

Random outcomes of allergen-specific responses in atopic families

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Summary

Background Allergens are common non-infectious antigens to which people will mount T cell dependent humoral responses. Among genetically susceptible individuals, an antigen-specific response results involving the production of allergen-specific IgE (atopy).

Objective Determine if this susceptibility is manifested as an inherited, allergen-specific trait or a random response to allergens among susceptible people.

Methods We evaluated allergen-specific outcomes in 1099 members of families with positive atopic history (26 multi-generation and 112 nuclear families). Each was tested for sensitivity to 14 common allergens by standardized skin prick test (SPT), a marker of specific IgE production. Over 15 000 individual SPT's were evaluated. Among five randomly selected multi-generation families ($N = 163$), semi-quantitative determinations of Amb a 1-specific IgA_{1,2} and IgG_{1–4} were determined in three groups: (A) Amb a SPT⁺/Amb a 1-IgE⁺, (B) Amb a SPT[−]/Amb a 1-IgE⁺, (C) Amb a SPT[−]/Amb a 1-IgE[−].

Results By rank correlation statistics, there were no discernible 'patterns' of specific SPT outcomes among any of the multi-generation families, suggesting that environmental exposure rather than allergen-specific inheritance determined the responses. This was confirmed among the nuclear families since the conditional SPT outcomes among children were independent of the SPT responses of their parents. Among five randomly selected multi-generation families, the relative proportionate concentrations of the Amb a 1-specific IgA and IgG subclasses were comparable, regardless of atopic sensitization to the ragweed allergen Amb a.

Conclusion While the general propensity for atopy may be inherited, an individual's specific atopic outcome is a random variable independent of familial sensitization patterns.

Keywords allergen, atopy, skin prick test

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Introduction

Allergens are ubiquitously distributed sources of non-infectious antigens (foods, plants, animals) to which all people are virtually continuously exposed. While it is thought that the immune system is educated to tolerate these essentially innocuous challenges, most people will mount a minimal CD4 T cell dependent humoral response to them involving all or a limited subset of antigen-specific immunoglobulin classes and subclasses [1–4]. Among genetically susceptible individuals an aberrant humoral response (atopy) results involving the production of allergen-specific IgE [5].

There are no known biochemical structural elements that determine whether or not a particular antigen will be allergenic. However, most allergens are composed of several major protein or glycoprotein antigens each of which, in turn, consists of several immunogenic (allergenic) epitopes [6, 7]. The enormous combinatorial possibilities of immunoglobulin

structure coupled with binding specificity changes via somatic hyper-mutation [8] and a catalogue of the libraries of T cell receptor (TCR) or MHC-II sequences in individuals [9–11] provide a great potential for the generation of a particular immunoglobulin binding site complementary to virtually any epitope encountered on natural or artificial surfaces. Yet this does not preclude the possibility of inherited restriction selectivity to certain allergen 'classes' among genetically susceptible people.

A number of investigators have observed HLA-based restrictions to recognition of small airborne proteins [12]. Epidemiological investigations have shown strong familial biases with regard to aberrant humoral responses to allergens [13], and further evidence of genetic pre-disposition derives from studies with twins in which it has been shown that the concordance of the 'atopic propensity' is higher among identical (monozygotic) twins compared with fraternal (dizygotic) twins [14, 15]. This has led to the belief that selectivity of response to allergens, or possibly tolerance, is inherited.

Two primary modes of information transmission have been forwarded to account for the adverse susceptibility to allergens. Either susceptible families inherit 'libraries' of

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immune response (Ir) genes (e.g. MHC-II polymorphisms) [12] or 'maternal imprinting' occurs by which allergen-specific information is transmitted from a sensitized mother to the fetus by transplacental passage of antigenic components or allergen-specific immunoglobulins [16]. In either case it is assumed that aberrant immune responses to allergen-specific information (e.g. allergenic epitopes) is transferred from one generation to the next and that subsequent immune responses are 'locked' into place.

This also implies that there are unique, inherited metabolic pathways leading to specific atopic outcomes. This is an Obligate Inheritance Model. While there is some evidence to suggest that either one or both of the aforementioned transmission mechanisms are operative, the scope of these investigations only account for limited 'sets' of small, low molecular weight allergenic determinants [17]. An alternative to this is that the general atopic tendency is inherited, but the specific outcome may be random. This is a Stochastic Bias Model.

It is estimated that if one parent is sensitized to some allergen (atopic), then there is about a 40% chance that a child will also be atopic. And, if both parents are atopic, there is about a 70% chance for atopic offspring [18]. Clearly, inheritance of atopy is not entirely obligatory as environmental factors also play crucial roles, although these details are not known to any degree of certainty including the possible selectivity of responses to specific allergens. However, these results derive from large samples from the at-large general population. A major hurdle for unraveling the details of atopic sensitivity has resulted from a lack of sufficiently large samples of families with atopic history.

