

A study of the human immune response to *Lolium perenne* (Rye) pollen and its components, *Lol p I* and *Lol p II* (Rye I and Rye II)

I. Prevalence of reactivity to the allergens and correlations among skin test, IgE antibody, and IgG antibody data*

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In a stratified random sample of 320 white adults, the prevalence of puncture skin test positivity (ST+) to Lolium perenne (rye grass)-pollen extract (LPE) was 16%. Fifteen percent of all subjects (or 84% of subjects classified LPE IgE antibody positive [Ab+]) was classified IgE Ab+ to highly purified Lol p I (Rye I), and 4% of all subjects (or 26% of subjects classified LPE IgE Ab+) was classified IgE Ab+ to highly purified Lol p II (Rye II). These data and similar results obtained in an allergy-enriched group of 361 subjects are consistent with previous studies that Lol I is a major allergen and Lol II is a minor allergen of LPE. Whether we studied LPE, Lol I, or Lol II, responder subjects were younger than nonresponder subjects and more male than female subjects were responders. We then investigated the quantitative interrelationships among ST, IgE, and IgG Ab responsiveness to LPE, Lol I, and Lol II in the allergy-enriched group. For each allergen, log-log correlations were strong and significant for ST versus IgE Ab and for IgE Ab versus IgG Ab. All subjects IgE Ab+ to Lol I or Lol II were IgG Ab+ to that allergen, supporting other evidence for a commonality in the genetic control influencing the production of IgE and IgG Abs to a given allergen. Log-log correlations among ST end points, IgE Ab levels, or IgG Ab levels were strong for LPE versus either Lol I or Lol II but weak between Lol I and Lol II, consistent with the reported lack of cross-reactivity between Lol I and Lol II. Despite these findings, almost all Lol II+ subjects were Lol I+ by ST (98%), IgE Ab (91%), and IgG Ab (83%), suggesting that the Ia-restricted immune recognition of both these molecules is at least in part under a common genetic control. (J ALLERGY CLIN IMMUNOL 78:1190-1201, 1986.)

The study of the prevalence of immune responsiveness toward well-characterized, highly purified allergens is of importance in understanding the genetics of specific immune response.^{1,2} Furthermore, in order to define the clinical relevance of individual components within an allergenic mixture, the quantitative contributions that responses toward highly purified components make in determining overall response to-

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Abbreviations used

Ab:	Antibody
Ag:	Antigen
CIE:	Crossed immunoelectrophoresis
IIEP:	Immunoelectrophoresis
Lol p I, Lol I:	Group I allergen from <i>Lolium perenne</i> pollen (Rye I)
Lol p II, Lol II:	Group II allergen from <i>Lolium perenne</i> pollen (Rye II)
LPE:	Dialyzed extract of <i>Lolium perenne</i> (rye grass) pollen
r:	Correlation coefficient
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gradient electrophoresis
ST:	Skin test
DEAE:	Diethylaminoethyl
RIA:	Radioimmunoassay

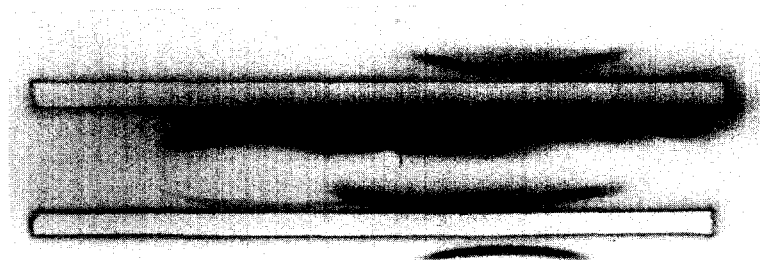


FIG. 1. IEP of *Lol* I (5 µg), LPE (140 µg), and *Lol* I (5 µg) in the Ag wells (top to bottom) against goat anti-LPE and goat anti-*Lol* I (impure) in the antiserum troughs (top to bottom). Electro-phoresis was carried out for 45 minutes at 8 V/cm at pH 8.6. The immunodiffusion was carried out for 18 hours at 23° C. The anode is to the right.

ward a complex allergenic extract merits study. Finally, the interrelationships among ST sensitivity, IgE Ab, and IgG Ab levels toward an allergenic extract and its purified components are of interest in furthering understanding of both the epidemiology and genetics of atopic disease.

The present study used a standardized LPE and two of its well-characterized components, *LOL* I* (rye I, molecular weight 27,000 daltons) and *Lol* II (rye II, molecular weight 11,000 daltons)²⁻⁶ to investigate the prevalence rates for IgE and IgG Ab positivity in a stratified random sample of 320 white adults. The quantitative contributions of *Lol* I and *Lol* II responses to LPE response as well as the interrelationships among ST, IgE, and IgG Ab responses were investigated in an allergy-enriched sample of 361 individuals from a population that is being used for ongoing genetic studies. Finally, we characterized the influences of both age and sex on these immunologic responses.

MATERIAL AND METHODS

Subject selection

We studied two groups of employees of the Westinghouse Electric Corporation, Hunt Valley, Baltimore Co., Md.⁷⁻⁹ A stratified random sample of 320 white subjects (40 persons of each sex for each of the age ranges, 20 to 30, 31 to 40, 41 to 50, and 51 to 60 years) was chosen in order to measure the prevalence of reactivity to the allergens.⁹ Nine subjects (3%) reported that they had received immunotherapy with grass-pollen extracts. The second group of 361 white subjects (271 male and 90 female subjects; mean age 39 ± 10 years; age range 20 to 64 years) was enriched for reported

allergy toward pollens and/or animals.⁹ The allergy-enriched group of subjects, all of whom were HLA typed, was included in order to facilitate genetic studies of specific response to allergens. Eighteen subjects (5%) reported that they had received immunotherapy with grass-pollen extracts. One hundred ninety-six employees were common to both the random group and the allergy-enriched group. All 485 subjects were skin tested by the puncture method, and blood samples were taken. Serum samples were stored at -70° C until they were used.

