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# Segregation Analysis Indicates a Major Gene in the Control of Interleukine-5 Production in Humans Infected with *Schistosoma mansoni*

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## Summary

The Interleukine-5 (IL-5) is a hormone of the immune system that is the main regulator of eosinopoiesis, eosinophil maturation and activation, and Immunoglobulin A production. Thus, IL-5 contributes in several ways to human immune defenses against various pathogens, including helminths and infectious agents of the digestive and respiratory tracts. On the other hand, the increase in eosinophil number and the activation of these cells, which both have been related to elevated IL-5 production, are the cause of severe pathological disorders, as in asthma or hypereosinophilic syndromes. Although the immunological pathways leading to IL-5 synthesis have been identified, the reasons for the large variability observed in IL-5 production among subjects exposed to comparable antigenic stimulation are unknown. To investigate the role of genetic factors in this variability, we conducted a segregation analysis in a Brazilian population infected by the helminth parasite *Schistosoma mansoni*. The analysis was performed on IL-5 levels produced by blood mononuclear cells of these subjects after in vitro restimulation with either parasite extracts (IL-5/schistosomula sonicates [SS] phenotype) or a T-lymphocyte mitogen (IL-5/phytohemagglutinin [PHA]). The results provide clear evidence for the segregation of a codominant major gene controlling IL-5/SS and IL-5/PHA production and accounting for 70% and 73% of the phenotypic variance, respectively; the frequency of the allele predisposing to low IL-5 production was ~.22 for both phenotypes. No significant relationship was found between these genes and the gene controlling infection intensities by *S. mansoni* detected in a previous study. Linkage studies are ongoing to locate those genes that would help to characterize the genetic factors involved in pathological conditions such as severe helminth infections and allergic diseases.

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## Introduction

Interleukines are hormones that regulate the immune system and determine the nature and the intensity of the immune response. Alterations in interleukine production may have severe deleterious effects on host tissues and on host capacity to fight aggressive pathogens. The Interleukine-5 (IL-5) is produced mostly by T-lymphocytes and eosinophils and plays a major role in human protection against helminth infections. In particular, IL-5 is the principal hormone controlling eosinopoiesis (Sanderson et al. 1985; Clutterbuck and Sanderson 1988; Coffman et al. 1989; Dent et al. 1990; Tominoga et al. 1991), which is markedly stimulated in these infections. Not only are eosinophil precursor multiplication and differentiation stimulated by IL-5 (Sanderson et al. 1985; Clutterbuck and Sanderson 1988; Yamaguchi et al. 1988*b*), but IL-5 also enhances eosinophil attraction (Wang et al. 1989; Nakajima et al. 1992; Sehmi et al. 1992) and activation (Lopez et al. 1988; Yamaguchi et al. 1988*a*; Cöeffier et al. 1991) in the tissues where activated eosinophils are the main helminthotoxic cells (Butterworth et al. 1974, 1975; Butterworth 1977; Dessein and David 1982; Dessein et al. 1982, 1983, 1984). On the other hand, high eosinophilia is a hallmark of allergic diseases, and eosinophils have been associated with the pathological processes occurring in these conditions, especially in subjects with asthma (Hamid et al. 1991; Broide et al. 1992; Chand et al. 1992; Robinson et al. 1992; Krishnaswamy et al. 1993; Mauser et al. 1993; Van Oosterhout et al. 1993). In this disorder, activated eosinophils accumulate in bronchial tissues, where they secrete a number of nonspecific toxic mediators (Gleich et al. 1979) that contribute to bronchial damage and hyperreactivity. Eosinophils are also the cause of major tissue damage in subjects with hypereosinophilia (Spry and Tai 1976; Olsson and Venge 1979; Guerra-Caceres et al. 1980; Spry 1981; Wassom et al. 1981; Desreumaux et al. 1993). Finally, IL-5 is the principal lymphokine directing the Immunoglobulin (Ig) isotype switch toward IgA (Murray et al. 1987; Harriman et al. 1988; Sonoda et al. 1989) and may therefore contribute to defense against mucosal and gastrointestinal

bacterial infections (Childers et al. 1989; Iscaki and Bouvet 1993; Underdown and Mestecky 1994).

