

EVIDENCE FOR BIOLOGICAL INHERITANCE OF THE EOSINOPHIL RESPONSE TO INTERNAL PARASITES IN SOUTHEASTERN BRAZIL

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ABSTRACT

One hundred and seventy-seven individuals belonging to 120 complete or incomplete nuclear families from Bambui, in the State of Minas Gerais, southeastern Brazil, were studied in order to examine causes of variation in the eosinophil rate among subjects infested by intestinal worms with an extra-digestive cycle. Segregation analysis without correction for skewness showed that the hypothesis of the presence of an additive major gene was consistent with the data, although a dominant, recessive, or a multifactorial hypothesis could not account properly for the observed significant familial aggregation. The most parsimonious correction for skewness showed similar results, but could not distinguish between dominant and recessive models, although co-dominance was rejected. Since these models assume that skewness was attributable to the commingling of two distributions, these results seem to agree with those for uncorrected data. These findings suggest that several genetically independent factors determine the resistance/susceptibility to helminth infestation mainly through their ability to influence the eosinophil response.

INTRODUCTION

Several studies have reported the existence of important genetic mechanisms acting on phenotypes involved in certain human responses possibly related to resistance/susceptibility to malaria (Abel *et al.*, 1992a), tegumentary (Shaw *et al.*, 1995; Alcaïs *et al.*, 1997) and visceral (Feitosa *et al.*, 1998) leishmaniasis, leprosy (Abel and Demenais, 1988; Abel *et al.*, 1989, 1992ab, 1995; Feitosa *et al.*, 1995a,b, 1996) and schistosomiasis mansoni (Abel *et al.*, 1991; Marquet *et al.*, 1996; Müller-Myhsok *et al.*, 1997).

Eosinophils are involved in the development and expression of antiparasite resistance and of parasite-induced pathology (Butterworth, 1977). One of the causes of eosinophilia is the presence of intestinal parasites with an extra-digestive phase in their life cycle. The activation of cells associated with eosinophilia, as well as alterations in functions, such as the synthesis and secretion of cationic proteins (Tai *et al.*, 1984), leukotriene C₄ (Silberstein *et al.*, 1986), major basic protein (Butterworth, 1977), and eosinophil peroxidase (Gruart *et al.*, 1989), are some of the host's mechanisms for fighting parasites. The capacity for eosinophilia probably has a major role in the resistance to parasitic infestations that trigger this response.

The presence of a major gene responsible for setting up the eosinophil response to helminthic infestation has been reported for rodents (Butterworth, 1977; Claas and

Deelder, 1979; Vadas, 1982; Hurley and Vadas, 1983; Wakelin and Donachie 1983; Lammas *et al.*, 1988, 1992). Studies in sheep selected for high and low responsiveness to helminths showed that this variation was under genetic control (Dawkins *et al.*, 1989). In humans, Moro-Furlani and Krieger (1992) demonstrated that in three Brazilian samples the familial resemblance of eosinophil levels resulted from biological transmission rather than shared environmental factors. These conclusions were based on a series of familial correlations.

In this study, we examined the causes of this biological mechanism, using commingling and segregation analyses, applied to one of the above Brazilian samples.

MATERIAL AND METHODS

Sample

The sample consisted of 350 individuals from 180 families living in Bambui (State of Minas Gerais) in southeastern Brazil, an area endemic for Chagas' disease (for more details see Dias *et al.*, 1983; Abreu *et al.*, 1989; Krieger *et al.*, 1992; Moro-Furlani and Krieger, 1992).

A sub-sample composed only of individuals infested by *Ascaris lumbricoides*, *Strongyloides stercoralis*, Ancylostomids and/or *Schistosoma mansoni* contained 177 subjects belonging to 120 complete or incomplete nuclear families. The presence of parasites in stool specimens was determined by standard parasitologic techniques and only helminths displaying extraintestinal cycles were considered. A clinical hemogram was obtained and the eosinophil rate was recorded as a percent of the total leukocytes.

Statistical analysis

In addition to the usual stepwise multiple regression, commingling and segregation analyses were performed.