Ags

Lolium perenne (perennial rye grass) pollen (200 gm) was kindly given by Bencard Allergy Unit, Betchworth, Surrey, U. K. The pollen was defatted, extracted, dialyzed, and lyophilized as previously described⁸ to provide the LPE used for subsequent STs and isolation of the *Lol* I and *Lol* II allergens. By radial immunodiffusion analysis, this LPE has previously been found to contain 96.0 ± 5.2 µg of *Lol* I per milligram and 48.7 ± 4.8 µg of *Lol* II per milligram.⁸

Lol I and *Lol* II allergens were purified by use of a methodology previously described^{2, 3} with some modifications. In brief, LPE was fractionated by a combination of DEAE cellulose (DE52, Whatman Ltd., Maidstone, England), and Sephadex G-75 chromatography.² *Lol* I was further purified by DEAE Sephadex chromatography, followed by Sephadex G-100 (twice), and *Lol* II was further purified by DEAE Sephadex chromatography and Sephadex G-50 (superfine) gel filtration (twice). The fractions at each stage of the purification were monitored by agarose electrophoresis at pH 8.6¹⁰ and by immunodiffusion, IEP, or rocket IEP against anti-LPE and anti-*Lol* I or anti-*Lol* II antisera.^{2, 3, 10, 11}

The resultant allergen fractions were examined for purity by agarose gel electrophoresis at pH 8.6, PAGE at pH 9.5,¹⁰ and SDS-PAGE¹⁰ as well as by IEP¹⁰ and CIE^{11, 12} against several hyperimmune rabbit and goat antisera raised against crude LPE and purified *Lol* I and *Lol* II fractions.^{6, 8} The goat anti-LPE used for experiments illustrated in Figs. 1 and 2 was prepared in three goats and is available through the Research Resources Branch, National Institute of Allergy and Infectious Diseases. For most IEP and CIE ex-

*The nomenclature for highly purified allergens corresponds to that recommended by the International Union of Immunological Societies Allergen Nomenclature Subcommittee (Marsh DG, Goodfriend L, King TP, et al: WHO Bull [in press]). *Lol p* I (also abbreviated as *Lol* I) is the major group I or Rye I allergen, and *Lol p* II (*Lol* II) is the group II or Rye II allergen from *Lolium perenne* pollen.

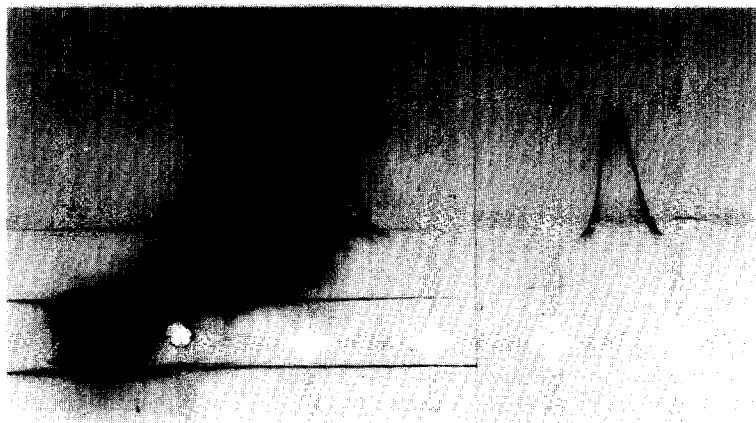


FIG. 2. CIE of LPE (300 μ g) and *Lol* II (7 μ g) against goat anti-LPE. Electrophoresis was carried out at pH 8.6 for 35 minutes and 45 minutes at 10 V/cm in the first dimension (left to right) and for 18 hours at 2 V/cm into the Ab-containing gel in the second dimension.

periments, the IgG fractions of the antisera were purified by ammonium sulfate precipitation¹¹ or DEAE cellulose chromatography.¹³

Several allergenic extracts, previously described,⁸ were used to assess ST reactivity to an array of other common environmental allergens (extracts of *Ambrosia artemisiifolia* [*A. elatior*, short ragweed] pollen, cat and dog danders, *Alternaria* spp., and house dust), as well as histamine and diluent (phosphate-saline-phenol-human serum albumin).

ST procedure

In all subjects, puncture STs were performed with the crude extracts.^{7-9, 14} For the group of 361 subjects, we also examined sensitivity to highly purified *Lol* I and *Lol* II at 10 μ g/ml in subjects ST+ to at least one of the crude allergenic extracts. Higher concentrations of *Lol* I or *Lol* II (300 μ g/ml) were used only when the reactions at the 10 μ g/ml level were negative or equivocal (<3 mm wheal diameter larger than any wheal response to diluent control).

RIAs of IgE and IgG Abs

Crude LPE. Analysis of IgE Ab levels toward crude LPE was performed by a modified RAST technique by use of LPE Sepharose beads. In brief, LPE (414 mg in 25 ml 0.1 mol/L NaHCO₃, and 1 mol/L NaCl at pH 8.5) was allowed to react with 25 ml of CNBr-activated Sepharose 4B-CL beads. The beads were washed and deactivated by further incubation with 1 mol/L of ethanolamine. For subsequent RAST assays, the washed beads were used at a 3% v/v. This bead concentration was determined to be optimal by means of a series of titrations against sera from five untreated patients with known high anti-LPE IgE Ab titers, and against a further serum (from a grass-treated patient) that was known to have high IgG Ab levels to LPE allergens.

The RAST analyses were performed as previously reported.^{15, 16} A serum pool of 10 untreated, highly grass-allergic subjects was used as a standard. The anti-LPE IgE

Ab level of this standard was estimated by determining the reduction in total IgE level caused by immunoabsorption with the LPE beads, according to the method of Gleich et al.¹⁷ Normally, two assays of triplicate samples of each serum were performed, and assays were repeated when the coefficients of variation for the mean values within or between assays were >10%. The serum blanks for patients who were ST- to LPE varied between 0.7% and 4% of "B_{max}" (maximum counts per minute bound for IgE Sepharose beads). Sera with low titers were often assayed three or four times in order to try to discriminate between IgE Ab+ and IgE Ab- levels.