To test these models, we evaluated the allergen-specific skin prick test (SPT) results to 14 common allergens from 1099 members of atopy-prone families (26 multi-generation and 112 nuclear families). If the Obligate Inheritance Model was correct, then we should have observed unique 'patterns' of allergen-specific responses among members of some of the families, but not all families combined. Alternatively, if the Stochastic Bias Model was correct, then allergen-specific outcomes among all the families should have been indistinguishable, reflecting random (stochastic) atopy outcomes to specific allergen challenges. Our results show that the Stochastic Bias Model best describes the allergen-specific outcomes in atopy-prone families.

Methods

Atopic families

Families that included at least one child with a physician's diagnosis of asthma were recruited as previously described [19]. Each person, or legal guardian, provided written, informed consent according to the guidelines of the Institutional Review Board of the University of Minnesota. We evaluated a total of 1099 family members. As detailed in results, 666 of the donors came from 26 families in which representatives of three or four generations were ascertained. These families have been followed in clinics of the University of Minnesota Medical School for several decades, and have been included in ongoing studies concerning the genetics of

asthma and other atopic disorders. An additional 433 came from 112 nuclear families that included both parents, the asthmatic child and their siblings. These families were recruited through media advertisements, also as part of studies for the genetics of asthma.

Participants were given a routine physical examination and an interviewer-monitored questionnaire, prepared according to American Thoracic Society guidelines, regarding asthma symptoms and other respiratory diseases, and allergic histories and symptoms. Baseline spirometry measures for FEV₁ were made according to American Thoracic Society guidelines [20], followed by provocative methacholine challenges as previously described [19]. Each was also tested for sensitivity to a battery of 14 allergens common to the Upper Midwest by standard SPTs (see below).

SPT for allergen sensitivities

Skin prick testing was administered with Duo-Tip Test Applicators (Lincoln Diagnostics Inc., Decatur, IL, USA) using standardized allergen extracts (Bayer Corporation, Elkhart, IN, USA). Positive histamine and negative saline controls were also administered. Weal areas (mm²) were measured with a planimeter. A positive response was recorded for any weal area ≥ 9 mm² above the negative saline control. The following list gives the allergen extracts tested including the allergen source, the systematic and scientific names [6].

Indoor allergens

1. Dust mite	Der p	<i>Dermatophagoides pteronyssinus</i>
2. Dust mite	Der f	<i>Dermatophagoides farinae</i>
3. Cat	Fel d	<i>Felis domesticus</i>
4. Dog	Can f	<i>Canis familiaris</i>
5. German cockroach	Bla g	<i>Blattella germanica</i>
6. American cockroach	Per a	<i>Periplaneta americana</i>
<i>Moulds</i>		
7. Mould	Cla h	<i>Cladasporium herbarum</i>
8. Mould	Alt a	<i>Alternaria tenuis (alternata)</i>
9. Mould	Asp f	<i>Aspergillus fumigatus</i>

Seasonal Allergens

10. Rye Grass	Lol p	<i>Lolium perenne</i>
11. Short Ragweed	Amb a	<i>Ambrosia artemisiifolia</i>
12. Birch	Bet v	<i>Betula verrucosa</i>
13. Mugwort	Art v	<i>Artemisia vulgaris</i>
14. Oak	Que a	<i>Quercus alba</i>

Serum total IgE assays

Whole blood was obtained by sterile venipuncture. Serum was separated and stored frozen (-80°C) until assayed. Quantitative serum total IgE determinations were made using the ACCESS Immunoassay System[®] developed by Sanofi Diagnostics Pasteur, Inc. (Division of Beckman Coulter, Chaska, MN, USA). Calibration was based upon commercial standards (Beckman Coulter).

Amb a 1-specific immunoglobulin profiles

Due to the unavailability of suitable standards, we devised a semi-quantitative screen for relative concentrations of Amb a 1-specific IgA1,2 and IgG1–4. The methods are described in detail elsewhere [1]. Although the method of detection for the different isotypes used the same purified allergen and reagents after the capture of the immunoglobulin isotype of interest, commercial standards are only available for IgE and IgG1 determinations. The concentrations of other isotypes can be estimated, however, by assuming that a similar signal response of relative luminometer unit's (RLU's) per bound antibody molecule is achieved for each isotype (RLU). The concentrations of isotypes other than IgG1 are thus nominal, and may be subject to small systematic errors. For purposes of this investigation, which centres upon patterns of responses, the numbers are adequate. All test results were within the range of 1–1000 ng/mL comparable with those reported by other investigators for these allergen-specific isotypes.

Statistics

Allergen rank correlations We ranked the allergens in order of how frequently they elicited an SPT⁺ result in this study population. Table 2 shows these results for the allergens ranked #1–#14 (most frequent to least frequent). Although these rankings were derived from all of the families' results, it was possible that individual families showed different 'patterns' of responses, possibly reflective of unique, genetically controlled allergen sensitivities. We tested this using the rank correlation method of Spearman [21].