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Commingling analysis

This analysis was carried out as described by MacLean *et al.* (1976) using the computer program SKUMIX (Morton *et al.*, 1983). The analysis assumes that the quantitative variable is influenced by a single major locus with two alleles (A , a) distributed in three possible genotypes (AA , Aa , and aa). This model, which may include an optional power transformation parameter (P) to reduce skewness, can contemplate a mixture of up to three distributions in Hardy-Weinberg proportions. In addition to P , other parameters include the overall mean (u), the within-component variance (E), which is assumed to be equal for each of the component distributions, the square root of the relative proportion of the component distribution with the highest mean (q), the displacement (t) between the means of the two extreme component means, and the relative position of the intermediate mean (d) from the mean of the lowest component.

The parameters were estimated by maximum likelihood. Six hypotheses were tested under one, two or three component distributions, each with (S = skewed) or without (N = normal) skewness. Tests of hypotheses for nested model comparisons were carried out using the likelihood ratio test ($-2\ln L$). For non-nested model comparisons, Akaike's information criterion (AIC), defined as the sum of $-2\ln L$ plus twice the number of estimated parameters, was used to assess the fit. The model with the smallest AIC value was accepted as the best model (Akaike, 1974).

Commingling analysis was used to determine: i) whether there were multiple distributions, consistent with (but not proof of) a major gene hypothesis, ii) whether the form of the distribution varied among parents and offspring (i.e., generation heterogeneity), and iii) whether there was skewness in the distributions.

Segregation analysis

While commingled distributions are consistent with the presence of a major gene, skewness can result from environmental effects and from causal heterogeneity. Segregation analysis was used to determine whether the major effect is transmitted in families according to Mendelian expectations. The model used for segregation analysis was the unified mixed model (Lalouel *et al.*, 1983), which combines the mixed model (Morton and MacLean, 1974) and the transmission probability parameters (Elston and Stewart, 1971) as implemented in the computer program POINTER (Lalouel and Morton, 1981; Morton *et al.*, 1983). The major gene effect results from segregation at a single locus with two alleles (A and a), with genotypes distributed in Hardy-Weinberg proportions. There are seven parameters in the model: the overall variance (V), the overall mean (u), the major locus gene frequency (q), the displacement between the two homozygous means (t), the relative position of the mean of the heterozygote,

or dominant (d), and two parameters representing the multifactorial heritabilities in children (H) and parents (HZ). The parameters d , t , and q are parallel to those outlined for commingling analysis.

Additional parameters, τ_{AA} , τ_{Aa} , τ_{aa} , can be estimated to test deviations from Mendelian transmission of the major effect from parent to offspring, and denote the probabilities of transmitting allele A for genotypes AA , Aa , and aa , respectively. Under Mendelian transmission, $\tau_{AA} = 1$, $\tau_{Aa} = 1/2$, $\tau_{aa} = 0$, while no major gene transmission is obtained when the three taus are equal. To infer the presence of a major locus, three conditions are usually required: i) rejection of the hypothesis $d = t = q = 0$ (no major effect), ii) no rejection of Mendelian transmission (when $\tau_{AA} = 1$, $\tau_{Aa} = 1/2$, $\tau_{aa} = 0$), and iii) rejection of the non-Mendelian transmission model (when $\tau_{AA} = \tau_{Aa} = \tau_{aa}$). Different hypotheses were tested by estimating or fixing parameters of the complete model.

RESULTS AND DISCUSSION

The eosinophil rate (ER) among individuals with and without internal parasites is shown in Table I, and the distribution of different parasites among the subjects is shown in Table II. The sample was divided into subjects without parasites and those with at least one of the internal parasites (*Ascaris lumbricoides*, *Strongyloides stercoralis*, Ancylostomids and/or *Schistosoma mansoni*). The mean ER among individuals infested with some form of internal parasite was significantly higher than among individuals with no signs of infestation ($t = -3.70$, $P < 0.001$). Similarly, the variance of ER was also significantly higher in the former sub-group ($F_{1,314} = 12.39$, $P < 0.001$), suggesting that since it was not restricted to a shift to the right, the response was not uniform among infested individuals, i.e., some individuals reacted more intensely than others.

