
Ra3 Skin Test Response and HLA-A2, Antigen E, and IgE: Evidence of Interactions Between Antigen E and HLA

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ABSTRACT: *A positive association of Ra3 skin test responses with HLA-A2 has previously been reported to be evident in individuals with low IgE levels and to a greater extent in these individuals than those with high IgE levels. We give evidence based on an analysis of data from 133 individuals that Ra3 response is positively correlated with HLA-A2 among individuals with low Antigen E response and negatively associated with HLA-A2 among individuals with high Antigen E response. Furthermore, we have evidence that any observed interaction between Ra3, IgE, and HLA-A2 can be explained by the correlation between IgE and Antigen E response, and that it is Antigen E response which interacts in the relationship between HLA-A2 and Ra3 skin test response.*

INTRODUCTION

Recent studies have reported a positive association of skin test response to purified extract of ragweed antigen Ra3 with HLA-A2 [1,2]. Furthermore, it has been proposed that this association is better observed in individuals with low total IgE levels [2]. In our analysis of Minnesota patients [3] using definitions of Ra3 positivity comparable to those of Marsh et al. [2] we observed no association between HLA-A2 and Ra3 positivity. This was true in the case of the entire sample of atopic individuals and in the subset of atopic individuals having low IgE levels.

In the study presented here we consider Ra3 response itself as the variable of interest (rather than dichotomizing the data) and we consider the Antigen E response in addition to the IgE response and HLA specificity as possible predictive variables. We give here evidence that the positive association between HLA-A2 and Ra3 response is instead observed among those individuals with low skin test response to Antigen E. That is, the association observed in individuals with low IgE level may be secondary due to the low Antigen E reduced IgE. Conversely, we also have observed a significant negative association between HLA-A2 and Ra3 response among those individuals with high Antigen E skin

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test response. Finally, our data indicate that the previously reported interaction between IgE, Ra3, and HLA-A2 results from the correlation between total IgE level (in nanograms) and Antigen E response and that it is Antigen E response which interacts in the relationship between HLA-A2 and Ra3 response, or, equivalently, that HLA-A2 interacts in the relationship between Antigen E and Ra3 skin test response.

MATERIAL AND METHODS

From the Minneapolis, Minnesota metropolitan area, 133 unrelated individuals were selected. Each had a clinical history of ragweed sensitivity characterized by respiratory symptoms of rhinitis or asthma, or both during August and September and by a positive intradermal skin test to crude ragweed (1:500 solution). All subjects had a medical evaluation followed by skin testing, measurement of serum IgE levels, and human leukocyte antigen (HLA) typing.

Skin testing using the intradermal titration method was performed using purified ragweed antigens E and Ra3 with solutions ranging in concentration from 10^{-1} to 10^{-12} of the stock solution. The stock solutions of Antigen E contained 140 $\mu\text{g/ml}$ and that of Ra3 contained 75 $\mu\text{g/ml}$. These antigens were standardized against known samples of Antigen E and Ra3 by skin test methods, radioallergosorbent test (RAST) inhibition assays, acrylamide gel analysis, isoelectric focusing, and immunodiffusion using specific antisera. IgE was determined by double antibody radioimmunoassay and reported in nanograms per milliliter [4]. The lymphocytotoxicity method was used to determine HLA antigens [5].

Individuals who responded with positive skin tests when the preparation was diluted to, or was more dilute than, that of the 33rd percentile of the responses (skin test dilutions) were categorized as high Antigen E responders; those whose skin tests were positive above the 66th percentile of test antigen concentrations were categorized as low Antigen E responders. Low and high IgE responders were defined in terms of those below the 33rd and those above the 66th percentile of IgE levels in the sample, respectively. Mean responses in Ra3 skin test (given as $-\log_{10} [\text{dilution}] \times 10$ whenever there was response and 0.0 when there was no response) were then obtained in the following groups:

1. Individuals having low IgE levels and low Antigen E skin test response
2. Individuals having low IgE levels and high Antigen E skin test response
3. Individuals having high IgE levels and low Antigen E skin test response
4. Individuals having high IgE levels and high Antigen E skin test response.