For each of the 26 multi-generation families, and the combined results for the smaller, nuclear families, we ranked the unique allergen SPT⁺ results numerically (1–14). When compared with the total population, this gave ranked response pairs (x_i, y_i) for the i th allergen tested (x , total population rank and y , individual family rank for the given allergen). The squared differences between these ranking pairs, $d_i^2 = (x_i - y_i)^2$, were used to determine the Spearman rank correlation co-efficient (r_s) for each family:

$$r_s = 1 - \{6\sum_i d_i^2\} / n(n^2 - 1).$$

These results approximately follow a t -distribution with $(n-2)$ degrees of freedom: $t = r_s\sqrt{(n-2)/\sqrt{(1-r_s^2)}}$. A t -test value less than tabulated values at a particular probability level (e.g. $P = 0.05$) would indicate significant deviations among the pair-wise rankings.

Conditional probabilities and independent variables

Based upon data we have for SPT results among small, nuclear families, we can ask a fundamental question: is the child's sensitivity to a specific allergen dependent or independent of whether or not one or both parents are sensitized to that specific allergen? The analytic approach is based upon theorems for conditional probabilities [22].

Let us say that we have two events of interest, A and B . For example, event A is the probability that a mother is sensitive to a specific allergen like Der p, and event B is the probability that a child is sensitive to the same specific allergen. By definition, the conditional probability of event B occurring given that event A occurred first is

- (1) $P[B|A] = P[A \cap B]/P[A]$, where $P[A \cap B]$ is the intersection probability of events A and B . However, if events A and B are independent of one another, then
- (2) $P[B|A] = P[B]$. The conditional probability of event B occurring is independent of whether event A occurred or not. Substituting this result into (1) gives
- (3) $P[B] = P[A \cap B]/P[A]$, or
- (4) $P[A \cap B] = P[A] \times P[B]$.

We had complete SPT results for 100 nuclear families that included the mother, father and two children. By simple enumeration we determined all of the probabilities of interest for each of 14 different allergen extracts listed above.

Results

Characteristics of the atopic families

A total of 1099 individuals from families with positive medical histories for atopic disorders were evaluated by standard SPT's (14 allergens listed in Methods) and serum total IgE concentrations. This study population comprised 26 multi-generation families ($N = 666$; mean family size = 25.6; range = 6–66 members), with representatives from three or four generations, and 112 nuclear families ($N = 433$; mean family size = 3.9) that included an asthmatic proband, both parents and the proband's siblings. In all, 69.3% (762/1099) had evidence of allergen sensitization by having one or more SPT⁺ reactions.

If the families, or possibly groups of families, differed with regard to sensitization to specific allergens, or groups of allergens (e.g. indoor allergens vs. seasonal allergens), then we might expect discernible 'patterns'. This could be reflected in the number of SPT⁺ reactions, the overall vigor of the SPT reactions, reflected by the average reaction weal areas, or general atopic propensity reflected by serum total IgE values.

In agreement with previous investigations [23], total IgE values in the entire study population were log-normally distributed (mean = 1.84; SD = 0.690), with no clear demarcation between those who were SPT⁺ and those who were SPT[−]. As expected, however, there was a significant difference between mean Log [Total IgE] for those who were SPT[−] (mean = 1.33; SD = 0.592) and those who were SPT⁺ (mean = 2.06; SD = 0.613) by t -test ($P \ll 0.0001$). Among all of the families, there were none demonstrating total IgE values significantly higher or lower than the overall population.

For test allergens listed in Methods, the %SPT⁺ results, the mean # SPT⁺ results and the mean weal areas (mm²) were calculated for the study population. Table 1 gives the results for each of the 26 multi-generation families and the combined results of the nuclear families. In no case was there a significant deviation from the expected values by χ^2 analysis, and all families showed similar 'trends' in responses to the particular allergens.

Family comparisons based upon allergen 'rank order'

We ranked the 14 allergens according to how frequently they induced an SPT⁺ reaction in the study population (Table 2). As described in Methods, we also determined the 'rank order'

Table 1. Atopic responses by family

Family	N	%SPT ⁺	Mean # SPT ⁺	Mean weal area (mm ²)
Nuclear (all)	433	69.2	4.8	36.6
0	46	68.0	4.7	28.6
1	66	66.7	4.6	32.3
2	43	81.3	6.9	36.9
6	14	71.4	4.6	27.7
7	33	56.8	4.9	42.5
11	8	69.2	4.7	33.5
17	47	51.1	3.8	27.3
18	18	61.1	7.4	38.0
20	10	71.4	5.5	33.4
22	16	62.5	4.3	25.9
23	57	74.5	4.5	30.6
24	25	80.6	4.7	24.5
25	21	57.1	6.8	39.4
26	56	67.3	5.4	33.0
27	12	33.3	3.8	17.6
28	38	81.6	6.1	34.9
29	13	92.3	5.8	27.8
30	33	86.8	7.4	36.4
31	13	70.6	3.8	23.6
32	19	78.3	6.9	31.0
33	17	76.5	6.5	24.7
35	6	66.7	4.5	19.4
36	10	70.0	5.9	23.5
41	14	75.0	6.3	28.1
42	18	68.2	5.5	37.1
44	13	52.9	4.7	29.5
Total	1099	69.3	5.2	33.1