Purified allergens. Measurement of IgE and IgG Abs to highly purified *Lol* I and to *Lol* II was performed by a double Ab RIA similar to that previously described.¹⁸ In brief, *Lol* I or *Lol* II was radiolabeled by the chloramine-T procedure with 2 mCi of ¹²⁵I per 40 μ g of Ag. Measurements of IgE and IgG Ab levels were performed by incubating 100 μ l of ¹²⁵I-labeled Ag (30 ng/ml in 5% bovine serum albumin) with 100 μ l human serum (1/10 to 1/50 dilution in borate-buffered physiologic saline for the IgG assay or 1/2 dilution for the IgE assay) for 4 hours at 23° C. For the RIA of IgG Ab, goat antihuman IgG (γ -chain specific) was added in slight Ab excess, and the mixture was allowed to incubate for 18 to 20 hours at 4° C. The resultant immunoprecipitates were washed and counted. For the IgE Ab RIA, 100 μ l of a freshly prepared mixture containing myeloma serum PS (1/100) and a slight excess of goat anti-IgE (ϵ -chain specific) was added at the second incubation step.¹⁸ (The inclusion of the myeloma serum was necessary to ensure an adequate amount of precipitate for subsequent washing.) Further procedures were performed as in the IgG RIA. After subtracting values for blank sera (containing no IgG or IgE Ab to *Lol* I or *Lol* II), the serum IgG and IgE Ab levels were computed by reference to an IgG Ab titration curve¹⁹ with a standard serum of known high IgG Ab content, determined by the procedure of Mulligan et al.²⁰ It has previously been

found^{19*} that IgE Ab measurements are not influenced by IgG Ab, except in the case of high IgG Ab levels (>1000 ng/ml) found in some subjects having had immunotherapy.

All analyses were performed in triplicate in at least two assays. Further experiments were performed when the coefficient of variation was >10% for the means of the first two assays. Sometimes as many as four or five assays were necessary to finalize low anti-*Lol* I or anti-*Lol* II Ab levels (0.3 to 1.0 ng of Ab per milliliter in diluted sera).

Total serum IgE

A variation of the direct radioallergosorbent test assay was used to determine total serum IgE levels with a high degree of precision.^{7-9, 15} The final results were expressed in nanograms per milliliter (1 IU = 2.42 ng). For both groups of subjects, the distribution of log [total serum IgE] was not different from a normal distribution.

Data analyses

All statistical analyses were performed with the Biomedical Data Programs package²¹ on an IBM 4341 computer. Responsiveness toward LPE, *Lol* I, or *Lol* II was evaluated according to ST and to serum IgE and/or IgG Abs. For parametric analyses, log₁₀ transformations were used for all ST sensitivities and total IgE, IgE Ab, and IgG Ab levels.

For nonparametric analyses, the cutoff point for ST positivity was a wheal diameter >2 mm after subtraction of any wheal response to diluent control. For parametric analyses, a subject's ST sensitivity was expressed by computing the concentration of that allergen that would elicit a 6 mm diameter wheal; subjects ST- to an allergen were assigned an end point 0.3 µg/ml greater than the maximum computed end point for subjects classified as ST+ to that Ag.⁸

In the case of nonparametric analyses of RAST data for anti-LPE IgE Ab, values ≥1.35 ng/ml were classified as positive, and values <1.35 ng/ml were classified as negative. The counts per minute corresponding to this Ab concentration were approximately twice the negative control counts per minute. In the case of parametric analyses, 1 ng/ml (log = 0) was used for all negative sera.

With the use of the double Ab RIAs for anti-*Lol* I or *Lol* II Abs, we were unable to discriminate between the counts per minute 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. Subjects having lower than these detectable limits were considered to be negative for all nonparametric statistical analyses and were characterized as having levels of 3 ng/ml and 0.6 ng/ml of IgG and IgE Ab, respectively, in the case of parametric tests.

For each subject, an index of skin sensitivity toward histamine, the Histamine Index,⁸ was expressed as the con-

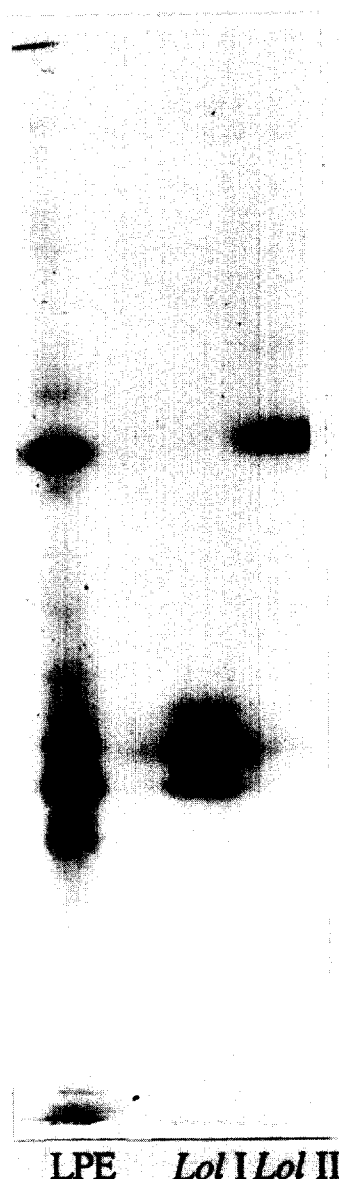


FIG. 3. Basic PAGE (pH 9.5) of LPE (70 µg), *Lol* I (7 µg), and *Lol* II (7 µg) for 5 hours at 10 V/cm. The anode is at the top of the gel.

centration of histamine that would elicit a wheal of 6 mm diameter. We used allergen titration data generated from an independent study of clinic patients to establish the dose-response relationship.⁸

RESULTS

Immunochemical and physicochemical analyses

The analyses of LPE by CIE and for content of *Lol* I and *Lol* II, previously presented,⁸ demonstrated that our LPE preparation met or exceeded the analytical specifications of three *Lolium perenne* refer-

*Marsh DG: Unpublished observation.

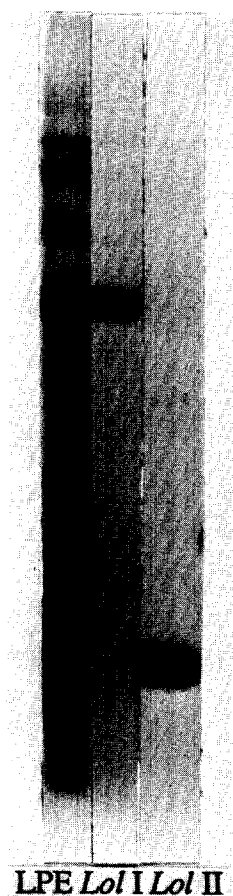


FIG. 4. Analysis of LPE, Lol I, and Lol II (left to right) by SDS-PAGE.

ence preparations of the Federal Drug Administration. Examples of IEP and CIE analyses of our Lol I and Lol II preparations, with a hyperimmune goat serum that was able to recognize at least 25 to 30 acidic antigens,⁸ are presented in Figs. 1 and 2. The Lol I sample consisted primarily of a single immunoprecipitate together with 2 to 3 trace antigenic impurities (Fig. 1), whereas the Lol II sample produced only a single immunoprecipitate under all conditions (Fig. 2). An anti-Lol I serum (prepared against a different, slightly less pure, Lol I sample) produced a single immunoprecipitate with the highly purified Lol I used for the present study (Fig. 2). Other antisera, prepared against crude or partially purified LPE Ags, also produced single immunoprecipitates with the Lol I and the Lol II samples (data not presented).