A number of studies have reported the variability of IL-5 production and have associated abnormal IL-5 levels, either too high or too low, with pathological disorders (Spry and Tai 1976; Guerra-Caceres et al. 1980; Spry 1981; Hamid et al. 1991; Chand et al. 1992; Mauser et al. 1993; Van Oosterhout et al. 1993) or with impairments of protective immunity (Korenaga et al. 1991; Sasaki et al. 1993; Lange et al. 1994). The origin of this variability, which is a central question to our understanding of the mechanisms of diseases caused by allergens or by certain pathogens, has not been addressed by these studies. The aim of the present work is to investigate this question, using segregation analysis, to evaluate whether the variability in IL-5 production could be accounted for by genetic factors. The data presented here provide evidence that IL-5 production, in a population exposed to a helminth infection (schistosomiasis), is markedly dependent on the effects of at least one major gene.

## Subjects, Material, and Methods

### *Study Subjects*

Study subjects live in a village of Bahia in Brazil, which is an endemic area for the parasite *Schistosoma mansoni*. The families and the conditions of parasite transmission including the measurements of individual exposure to infection have been extensively described in previous papers (Dessein et al. 1992; Demeure et al. 1993). The families studied here were part of a sample used in our previous segregation analysis on *S. mansoni* infection levels (Abel et al. 1991). IL-5 production was assessed on cells of 107 of 269 subjects of the first study. The selection among these 269 subjects was made on a voluntary basis, and these 107 subjects belong to 13 families (7 nuclear families and 6 pedigrees). The information available for each family member includes (1) IL-5 levels in supernatants of peripheral blood mononuclear cells (PBMCs) stimulated with either phytohemagglutinin (PHA), noted as IL-5/PHA, or with a helminth antigenic preparation, the soluble fraction of schistosomula sonicates (SS), noted as IL-5/SS; (2) the phenotype measuring susceptibility to *S. mansoni* infection determined from the fecal egg counts in our previous study (Abel et al. 1991), noted as SCHISTO phenotype; (3) epidemiological covariates as sex, age, and exposure to infection quantified by three classes of water contact (W) determined as described elsewhere (Demeure et al. 1993).

### *Immunological Methods*

*Cell culture.*—PBMCs were isolated from heparinized blood by centrifugation (400 g, 20 min at room temperature) on ficoll-hypaque gradient ≤3 h after blood collection. PBMCs were collected, were washed three times in RPMI medium (Gibco BRLK), and were resuspended in RPMI containing 50 μg/ml gentamicin (Gibco BRLK), 50 μM 2-Mercapto-Ethanol (Merck), 10% FCS (Gibco BRLK). Then, cells were distributed at a concentration of $2 \times 10^6$ cells per well in 24-well culture plates (Becton Dickinson) in presence of 2 μg/ml PHA (Sigma) or 5 μg/ml SS. They were cultured at 37°C in a 5% $CO_2$ atmosphere for 120 h; these culture conditions were found to allow optimal IL-5 production by PBMCs. Then, the supernatants were collected, centrifuged (400 g, 15 min at 4°C) and stored at −85°C until titration.

*IL-5 titration.*—Mouse monoclonal antibody (mAb), anti-human IL-5 (Genzyme), and rabbit antihuman IL-5 antibody (Genzyme) were used. High-affinity microplates (Nunc) were sensitized overnight with mouse antihuman IL-5 mAb diluted in carbonate buffer, pH 9.2. Nonspecific binding was reduced by incubating the plates with 3% bovine serum albumin (BSA) (Sigma) in phosphate buffer saline (PBS). Graded dilutions of culture supernatants and serial dilutions of standard human IL-5 were incubated overnight at 4°C. Plates were washed four times with PBS containing 0.05% Tween 20 and 0.1% BSA (PBS/Tween) and then incubated for 4 h with rabbit antihuman IL-5 antibody. After four washes with PBS/Tween, plates were incubated for 2 h with alkaline phosphatase conjugated goat antirabbit IgG. Finally, after four washes with PBS/Tween, enzymatic activity was revealed by incubation with 1 mg/ml p-nitrophenil phosphate in diethanolamine buffer pH 9.8. Absorbance at 495 nm was determined with a microplate reader (Biorad). The sensitivity of the IL-5 titration assay was 2 pg/ml. The reproducibility of the measurement method could not be extensively assessed, since repeat samplings of the same individuals were not possible for ethical and practical reasons. However, eight subjects who have been studied two or three times indicated a high reproducibility of IL-5 measurements, considering that these samplings were spaced several months apart. For IL-5/SS, the measurements were within a twofold dilution in seven subjects and within a threefold dilution in the remaining individual, whereas variations were slightly larger for IL-5/PHA with measurement values within a twofold to fourfold dilution.