Table 2. Allergen response rank

Allergen rank	Systematic name	# SPT ⁺	Proportion all SPT ⁺
1	Amb a	504	0.120
2	Der p	435	0.103
3	Fel d	407	0.097
4	Der f	399	0.095
5	Bet v	360	0.085
6	Art v	343	0.081
7	Que a	315	0.075
8	Lol p	304	0.072
9	Can f	301	0.071
10	Bla g	206	0.049
11	Alt a	199	0.047
12	Per a	182	0.043
13	Cla h	147	0.035
14	Asp f	113	0.027

for the SPT⁺ responses for each of the 26 multi-generation families and the smaller, nuclear families combined. We compared these family rank order results with the expected results for the entire study population (Table 2). If there were significant deviations from the expected results among any of the families, this would be indicated by the Spearman rank correlation co-efficient (r_s ; see Methods).

Table 3 lists the results for the correlation co-efficients (r_s) and the corresponding t -test values. With $n-2 = 12$ degrees of

freedom, a t -test value less than 1.78 would indicate a significant difference between the expected (population) co-efficient and the observed (family) co-efficient at $P = 0.05$. This corresponds to a 'critical' $r_s = 0.458$. (a 'perfect' value, $r_s = 1.0$, translates to a t -test value $= \infty$).

There was no relationship between the estimated r_s values and the number of members in each family that were used to determine the proportionate SPT⁺ responses for each allergen. In fact, only three of the multi-generation families (41, 42 and 44 in Table 3) showed significant deviations from the population's expected values, and this was due primarily to limited responses to the allergens from dog (Can f) and cockroach (Bla g and Per a) in these families. (The limited responses to these particular allergens may have been due to living conditions, although this was not known.) In general, however, the vast majority of families showed no significant differences from the overall population SPT responses, and there were no indications of unique family response patterns to specific allergens or groups of allergens. These results suggest that the specific responses to allergens by atopy-prone families are random environmental variables.

Equiprobable humoral responses to allergen by atopic and non-atopic individuals

We have previously shown among unrelated people that both atopic and non-atopic individuals can mount an equally

Table 3. Spearman's rank correlation results

Family number	Family size	r_s	t -test
Nuclear (all)	433	0.978	16.24
0	46	0.912	7.70
1	66	0.965	12.75
2	43	0.912	7.70
6	14	0.873	6.20
7	33	0.873	6.20
11	8	0.754	3.98
17	47	0.604	2.63
18	18	0.965	12.75
20	10	0.767	4.14
22	16	0.943	9.82
23	57	0.881	6.45
24	25	0.723	3.63
25	21	0.881	6.45
26	56	0.697	3.37
27	12	0.767	4.14
28	38	0.912	7.70
29	13	0.631	2.82
30	33	0.864	5.94
31	13	0.552	2.29
32	19	0.934	9.06
33	17	0.789	4.45
35	6	1.000	∞
36	10	0.974	14.07
41	14	0.363	1.35**
42	18	0.248	0.89**
44	13	-0.046	-0.16**

r_s , Spearman's rank correlation co-efficient $= 1 - (6\sum d_i^2)/n(n^2 - 1)$.

$t = r \times \sqrt{(n-2)/\sqrt{(1-r^2)}}$; d.f. = $n-2$.

$t(12) = 1.78$ at $P = 0.05$ (r_s (critical value) = 0.458).

**Significant difference at $P = 0.05$.

vigorous humoral response to allergens [1]. Save for production of allergen-specific IgE among atopics, the relative proportionate amounts of the specific IgA and IgG subclasses produced were comparable for these groups. To determine if this was also apparent among related individuals, with or without atopy, we randomly selected five of the multi-generation families from those with 15 or more representatives (Table 1).

The families selected were numbered 2, 18, 24, 25 and 26 ($N = 163$). Because ragweed allergen (Amb a) elicited the highest proportion of SPT⁺ reactions in the entire study population (Table 2), we chose this as a model. We categorized the members of the five families based upon whether or not they had an SPT⁺ reaction to the Amb a extract or detectable amounts of serum Amb a 1-specific IgE.

Of the 163 people evaluated by these criteria:

Group A. Amb a SPT⁺/Amb a 1-IgE⁺; $N = 84$ (51.5%).

Group B. Amb a SPT⁻/Amb a 1-IgE⁺; $N = 54$ (33.1%).

Group C. Amb a SPT⁻/Amb a 1-IgE⁻; $N = 25$ (15.3%).

We also screened their sera for the Amb a 1-specific IgA_{1,2} and IgG1–4 isotype subclasses. Figure 1 shows the average proportionate amounts of these subclasses for the Groups A, B and C. By ANOVA, there were some slight, although statistically significant differences for some of the subclasses (marked by asterisks *** – in Fig. 1). However, regardless of atopic sensitivity to the ragweed allergen, the average proportionate responses among the three groups from these atopic families were comparable with results we have previously reported among unrelated people [1].

