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$Fc_\varepsilon R1$-β Polymorphism and Total Serum IgE Levels in Endemically Parasitized Australian Aborigines

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Summary

Endemic helminthic infection is a major public-health problem and affects a large proportion of the world's population. In Australia, helminthic infection is endemic in Aboriginal communities living in tropical northern regions of the continent. Such infection is associated with nonspecific (polyclonal) stimulation of IgE synthesis and highly elevated total serum IgE levels. There is evidence that worm-infection variance (i.e., human capacity of resistance) and total serum IgE levels may be related to the presence of a major codominant gene. The beta chain of the high-affinity IgE receptor, $Fc_\varepsilon R1$-β, has been previously identified as a candidate for the close genetic linkage of the 11q13 region to IgE responses in several populations. We show a biallelic *Rsa*I polymorphism in $Fc_\varepsilon R1$-β to be associated with total serum IgE levels ($P = .0001$) in a tropical population of endemically parasitized Australian Aborigines ($n = 234$ subjects). The polymorphism explained 12.4% of the total residual variation in serum total IgE and showed a significant ($P = .0000$) additive relationship with total serum IgE levels, across the three genotypes. These associations were independent of familial correlations, age, gender, racial admixture, or smoking status. Alleles of a microsatellite repeat in intron 5 of the same gene showed similar associations. The results suggest that variation in $Fc_\varepsilon R1$-β may regulate IgE-mediated immune responses in this population.

Introduction

Endemic helminthic infection is a major public-health problem in developing tropical countries and affects a large proportion of the world's population (Grove 1991). In Australia, helminthic infection is endemic in Aboriginal communities living in tropical northern regions of the continent (Gracey 1992; Meloni et al. 1993). Such infection is associated with nonspecific (polyclonal) stimulation of IgE synthesis and with highly elevated total serum IgE levels (Lynch et al. 1983; King et al. 1993; Pritchard et al. 1995). IgE antibodies may protect chronically exposed humans from reinfection by parasites (Sher and Coffman 1992; Watanabe et al. 1993), and there is evidence that both worm-infection variance (i.e., human capacity of resistance) (Dessein et al. 1992; Rodrigues et al. 1996) and total serum IgE levels (Meyers et al. 1982; Martinez et al. 1994) may be related to the presence of a major codominant gene.

Close genetic linkage of the 11q13 region to serum IgE responses has been reported in several populations (Cookson et al. 1989; Young et al. 1992; Collée et al. 1993; Shirakawa et al. 1994*a;* Hizawa et al. 1995; Boguniewicz and Hayward 1996; Daniels et al. 1996). The gene for the beta chain of the high-affinity receptor ($Fc_\varepsilon R1$-β) has been identified as a candidate (Sandford et al. 1993; Shirakawa et al. 1994*b*) for the observed linkage, and coding and noncoding polymorphisms within the gene have been related to serum total IgE levels and other measures of atopy (Shirakawa et al. 1994*b*, 1996; Hill et al. 1995; Hill and Cookson 1996).

The aims of this study were to investigate the genetic regulation of IgE immune responses in a non-Caucasian population and to explore possible associations between the pathogenic mechanisms underlying atopic disease and immune responses to parasitization. We therefore investigated polymorphisms in the $Fc_\varepsilon R1$-β gene, for associations with total serum IgE titers in an isolated and endemically parasitized indigenous Australian population.

Subjects and Methods

Study Population

The indigenous population studied came from a tropical Aboriginal community in the coastal Kimberley region of northwestern Western Australia (14°18′ south

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latitude, 126°139′ east longitude). A survey of the community was performed in April 1993. All individuals >4 years of age who were present during the survey were studied, a total of 234 subjects, of whom 171 were from 19 interrelated pedigrees and 63 were genetically unrelated individuals.

Pedigrees were constructed on the basis of histories given by the subjects, and inheritance was checked by the genotyping of all subjects by polymorphic genetic markers (data not shown). The 19 pedigrees studied comprised 5 two-generation families and 14 three-generation families. On average, families comprised 9 members (range 2–37). On average, each father had 2.0 offspring and each mother 2.8 offspring; multiple paternity was present within nine sibships.