Mean Ra3 responses were calculated separately for those positive for HLA-A2 and for those negative for HLA-A2. Thus, in this first analysis, individuals having intermediate response in the Antigen E skin test or having intermediate IgE levels were not included.

A three-way analysis of variance was done to compare the eight observed means and to evaluate the effect of HLA-A2, low versus high IgE levels, and low versus high Antigen E responses on the Ra3 titer. All possible two- and three-way interactions between the above three variables were also evaluated. Following this, the response categories to Antigen E and IgE were ignored and the relationship among Ra3 skin test response and IgE, Antigen E skin test response, and HLA-A2 was evaluated through stepwise regression analysis first considering only HLA-A2 and IgE and the interaction between HLA-A2 and $(\log_{10})$ IgE, and then considering the observed Antigen E response and its interaction with HLA-A2 as possible independent variables. In this case all individuals in the study were included (i.e., individuals with intermediate responses were not deleted). Further evidence for evaluating the correlations between IgE levels, Ra3 skin

TABLE 1 Definition of responder groups used to evaluate the relationship of IgE levels, Antigen E response and HLA-A2 with Ra3 skin test response

IgE Group	Low IgE	High IgE
Range of Values	0-299 ng	>878 ng
Sample Size of Group	37	35
Antigen E Group	Low E	High E
Range of Values	dilution ^a of 10^{-1} , 10^{-2} , or no response	dilution $\leq 10^{-4}$
Sample Size of Group	35	37
HLA-A2 Group	A2 +	A2 -
Sample Size of Group	39	33

^aConcentration of stock solution required for a 2+ response.

test response, and Antigen E skin test response was obtained through comparing the appropriate partial correlations and nonparametric correlations.

RESULTS

In Table 1 we present the observed 33rd and 66th percentiles of IgE and Antigen E skin test response and the resulting definition of the responder groups compared in the analysis of variance. We also present in Table 1 the number of individuals who were in each group. In Table 2 rows 1 through 4, columns 1 and

TABLE 2 Mean Observed Ra3 Dilution^a in Atopic Individuals: Its Relationship to HLA-A2, IgE, and Antigen E Skin Test Response

IgE	HLA-A2	Antigen E Skin Test		
		1 Low ^b	2 High ^c	3 Total sample
1. Low ^d	+	5.0 ± 2.2(16) ^e	20.0 ± 5.7(8)	10.0 ± 2.8(24)
2. Low	-	1.2 ± 1.2(8)	34.0 ± 7.5(5)	13.8 ± 5.4(13)
3. High ^f	+	18.0 ± 6.6(5)	14.0 ± 3.1(10)	15.3 ± 2.9(15)
4. High	-	1.7 ± 1.7(6)	27.1 ± 6.3(14)	19.5 ± 5.2(20)
5. Ignored	+	8.1 ± 2.6(21)	16.7 ± 3.0(18)	12.0 ± 2.0(39)
6. Ignored	-	1.4 ± 1.0(14)	29.0 ± 5.0(19)	17.3 ± 3.8(33)
7. Low	Ignored	3.8 ± 1.6(24)	25.4 ± 4.8(13)	11.4 ± 2.6(37)
8. High	Ignored	9.1 ± 3.9(11)	21.8 ± 4.1(24)	17.0 ± 3.2(35)
9. Ignored	Ignored	5.4 ± 1.7(35)	23.0 ± 3.1(37)	14.4 ± 2.1(72)

^aMean of $-10 \times \log_{10}$ (dilution) at which response of 2+ is observed in Ra3 skin test. If no response observed at any concentration then $-10 \times \log_{10}$ (dilution) = 0.0.

^bLow Antigen E: no response to a dilution of 10^{-2} or more.

^cHigh Antigen E: response to a dilution of 10^{-4} or less.

^dLow IgE: IgE < 300 ng.