By agarose gel or basic PAGE electrophoresis, Lol I produced two prominent and one faint band corresponding to the different isoallergens,⁴ and Lol II produced a single band (Fig. 3). Similar results were obtained by IEF. By gel filtration, both the Lol I

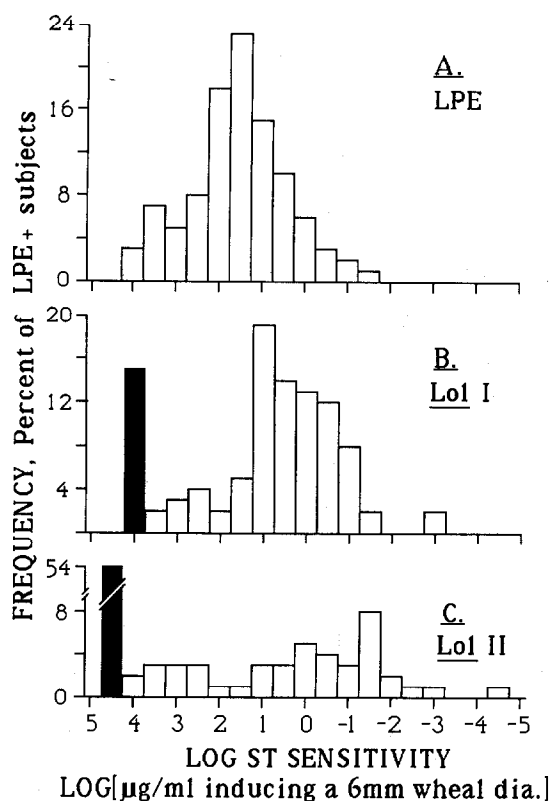


FIG. 5. Distributions of log [puncture ST sensitivity end points] toward rye antigens in 115 subjects classified ST+ to LPE. A, Distribution of LPE sensitivity. B, Distribution of Lol I sensitivity. Subjects ST- to Lol I are designated by the solid bar. C, Distribution of Lol II sensitivity. Subjects ST- to Lol II are designated by the solid bar.

and Lol II preparations produced single symmetrical elution peaks, suggesting molecular size homogeneity. This was confirmed by SDS-PAGE (Fig. 4).

Stratified random subjects

Qualitative relationships among ST reactivity and Abs to LPE, Lol I, and Lol II. Of 320 study subjects, 90 (28%) were ST+ to at least one of six crude allergens.⁹ As assessed by ST+ and IgE Ab+ toward LPE and IgE, and IgG Ab+ toward Lol I and Lol II, the proportions of responders to LPE, Lol I, and Lol II are presented in Table I. Sixteen percent of our random sample was ST+ to LPE. Eighty-two percent of subjects ST+ to LPE had detectable IgE Ab to this allergen. Only two (5%) of the subjects with IgE Ab to LPE were ST minus to LPE.

For Lol I and Lol II, all subjects IgE Ab+ had IgG Ab to that allergen. However, among subjects who had never received grass immunotherapy, only 40% and 38% of subjects with IgG Ab to Lol I and Lol II, respectively, had IgE Ab to that allergen. Among the 43 subjects who were LPE IgE Ab+, 84% were

TABLE I. Means and SDs for distributions of total IgE and of responses to LPE, *Lol* I, and *Lol* II in 320 random subjects

	No.	%	Data for responders	
			Log[mean $\pm$ SD]*	Geometric mean
ST sensitivity to†				(μ g/ml)
LPE	50	16	1.88 $\pm$ 1.11	76
Total serum IgE	320		1.88 $\pm$ 0.71	(ng/ml) 76
IgE Ab to‡				
LPE	43	13	1.33 $\pm$ 0.74	21
<i>Lol</i> I	47	15	1.13 $\pm$ 0.79	13
<i>Lol</i> II	12	4	0.97 $\pm$ 0.75	9
IgG Ab to§				
<i>Lol</i> I	107	33	1.47 $\pm$ 0.75	30
<i>Lol</i> II	30	9	1.41 $\pm$ 0.82	26

*Log₁₀ (micrograms per milliliter eliciting a 6 mm wheal diameter) in the case of ST sensitivities and log₁₀ (nanograms per milliliter) in the case of total IgE and the specific IgE Ab and IgG Ab levels.

†The distribution of ST end points was not different from a normal distribution.

‡Each of the frequency distributions of IgE Ab was not significantly different from a normal distribution; there were no differences by t test among the geometric means of these distributions.

§Among subjects with IgG Ab to *Lol* II, the distribution of log[IgG Ab] to *Lol* II was not different from a normal distribution. In contrast, only among subjects with both IgG and IgE Abs to *Lol* I was the distribution of log[IgG Ab] to *Lol* I not different from a normal distribution.

Lol I IgE Ab+ and 26% were *Lol* II IgE Ab+. Nineteen percent of 47 subjects who were *Lol* I IgE Ab+ were also *Lol* II IgE Ab+.

Age and sex effects. As previously found for LPE ST reactivity,⁹ the group of subjects who were *Lol* I or *Lol* II IgE Ab+ had a significantly lower mean age than the group who were *Lol* I or *Lol* II IgE Ab- ($p < 0.01$, in both cases). This was also true for subjects who were IgG Ab+ versus subjects who were IgG Ab- ($p < 0.02$). More male than female subjects were ST+ to LPE (21% versus 10%, respectively; $p = 0.008$). Although there were also about twice as many male as female subjects who were IgE Ab+ to *Lol* I or *Lol* II, the differences were not significant with the smaller numbers of subjects *Lol* I+ or *Lol* II+.