### *Statistical Methods*

All analyses were performed on the logarithmic transformation of both the IL-5/SS and the IL-5/PHA levels as classically done for data measured using successive dilutions. Prior to segregation analysis, the influence of measured covariates on IL-5 phenotypes was assessed using analysis of variance for sex and W, and regression analysis for age. Covariates having a significant effect on a given IL-5 phenotype were retained for segregation

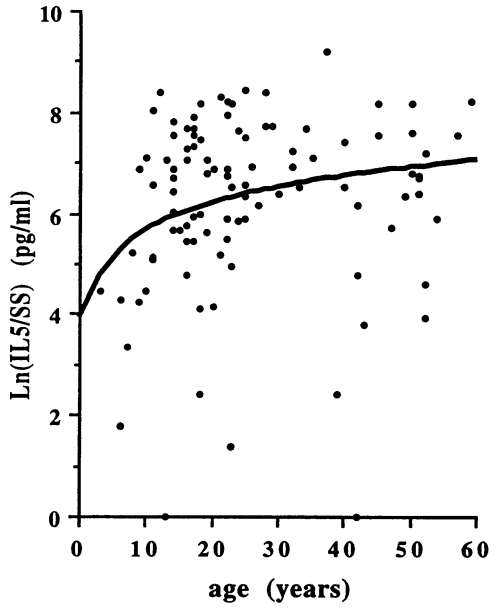


**Figure 1** Influence of age on ln(IL-5/SS). Results are presented as the individual ln(IL-5/SS) observations by age, and the curve represents the values predicted by the following regression on ln(age): ln(IL-5/SS) = 3.92 + .76ln(age).

analysis of this phenotype. Correlation between IL-5 phenotypes and the SCHISTO phenotype were also computed. All these analyses were carried out using the SAS software (SAS Institute).

Segregation analyses were performed using the regressive models for continuous phenotypes developed by Bonney (1984). These models are constructed by specifying a regression relationship between the phenotype of an individual and (1) a major effect, (2) the phenotypes of his preceding relatives, and (3) measured covariates. Under the hypothesis that there is a major gene, the major effect results from the segregation of two alleles (A, a) at a single locus. The parameters of the major gene effect are the frequency, $q$, of A, which will represent the allele predisposing to low IL-5 levels, and the three means of the phenotype, $\mu_{AA}$, $\mu_{Aa}$, $\mu_{aa}$, corresponding to the three genotypes AA, Aa, aa; for a given genotype, the distribution of the phenotype is assumed to be normal with residual variance $\sigma^2$. Parent-offspring transmission at the major locus is parameterized in terms of $\tau_{AAA}$, $\tau_{AaA}$, and $\tau_{aaA}$, which denote the probabilities of transmitting allele A for individuals AA, Aa, and aa, respectively. Mendelian transmission corresponds to $\tau_{AAA} = 1$, $\tau_{AaA} = 0.5$, and $\tau_{aaA} = 0$, and no parent-offspring transmission is represented by $\tau_{AAA} = \tau_{AaA} = \tau_{aaA}$. Besides the major effect, different patterns of dependence between an individual and preceding relatives can be considered and are simply expressed in terms of phenotypic correlations. In the class D model (Bonney 1984, 1986), used in this analysis, four types of correlations are defined: the father-mother, $\rho_{FM}$; the father-offspring, $\rho_{FO}$; the mother-offspring, $\rho_{MO}$; and the sib-sib, $\rho_{SS}$. The class D regressive model has been shown to include several patterns of dependence as particular cases, for example, the pure polygenic model when $\rho_{FM} = 0$ and $\rho_{FO} = \rho_{MO} = \rho_{SS}$ (Demenais and Bonney 1989). As we never observed a significant difference between $\rho_{FO}$ and $\rho_{MO}$, these parameters were set equal to a unique parameter, $\rho_{PO}$, representing the parent-offspring correlation. The effects of covariates are parameterized in terms of regression coefficients that can be genotype dependent and that will be noted as $\beta_g$.

The likelihood of the sample was computed by means of the computer program REGRESS (Demenais and Lathrop 1994), which incorporates the regressive approach into the LINKAGE package (Lathrop et al. 1984), as proposed by Bonney et al. (1988). Computations of the likelihood were performed under different hypotheses (or models) by using the approximation proposed by Demenais et al. (1990) to lessen the computing time under the class D model. Nested models were compared by means of the likelihood-ratio test. Evidence for a major gene effect is obtained by rejecting the null hypothesis of familial correlations without major gene against a model including a major gene and residual familial correlations. Two additional criteria concerning the parent-offspring transmission of the major effect are needed to confirm the presence of a major gene: (1) failure to reject the Mendelian transmission (Mendelian $\tau$'s) and (2) rejection of the no parent-offspring transmission (equal $\tau$'s) when compared with the general transmission model (free $\tau$'s).