To investigate the effects of sex and age on eosinophil rate, regression analyses were done on the entire sample and on the parasitized sub-group. Because of the skewness of the distributions and the large variance values, the stepwise multiple regressions were done using the natural logarithms of the eosinophil rate (LER) and the independent variables sex, age, age squared and sex-age interactions. None of these independent variables had a significant effect on the LER of the whole sample or the parasitized sub-group.

Tests of generation heterogeneity for each of the six commingling models (1N, 2N, 3N, 1S, 2S and 3S distributions) showed no heterogeneity between parents and offspring ($P > 0.05$). Thus, all subsequent analyses were applied only to combined generations. Table III shows that there was skewness under one (1N-1S; $\chi^2_1 = 22.23$, $P < 0.001$), two (2N-2S; $\chi^2_1 = 16.89$, $P < 0.001$) and three (3N-3S; $\chi^2_1 = 16.89$, $P < 0.001$) distributions. Two-skewed distributions fitted better than one-skewed (1S-2S; $\chi^2_2 = 7.73$,

$P = 0.02$) and was more parsimonious than three-skewed distributions (3S-2S; $\chi^2_1 = 0.0$, $P > 0.99$). The AIC test indicated two-skewed distributions to be the best model.

Segregation analyses were applied to both the untransformed and transformed data to avoid skewness *per se*, using the best model suggested by the commingling

Table I - The distribution of the eosinophil rate among parasitized and non-parasitized individuals.

Eosinophil rate	Non-parasitized	Parasitized	Total
0-2	35	35	70
3-7	65	58	123
8-12	24	39	63
13-17	9	23	32
18-22	4	16	20
23-27	1	2	3
28-32	0	3	3
33-37	1	0	1
>37	0	1	1
Total	139	177	316
Mean	6.06	8.72	7.55
Standard deviation	5.60	7.22	6.68
Variance	31.37	52.10	44.61
Skewness	1.94	1.15	1.44
Kurtosis	5.47	1.53	2.55

t -value = -3.70, degrees of freedom = 314.

Table II - Distribution of different parasites among individuals in a sample from Bambui, MG, Brazil.

Parasites	Number of individuals
<i>Ascaris lumbricoides</i>	43
<i>Strongyloides stercoralis</i>	11
Ancylostomids	90
<i>Schistosoma mansoni</i>	4
<i>A. lumbricoides</i> and <i>S. stercoralis</i>	2
<i>A. lumbricoides</i> and Ancylostomids	13
<i>S. stercoralis</i> and Ancylostomids	7
<i>S. mansoni</i> and Ancylostomids	4
<i>A. lumbricoides</i> , <i>S. stercoralis</i> and Ancylostomids	2
<i>A. lumbricoides</i> , <i>S. mansoni</i> and Ancylostomids	1

Table III - Commingling analysis of eosinophil's levels among parasitized individuals.

Distribution	E	u	d	t	q	P	$-2\ln L$	AIC
1 Normal	1.230	0.067	[0]	[0]	[0]	[1]	269.57	273.57
1 Skewed	0.981	-0.181	[0]	[0]	[0]	-1.815	247.34	253.34
2 Normal	0.525	0.067	[0]	2.106	0.446	[1]	256.50	264.50
2 Skewed	0.351	-0.190	[0]	1.592	0.687	-1.925	239.61	249.61
3 Normal	0.525	0.067	0.0*	2.106	0.446	[1]	256.50	266.50
3 Skewed	0.351	-0.190	0.0*	1.535	0.687	-1.925	239.61	251.61

E = Variance, u = mean, d = dominance, t = displacement, q = major gene frequency, P = power transform, $-2\ln L$ = likelihood ratio test, AIC = Akaike's information criterion.