^eHigh IgE: IgE > 878 ng.

^fMean ± standard error; sample size in parentheses.

2, we present the mean Ra3 skin test response observed in the eight groups of responders on considering Antigen E response, IgE response, and HLA-A2 phenotype simultaneously. We also present the number of individuals in each group and the standard error. In rows 5 and 6 we present the results on considering only HLA-A2 and Antigen E response, and in column 3 we compare the mean Ra3 response considering only HLA-A2 and IgE. We see that in individuals with low Antigen E response (column 1), Ra3 skin test response is *positively* associated with HLA-A2 regardless of whether IgE is low or high. On comparing the final column of row 1 with the last column of row 2 we see that, regardless of whether an individual's IgE is low or high, HLA-A2 is not at all associated with Ra3 response. The fact that Ra3 is positively associated with Antigen E response *independent* of IgE is demonstrated by comparing E high with E low among low IgE and comparing E high with E low among high IgE (i.e., column 1 to column 2 in rows 7 and 8). We see that regardless of the IgE level, Ra3 response is much higher for high Antigen E skin test responders—a difference of 21.6 among those with low IgE and of 12.7 among those with high IgE. However, on comparing row 7 and row 8 within column 1 and comparing row 7 and row 8 within column 2, we see that the weakly positive association between Ra3 and IgE observed among low Antigen E skin test responders is not observed among high Antigen E skin test responders.

The statistical significance of these observations was evaluated through a three-way analysis of variance. This analysis compares the mean Ra3 responses of high IgE responders with low IgE responders, of high Antigen E skin test responders with low Antigen E skin test responders, and of HLA-A2 positive individuals with HLA-A2 negative individuals; in doing so, this analysis adjusts for any associations in the sample between IgE response, Antigen E response, and presence of HLA-A2. The resulting significant variables were the HLA-A2 Antigen E interaction and Antigen E effect indicating that (a) Ra3 response is associated with Antigen E response (Antigen E effect significant, $p < 0.001$), (b) the rela-

TABLE 3 Regression analysis of Ra3 skin test response^a considering IgE ($\log_{10}$), HLA-A2, and IgE $\times$ HLA-A2 interaction as the independent variables^b

Independent variable	Slope (B)	Standard error of B	F(1,121) ^c	Significance of variable
IgE($\log_{10}$)	7.42	4.78	2.41	>0.1
HLA-A2	-1.68	6.29	0.07	>0.7
IgE($\log_{10}$) $\times$ HLA-A2 interaction ^d	2.24	17.46	0.02	>0.9
Intercept	-1.40			

^aAnalysis of y where:

$$y = -\log_{10}(\text{dilution}) \times 10 \quad \text{if there is a response to Ra3 of 2+ or more} \\ = 0.0 \quad \text{otherwise.}$$

^bDone using the REGRESSION procedure of SPSS [6]. This procedure does a regression of any dependent variable with up to 100 independent variables, deleting all cases with missing values on any of the variables specified.

^cValue of the F statistic used to test the significance of the independent variable. The numbers in parentheses denote the degrees of freedom.

^dEstimated by creating a variable:

$$X_1 = \log_{10}(\text{IgE}) \quad \text{for case } j \text{ if he is HLA-A2 +;} \\ = 0.0 \quad \text{if case } j \text{ is HLA-A2 -}.$$

TABLE 4 Regression analysis of Ra3 skin test response^a considering Antigen E response, IgE, HLA-A2, IgE $\times$ HLA-A2, and Antigen E $\times$ HLA-A2 interactions as independent variables^b

Independent Variable	Slope(B)	Standard error of B	F(1,119) ^c	Significance of variable
Log ₁₀ (IgE)	-0.966	4.26	0.05	>0.8
Antigen E titer ^d	5.38	0.85	40.41	<0.001
HLA-A2	-4.18	14.83	0.08	>0.7
Interactions				
HLA-A2 $\times$ IgE(log ₁₀) ^e	3.90	5.57	0.49	>0.25
HLA-A2 $\times$ Antigen E titer ^e	-3.43	1.02	10.35	<0.005
Intercept	5.06			

^ay = -log₁₀(Ra3 dilution) $\times$ 10 if there is response of 2+ or more; = 0.0 otherwise.