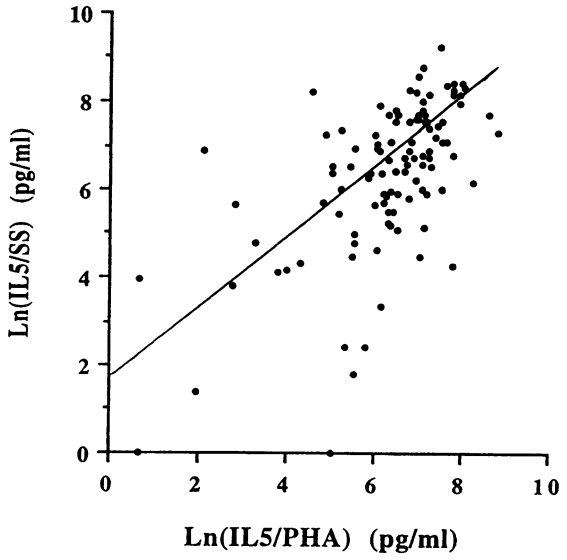


**Figure 2** Linear regression analysis between ln(IL-5/PHA) and ln(IL-5/SS) levels. The correlation coefficient is equal to .60, and the regression equation is the following: ln(IL-5/SS) = 1.78 + 1.05ln(IL-5/PHA)

**Table 1**

**Segregation Analysis of ln(IL-5/PHA)**

| Model and Hypothesis[a] | Parameters | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\mu_{AA}$ | $\mu_{Aa}$ | $\mu_{aa}$ | $\sigma^2$ | $q$ | $\tau_{AAA}$ | $\tau_{AaA}$ | $\tau_{aaA}$ | $\rho_{FM}$ | $\rho_{PO}$ | $\rho_{SS}$ | $-2\ln L + c$ |
| I. Sporadic | 6.27 | (=$\mu_{AA}$) | (=$\mu_{AA}$) | 2.21 | (0) | ... | ... | ... | (0) | (0) | (0) | 70.2 |
| II. FC | | | | | | | | | | | | |
| 1. All FC | 6.38 | (=$\mu_{AA}$) | (=$\mu_{AA}$) | 2.09 | (0) | ... | ... | ... | −.03 | .31 | .34 | 46.2 |
| 2. PO and SS | 6.37 | (=$\mu_{AA}$) | (=$\mu_{AA}$) | 2.06 | (0) | ... | ... | ... | (0) | .32 | .34 | 46.3 |
| III. Mendelian major gene | | | | | | | | | | | | |
| 1. Residual PO-SS | | | | | | | | | | | | |
| a. Codominant | 2.27 | 5.87 | 7.09 | .60 | .226 | (1) | (.5) | (0) | (0) | .12 | .00 | 4.8 |
| b. Dominant | 2.13 | (=$\mu_{AA}$) | 6.47 | 1.38 | .029 | (1) | (.5) | (0) | (0) | .19 | .43 | 21.5 |
| c. Recessive | 2.74 | 6.65 | (=$\mu_{Aa}$) | .98 | .282 | (1) | (.5) | (0) | (0) | .32 | .22 | 11.4 |
| 2. Codominant, no residual FC | 2.22 | 5.83 | 7.10 | .58 | .222 | (1) | (.5) | (0) | (0) | (0) | (0) | 5.1 |
| IV. Nontransmitted major effect | | | | | | | | | | | | |
| 1. Codominant + residual PO-SS | 2.59 | 6.80 | 6.46 | .97 | .332 | .23 | (=$\tau_{AAA}$) | (=$\tau_{AAA}$) | (0) | .32 | .29 | 15.2 |
| 2. Codominant | 2.24 | 6.94 | 6.31 | .85 | .366 | .25 | (=$\tau_{AAA}$) | (=$\tau_{AAA}$) | (0) | (0) | (0) | 29.7 |
| V. General transmitted major effect | | | | | | | | | | | | |
| 1. Codominant + residual PO-SS | 2.96 | 5.93 | 7.22 | .58 | .203 | .82 | .70 | .00 | (0) | .15 | .00 | .0 |
| 2. Codominant | 2.24 | 5.80 | 7.11 | .55 | .197 | .93 | .58 | .01 | (0) | (0) | (0) | 4.4 |

NOTE.—All parameters in parentheses are fixed at the value shown.
[a] FC = familial correlation; PO = parent offspring; and SS = sib-sib.