Children's allergen-specific responses are independent of their parents' atopic responses

The results in the previous sections show that the exposures and potential responses to allergens among atopy-prone families are random variables. These results were based upon overall response trends in the study population. However, this does not exclude the possibility that parents might 'pass on'

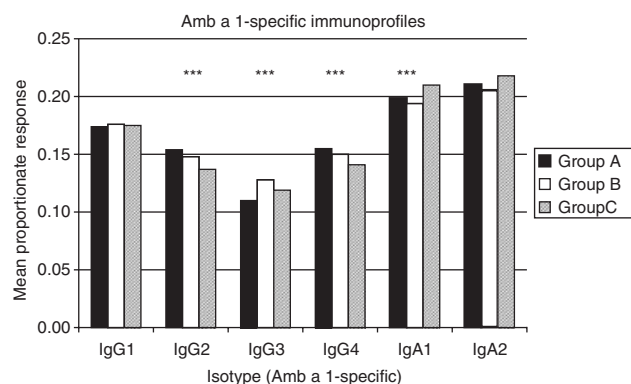


Fig. 1. Amb a 1-specific immunoglobulin isotype profiles. Among members of five randomly selected multi-generation families, proportions were found for each member by summing all values for Amb a 1-specific immunoglobulins (except IgE), dividing the result for each immunoglobulin by this sum and averaging the values for each donor group. Values on Y-axis are dimensionless. Group A: atopic, ragweed sensitive. Group B: atopic, not ragweed sensitive. Group C: not atopic. For those cases highlighted by asterisks (***), there were significant differences between the mean values of the three subject groups by ANOVA ($P < 0.05$).

specific allergen response patterns to their children. In this case, the allergen-specific atopic response 'patterns' among atopic children would be identical to those of their atopic parents. To test this, we used the response patterns based upon the SPT results for 100 of the nuclear families for which we had complete results for both parents and two children.

We define an event A to be the occurrence of sensitization to a particular allergen, like Amb a, by one or both parents. The probability of this occurrence is $P[A]$. Similarly, an event B is that a parent's child is also sensitized to the same allergen, with the associated probability of occurrence $P[B]$. We wish to determine if the conditional probability, $P[B|A]$, of event B occurring is dependent or independent of event A occurring first.

As shown by (4) in Methods, the critical relation is to determine whether or not the intersection probability between events A and B , $P[A \cap B]$ is or is not equal to $P[A] \times P[B]$. If event B is dependent upon event A occurring first, then: $P[A \cap B] \neq P[A] \times P[B]$. In contrast, if event B is independent of event A , then $P[A \cap B] = P[A] \times P[B]$. It is this latter condition that we wish to test.

Null Hypothesis, H_0 : there is no difference between the intersection probability and the product of the probabilities for events A and B .

Alternate Hypothesis, H_1 : there is a difference between the intersection probability and the product of the probabilities for events A and B .

Table 4, Part I lists the four possible probabilities for parents' event A , $P[A]$, the two probabilities for children's event B , $P[B]$, and the $4 \times 2 = 8$ possible intersection probabilities, $P[A \cap B]$. All of these probabilities can be estimated by simple enumeration of the number of positive responses in each of the categories and converting to proportionate values (estimates of the true population probabilities).

For each of the 14 allergens, there are eight separate relationships to test: $P[A \cap B]$ vs. $P[A] \times P[B]$. A simple correlation analysis will reveal whether or not these probabilities are or are not related (equal) to one another. These analyses were done after defining two variables: $Y = P[A \cap B]$ and $X = P[A] \times P[B]$. The regression of variable Y on X gave the correlation co-efficient (R), whose significance could be evaluated by t -test and associated P -value. The statistical results are listed in Table 4, Part II.

From the allergen list in Methods, for those numbered 1–3 and 10–14, the corresponding correlation co-efficients are all approximately equal to 1.0. These were also the allergens that produced the most frequent SPT⁺ results in the study population (Table 2). However, for the allergens numbered 4–9 the correlation co-efficients are only in the range: $0.8 \leq R \leq 0.9$. These allergens were the least likely to induce SPT⁺ results in the population (Table 2). The fewer numbers of positive cases lowered the statistical power of the estimates.

In any event, there is no evidence to reject the Null Hypothesis (H_0), and it would appear that the probability that a child is atopically sensitized to a specific allergen is independent of whether or not one or both of his/her parents was sensitized to the same particular allergen.

