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Analysis of the 5q31-q33 Locus Shows an Association between IL13-1055C/T IL-13-591A/G Polymorphisms and *Schistosoma haematobium* Infections[1]

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Millions of humans are exposed to schistosome infections, which cause severe kidney and liver disease and 280,000 deaths annually. Th2-mediated immunity is critical to human defenses against this pathogen and susceptibility to infection is controlled by a major genetic locus that includes IL4, IL5, and IL13 genes. These observations led us to evaluate whether certain polymorphisms in IL4, IL5, or IL13 determine schistosome infection. The study was performed in two Dogon villages where *Schistosoma haematobium* is endemic. Schistosome infections were evaluated by counting eggs and measuring worm Ags in urine. Genetic polymorphisms were determined by restriction enzyme analysis or by primer extension and denaturing high-performance liquid chromatography analysis. Associations were tested using family-based association tests and logistical regression analysis. The alleles IL13-1055C ($p = 0.05$) and IL13-591A ($p = 0.01$) are shown, by family-based association test, to be preferentially transmitted to children with the 10% highest infections. A logistic regression analysis that included IL13-1055 G/G, G/T and T/T genotypes, age, gender, and village of residency, applied to the whole study population, showed that subjects bearing the IL13-1055T/T genotype were on average much less infected than individuals with other genotypes. Previous studies on asthma indicated that the IL13-1055T allele increased gene transcription, which is in agreement with the fact that this cytokine enhances resistance to infection by schistosome in humans. *The Journal of Immunology,* 2005, 174: 6274–6281.

Schistosomiasis is the fourth most prevalent disease in the world and the second leading parasitic disease after malaria (1). It comprises a group of chronic diseases caused by helminth digenetic trematode of the *Schistosoma* genus. *Schistosoma mansoni* and *Schistosoma haematobium* inhabit the mesenteric and vesical venous plexus, respectively, where female worms lay on average 300–3,000 eggs a day. Eggs may pass into feces (*S. mansoni*) or urine (*S. haematobium*) or remain trapped in the tissues, mainly in liver or in the bladder and ureter walls, and cause major pathological disorders. *S. mansoni* causes hepatic fibrosis associated with portal blood hypertension that is often lethal. *S. haematobium* causes obstructive nephropathy that is often aggravated by urinary bacterial infections (2–4). In endemic regions, 5–20% of the population is affected by severe schistosomiasis and the annual number of deaths is 250,000–300,000 worldwide (5).

In a previous report, we have studied the prevalence and intensities of infection by *S. haematobium* in two Dogon communities of Mali and show that high infections are clustered in certain families suggesting some hereditary components in the control of infection. In contrast, we have also shown that infection levels by *S. mansoni* in a Brazilian population are controlled by a major locus on chromosome 5q31-q33 (6, 7). This result was further confirmed by an independent study in Senegal (8). This genetic region contains several genes related to immune function including the Th2 cluster that contains IL4, IL5, and IL13 genes (9–11). We and others have shown that sterile immunity in human schistosomiasis is dependent on IgE levels and eosinophils (12–15) and on the Th1/Th2 balance (16, 17). Altogether, these observations led us to test whether any allelic variants in the 5q31-q33 region would predispose to high *S. haematobium* infection and the IL4, IL5, and IL13 genes were excellent candidates. Our data confirm that polymorphisms in the IL13 gene promoter are associated with susceptibility to *Schistosoma* infection.

Materials and Methods

Study area-study population

This study was conducted in the populations of two Dogon villages (Boul and Ségué) in the district of Bankass, 200 km from Mopti in Mali. All study subjects were Dogon. Boul and Ségué were built up a hillside and at the top of the hill, respectively. The populations of these two villages are

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Table I. *Description of the primer sets used in the characterization of the promoters of the IL4, IL5, and IL13 genes*[a]