Linkage analyses using the LOD score method were conducted between the two major genes detected in the present segregation analysis controlling IL-5/SS and IL-5/PHA levels, respectively. Linkage analyses were also performed between these two genes and the major gene controlling the SCHISTO phenotype found in our previous segregation analysis (Abel et al. 1991). The major gene parameters used for these studies were the maximum likelihood estimates obtained by segregation analysis. All computations were performed using the LINKAGE package (Lathrop et al. 1984).

## Results

### *Influence of Age, Sex, and W on IL-5 Levels*

The mean (SD) of ln(IL-5/PHA) was 6.26 (1.49). No significant effects of sex ($P > .39$) and W ($P > .7$) were found. Age had no significant influence on ln(IL-5/PHA) levels. Therefore, no covariates were included in segregation analysis of ln(IL-5/PHA). The mean (SD) of ln(IL-5/SS) was 6.32 (1.76). Using two-way analysis of variance there was no significant effect on ln(IL-5/SS) levels of either sex ($P > .46$) or W ($P > .25$). Different functions of age were used to account for the dependence of age on ln(IL-5/SS), shown in figure 1: polynomial in age and logarithm of age since the major increase was observed for young ages. The best fitting function was a regression on ln(age) with the following equation: ln(IL-5/SS) = 3.92 + .76 ln(age); the values predicted by this equation are shown in figure 1. Consequently, the only covariate retained for segregation analysis was ln(age). As shown in figure 2, there was a highly significant correlation ($r = .60$, $P < 10^{-4}$) between ln(IL-5/SS) and ln(IL-5/PHA).

### *Segregation Analysis of IL-5/PHA*

Results are presented in table 1. The hypothesis of no familial correlations is rejected ($\chi^2 = 24.0$, 3 df, $P < .001$), and the spouse correlation is not significantly different from 0. In presence of parent-offspring and sib-sib correlations, there is strong evidence for a codominant major gene effect (model $II_2$ vs. $III_{1a}$, $\chi^2 = 41.5$, 3 df, $P < .001$). Both the recessive ($III_{1c}$ vs. $III_{1a}$) and the dominant ($III_{1b}$ vs. $III_{1a}$) hypothesis for the major gene effect are rejected. Familial correlations residual from the codominant major gene ($III_2$ vs. $III_{1a}$) are not significant ($\chi^2 = .4$, 2 df, $P > .5$). The Mendelian transmission of the codominant major effect ($III_2$ vs. $V_2$) is compatible with the data ($\chi^2 = .7$, 3 df, $P > .4$), and its nontransmission ($IV_2$ vs. $V_2$) is rejected ($\chi^2 = 25.3$, 2 df, $P < .001$). The same results concerning the transmission of the major effect are observed when $\rho_{PO}$ and $\rho_{SS}$ are estimated. In conclusion, a codominant major gene without residual familial correlations accounts for the familial distribution of ln(IL-5/PHA) and explains ~73% of its variance. The frequency of the allele predisposing to low IL-5/PHA production was estimated at .222 with a standard error (SE) of .056. The curves of figure 3*A* show the predicted distribution of ln(IL-5/PHA) under this model; the proportion of AA homozygotes with low production