Table 4. Tests of independence

I. Probabilities for tests of independence			
$P[A]$	$P[B]$	$P[A \cap B]$	
1. Mother (M)	1. First child (C1)	1. (M $\cap$ C1)	
		2. (M $\cap$ C2)	
2. Father (F)	2. Second child (C2)	3. (F $\cap$ C1)	
		4. (F $\cap$ C2)	
3. Mother or Father (M or F)		5. (M or F $\cap$ C1)	
		6. (M or F $\cap$ C2)	
4. Mother and father (M and F)		7. (M and F $\cap$ C1)	
		8. (M and F $\cap$ C2)	
Null Hypothesis (H_0): $P[A \cap B] = P[A] \times P[B]$. Alternate Hypothesis (H_1): $P[A \cap B] \neq P[A] \times P[B]$			
II. Correlation results for tests of independence			
Allergen #	R	$t(6)$	P -value
<i>Indoor</i>			
1. Der p	0.960	8.40	<0.005
2. Der f	0.955	7.89	<0.005
3. Fel d	0.991	18.13	<0.001
4. Can f	0.826	3.59	<0.01
5. Bla g	0.871	4.34	<0.005
6. Per a	0.831	3.66	<0.01
<i>Moulds</i>			
7. Cla h	0.805	3.32	<0.01
8. Asp f	0.816	3.46	<0.01
9. Alt a	0.829	3.63	<0.01
<i>Seasonal</i>			
10. Lol p	0.985	13.98	<0.001
11. Amb a	0.979	11.76	<0.001
12. Bet v	0.974	10.53	<0.001
13. Art v	0.995	24.40	<0.001
14. Que a	0.977	11.22	<0.001

Discussion

Numerous sources with allergenic potential exist in the environment (trees, grasses, insects, animal furs, etc.) to which all people are virtually continuously exposed. Yet, only a limited proportion of the population responds adversely ($\sim 20\%$ – 30% by current estimates), and then to only a limited number of stimuli with the allergenic potential. At a minimum, the response to allergen requires an initial immune recognition and activation phase, followed by a consolidation phase leading finally to the effector phase. Metabolic errors in either one or both of the preceding phases will ultimately lead to the effector phase. What is not known with any degree of certainty is which among the multitude of regulatory steps involved with immune responses to allergens are essentially required and lead to the aberrant production of IgE [24].

The difference between those who respond adversely and those who do not could be due to unique recognition of specific allergen epitopes residing in an inherited library of V_H genes [25]. Or, as has been suggested, those with the 'atopic propensity' mount a vigorous humoral response while the majority of the population remains tolerant through education of their immune systems to essentially ignore these

otherwise benign challenges [26]. Alternatively, atopy defined as a hyper-reactive condition could result in a pre-mature or inappropriate production of IgE during secondary responses and immunoglobulin isotype switching.

The earliest investigations into the inheritance of atopic disorders postulated that there might be an MHC restriction involved. Limited evidence for this was found using small molecular weight allergens, such as the minor ragweed allergen Amb a V (MW ~ 5000) [27, 28]. However, attempts to extend these analyses using larger molecular weight allergens showed that there was no convincing evidence for an MHC restriction [17]. Many of the reports suggesting an MHC restriction to allergens were based upon very small study populations that may have 'skewed' the results [12]. Thus, the earlier observations were more likely due to chance rather than evidence of an inherited mechanism.

More recent investigations have suggested that 'maternal imprinting' may play an important role in the transmission of atopic information [16]. This model implies that allergenic information is transferred across the placenta from a sensitized mother to the fetus. The information may be either in the form of allergenic epitopes or allergen-specific immunoglobulins. Again, the information is limited to small molecular weight subsets of potentially allergenic materials.

Both of these suggested modes of information transfer leads to the question of whether all atopic responses are essentially the same, or are there unique inherited pathways with characteristic regulatory errors that lead, generally, to similar atopic outcomes? It is this question that we have attempted to address in this paper. Due to the tendency to 'cluster' within families, one of two models could explain atopic progression among members of atopy-prone families.

The atopic humoral response may be an obligatory inherited pre-disposition. An Obligate Inheritance Model postulates that there are specific, inherited errors in recognition and/or response to allergen. Given the appropriate circumstances (e.g. allergen exposure), errors at one or several key regulatory checkpoints are involved. Thus, an individual's humoral response to allergen is genetically 'locked into' progression along a particular pathway and its associated metabolic errors. As a consequence, among some atopy-prone families we would expect to see distinct 'patterns' of responses to specific allergens or sets of allergens that would distinguish them from the 'patterns' of other atopy-prone families.

In contrast, a Stochastic Bias Model proposes that the metabolic regulatory pathways involved with the humoral responses to allergens are essentially the same among all who are clinically affected. Errors might arise due to temporary changes in physiological status (e.g. immunological skewing) or certain collateral effects that alter the probabilities of progressing along a particular metabolic pathway. Hence, the end-point will be reflected by the degree of statistical weighting effects that occurred at key regulatory points, with inheritance being one weighting factor.

The results presented here derive from a large cross-section of predominantly Caucasian families residing in Minnesota with positive histories of atopic disorders. Our intention was to address the specific question of whether there are unique pathways among some families that obligately lead to atopic

progression to certain allergens or is the propensity for atopy shared among all family members with the atopic outcome derived primarily from probabilistic contingencies? Our results differ in certain key aspects to the results and conclusions drawn, or implied, from previous investigations.

Among 1099 people from families with positive atopic histories, it would appear, generally, that the response to allergens among atopy-prone people is a random (stochastic) variable that derives from environmental exposure. This provides considerable evidence against MHC restriction as a primary motive force in the aberrant response to allergens. However, given that small nuclear families share similar intimate living conditions, including the kinds of allergens to which they would be exposed, it was possible that atopic sensitivity to specific allergens might be 'passed on' from parents to children. This would not necessarily alter the results of the proportionate responses to allergens in the at-large study population.