Informed, personal, or parental consent was obtained from all subjects, with the assistance of local community-health workers who were employed in the project. This study was approved by the Human Rights Committee of the University of Western Australia and by the Council of the Aboriginal community (National Health and Medical Research Council 1991).

Data Collection

Individual and family histories of respiratory symptoms, demographic information, and smoking were assessed at interview using the British Medical Research Council questionnaire (Medical Research Council 1965). Questionnaires relating to children were administered to a parent (generally the mother).

Modifications to the questionnaire were made with the aid of the local medical officer, by translation of questions into the local idiom, as required. Stated age was verified by community health records and census data. Ancestry was defined as "Aboriginal" if there were no known non-Aboriginal ancestors. "Admixture" was defined as one or more non-Aboriginal parents or grandparents. "Smoking" in subjects was defined as current cigarette smoking, assessed by questionnaire.

Blood was taken by venipuncture from all subjects (n = 234), for IgE assays and DNA studies. Venous blood was collected into polypropelene centrifuge tubes containing ETDA (anticoagulant).

IgE Assays

Measurement of total IgE was undertaken by use of the Pharmacia FEIA CAP system (Pharmacia Diagnostics Sweden). Standard controls were included in the assays. Because of the presence of very high titers in most of the samples, sera for the determination of total IgE were diluted 1:5 and were assayed, giving a range of 10–10,000 kU/liter. Twenty sera were diluted 1:21 and were reassayed.

Molecular Analysis

DNA was extracted from whole-blood samples by standard phenol-chloroform extraction. The genomic DNA of all individuals was genotyped for three polymorphisms within the $Fc_\varepsilon R1$-β gene (table 1): (1) biallelic *Rsa*I polymorphism in intron 2 (Shirakawa et al. 1996) ($Fc_\varepsilon R1$-β_*Rsa*I[in2]), (2) biallelic polymorphism in the UTR of exon 7 ($Fc_\varepsilon R1$-β_*Rsa*I[ex7]), and (3) a CA-repeat microsatellite marker in intron 5 (Daniels and Shirakawa 1994) ($Fc_\varepsilon R1$-β_CA). The $Fc_\varepsilon R1$-β_*Rsa*I(ex7) polymorphism has not been described elsewhere.

All PCR amplifications were performed in a Hybaid Omnigene™ thermal cycler (block control). Genotyping and phenotyping were performed double blind. $Fc_\varepsilon R1$-β *Rsa*I (intron 2) detection was performed by PCR with the following oligonucleotide primers: (*a*) 5′-TCT GTC TGT CGA GAA TGT TGC-3′ and (*b*) 5′-CTG GTT AGA TCT GAG AAA GAG-3′. Genomic DNA samples (150 ng) were amplified in a total volume of 15 μl containing 0.3 μM each primer, 200 μM each dNTP, 2.0 mM Mg^{2+}, 67 mM Tris-HCl (pH 8.0), 16 mM $(NH_4)_2SO_4$, 0.01% TWEEN-20, and 0.5 units *Taq* DNA polymerase (Bioline UK), overlaid with mineral oil. Amplification conditions were 34 cycles of 94°C for 1 min, 59°C for 1 min, and 72°C for 2 min. After confirmation of successful PCR, 5 μl PCR product was digested in a final volume of 10 μl with 0.75 U *Rsa*I enzyme (New England Biolabs), with the recommended buffer, for 1 h at 37°C. Digested products were then separated on 2% agarose gels. Three bands potentially resulted from the digestion of PCR products: *AA* (absence of restriction site on both alleles), *AB* (heterozygous), or *BB* (presence of restriction site on both alleles) (Shirakawa et al. 1996). An additional, constant 148-bp band was present as a result of the primers used; the predigestion PCR product contained a second, nonpolymorphic *Rsa*I restriction-site (confirmed by sequencing).