## Table 2

**Segregation Analysis of ln(IL-5/SS)**

| Model and Hypothesis | Parameters | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\mu_{BB}$ | $\mu_{Bb}$ | $\mu_{bb}$ | $\sigma^2$ | $\beta_{BB}$[a] | $\beta_{Bb}$ | $\beta_{bb}$ | $q$ | $\tau_{BBB}$ | $\tau_{BbB}$ | $\tau_{bbB}$ | $\rho_{FM}$ | $\rho_{PO}$ | $\rho_{SS}$ | $-2\ln L + c$ |
| I. Sporadic | 3.92 | (=$\mu_{BB}$) | (=$\mu_{BB}$) | 2.85 | .76 | (=$\beta_{BB}$) | (=$\beta_{BB}$) | (0) | ... | ... | ... | (0) | (0) | (0) | 63.6 |
| II. Familial correlations (FC) | | | | | | | | | | | | | | | |
| 1. All FC | 5.03 | (=$\mu_{BB}$) | (=$\mu_{BB}$) | 2.55 | .45 | (=$\beta_{BB}$) | (=$\beta_{BB}$) | (0) | ... | ... | ... | .03 | .35 | .21 | 35.1 |
| 2. PO and SS | 5.01 | (=$\mu_{BB}$) | (=$\mu_{BB}$) | 2.54 | .47 | (=$\beta_{BB}$) | (=$\beta_{BB}$) | (0) | ... | ... | ... | (0) | .34 | .21 | 35.2 |
| III. Mendelian major gene | | | | | | | | | | | | | | | |
| 1. Residual PO-SS | | | | | | | | | | | | | | | |
| a. Codominant | .31 | 4.42 | 5.95 | .82 | .41 | (=$\beta_{BB}$) | (=$\beta_{BB}$) | .210 | (1) | (.5) | (0) | (0) | .23 | .19 | 3.3 |
| b. Codominant, interaction | 3.23 | 4.14 | 6.19 | .82 | −.57 | .51 | .34 | .219 | (1) | (.5) | (0) | (0) | .31 | .25 | .0 |
| c. Recessive | .67 | 5.27 | (=$\mu_{Bb}$) | 1.43 | .44 | (=$\beta_{BB}$) | (=$\beta_{BB}$) | .228 | (1) | (.5) | (0) | (0) | .34 | .33 | 9.6 |
| d. Dominant | .20 | (=$\mu_{BB}$) | 5.02 | 1.53 | .52 | (=$\beta_{BB}$) | (=$\beta_{BB}$) | .026 | (1) | (.5) | (0) | (0) | .34 | .29 | 11.6 |
| 2. No residual FC | | | | | | | | | | | | | | | |
| a. Codominant | −.21 | 3.88 | 5.65 | .74 | .53 | (=$\beta_{BB}$) | (=$\beta_{BB}$) | .209 | (1) | (.5) | (0) | (0) | (0) | (0) | 9.1 |
| b. Codominant, interaction | 1.85 | 3.62 | 5.64 | .71 | −.17 | .61 | .54 | .212 | (1) | (.5) | (0) | (0) | (0) | (0) | 7.3 |
| IV. Nontransmitted major effect | | | | | | | | | | | | | | | |
| 1. Codominant + residual PO-SS | 1.03 | 4.66 | 6.21 | 1.09 | .30 | (=$\beta_{BB}$) | (=$\beta_{BB}$) | .098 | .24 | (=$\tau_{BBB}$) | (=$\tau_{BBB}$) | (0) | .44 | .62 | 9.8 |
| 2. Codominant | .25 | 4.22 | 6.07 | .76 | .37 | (=$\beta_{BB}$) | (=$\beta_{BB}$) | .099 | .24 | (=$\tau_{BBB}$) | (=$\tau_{BBB}$) | (0) | (0) | (0) | 31.0 |
| V. General transmitted major effect | | | | | | | | | | | | | | | |
| 1. Codominant + residual PO-SS | .78 | 4.43 | 6.16 | .97 | .32 | (=$\beta_{BB}$) | (=$\beta_{BB}$) | .104 | .67 | .43 | .12 | (0) | .41 | .58 | 2.4 |
| 2. Codominant | −.24 | 3.85 | 5.63 | .74 | .54 | (=$\beta_{BB}$) | (=$\beta_{BB}$) | .207 | 1.00 | .48 | .00 | (0) | (0) | (0) | 9.1 |

NOTE.—All parameters in parentheses are fixed at the value shown.
[a] Regression coefficient on ln(age), which can be genotype dependent.

of IL-5/PHA is ~5%, and levels of Aa heterozygotes are close to those of aa homozygotes.