Genes	Primer Set	Primer Sequences 5′-3′ (Forward and Reverse)	Position on the Reference Sequence (NT_007072)
IL4	First	AAA CAA GCA GGG CGC	1937486/1937501
		TAG CAG TAG GTG CGT GGT	1937771/1937788
	Second	AAA TGA GCC AGG CAT GGT GC	1937747/1937766
		CTG CAT AGA GGC AGA ATA ACA GGC	1938083/1938107
	Third	TTG CCA AGG GCT TCC TTA TGG G	1937944/1937965
		CAG TGT CCG AAT TTG TTG TAA TG	1938241/1938265
	Fourth	CTT GAT ATT ACT CTA TCT TTC C	1938207/1938228
		CCA ATC AGC ACC TCT CTT CAG	1938453/1938475
IL5	First	GGA TCC TAA TCA AGA CCC CA	1808605/1808625
		ACA TCA TTG CCC CAC ATT TG	1808339/1808359
	Second	ATT CTG TTT AGT GGT CT	1808422/1808439
		TTC TGC GTT TGC CTT TG	1808086/1808103
IL13	First	CTG GCG CTC GGA CCG CCA CCT TT	1920888/1920911
		TTA TCC CCC ATG GCC ACC AAA	1921064/1921084
	Second	CCC TAT CTC CGC GGG CCA TC	1921157/1921178
		GAC CCC TCT ACG GGC CTGTTC	1921297/1921319
	Third	GGC CCT CTA CTA CAG ATT AGG AAA	1921277/1921301
		CCG TCC TGT CCT CGA TGG GGC T	1921546/1921568
	Fourth	TGA CAT CAA CAC CCA ACA GGC AAA	1921646/1921666
		ATG GGC CAT CCT TCT GGA AAC	1921933/1921955
	Fifth	TAG GGG AGA AAT CTT GAC ATC AAC	1921628/1921651
		ATC CTT CTG CCA AAC TCT TAA C	1921926/1921948
	Sixth	CCA GCC TGG CCC AGT TAA GAG TTT	1921913/1921937
		CTA ATT CCT CCT TGG CCC CAC T	1922204/1922226
	Seventh	CAC AGT GGG GCC AAG GAG GAA TTA	1922201/1922225
		TGC CCT GAG TTC CAG CCA GCT TT	1922482/1922506
	Eighth	TAC TCA GGG TTG TCA CAG GCA AAA	1922460/1922483
		CCT GGC GTC TTG TGG CAG CTT T	1922741/1922764

[a] The sets of primers used to amplify the human IL4, IL5 and IL13 genes were designed with the Oligo.O.4 software. The sequence and the position of each primer are indicated (reference sequence used GenBank: NT_007072).

interrelated because marriages between subjects from the two villages are frequent. All health and educational equipments (a primary care unit and a primary school) are located in Ségué. A seasonal (June to January) river provides water for drinking, domestic use, and irrigation to both villages. The inhabitants of Ségué also have access to water from a deep well. Small dams have been built in the river to allow the cultivation of vegetables from October to June. Agriculture, gathering, and breeding are the main economical activities. The study was performed on all subjects older than 4 years (693 subjects in Ségué and 148 in Boul) except those (2%) who refused to participate or were traveling. All the subjects included in this study have signed a consent form.

Parasitological methods

S. haematobium infections were quantified by counting eggs in urine (18) and by measuring serum levels of circulating anodic Ag (CAA)[4] produced by the adult worms (19). Individual infection levels are the arithmetic mean egg counts on three to seven samples. To ensure quality control of the egg count, 10% of the filters were randomly selected and recounted by another microscopist. No *S. mansoni* eggs were encountered in feces samples taken on 2 different days from 200 young subjects.

Blood samples and DNA preparation

Five to 15 ml of blood were collected on sodium citrate and kept at −20°C until DNA was extracted using the standard salting-out method (20).

PCR conditions

PCR amplifications were conducted on a robocycler gradient 96 (Stratagene) in a 30-μl reaction containing 100 ng of DNA, 10 mM Tris-HCl, pH 9, 0.1% Triton X-100, 50 mM KCl, 0.2 mg/ml BSA, 1.5 mM MgCl2, 1 μM of each primer, 1 mM of dNTP and 1.5 U of *Taq* polymerase (Appligene). Following the initial denaturation step (94°C, 5 min), samples were subjected to 35 cycles of PCR consisting of 94°C for 1 min, annealing temperature for 45 s and 72°C for 45 s.

[4] Abbreviations used in this paper: CAA, circulating anodic Ag; SSCP, single-stranded conformational polymorphism; TEAA, triethylamine acetate buffer; SNP, single nucleotide polymorphism; dd, dideoxy.