$Fc_\varepsilon R1$-β *Rsa*I (exon 7) detection was performed by PCR with the following oligonucleotide primers: (*a*) 5′-TCA CTG TGT ATC ATG CTA AGC-3′ and (*b*) 5′-TGA TAC AAT ACT GCA TCG TGG-3′. Genomic DNA samples (100 ng) were amplified in a total volume of 15 μl containing 0.5 μM each primer, 200 μM dNTPs, 1.5 mM Mg^{2+}, 67 mM Tris-HCl (pH 8.0), 16 mM $(NH_4)_2SO_4$, 0.01% TWEEN-20, and 0.75 units DNA *Taq* Polymerase (Bioline UK), overlaid with mineral oil. Amplification conditions were 32 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. After confirmation of successful PCR, 5μl of PCR product was digested in a final volume of 10 μl with 0.75 U *Rsa*I enzyme (New England Biolabs), with the recommended buffer, for 1 h at 37°C. Digested products were then separated on 2% agarose gels. Three sets of bands potentially resulted from the digestion of PCR products: two 481-bp bands (*AA* genotype), a 481-, a 295-, and a 187-bp band (*AB* genotype), or a 295- and a 187-bp band (*BB* genotype).

Table 1

Allele Frequencies of the $Fc_{\varepsilon}R1$-β Polymorphism in an Aboriginal Community

Marker and Allele	Allele Size (bp)	Allele Frequency in (%)	
		Aborigines	Caucasians[a]
*Rsa*I exon 7 UTR (*Rsa*I_ex7):			
A	...	92.3[b]	59
B	...	7.7	41
*Rsa*I intron 2 (*Rsa*I_in2):			
A	...	35[c]	12
B[c]	...	65	88
Intron 5 microsatellite repeat ($Fc_{\varepsilon}R1$-β_CA):			
A	116	1.2[d]	41
B	118	15.5	1
C	120	4.2	31
D	122	62	22
E	124	4.9	1
F	126	.7	...
G	128	.5	2
H	130	.2	...
I	132	10.8	...

[a] General-population sample of 230 Western Australian Caucasian nuclear families (1,000 subjects genotyped). The population and field methods used have been described in detail elsewhere (Hill et al. 1995).
[b] Genotypes obtained on 232 subjects.
[c] *Rsa*I(in2) allele *B* prevalence ≈18% in a Japanese population (Shirakawa et al. 1996).
[d] Genotypes obtained on 227 subjects.

Genotyping for the microsatellite $Fc_{\varepsilon}R1$-β_CA (Daniels and Shirakawa 1994) was performed by PCR with the following oligonucleotide primers: (*a*) GT strand, 5′-ATC TAC TGC AAG TGA CGA TC 3′; and (*b*) CA strand, 5′-CAT CTC CCT ACC ATC TGA CC-3′. The GT strand was fluorescently labeled with tetrachloro-6-carboxyfluorescein phosphoramidite. Genomic DNA samples (50 ng) were amplified in a total volume of 10 μl containing 0.5 μM each primers, 200 μM each dNTP, 1.5 mM Mg^{2+}, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 0.1% Triton X-100, and 0.25 units *Taq* DNA Polymerase (Bioline), overlaid with mineral oil. Amplification conditions were 28 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 45 s. Fluorescently labeled PCR products were then genotyped as described elsewhere (Reed et al. 1994).

Statistical Analysis

The primary response variable modeled was total serum IgE titer. Smoking (current smoker = 1; nonsmoker = 0), admixture status (one or more Caucasian parents or grandparents = 1; no Caucasian parent or grandparent = 0), and gender (male = 1; female = 0) were analyzed as binary covariates. Age was analyzed as a continuous covariate. Total serum IgE titers exhibited a skewed distribution with a long right-hand tail and were $\log_e$ transformed prior to analysis.

Linear regression was used to identify significant predictors of total serum IgE levels. Analysis of variance (ANOVA) was used to evaluate differences in genotype-specific means for total serum IgE levels. Statistical significance was taken at the 5% level.

