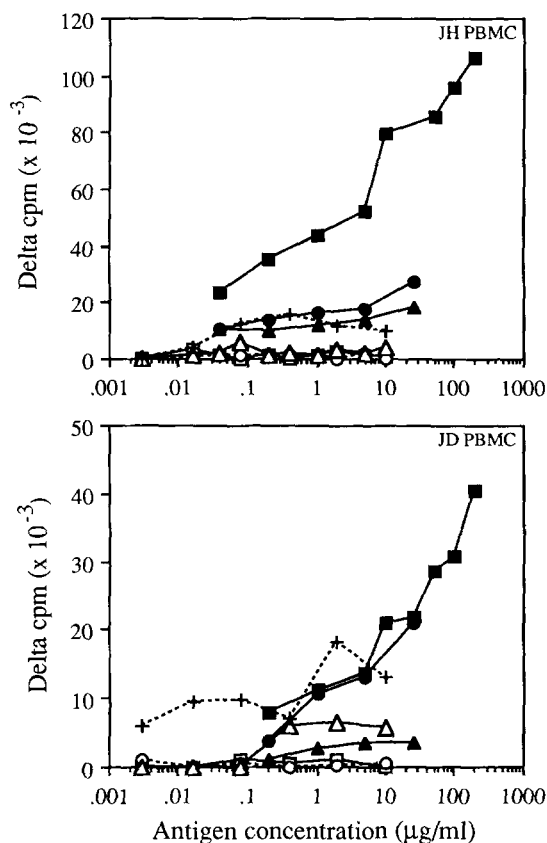
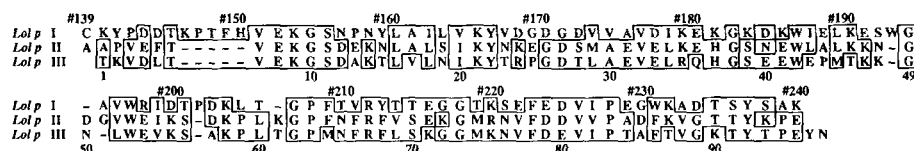


Figure 5. PBMC response to whole proteins and synthetic peptides of *Lol p* Ag. The PBMC (5×10^5) from two subjects (JH and JD) were cultured in the absence or presence of different doses of *Lol p* I (■), *Lol p* II (▲), *Lol p* III (●), and synthetic peptides of *Lol p* II [p72–86, (□)], and *Lol p* III [p1–20 (+), p20–34 (Δ) and p70–84 (○)]. The background response in the absence of Ag (<1550 cpm) was subtracted and the values shown are the arithmetic mean Δ cpm in triplicate cultures.

of the response varied among different individuals, the overall strength of the response to the three *Lol p* allergens was in the following order: *Lol p* I > *Lol p* III > *Lol p* II. This pattern of T cell reactivity seems to correlate very well with antibody responses to these proteins in these subjects (S. Baskar, R. G. Hamilton, P. S. Norman, and A. A. Ansari, manuscript in preparation). It is our contention that the difference in responses to individual proteins is a reflection of the respective frequencies of specific T cells recognizing each of these Ag, which might vary among individuals with different MHC haplotypes.

PBMC from majority of the subjects responded to *Lol p* II very poorly or not at all (Table I). One possibility is that this could be due to low affinity of *Lol p* II-specific T cells. However, we believe that is unlikely for the following reasons: 1) no improvement in the response to *Lol p* II was seen with a wide range of antigen doses used (Fig. 1, LGe and KM); 2) those who responded to *Lol p* II showed a dose-dependent increase in the response within the same range of Ag concentrations (Fig. 1, TS); and 3)

furthermore, one of the subjects (JH) who responded to all the three *Lol p* allergens showed similar dose response curves for *Lol p* II and *Lol p* III (Fig. 4). Although *Lol p* II and *Lol p* III showed significant structural similarities and antibody cross-reactivity (16), it is intriguing that only *Lol p* III was able to induce significant T cell response in majority of the subjects. Alternatively, it is possible that the *Lol p* II, being the most acidic protein (pI 5.0–5.3) among the three allergens (15), was processed differently and the derived peptide was unable to form a stable complex with class II MHC molecule available in the APC of the *Lol p* II low-responders, and consequently failed to stimulate the specific T cells (26–28).