analysis. Table IV shows the results of segregation analyses for 177 individuals from 120 incomplete families, using only individuals with a positive parasite test, and untransformed data. The mixed model (model 1) did not converge when the usual iterative procedure was used. The parameter H tended towards zero. Thus, a gradient of fixed values of H was used and the model that provided the smallest value of $-2\ln L$ was considered the minimum chi-square estimator (confirming $H = 0$). Using this approach, there was no generational difference in the multifactorial component (model 2 vs. model 3: $\chi^2_1 = 0.50$; $P = 0.48$) and the model without a multifactorial component (4) was not rejected (model 4 vs. model 1: $\chi^2_1 = 0.0$, $P > 0.99$). The hypothesis of no familial aggregation (model 8) was rejected (model 8 vs. model 1: $\chi^2_4 = 46.30$, $P < 0.001$), as was the hypothesis of no major effect (model 2 vs. model 1: $\chi^2_3 = 45.74$, $P < 0.001$). The data were consistent with an additive model of inheritance (model 6 vs. model 4: $\chi^2_1 = 2.24$, $P = 0.13$), while the recessive and dominant models were rejected (model 5 vs. model 4: $\chi^2_1 = 17.77$, $P < 0.001$; model 7 vs. model 4: $\chi^2_1 = 16.67$, $P < 0.001$). The models that estimate taus (free and equal τ_g) did not reach convergence. Consequently, the presence of a major gene, based on the untransformed data, although suggestive, could not be further tested.

The results for segregation analyses after correction for skewness ($P = -1.925$) are shown in Table V. No generational difference was detected in the multifactorial component (model 2 vs. model 3: $\chi^2_1 = 0.0$, $P > 0.99$). There was a significant familial resemblance (model 8 vs. model 1: $\chi^2_4 = 18.11$, $P = 0.001$) and a strict multifactorial model could not explain the familial aggregation (model 2 vs. model 1: $\chi^2_3 = 14.01$, $P = 0.003$). Neither the recessive (model 5 vs. model 4: $\chi^2_1 = 1.40$, $P = 0.24$) nor dominant (model 7 vs. model 4: $\chi^2_1 = 0.0$, $P > 0.99$) model was rejected, whereas the additive model was rejected (model 6 vs. model 4: $\chi^2_1 = 6.95$, $P = 0.008$). Additionally, the dominant Mendelian model was not rejected (model 1 vs. model 9: $\chi^2_3 = 2.11$, $P = 0.55$), although a model with equal taus, i.e., equal transmission rates but different from half (model 10 vs. model 9: $\chi^2_2 = 2.00$, $P = 0.37$) fitted to the data. The AIC criterion also indicated the dominant Mendelian model as the most parsimonious and best model. Thus, a major

Table IV - Segregation analysis of eosinophil levels among parasitized individuals.

Model	V	u	d	t	q	H	Z	τ_{AA}	τ_{Aa}	τ_{aa}	-2lnL	AIC
1. Mendelian mixed	1.25	0.07	0.425	4.255	0.145	0.0*	[1]	[1]	[0.5]	[0]	492.84	504.84
2. No major gene (Z = 1)	1.21	0.03	[0]	[0]	[0]	0.106	[1]	[1]	[0.5]	[0]	538.58	544.58
3. No major gene	1.21	0.05	[0]	[0]	[0]	0.206	0.264	[1]	[0.5]	[0]	538.08	546.08
4. No multifactorial component	1.25	0.07	0.425	4.255	0.145	[0]	[1]	[1]	[0.5]	[0]	492.84	502.84
5. Recessive Mendelian	1.22	0.06	[0]	2.117	0.437	[0]	[1]	[1]	[0.5]	[0]	510.61	518.61
6. Additive Mendelian	1.22	0.07	[0.5]	3.780	0.140	[0]	[1]	[1]	[0.5]	[0]	495.08	503.08
7. Dominant Mendelian	1.22	0.06	[1]	2.123	0.099	[0]	[1]	[1]	[0.5]	[0]	509.51	517.51
8. Sporadic	1.24	0.07	[0]	[0]	[0]	[0]	[0]	[1]	[0.5]	[0]	539.14	543.14

* The parameter tended towards zero. H = Multifactorial component, Z = generation component for the multifactorial component. For other abbreviations see Table II.

Table V - Segregation analysis of eosinophil levels among parasitized individuals, after correction for skewness (P = -1.925).