Association analysis of the three markers with total serum IgE levels was performed by use of a maximum-likelihood technique that allows for familial correlations of genotypes and phenotypic traits (George and Elston 1987). The ASSOC module of the software package S.A.G.E. (SAGE 1994) was used to estimate fixed effects and to partition observed phenotypic variance into "polygenic" and residual environmental components, by use of maximum likelihood. Models reported in this paper invoke the assumption that the distribution of the response phenotype is multivariate normal, with a mean that depends on the particular set of explanatory covariates that it includes. Another assumption of this method is the presence of polygenic correlations in families; the residual error variance is partitioned into polygenic and environmental components.

The biallelic $Fc_{\varepsilon}R1$-β *Rsa*I polymorphism (intron 2) and the biallelic $Fc_{\varepsilon}R1$-β *Rsa*I polymorphism (exon 7) were coded into three classes (*AA* = 1; *AB* = 2; *BB* = 3) and were analyzed categorically as two binary (1,0) dummy variables relative to genotype *AA*. The alleles of the $Fc_{\varepsilon}R1$-β_CA polymorphism were coded into two

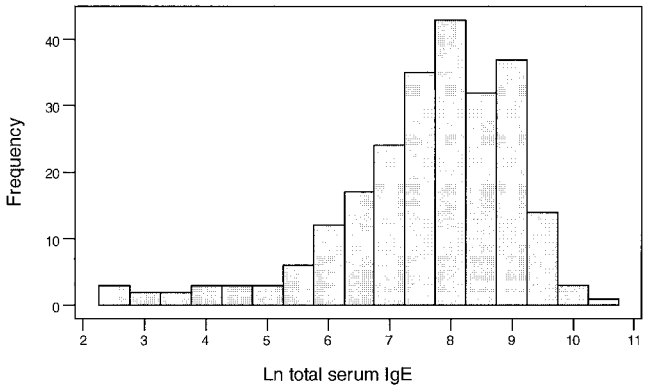

Figure 1 Distribution of ln total serum IgE levels in Aboriginal study population.

classes, separately for each allele (allele present = 1; allele absent = 0). Variables potentially influencing serum total IgE levels, such as smoking and age, were included as covariates in the models. Age was centered at its mean and was modeled as a linear, quadratic, and cubic fixed effect, in order to adjust for any nonlinear effects on total serum IgE levels. ASSOC was also used to estimate genotype-specific mean differences and SDs relative to a baseline genotype.

The statistical association of covariates entered as fixed effects and total serum IgE levels was formally assessed by removal of terms from the mean model, followed by calculation of the likelihood-ratio test statistic (Khoury et al. 1993). The asymptotic distribution of the test statistic was assumed to be $\approx\chi^2_p$ when p terms were removed from the model.

Results

Characteristics of Study Population

The gender ratio was balanced; 108 male (46.1%) and 126 female subjects were studied. The mean age was 25.1 years (standard error [SE] = 1.2 years). Twenty-eight subjects (12.0% of the population) had a Caucasian parent or grandparent. Among adults (i.e., individuals ⩾18 years of age), 54% were current cigarette smokers and 26.2% were current chewers of tobacco. Among children (i.e., individuals <18 years of age), 7% currently smoked and 7% chewed tobacco. The prevalence of physician-diagnosed asthma among adults was 15.0%, and that among children was 14.8%.

As with other remote Aboriginal communities in Western Australia (Meloni et al. 1993), living conditions and hygiene remain poor and intestinal parasites are common. A comprehensive parastite survey of the entire community was conducted in February 1993 (Hopkins et al., in press). *Ancylostoma duodenale* (hookworm) infection was found in 77% of the population, and *Hymenolepis nana* (tapeworm) was found in 23%. Among children 5–14 years of age, these prevalences rose to 93% and 53%, respectively. Given these prevalences, it is likely that most or all individuals in the population would have been exposed to recurrent helminthic infection. Furthermore, these estimates of prevalence are likely to underestimate the true prevalence, since they were based on only one fecal examination. Hookworm infection was associated with substantial morbidity in this population, particularly with iron-deficiency anemia (Hopkins et al., in press). The total IgE levels of the subjects (n = 233; geometric mean total IgE = 2,008 kU/liter; SE = 289 kU/liter) were, on average, ~40-fold higher than those in Western Australian Caucasian general population samples (e.g., see Hill et al. 1995) (fig. 1).