Study group of 361 subjects (allergy-enriched)

Qualitative relationships among reactivity and Abs to LPE, *Lol* I, and *Lol* II. Forty-seven percent of subjects were ST+ to any one of six allergens. The proportions of subjects who were ST+ or who had IgE Ab or IgG Ab to LPE, *Lol* I, and *Lol* II are presented in Table II. The proportions of subjects ST+ or IgE Ab+ among LPE+ subjects in each category are presented in parentheses. Fifty-three percent of subjects *Lol* I ST+ were *Lol* II ST+, and 40% of subjects *Lol* I IgE Ab+ were *Lol* II IgE Ab+.

Conversely, 52/53 (98%) of subjects *Lol* II ST+ were *Lol* I ST+, and 40/44 (91%) of subjects *Lol* II IgE Ab+ were *Lol* I IgE Ab+. Among the four exceptions, only one subject, currently receiving grass immunotherapy, had no detectable responsiveness to *Lol* I by IgG Ab or ST.

In subjects ST+ to a given allergen, we were generally able to detect both IgE and IgG Abs to that allergen (Table III). For example, among subjects *Lol* II ST+, 79% and 87% had detectable levels of IgE and IgG Abs, respectively. Furthermore, most subjects who were classified as IgE Ab+ to a given allergen had been classified as ST+ to that allergen (97% for LPE, 96% for *Lol* I, and 95% for *Lol* II).

For *Lol* I or *Lol* II, all subjects IgE Ab+ had IgG Ab to that allergen. However, among subjects who had never received grass immunotherapy, only 56% and 68% of subjects with IgG Ab to *Lol* I and *Lol* II, respectively, had IgE Ab to that allergen.

Age and sex effects. Similar age and sex effects were noted for the 361 allergy-enriched subjects as for the 320 random subjects. For each allergen, age effects were significant when reactivity was on the basis of ST as well as IgE or IgG Abs. Sex effects were significant when reactivity was on the basis of not only ST or IgE Ab to LPE, *Lol* I, or *Lol* II but also IgG Ab to *Lol* I. For IgG Ab positivity to *Lol* II, the male to female ratio was 1.75:1 ($p = 0.06$).

TABLE II. Means and SDs for distributions of total IgE and of responses to LPE, *Lol* I, and *Lol* II in 361 white subjects

	Total		Data for responders*		Geometric mean
	No.	%	Of LPE+ (%)	Log[mean $\pm$ SD] [†]	
ST sensitivity to			Of ST+		(μ g/ml)
LPE	115	32	(100)	1.52 $\pm$ 1.12	33
<i>Lol</i> I	98	27	(85)	0.45 $\pm$ 1.20	3
<i>Lol</i> II	53	15	(46)	0.24 $\pm$ 1.91	2
					(ng/ml)
Total serum IgE	361			2.05 $\pm$ 0.70	112
IgE Ab to [‡]			Of IgE Ab+		
LPE	97	27	(100)	1.28 $\pm$ 0.64	19
<i>Lol</i> I	99	27	(90)	1.14 $\pm$ 0.64	14
<i>Lol</i> II	44	12	(44)	1.10 $\pm$ 0.57	13
IgG Ab to [§]					
<i>Lol</i> I	166	46		1.68 $\pm$ 0.70	48
<i>Lol</i> II	69	19		1.68 $\pm$ 0.74	48

*The large proportions of positive subjects in our study group reflect the selection criteria used.

[†]Log₁₀ (micrograms per milliliter eliciting a 6 mm wheal diameter) in the case of ST sensitivities and log₁₀ (nanograms per milliliter) in the case of total IgE and the specific IgE Ab and IgG Ab levels.

[‡]Each of the frequency distributions of log[IgE Ab] among responders was not different from a normal distribution.

[§]Only among subjects with both IgG and IgE Abs to *Lol* I was the distribution of log[IgG Ab] to *Lol* I not different from a normal distribution. The distribution of log[IgG Ab] to *Lol* II was not different from a normal distribution.

TABLE III. Percent of responders to LPE, *Lol* I, and *Lol* II in 361 white subjects

Subjects	Percent responding to		
	LPE	<i>Lol</i> I	<i>Lol</i> II
170 ST+ to at least 1 of 6 allergens			
By ST reactivity	68	58	31
By IgE Ab	56	57	26
By IgG Ab	ND	71	38
115 ST+ to LPE			
By ST reactivity	100	85	46
By IgE Ab	82	83	38
By IgG Ab	ND	90	52
98 ST+ to <i>Lol</i> I			
By ST reactivity	100	100	53
By IgE Ab	91	97	43
By IgG Ab	ND	98	54
53 ST+ to <i>Lol</i> II			
By ST reactivity	100	98	100
By IgE Ab	94	94	79
By IgG Ab	ND	96	87

ND = not done.

Quantitative relationships among total serum IgE, ST sensitivity, and Abs to LPE, Lol I, and Lol II. Fig. 5, A through C, illustrate the frequency distributions of ST end points (microgram of Ag per mil-

liliter producing 6 mm wheal diameter) for LPE, *Lol* I, and *Lol* II, respectively, among 115 subjects LPE ST+. These distributions have been plotted in order that the more sensitive subjects are on the right. Among the responders (*open bars*), each of the frequency distributions of log [ST sensitivity] was not significantly different from a normal distribution. By t test, the geometric means for sensitivity to *Lol* I and *Lol* II (Fig. 5, B and C) were not different from each other, but the geometric mean sensitivities to both of the purified allergens were significantly lower ($p < 0.001$) than that to crude LPE (Table II).

For subjects who had never received grass immunotherapy but who had both IgE and IgG Abs to *Lol* I or *Lol* II, the geometric mean IgG Ab was significantly higher than the geometric mean IgE Ab ($p < 0.001$).^{*} The corresponding ratios of the geometric means (IgG:IgE) were 7.3:1 and 8.5:1 for *Lol* I and *Lol* II, respectively.

Table IV and Figs. 6A, 6B, and 6C illustrate log-log correlations among responses to LPE, *Lol* I, and *Lol* II. The persons who had received grass immunotherapy (*open symbols* in the figures) have been omitted from the correlations illustrated in the last two columns of Table IV. Section A of Table IV examines

^{*}For *Lol* I, geometric mean = 95 ng/ml (mean log = 1.98 $\pm$ 0.54) and 13 ng/ml (1.11 $\pm$ 0.62), respectively; for *Lol* II, geometric mean = 93 ng/ml (1.97 $\pm$ 0.43) and 11 ng/ml (1.05 $\pm$ 0.56), respectively.