^bDone using SPSS REGRESSION procedure (see Table 3, footnote b).

^cSee Table 3, footnote c.

^dx = -log₁₀ (Antigen E dilution) if there is a response of 2+ or more; = 0.0 otherwise.

^eSee Table 3, footnote d.

tionship between Ra3 response and HLA-A2 depends on whether the Antigen E titer is low or high (HLA-A2 effect alone is not significant, $p > 0.6$; HLA-A2 Antigen E interaction is highly significant, $p < 0.0002$), (c) the Ra3 response is not related to IgE independent of Antigen E response. (All effects involving IgE are not significant, $p > 0.05$).

In Table 3 we present the results of stepwise regression on considering Ra3 response as a function of IgE (log₁₀), presence of HLA-A2, and the interaction between them. We see that none of these variables, either alone or in combination, are significant. However, in Table 4 where we also consider Antigen E

TABLE 5 Observed correlation between IgE (log₁₀), Antigen E skin test response, and Ra3 skin test response

	Pearson Correlation:	
	Ra3 response	IgE (log ₁₀)
Ra3 response ^a	X	0.20* (130)
IgE (log ₁₀) ^a	0.20* (130)	X
Antigen E response ^a	0.49*** (126)	0.24** (125)
Partial Correlation with Ra3		
IgE (log ₁₀) controlling for Antigen E ^b	0.10 NS (124)	X
Antigen E controlling for IgE (log ₁₀) ^b	0.47*** (124)	X

Note: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. NS, not significant; degrees of freedom in parentheses.

^aObtained using SPSS procedure PEARSON CORR with option 3, which gives a two-sided test of significance or r [6].

^bObtained using SPSS procedure PARTIAL CORR with option 3, which gives a two-sided test of significance or r [6].

skin test response and the interaction of Antigen E skin test response with HLA-A2 as independent variables, these variables are significant.

In Table 5 we present the observed Pearson correlation between Ra3 response, Antigen E response, and ($\log_{10}$) IgE. Positive correlations are observed both between Ra3 response and Antigen E response and between Ra3 response and ($\log_{10}$) IgE. However, the partial correlation between Ra3 response and ($\log_{10}$) IgE is not significant; the partial correlation between Ra3 response and Antigen E response is significant. Nonparametric correlation coefficients were also computed. Although Ra3 had a significant positive correlation with both IgE response (Spearman $r = 0.19$, $p < 0.05$), a much higher correlation is observed between Ra3 response and Antigen E response (Spearman $r = 0.55$, $p < 0.001$).

DISCUSSION

The data in our study point up three important issues.

1. No matter how we consider the IgE level (either by defining high and low groups and discarding intermediate responses or by considering the logarithms of the values), any apparent association between Ra3 response and IgE is not independent of the association between IgE and Antigen E skin test response.
2. There is an association between Ra3 response and Antigen E response independent of IgE.
3. The relationship between Ra3 response and HLA-A2 phenotype is in some way affected by Antigen E response.

Points 1 and 2 are most powerfully illustrated through comparing the appropriate partial correlations and noting that the partial correlation between Ra3 titer and Antigen E controlling for ($\log_{10}$) IgE is significant and positive; whereas, the association between Ra3 response and ($\log_{10}$) IgE controlling for Antigen E response is not significant. Similarly, the interaction between Antigen E response and HLA-A2 response is most powerfully detected in the regression analysis where we consider all the information in the data rather than categorizing our responder groups and deleting intermediate responders. However, it is reassuring to see that the same conclusions are obtained regardless of how we approach these data, i.e., comparison of means, nonparametric correlations, or multiple regression.