Interrelationships between Total IgE Levels and Other Factors

Linear regression indicated that age (regression coefficient [β] = −.023; SD = .006; P = .000) and smoking status (β = .593; SD = .218; P = .007) were significant predictors of $\log_e$ serum total IgE levels. Gender (β = .096; SD = .204; P = .640), admixture status (β = −.470; SD = .305; P = .124), and the presence of physician-diagnosed asthma (β = .463; SD = .276; P = .094) were not significantly associated with $\log_e$ total serum IgE levels.

Genetic Analysis

The allele frequencies for all three polymorphisms in the Aboriginal population were markedly different from those found in a comparison Australian Caucasian general-population sample (table 1). Association analysis using the ASSOC module of S.A.G.E indicated a significant association between $\log_e$ total serum IgE levels adjusted for familial correlations and both the $Fc_\epsilon R1$-β_CA polymorphism and the $Fc_\epsilon R1$-β_*Rsa*I(ex7) polymorphism (table 2). The $Fc_\epsilon R1$-β_*Rsa*I(ex7) polymorphism

Table 2

Association between $Fc_\epsilon R1$-β Polymorphisms and $\log_e$ Total Serum IgE

Polymorphism	χ^2 [a]	P
*Rsa*I_in2	1.30[b]	.5
CA repeat:		
Allele *A*	8.57[c]	.003
Allele *E*	8.69[c]	.003
Alleles *A* and *E*	15.8[c]	.0001
*Rsa*I_ex7	25.8[b]	.0000

[a] Derived from likelihood-ratio statistic.
[b] With 2 df (marker coded into three classes).
[c] With 1 df (marker coded into two classes).

Table 3

Genotype-Specific Mean Values and Mean Differences for Log_e Total Serum IgE Levels for $Fc_\varepsilon R1$-β *Rsa*I_ex7 Genotypes

Genotype (*N*)	Genotype-Specific Mean (SD)[a]	Mean Difference[b]	Adjusted Mean Difference[c] (SD)
AA (200)	7.806 (1.255)	Reference	Reference
AB (28)	6.580 (2.002)	−1.226	−.846 (.276)
BB (4)	4.655 (1.036)	−3.151	−2.784 (1.077)

[a] Unadjusted, from ANOVA.
[b] Unadjusted, from ANOVA; data are relative to *AA* genotype.
[c] Relative to *AA* genotype, from ASSOC model, adjusted for age, smoking status, admixture status, and familial correlations.

showed the strongest evidence of association and was therefore investigated further in extended analyses.

Total serum IgE levels were stratified by the genotypes of the $Fc_\varepsilon R1$-β_*Rsa*I(ex7) polymorphism (table 3). ANOVA indicated that the $Fc_\varepsilon R1$-β_*Rsa*I(ex7) genotype was significantly associated with the unadjusted total serum IgE level ($F_{2,236} = 19.656$; $P = .0000$). A formal test for linear trend, in total serum IgE level, across the three genotypes of $Fc_\varepsilon R1$-β_*Rsa*I(ex7) indicated a significant ($F_{1,237} = 38.703$; $P = .0000$) additive relationship with total serum IgE levels: mean total serum IgE levels were highest for the *AA* genotype, lower for the *AB* genotype, and lowest for the *BB* genotype.

Extended modeling using the ASSOC module of S.A.G.E. included age, gender, smoking status, racial admixture, and $Fc_\varepsilon R1$-β_*Rsa*I(ex7) as covariates. The most reasonable parsimonious model, estimated from the Akaike (1977) information criterion, was the model including age, smoking status, admixture, and $Fc_\varepsilon R1$-β_*Rsa*I(ex7) as covariates (table 4). The $Fc_\varepsilon R1$-β_*Rsa*I(ex7) genotype remained significantly associated with total serum IgE level, after adjustment for age, smoking status, admixture status, and familial correlations ($\chi^2_2 = 17.73$; $P = .0000$). The additive trend in genotype-specific mean total serum IgE also remained after adjustment for these factors (table 3). Estimation of the familial and nonfamilial (environmental) components of residual variance (George and Elston 1987) indicated that the inclusion of the $Fc_\varepsilon R1$-β_*Rsa*I(ex7) polymorphism in the model explained ~12.4% of the total residual IgE variance and that this was associated with a reduction in the "polygenic" (familial) component of residual variance (table 4).