TABLE IV. Log-log correlations among total IgE levels and responses toward LPE, *Lol* I, and *Lol* II as measured by skin reactivity and IgE and IgG Abs in 361 allergy-enriched subjects*

Logarithmic comparisons	All responders		Responders never having immunotherapy	
	N†	r	N	r
A. ST‡: between allergens				
LPE vs <i>Lol</i> I	115	0.66	100	0.63
LPE vs <i>Lol</i> II	115	0.58	100	0.62
<i>Lol</i> I vs <i>Lol</i> II	99	0.21§	86	0.18
<i>Lol</i> I vs <i>Lol</i> II (both)¶	52	0.25	45	0.17
B. Total IgE vs IgE Ab#				
LPE (both)	97	0.38	ND	
<i>Lol</i> I (both)	99	0.43	ND	
<i>Lol</i> II (both)	44	0.47	ND	
C. IgE Ab: between allergens				
LPE vs <i>Lol</i> I	109	0.79	92	0.78
LPE vs <i>Lol</i> II	98	0.46	83	0.44
LPE vs <i>Lol</i> I + <i>Lol</i> II	110	0.83	93	0.82
<i>Lol</i> I vs <i>Lol</i> II	103	0.38	87	0.37
<i>Lol</i> I vs <i>Lol</i> II (both)	40	0.51	35	0.49
D. IgG Ab: between allergens#				
<i>Lol</i> I vs <i>Lol</i> II	178	0.49	160	0.40
<i>Lol</i> I vs <i>Lol</i> II (both)	57	0.40	47	0.17
E. ST vs IgE Ab				
LPE	118	0.56	103	0.61
<i>Lol</i> I	102	0.49	87	0.50
<i>Lol</i> II	55	0.79	47	0.80
F. IgE Ab vs IgG Ab				
<i>Lol</i> I	166	0.82	149	0.83
<i>Lol</i> II	69	0.73	58	0.80

ND = not done.

*All *p* values are <0.001 unless it is otherwise indicated.

†N is the number of responders to either of the two variables being correlated unless it is otherwise indicated.

‡Log₁₀ (micrograms per milliliter eliciting a 6 mm wheal diameter).§0.02 < *p* < 0.05.

||Not significant.

¶Both signifies comparisons of responders to both variables (e.g., *Lol* I and *Lol* II).

#Total IgE and specific IgE and IgG Abs measured in nanograms per milliliters.

the relationships among ST sensitivities. Correlations between ST end points for LPE versus ST end points for either *Lol* I or *Lol* II were strong and significant. In contrast, correlations between ST end points for *Lol* I versus *Lol* II were weak and generally not significant (e.g., Fig. 6A).

The correlations among total serum IgE and IgE Abs were significant, although they were relatively weak, for each allergen (Table IV, section B). This is not unexpected because we were examining only the subset of subjects IgE Ab+. The correlations among responses to LPE, *Lol* I, and *Lol* II were generally stronger for the IgE Ab data than for the ST data (Table IV, sections C and A). The correlations of IgG Ab between *Lol* I and *Lol* II were comparable to those for IgE Ab levels (Table IV, sections D and C). For

subjects with IgG Abs to both *Lol* I and *Lol* II, the deletion of subjects having had immunotherapy decreased the *r* value from 0.40 (*p* < 0.001) to 0.17 (*p* = not significant).

Section E of Table IV presents the correlations between ST sensitivity IgE Ab in subjects who were classified LPE+, *Lol* I+ (Fig. 6B), or *Lol* II+. This correlation was strongest and most significant for the *Lol* II Ag. We also investigated whether this relationship might be dependent on the influences of total IgE and other factors. The log-log correlations for ST sensitivity versus specific IgE Ab divided by total IgE were essentially unchanged from those presented for LPE, *Lol* I, and *Lol* II in section E of Table IV. Stepwise multiple regression analysis was also used to examine the influences of the variables

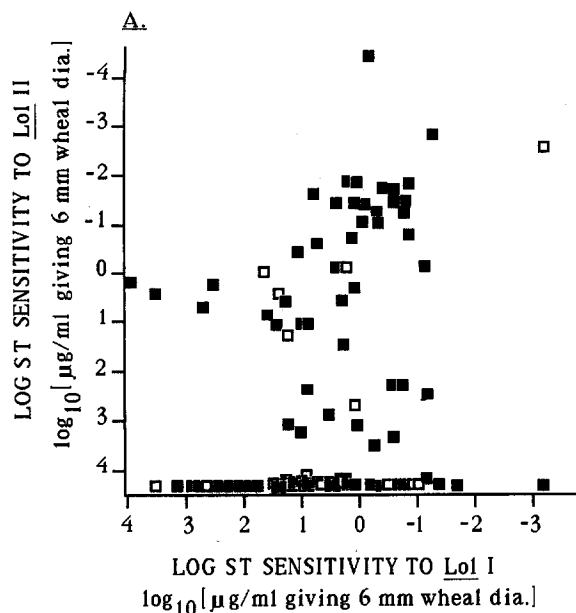


FIG. 6A. Log correlation between ST sensitivity to *Lol* I vs ST sensitivity to *Lol* II in 99 subjects ST+ to *Lol* I and/or *Lol* II ($r = 0.21$ and $0.02 < p < 0.05$). Open symbols indicate subjects who reported having had grass-pollen immunotherapy.

$\log$ [specific IgE Ab], $\log$ [total IgE], age, sex and Histamine Index on $\log$ [ST sensitivity] for each of the allergens. In each case, $\log$ [specific IgE Ab] was the best predictor of $\log$ [ST sensitivity] ($p < 0.0001$). $\log$ [total IgE] never entered the regression equation. Although the final regression equation for LPE ST sensitivity also included the variable "sex" ($p < 0.05$), the final equation for *Lol* I ST sensitivity also included the Histamine Index ($p < 0.05$). We have demonstrated previously that the variables, Histamine Index and sex, are not independent. Histamine Index for male subjects was significantly greater than for female subjects and significantly greater for ST+ male than for ST+ female subjects.⁹

Fig. 6C illustrates a strong correlation between IgE Ab and IgG Ab in subjects having IgE Ab and/or IgG Ab for *Lol* I ($r = 0.82$; $p < 0.001$). This correlation is similarly strong for *Lol* II (Table IV, section F).