Employing a simple argument derived from theorems of conditional probabilities [22], we tested two alternate hypotheses. H_0 : the probability that a child was sensitized to a particular allergen was independent of whether or not one or both parents were sensitized to the same specific allergen. H_1 : the probability of a child's specific sensitization was dependent upon the parent's history of specific sensitization. Based upon 100 nuclear families, including the mother, father and two children in each, there is no evidence to reject our null hypothesis (H_0). In addition to lack of evidence for an MHC restriction, these results also call into question the degree to which 'maternal imprinting' plays a role in sensitization to specific allergens.

It has been observed that the incidence of atopic disease has increased dramatically in recent decades [13]. An increased incidence of sensitivity to allergens among the children in this study during this period of general increased sensitivity could potentially 'mask' the effects observed. That is, the specific 'sensitivity patterns' of parents might be masked in their offspring due to the increased likelihood of a more general increase in allergen sensitivity. However, as we have recently shown for this study population [29], the proportion of those with any SPT⁺ result was lower among children 10 years of age or younger compared with the older age groups. This was due to a mechanism independent of IgE production.

It should be emphasized that the study population used here is extremely biased towards the presence of atopy and asthma; the results may not be applicable to the population at large. In the at-large population significant genetic and environmental effects must be taken into account, whereas in the population studied here most of the effects are presumably genetic. However, knowledge of response(s) to specific allergens is essential for understanding the pathologies among those families with pre-dispositions to atopic disorders.

Recent thought on general immune surveillance argues that finer and finer dissection of the behaviors of immunological components has led to ever increasing uncertainty regarding the specific outcomes derived from them [30]. With regard to the immune system in atopic disorders, at least three principal components are responsible for cognate interactions leading to a particular allergen-specific response: T and B lympho-

cytes and antigen-presenting cells (APCs). Presumably, the atopic response may be considered as one kind of 'polarized response' that is either distinct from a normative response or is unique only to those who respond adversely to common environmental stimuli.

'Polarized' responses could result from physiological propensities among the components themselves. Yet, this would not appear to be the case in this study population. Among five randomly selected multi-generation families, save for the production of allergen-specific IgE among atopically sensitized people, it is equally likely that the same relative proportion amounts of allergen-specific IgA and IgG subclasses are produced regardless of atopic status (Fig. 1). We have previously proposed a mechanistic distinction between the atopic and non-atopic responses to allergens based upon allergen-antibody binding affinities [1].

Alternatively, the polarization could arise from tissue-specific cues that direct the 'education' of these immune components [31]. This proposition implies that errors would arise at the interface between the entry sites of allergens (mucosa, epithelia, etc.) and the adaptive immune responses generated against them.

From this we might differentiate between two operational modes: those that directly involve immune system components and their responses, and those that impinge upon the immune system components and 'educate' them. For an immune deviation state such as atopy, the former would imply inherited defects in components or pathways of the immune system leading to the pathological outcome, while the latter would infer temporary skewing effects, some of which might be inherited while others may be context dependent such as collateral effects of generalized inflammatory responses or temporary enhancement of inflammatory processes during secondary infections unrelated to atopy.

In summary, in a study population of 1099 members from 138 atopy-prone families, we can find no compelling evidence for inheritance of sensitization to specific allergens. Rather, although the 'propensity for atopy' may be passed on from generation to generation, the exposure to and possible atopic response to specific allergens are random variables independent of any patterns of familial sensitization. The evidence supports our Stochastic Bias Model.

Among members of atopy-prone families, the likelihood of exposure and adverse response to common environmental allergens are random variables. Presumed metabolic errors that lead to the adverse atopic response are probably not incorporated in the components of the adaptive immune system themselves. Rather, it is more likely that errors arise during initial allergen recognition and activation of the adaptive immune components that derive from enhanced collateral effects that 'educate' the adaptive components.