### *Segregation Analysis of IL-5/SS*

Results are presented in table 2 with a pattern similar to IL-5/PHA. For this analysis, the alleles of the major gene will be noted "B" and "b," "B" being the allele predisposing to low IL-5/SS levels. There is strong evidence for a codominant major gene effect (model $II_2$ vs. $III_{1a}$, $\chi^2 = 31.8$, 3 df, $P < .001$). Both the recessive ($III_{1c}$ vs. $III_{1a}$) and the dominant ($III_{1d}$ vs. $III_{1a}$) hypotheses for the major gene effect are rejected with $\chi^2 = 6.3$ (1 df, $P < .01$) and $\chi^2 = 8.3$ (1 df, $P < .005$), respectively. Familial correlations residual from the codominant major gene ($III_{2a}$ vs. $III_{1a}$) are borderline nonsignificant ($\chi^2 = 5.8$, 2 df, $P > .05$). No significant interaction between the major gene and ln(age) is found whether residual familial correlations are taken into account ($III_{1a}$ vs. $III_{1b}$, $\chi^2 = 3.3$, 2 df, $P > .15$) or not ($III_{2a}$ vs. $III_{2b}$, $\chi^2 = 1.8$, 2 df, $P > .3$). In the presence of residual familial correlations, the Mendelian transmission of the codominant major effect ($III_{1a}$ vs. $V_1$) is compatible with the data ($\chi^2 = .9$, 3 df, $P > .5$), and its nontransmission ($IV_1$ vs. $V_1$) is rejected ($\chi^2 = 7.4$, 2 df, $P < .05$). The same pattern of results concerning the transmission of the major effect are observed when $\rho_{PO}$ and $\rho_{SS}$ are fixed at 0. Although the interaction between a Mendelian major gene and ln(age) was not significant, tests of transmission were also performed including this interaction and led again to both failure to reject the Mendelian transmission and rejection of the nontransmission hypothesis (not shown in table 2). In conclusion, a codominant major gene with or without residual familial correlations accounts for the familial distribution of ln(IL-5/SS) and explains ~70% of its variance. The allelic frequency estimated at .209 (SE = .057) indicates that ~4% of the subjects are BB homozygotes predisposed to low levels of IL-5/SS, and 32% are Bb heterozygotes with IL-5/SS levels much closer to bb than to BB homozygotes (fig. 3*B*).

### *Linkage Analyses*

LOD score results are presented in table 3. For linkage between the major genes controlling IL-5/SS and IL-5/PHA levels, the highest LOD score is +1.27 at a recombination rate of 0%. However, it should be said that most of the linkage information came from one large pedigree (+1.05 at 0%). Two smaller families provided some information in favor of linkage (total value of +.29 at 0%), whereas the remaining families yielded LOD scores close to 0. This lack of information was due to the small size of some nuclear families and to the fact that parents were likely homozygous according to their IL-5 values. Families were also not very informative for linkage with the SCHISTO phenotype providing slightly negative LOD scores so that no definitive conclusion could be reached. It can be also noted that no significant correlation was found between the SCHISTO phenotype and ln(IL-5/SS) and ln(IL-5/PHA) ($r = .08$ and $r = -.02$, respectively).

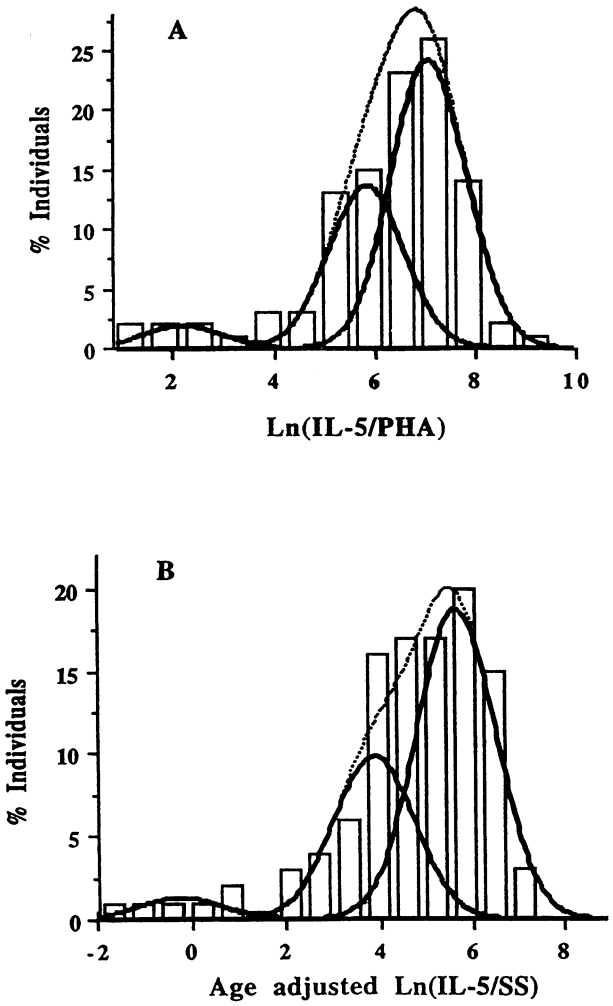


**Figure 3** Histogram of (*A*) the ln(IL-5/PHA) and (*B*) the ln(IL-5/SS) values. The three plain curves represent the predicted normal distribution of the trait within each genotype under a codominant major gene model for (*A*) ln(IL-5/PHA) (from model $III_2$ in table 1) and (*B*) ln(IL-5/SS) (from model $III_{2a}$ in table 2). The dotted line corresponds to the mixture of the three normal distributions.