Finally, we examined the relationship between ST sensitivity to LPE and the combined ST sensitivities to *Lol* I and *Lol* II with the aim of assessing the contribution that the sensitivities toward the two purified allergens made in determining overall sensitivity to LPE (Fig. 7). The ST sensitivities to *Lol* I and to *Lol* II have been weighted by their respective contents in LPE (legend, Fig. 7). In general, the subjects most

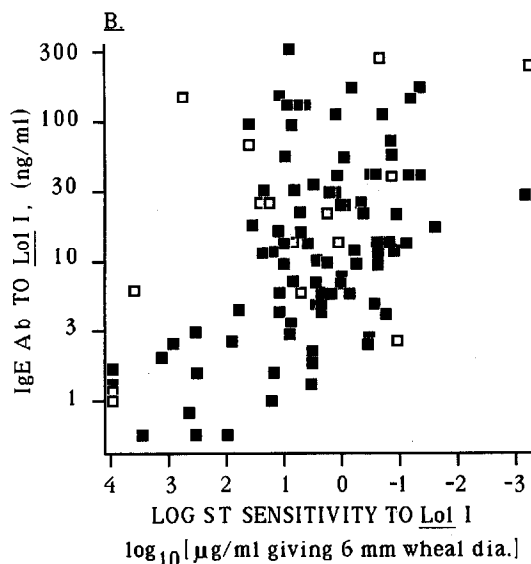


FIG. 6B. Log correlation between ST sensitivity to *Lol* I vs IgE Ab to *Lol* I in 102 subjects ST+ to *Lol* I and/or having IgE Ab to *Lol* I ($r = 0.49$; $p < 0.001$). Open symbols indicate subjects who reported having had grass-pollen immunotherapy.

sensitive to LPE were ST+ to both *Lol* I and *Lol* II (indicated by B in Fig. 7).

DISCUSSION

These studies provide the first detailed epidemiologic data concerning human immune responsiveness toward a well-characterized allergenic extract (LPE) and two of its highly purified allergenic components, *Lol* I and *Lol* II. The *Lol* I preparation consisted almost entirely of a single antigenic component, trace antigenic impurities being detected only with one hyper-immune goat anti-LPE serum at high levels of Ag and Ab (Fig. 1). No impurities could be detected in the *Lol* II preparation (Fig. 2).

Our data confirm and extend previous findings that, in adults, responsiveness to allergens decreases significantly with age^{9, 22, 23} and is significantly more marked in male than in female subjects.^{9, 23, 24} Our additional findings are that these effects are observed for purified allergens as well as crude allergens, and for IgE Ab and IgG Ab as well as ST responsiveness.

Eighty-five percent of 115 subjects classified LPE ST+ was *Lol* I ST+, and a similar proportion (90%) of 97 subjects classified LPE IgE Ab+ was *Lol* I IgE Ab+. Furthermore, the quantitative levels of ST sensitivity and IgE Ab to LPE and *Lol* I were strongly correlated (r values of 0.6 to 0.8; Table IV). Although ST sensitivity and IgE Ab to LPE and *Lol* II were also correlated ($r = 0.4$ to 0.6; Table IV), *Lol* II is

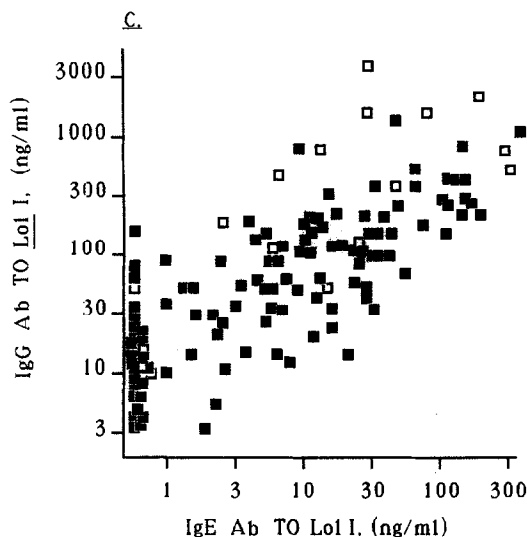


FIG. 6C. Log correlation between IgE Ab to *Lol* I and IgG Ab to *Lol* I in 166 subjects having IgE Ab to *Lol* I and/or IgG Ab to *Lol* I ($r = 0.82$; $p < 0.001$). Open symbols indicate subjects who reported having had grass-pollen immunotherapy.

clearly less important allergenically than *Lol* I.^{2, 25} Thus, our data confirm and extend previous conclusions: *Lol* I and *Lol* II are both clinically relevant allergens, but the structurally more complex allergen, *Lol* I (27,000 daltons), is more immunogenic than the less complex allergen, *Lol* II (11,000 daltons).

Our study further confirms previous findings^{26, 27} that the degree of ST sensitivity to a given allergen is significantly related to the amount of specific serum IgE Ab; this was found for LPE as well as its two components (Table IV and Fig. 6B). Interestingly, this correlation was much stronger for the minor allergen *Lol* II than for the major allergen *Lol* I. Subjects who were ST+, but IgE Ab-, to a given allergen were found to have significantly ($p < 0.001$) lower levels of ST sensitivity than IgE Ab+ subjects. The respective geometric mean concentrations required to induce a 6 mm wheal were 646 $\mu\text{g/ml}$ for LPE, 389 $\mu\text{g/ml}$ for *Lol* I, and 1318 $\mu\text{g/ml}$ for *Lol* II. These and other data^{27, 28} demonstrate that the ST assay is more sensitive than either of the RIAs for IgE Ab.