There are several possible ways of interpreting the observed interaction between HLA-A2 and Antigen E. To some extent our conclusions depend on whether we wish to consider the Ra3 association with HLA-A2 as being dependent on the level of Antigen E response or whether we wish to interpret it as indicating that the positive association between Ra3 response and Antigen E response is modified by the presence or absence of the HLA-A2 allele. We cannot in a population study discriminate between the two. One conclusion from the first approach would be to hypothesize three linked genes on the sixth chromosome: one which might be near HLA-A and in linkage disequilibrium with HLA-A2, one for Antigen E response having two alleles, and one for Ra3 response having two alleles (E and 3). If we assume that the alleles for high response to both Antigen E and Ra3 (E_h and 3_h) are less frequent and recessive to the alleles for low response (E_l and 3_l) than these results are consistent with the orderings of HLA-A Ra3-E, a strong linkage disequilibrium between 3_l and E_l , and a weaker positive disequilibrium between 3_h and A2+ among chromosomes having E_l . Thus the four most common combinations would be A2- 3_l E_l , A2- 3_h E_h , A2+ 3_l E_h , and A2+ 3_h E_l . Such genetic hypotheses must be tested in appropriate family studies. A second approach to these results, which we

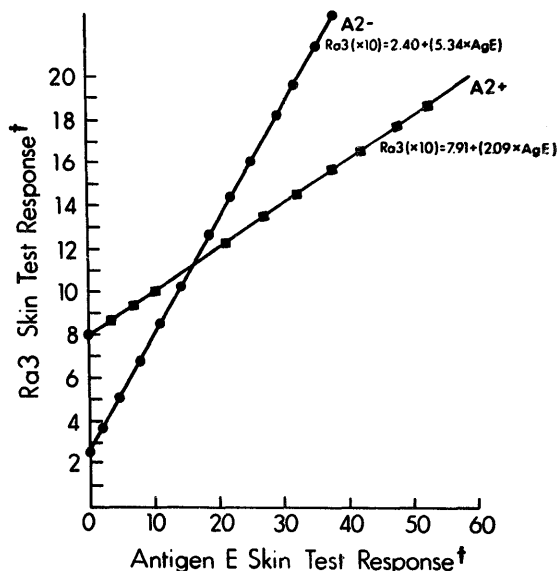


FIGURE 1 Results of regression analysis of Ra3 skin test response with Antigen E skin test response: A comparison of the relationship in HLA-A2+ and HLA-A2- individuals. Results were obtained through SPSS REGRESSION procedure with Ra3 Skin test response as the dependent variable and Antigen E skin test response as the independent variable [6]. ■ best-fitting line for HLA-A2+ responders; ● best-fitting line for HLA-A2- Responders; † = $-10 \log_{10}$ (dilution); 0 = no response.

prefer, is to consider the level of Ra3 response to be positively associated with Antigen E skin test response, but the extent of this association to be modified by presence of HLA-A2 or a gene in linkage disequilibrium with A2. This is illustrated by the two regression lines in Figure 1. On comparing the estimated slopes of the two lines we might conclude that HLA-A2-negative individuals are affected (as measured by their Ra3 sensitivity) more by their sensitivity to Antigen E than HLA-A2-positive individuals. This difference is so great that it counterbalances the association between HLA-A2 and Ra3 response observed among nonresponders to Antigen E (comparison of intercepts). In any case, additional family studies need to be done to examine the relationship between these three variables.

CONCLUSIONS

Data on HLA-A2, Ra3 skin test sensitivity, Antigen E skin test sensitivity, and IgE levels taken from 133 atopic individuals were evaluated. We observed through both regression analysis and comparison of the appropriate means that

Ra3 skin test response is associated with Antigen E response independent of the IgE levels, but that it is not associated with IgE levels independent of the Antigen E response. We also observed that the nature of the association between HLA-A2 and Ra3 response was interrelated with an individuals' Antigen E response. This observation could indicate that the extent to which Ra3 sensitivity depends on Antigen E sensitivity is in some way modified by the presence of HLA-A2.

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