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References

- 1 Jackola DR, Pierson-Mullany LK, Liebeler CL, Blumenthal MN, Rosenberg A. Variable binding affinities for allergen suggest a 'selective competition' among immunoglobulins in atopic and non-atopic humans. *Molec Immunol* 2002; 39:367–77.
- 2 Allauzen S, Blocki F, Pierson-Mullany L et al. Expression of IgH chain genes in antibody response to ragweed and purified Amb a 1: evidence for differential regulation of isotype production. *Molec Immunol* 1999; 36:587–98.
- 3 Batard T, Basuyaux B, Lambin P, Brémard-Oury C, Hamilton RG, Peltre G. Isotype analysis of grass pollen-specific immunoglobulins in human plasma. I. Specialization of certain classes and subclasses in the immune response. *Int Arch Allergy Immunol* 1993; 100:68–78.
- 4 Batard T, Basuyaux B, Laroze A et al. Isotype analysis of grass pollen-specific immunoglobulins in human plasma. II. Quantification of the IgE, IgM, IgA class and the IgG subclass antibodies. *Int Arch Allergy Immunol* 1993; 102:279–87.
- 5 Blumenthal MN, Björkstén B., (eds.). *Genetics of Allergy and Asthma*. New York: Marcel Dekker, Inc., 1997.
- 6 Marsh DG. Allergens and allergen nomenclature. In: Marsh DG, Blumenthal MN, eds. *Genetic and Environmental Factors In Clinical Allergy*. Minneapolis: University of Minnesota Press, 1990; 20–31.
- 7 Blumenthal MN, Rosenberg A. Definition of an allergen (immunobiology). In: Lockey RF, Bukantz SC, eds. *Allergens and Allergen Immunotherapy*. New York: Marcel Dekker, Inc., 1999; 39–51.
- 8 Wabl M, Steinberg C. Affinity maturation and class switching. *Curr Opin Immunol* 1996; 8:89–92.
- 9 Sebзда E, Mariathasan S, Ohteki T, Jones R, Bachmann MF, Ohashi PS. Selection of the T cell repertoire. *Ann Rev Immunol* 1999; 17:829–74.
- 10 Rudolph MG, Wilson IA. The specificity of TCR/pMHC interaction. *Curr Opin Immunol* 2002; 14:52–65.
- 11 Rhodes DA, Trowsdale J. Genetics and molecular genetics of the MHC. *Rev Immunogenetics* 1999; 1:21–31.
- 12 Moffat MF, Cookson WOCM. The genetics of specific allergy. In: Hall IP, ed. *Genetics of Asthma and Atopy*. Basel: Karger. *Mongr Allergy* 1996; 33:71–96.
- 13 ISAAC (International Study of Asthma and Allergies in Childhood Steering Committee). Worldwide variations in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis and atopic eczema. *Lancet* 1998; 351:1225–32.
- 14 Hanson B, McGue M, Roitman-Johnson B, Bouchardt TJ Jr., Blumenthal MN. Atopic disease and immunoglobulin E in twins reared apart and together. *Am J Human Genet* 1991; 48:873–9.
- 15 Schultz-Larsen F. A genetic-epidemiological study in a population-based twin sample. *J Am Acad Dermatol* 1993; 28:719–23.
- 16 Jones CA, Kilburn SA, Warner JA, Warner JO. Intrauterine environment and fetal sensitization. *Clin Exp Allergy* 1998; 28:655–9.
- 17 Marsh DG. Approaches toward the genetic analysis of complex traits: asthma and atopy. *Allergy* 1999; 54:198–205.
- 18 De Swert LFA. Risk factors for allergy. *Eur J Pediatr* 1999; 158:89–94.
- 19 CSGA (Collaborative Study on the Genetics of Asthma). A genome-wide search for asthma susceptibility loci in ethnically diverse populations. *Nat Genet* 1997; 15:389–92.
- 20 American Thoracic Society. Standardization of spirometry – 1987 update. *Am J Respir Crit Care Med* 1987; 133:1285–98.
- 21 Keeping ES. *Introduction to Statistical Inference*. New York: Dover Publications, Inc, 1995; 308–14.
- 22 Feller W. Chapter V. Conditional probability. Stochastic independence. In: *An Introduction To probability Theory and its Applications*, Vol. I. New York: John Wiley & Sons, Inc., 1968; 114–45.
- 23 Barbee RA, Halonen M, Lebowitz M, Burrows B. Distribution of IgE in a community population sample: correlations with age, sex, and allergen skin test reactivity. *J Allergy Clin Immunol* 1981; 68:106–11.
- 24 Sutton BJ, Gould HJ. The human IgE network. *Nature* 1993; 366:421–8.
- 25 Snow RE, Djukanovic R, Stevenson FK. Analysis of immunoglobulin E VH transcripts in a bronchial biopsy of an asthmatic patient confirms bias toward VH5, and indicates local clonal expansion, somatic mutation and isotype switch. *Immunology* 1999; 98:646–51.
- 26 Aalberse RC. Specific IgE and IgG responses in atopic versus nonatopic subjects. *Am J Respir Crit Care Med* 2000; 162:S124–7.
- 27 Marsh DG, Rafnan T, Ghosh B, Huang S-K. Specific immune responses to purified allergen. In: Blumenthal MN, Björkstén B, eds. *Genetics of Allergy and Asthma*. New York: Marcel Dekker, Inc., 1997; 197–210.
- 28 Marsh DG, Bias WB, Ishizaka K. Genetic control of basal serum immunoglobulin E and its effect on specific reaginic sensitivity. *Proc Natl Acad Sci USA* 1974; 71:3588–92.
- 29 Jackola DR, Pierson-Mullany L, Blumenthal MN, Rosenberg A. Allergen skin test reaction patterns in children (≤ 10 y.o.) from atopic families suggest age-dependent changes in allergen-IgE binding in early life. *Int Arch Allergy Immunol* 2003; 132:364–72.
- 30 Germain RN. The art of the probable: system control in the adaptive immune system. *Science* 2001; 293:240–5.
- 31 Matzinger P. Tolerance, danger and the extended family. *Ann Rev Immunol* 1994; 12:991–1045.