Model	V	u	d	t	q	H	Z	τ_{AA}	τ_{Aa}	τ_{aa}	-2lnL	AIC
1. Mendelian mixed	0.99	-0.13	1.0*	1.603	0.254	0.102	[1]	[1]	[0.5]	[0]	482.62	494.62
2. No major gene (Z = 1)	0.98	-0.14	[0]	[0]	[0]	0.336	[1]	[1]	[0.5]	[0]	496.63	502.63
3. No major gene	0.89	-0.14	[0]	[0]	[0]	0.346	0.921	[1]	[0.5]	[0]	496.63	504.63
4. No multifactorial component	1.00	-0.13	1.0	1.590	0.244	[0]	[1]	[1]	[0.5]	[0]	483.70	493.70
5. Recessive Mendelian	0.99	-0.14	[0]	1.582	0.649	[0]	[1]	[1]	[0.5]	[0]	485.10	493.10
6. Additive Mendelian	1.00	-0.14	[0.5]	2.410	0.651	[0]	[1]	[1]	[0.5]	[0]	490.65	498.65
7. Dominant Mendelian	0.99	-0.13	[1]	1.589	0.244	[0]	[1]	[1]	[0.5]	[0]	483.70	491.70
8. Sporadic	0.99	-0.15	[0]	[0]	[0]	[0]	[0]	[1]	[0.5]	[0]	500.73	504.73
9. Free τ_s	1.00	-0.17	1.0*	1.606	0.304	0.117	[1]	1.0*	0.647	0.0*	480.51	498.51
10. Equal τ_s	0.99	-0.11	1.0*	1.600	0.264	0.101	[1]	0.102	0.102	0.102	482.51	496.51

* The parameter reached its bound. Abbreviations as in Tables III and IV.

effect acting on eosinophil levels could be inferred, but not proved. Analyses of the data without correction for skewness indicated the presence of a co-dominant mechanism while a model corrected for two distributions did not take into account the three genotypes. This probably explains why dominant or recessive models fit the data.

The genetic basis for the capacity to develop an eosinophilic response to helminthic infections may depend on the eosinophils themselves and/or on some external factor. Eosinophilia is lymphocyte T-cell dependent (Basten and Beeson, 1970), with interleukin 5 (IL-5) playing a major regulatory role, not only in eosinopoiesis, but also in eosinophil maturation/activation and in immunoglobulin A production. Segregation analysis has indicated the presence of a major gene controlling IL-5 production in humans infected with *S. mansoni* (Rodrigues *et al.*, 1996), while Rioux *et al.* (1998) showed that familial eosinophilia maps to the cytokine gene cluster. Using a similar approach, Abel *et al.* (1991) showed that the intensity of infection by *S. mansoni*, measured by fecal egg counts, is under the control of a major gene. These observations were latter confirmed by mapping this gene to chromosome 5q31-q33 (Marquet *et al.*, 1996; Müller-Myhsok *et al.*, 1997).

If the present results represent the individual's capacity to produce an efficient eosinophil response to in-

festation by helminths, then the factors involved in this phenomenon may be potentially separable by complex segregation and linkage analyses in an appropriate population, particularly since past contact with parasites and the population's demographic history should indicate the factor determining this phenotype.

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RESUMO

Uma amostra de 177 indivíduos pertencentes a 120 famílias nucleares, completas ou incompletas, de Bambuí, Estado de Minas Gerais, sudeste do Brasil, foi estudada com o objetivo de apurar algumas das causas da variabilidade da taxa de eosinófilos em pessoas parasitadas por vermes intestinais com ciclo de vida extra-digestivo. A análise de segregação, aplicada aos dados sem correção para a assimetria, mostrou que a hipótese de um gene principal aditivo é consistente com os dados, enquanto que as hipóteses que supõem a ação de um gene dominante, de um gene recessivo ou ainda herança multifatorial não explicam, adequadamente, a significante agregação familiar observada. A

correção mais parcimoniosa para assimetria mostrou resultados semelhantes, mas não permitiu a distinção entre os modelos dominante e recessivo, embora permitisse a rejeição do modelo codominante. Considerando que esse modelo supõe ser a assimetria devida ao entrelaçamento de duas distribuições, esses resultados parecem concordar com aqueles obtidos quando os dados não foram corrigidos. Pode-se sugerir que o papel desempenhado por vários fatores genéticos independentes na resistência/suscetibilidade à infestação por helmintos seja determinado, principalmente, por suas capacidades de agir no estabelecimento de uma resposta eosinofílica.

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