The fact that we found no significant influence of total IgE level on the relationship between specific IgE Ab and ST sensitivity is of interest in regard to the variability of the number of IgE receptors on skin mast cells. If the number of IgE receptors per mast cell were constant, a significant influence of total IgE level on ST sensitivity might be anticipated, since both specific and irrelevant IgE molecules would be competing for a fixed number of receptors. However, if

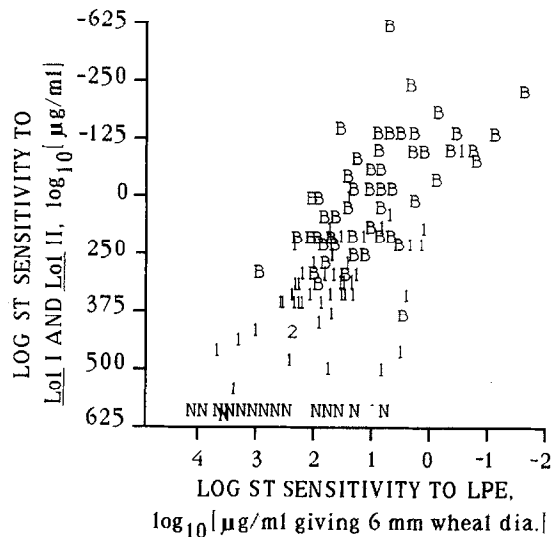


FIG. 7. Log correlation between ST sensitivity to LPE and weighted combined sensitivity toward *Lol* I and *Lol* II ($N = 115$; $r = 0.75$; $p < 0.001$). Combined sensitivity was determined as follows:

$$(96.0) \times (\log_{10} [\mu\text{g/ml eliciting 6 mm wheal dia. for } Lol\text{ I}]) + (48.7) \times (\log_{10} [\mu\text{g/ml eliciting 6 mm wheal dia. for } Lol\text{ II}])$$

where 96.0 and 48.7 ($\mu\text{g/mg}$) are the contents of *Lol* I and *Lol* II in LPE, respectively.⁸ ST sensitivities are indicated by symbols: N, not ST+ to *Lol* I or *Lol* II; 1, ST+ to *Lol* I but not to *Lol* II; 2, ST+ to *Lol* II but not to *Lol* I; B, ST+ to both *Lol* I and *Lol* II.

the number of mast cell receptors were positively correlated with total IgE concentration as in the case of the basophil,²⁹ a significant influence of total IgE would not be expected, in agreement with the present findings. Thus, our data provide indirect evidence for a positive relationship between total IgE concentration and the number of IgE receptors on mast cells. More direct measurement of receptor numbers is, however, needed to confirm this prediction.

The strong correlations between $\log[\text{IgE Ab}]$ and $\log[\text{IgG Ab}]$ reported here confirm those found in previous studies involving *Lol* I and other purified allergens.^{18, 19, 27, 30, 31} Furthermore, the geometric mean level of IgG Ab was significantly higher than that of IgE Ab among subjects classified IgE Ab+ (7.3:1 for *Lol* I and 8.5:1 for *Lol* II; 7.1:1 for short ragweed allergen *Amba* V [Ra5]¹⁸). Although low levels of IgG Ab were sometimes found without detectable IgE Ab, IgE Ab was never present without IgG Ab.^{18, 27, 31} These results support other evidence for a commonality in the genetic control influencing the production of IgE and IgG Abs in both mice³² and humans.^{18, 33}

The quantitative correlations for responsiveness to *Lol* I versus *Lol* II were weak overall, among ST, IgE

Ab, or IgG Ab measurements (Table IV). These findings are consistent with the previously reported lack of antigenic and allergenic cross-reactivity between *Lol* I and *Lol* II^{2,3,34} although there is one preliminary finding suggesting allergenic cross-reactivity between partially purified *Lol* I and *Lol* II fractions.³⁵ Conversely, we found that almost all subjects classified *Lol* II+ are *Lol* I+ by ST (98%), IgE Ab (91%), and IgG Ab (83%). The latter finding suggests the possibility that the primary immune recognition of both of these molecules is at least, in part, under a common genetic control. This would be consistent with the postulate that the same immune-associated (Ia) molecule (HLA-D gene product) controls the recognition of a structurally related element on both *Lol* I and *Lol* II. We already have presented evidence^{36,37} that responses toward both allergens are associated with HLA-Dw3, as well as evidence for structural similarity between the two molecules from high-performance liquid chromatography analysis of tryptic digests.³⁷ These data will be presented in detail in a future publication.

We conclude that sensitivities to *Lol* I and *Lol* II are prominent in subjects sensitive to LPE. *Lol* I sensitivity is the more important of the two, consistent with its designation as a *major* allergen. Furthermore, although *Lol* I and *Lol* II generally appear to be non-cross-reactive antigenically and allergenically, responsiveness to *Lol* II is significantly more common in subjects classified *Lol* I+ than in subjects classified *Lol* I-.

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Correction

The corrections shown here apply to the listings of Workshop Members and Task Force that appear on pages 3A through 6A of the September 1986, Part 2, issue of the JOURNAL.

The following entries under Workshop Members are correct: .

Robert A. Goldstein, M.D., Ph.D., NIH/NIAID
 Robert A. Kinsman, Ph.D., National Jewish Hospital (Denver)
 Peter A. König, M.D., Ph.D., University of Missouri School of Medicine (Columbia)
 Richard F. Lockey, M.D., University of South Florida College of Medicine
 Richard A. Nicklas, M.D., George Washington University School of Medicine
 John A. Sbarbaro, M.D., M.P.H., University of Colorado School of Medicine
 Gail G. Shapiro, M.D., University of Washington School of Medicine
 Thomas J. Uryniak, M.A., Fisons Corporation

The following entries under the Task Force listing are correct:

(Organizing Committee) Members: Mr. Harry DeLozier, Thomas J. Fischer, M.D. (addition), Mr. John J. Maddox
 (Workshop 3) Chairperson: Richard A. Nicklas, M.D.; Cochairpersons: N. Franklin Adkinson, Jr., M.D., Albert L. Sheffer, M.D.; Members: Thomas J. Uryniak, M.A.
 (Workshop 5) Members: Saul S. Bodenheimer, M.D., Patricia H. Russell, M.D., David L. Winter, M.D.
 (Workshop 6) Cochairperson: Charles E. Lewis, M.D.; Members: Charles H. Feldman, M.D., Michele Hindi-Alexander, Ph.D., Robert A. Kinsman, Ph.D., Roger A. Menendez, M.D., John A. Sbarbaro, M.D., M.P.H.
 (Workshop 7) Members: William F. Westlin, M.D.
 (Workshop 8) Cochairperson: Richard R. Evans III, M.D., M.P.H.; Members: C. Ralph Buncher, Sc.D.