Discussion

The marked elevation of total serum IgE levels in this Aboriginal population compared with Australian Caucasian populations suggests the polyclonal IgE stimulation characteristic of helminthic infestation. Total serum IgE levels are closely associated with clinical asthma in nonparasitized Caucasian populations (Sears et al. 1993; Sunyer et al. 1995). In contrast, highly elevated total serum IgE levels that exhibit reduced associations with atopic disease are characteristic of endemically parasitized tropical populations (Perdomo de Ponce et al. 1991; Lynch et al. 1992; Hagel et al. 1993). Our results are consistent with these findings. However, although the

Table 4

Association between Log_e Total Serum IgE and $Fc_\varepsilon R1$-β *Rsa*I Exon 7 Polymorphism, with Age, Smoking, and Racial Admixture Included in Model

		Variance (SD)					
Model	Variables Included	Familial ("Polygenic")	Nonfamilial	Total Residual Variance (% Previous Model)	Log_e Liklihood	$\chi^{2\,a}$	*P*
1	. . .	1.033 (.391)	.808 (.296)	1.8421 (100.0)	−391.360	. . .	. . .
2	Age, smoking, admixture	1.100 (.426)	.600 (.313)	1.700 (92.3)	−353.771	75.19[b]	.0000
3	Age, smoking, admixture, *Rsa*I_ex7	.676 (.391)	.814 (.318)	1.490 (87.6)	−344.904	17.73[c]	.0001

[a] Derived from likelihood-ratio statistic.
[b] With 3 df; model 2 vs. model 1.
[c] With 2 df (marker coded into three classes); model 3 vs. model 2.

raised IgE levels were most likely a response to endemic helminthic infestation, a direct causal association was not able to be proved.

There is evidence that total serum IgE levels are controlled by a major gene acting independently of specific responses to allergens (Dizier et al. 1995). Work at the cellular level has suggested that $Fc_\varepsilon R1$-β genetic variants on mast cells may have a direct regulatory effect on IgE synthesis, through enhanced IL-4 production, and may also make the receptor more sensitive to ligand (Ravetch 1994). Our study demonstrates association between polymorphism in the $Fc_\varepsilon R1$-β gene and total serum IgE levels in an isolated, non-Caucasian population. These results suggest that a functional polymorphism in linkage disequilibrium with $Fc_\varepsilon R1$-β_CA and $Fc_\varepsilon R1$-β_*Rsa*I(ex7) has exerted a significant regulatory influence on serum total IgE levels in the presence of endemic parasitization. The results may thus represent molecular evidence of a gene with a major role in the regulation of human immune response to helminthic infection. However, further genetic studies, which include direct measures of helminthic infection from fecal samples, are necessary to clarify the role of $Fc_\varepsilon R1$-β in the regulation of human immune response to parasitization.

Segregation analysis has suggested the presence of major genes influencing serum total IgE levels not only in parasitized peoples but also in nonparasitized Caucasians (Dessein et al. 1992; Martinez et al. 1994; Dizier et al. 1995). Life-style changes and improved public health in developed nations such as Australia have resulted in a relatively low prevalence of parasitic infection (Grove 1991; Gracey 1992) but have also been associated with a marked increase in the incidence and severity of asthma and other atopic disorders (Ninan and Russell 1992; Peat et al. 1994). Our study raises the possibility that immune responses to parasitization and the predisposition to atopic disease may share common genetic mechanisms.

Together with recent molecular studies of the genetic regulation of human resistance to parasites (Marquet et al. 1996), the results of the current study are consistent with the hypothesis that human immune responses to parasitic infections are under a significant level of genetic control. The results are also consistent with the hypothesis that helminthic infections may have provided the selective pressure for high-IgE-response alleles that now predispose to atopic disease in nonparasitized populations.

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