A significant finding of this work is the cross-reactivity at the T cell level among the three *Lol p* allergens, which is evident from the studies employing *Lol p* I- and *Lol p* III-specific T cell lines and T cell clones. A few lines of evidence suggest that the observed cross-reactivity is not an artifact. 1) The Ag used in this study are highly purified (9) and therefore, the cross-reactivity is not due to contaminating proteins. 2) It is unlikely that the cross-reactivity observed between *Lol p* III and *Lol p* I is due to their mitogenic property because only some of the T cell clones exhibited such cross-recognition in either case whereas the other clones derived from the same individuals did not (Tables II and III). 3) It is possible that the cross-reactivity seen with the T cell lines might be due to the oligoclonality of these T cell lines and actually separate population of T cells recognizing *Lol p* I, II, or III existed. However, data on the cross-reactivity expressed by the monoclonal population of T cells derived from different donors strongly supports the view that the same T cells were able to recognize, in addition to their specific Ag, peptide fragments from other *Lol p* proteins (Fig. 3; Tables II and III). The authenticity of these T cell clones is supported by the following: 1) the clones shown in Table II and Figure 3 were derived by limiting dilution method at 1 cell/well and the cloning efficiency was <25% and 2) the clones depicted in Table III did not show any evidence of mixed cells in a Southern blot analysis of the TCR β -chain gene rearrangements in these cells (data not shown). It is, therefore, possible that the cross-reacting T cells recognized perhaps similar residues on *Lol p* I, *Lol p* II, and *Lol p* III. Additional evidence for this notion comes from the very similar dose response curves generated by *Lol p* III and *Lol p* I in the three different T cell clones that have been studied (Fig. 3). This would imply that perhaps the TCR of those T cells have similar affinities for the relevant peptides derived from the cross-reacting Ag. However, the other T cell clones derived from the same individuals or other subjects did not show such cross-recognition (Tables II and III). Therefore, we suggest that the cross-reactivity is conferred by T cells with a specially rearranged TCR and the proportion of such cells may vary among individuals. In other words, individuals

allergic to grass pollens might have certain T cells that are unique to a particular allergen and other T cells that cross-react with one or more related Ag.

Nevertheless, the observed cross-reactivity can be explained based on the structural analysis of the three *Lol p* proteins that revealed several homologous segments among them (Fig. 4). A recent report identifies one such segment in *Lol p I* (residues 191–210) as the immunodominant peptide for T cell response (14). However, subsequent analysis has revealed that several peptides present all along the *Lol p I* molecule were able to stimulate specific T cells (G. Y. Ishioka and R. W. Chestnut, personal communication). Therefore, it is possible that while some of these peptides will induce unique T cell response, others will induce T cells that might cross-react with the other *Lol p* proteins. Based on the sequence homology and amphipathic nature a few of the segments of *Lol p I*, II, and III were predicted to contain T cell epitopes (16). These predictions, however, are not infallible and the data given in Figure 5 indicated that three such segments do not constitute a complete peptide capable of stimulating the PBMC from two individuals over a wide range of Ag concentrations tested. It is unlikely that higher concentration (>10 µg/ml) of these peptides would stimulate because equal amount of the whole proteins (much less immunogenic peptide on a molar basis) induced significant PBMC response. Nonetheless, the 20mer peptide of *Lol p III* induced appreciable response in both the subjects suggesting that many other peptides may be involved in the observed cross-reactivity. Additional experiments using a series of overlapping peptides of the *Lol p* proteins need to be performed in order to identify the immunodominant peptides of each of these proteins and to map those involved in T cell cross-reactivity. To our knowledge, this is the first report addressing the relationship between structural homology of related, but yet different, allergenic proteins and their ability to elicit specific T cell responses. Identification of the different segments of allergenic proteins with reference to their ability to stimulate or block activation of specific T cells will lead to better understanding of the regulatory mechanisms involved in human allergic responses and help improve therapeutic treatment.

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