## Discussion

The results presented here provide clear evidence for the segregation of a codominant major gene controlling the production of IL-5 by blood mononuclear cells stimulated by either a polyclonal activator (PHA) or an helminth antigenic preparation (SS). For both IL-5/PHA and IL-5/SS, this gene has a major effect, since it accounts for 73% and 70% of the phenotypic variance, respectively. The frequency of the allele predisposing to low IL-5 production was close for both stimulation conditions and estimated around .22. The similarity of major gene parameters raises the possibility that the two detected genes could be a single gene. Linkage analysis showing a positive LOD score of 1.27 at 0% recombination between these two genes is consistent with this view. However, there were very few informative families for this linkage analysis, and whether these two genes are identical will be decided by further linkage analyses with genetic markers. In this view, the first candidate for the detected gene could be the gene encoding IL-5, which comprises four exons and is mapped on chromosome 5 close to *IL-13, IL-4, IL-3,* and *GM-CSF* genes (Campbell et al. 1987; Van Leeuwen et al. 1989) in a region shown to be linked with the genetic control of asthma (Postma et al. 1995) and Immunoglobulin E levels (Marsh et al. 1994; Meyers et al. 1994).

IL-5 is the major eosinopoietic lymphokine (Sanderson et al. 1985; Clutterbuck and Sanderson 1988; Coffman et al. 1989; Dent et al. 1990; Tominoga et al. 1991), and eosinophils are produced in large numbers in helminth-infected subjects by IL-5-dependent mechanisms (Coffman et al. 1989; Tominoga et al. 1991; Sasaki et al. 1993). A number of in vitro and in vivo observations indicate that these cells may play a critical role in protection against these parasites by destroying their larvae (Butterworth et al. 1977; Dessein et al. 1982). We have shown in a previous study carried out on the same subjects that human susceptibility to infection by the helminth *S. mansoni* is controlled by a major gene (Abel et al. 1991); this genetic effect has been associated with the regulation of the balance between T lymphocytes producing mostly interferon γ (Th1-like T-cell subset) and those producing mostly IL-4 and IL-5 (Th2-like T-cell subset) (Demeure et al. 1993; Couissinier-Paris and Dessein 1995); thus, this gene might have some relationship to the gene described in the present study. The linkage analyses study between the gene controlling susceptibility to infection and the one controlling IL-5 levels is, however, not conclusive, and further linkage work with genetic markers will be required before any conclusion can be reached.

Although the heterogeneity of IL-5 production has been recognized in human population studies including subjects with schistosomiasis (Roberts et al. 1993), the possible genetic origin of this heterogeneity has never been analyzed and this report is, to our knowledge, the

**Table 3**

**Pairwise LOD Score Analysis Between the Major Genes Detected by Segregation Analysis Controlling the Three Following Traits: IL-5/PHA, IL-5/SS, and the SCHISTO Phenotype Assessing the Levels of Infection by *S. mansoni***

| | LOD Score at θ = | | | | | |
|---|---|---|---|---|---|---|
| Tested Linkage | .00 | .05 | .10 | .20 | .30 | .40 |
| IL-5/PHA and IL-5/SS | +1.27 | +1.08 | +.89 | +.55 | +.27 | +.08 |
| IL-5/PHA and SCHISTO | −.26 | −.20 | −.16 | −.09 | −.05 | −.02 |
| IL-5/SS and SCHISTO | −.63 | −.48 | −.36 | −.18 | −.07 | −.02 |

first study searching for major gene control of interleukine production in humans. The fact that this work was performed on a population of an endemic area of schistosomiasis does not imply that the conclusions are only valid under these specific study conditions. Helminth infections strongly stimulate IL-5 and more generally Th2-type lymphokine production; these strong stimulation conditions, which are relatively uniform among study subjects, who are permanently exposed to infection by schistosomes (Dessein et al. 1992), increase the probability of detecting genetic effects by reducing the effect of environmental variables on IL-5 phenotype. Finally, the in vitro restimulation of the cells with a mitogen, PHA, which is a polyclonal activator for T-lymphocytes, allows evaluation of the total capacity of IL-5 production by the patient T-cells irrespective of their antigen specificity.

In conclusion, the detection by segregation analysis of major gene(s) controlling IL-5 production is an important step in the characterization of genetic factors that can influence pathological conditions such as severe helminth infections and allergic diseases. To investigate this question further, studies are ongoing in the same Brazilian population, including comparable genetic studies on other lymphokines and genotyping of the population.

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