Single-stranded conformational polymorphism (SSCP) analysis

PCR products were analyzed by SSCP (21). A total of 20 μl of PCR product was mixed with 20 μl of 0.2N NaOH solution and denatured at 95°C for 5 min. Twenty microliters of loading buffer were added to the denatured products before electrophoresis on acrylamide gel in 1× Tris boric acid EDTA buffer at 7 mA (constant amperage) for 16 h at 4°C or room temperature. After which the gel was stained for 10 min in ethidium bromide solution (1 μg/ml). The analysis was performed at 4°C and at room temperature to increase the detection power of the analysis. The overlapping sets of primers, used in this analysis, are described in Table I.

Sequencing

Purified PCR products were sequenced using ABI Prism BigDye Terminator cycle sequencing system (Applied Biosystems) on the ABI Prism 310 automatic sequencer. Sequencing reactions were performed on both strands.

Allelic typing

Five polymorphisms have been identified in this study. The IL4-590T/C polymorphism revealed by SSCP analysis as previously described. The IL13-1258A/G polymorphism destroys a *Bse*RI restriction site. So, population typing was performed by restriction analysis of the corresponding PCR products as specified by the enzyme manufacturer (New England Biolabs).

The polymorphism IL13 arg130gln was previously described by Graves et al. (22). A 236-bp PCR fragment including the IL13 arg130gln polymorphism was generated with use of the primers 5′-GCAAATAATGA GCTTTCGAAGTTTCAGTGG-3′ and 5′-CTTCCGTGAGGACTGAAT GAGACGGTC-3′. The underlined bases were modified to create *Nla*IV restriction sites. The *Nla*IV restriction analysis of the PCR product obtained from subjects bearing the IL13 *arg130glnA* allele gives two bands (210 and 26 bp) on acrylamide gel whereas the same analysis performed on subjects bearing the IL13 *arg130glnG* allele gives three bands (178, 32, and 26 bp).

The IL5-202C/T, IL13-1055C/T, and IL13-591A/G polymorphisms were typed by primer extension reactions and denaturing high-performance liquid chromatography analysis of the products. Previous to the primer extension reaction, unincorporated primers and dNTPs were removed by

treating 5 μl of each PCR with 0.5 U of shrimp alkaline phosphatase and 5 U of exonuclease I (both from Amersham). The reactions were incubated 20 min at 37°C, after which the enzymes were inactivated by incubation at 80°C for 15 min. Primer extension reactions were conducted in 20 μl containing 50–60 ng of the template fragment (the purified PCR product), 1 μM of the appropriate dideoxy (dd) NTPs, 15 pmol primer, and 0.5 U of Thermo Sequenase (Amersham), in the buffer provided by the manufacturer. The reaction was performed in a thermal cycler with an initial denaturation step of 1 min at 96°C followed by 50 cycles of 96°C for 10 s, 43°C for 15 s, and 60°C for 1 min. At the end of thermal cycling, the reaction was heated to 96°C for 30 s and immediately placed on ice. Separation of the extended primers was then performed by denaturing high-performance liquid chromatography on a Wave HPLC instrument (Transgenomic). Fifteen microliters of the primer extension reaction were loaded on a SaraSep DNASep column (Transgenomic) at 70°C. Samples were eluted from the column using a linear acetonitrile gradient in a 0.1 M triethylamine acetate buffer (TEAA), pH 7, at a constant flow rate of 0.9 ml/min. The gradient was created by mixing eluents A (0.1 M TEAA) and B (25% acetonitrile in 0.1 M TEAA). The gradient was 18–28% buffer B over 5 min for both single nucleotide polymorphisms (SNPs). Data were acquired using a UV detector at 260 nm. The extension primers were 5′-AGGGATTGTCAAAGTTCA-3′ for IL13-591A/G, 5′-ATTAACCC AAAGATTCTT-3′ for IL5-202C/T, and 5′-GGGTTTCTGGAGGAC TTC-3′ for IL13-1055C/T. The appropriate ddNTPs were ddCTP for IL13-1055C/T and IL13-591A/G whereas ddGTP was used for the IL5-202C/T.

Statistical analysis

Linkage disequilibrium analysis was performed on the Genepop web site (⟨http://wbiomed.curtin.edu.au/genepop/index.html⟩). Associations between individual polymorphisms and infection levels were sought by use of the Family-Based Association Test Package (FBAT; version 1.2) (23). As other family-based association tests, this method is not susceptible to bias due to population admixture and tests for excess transmission of a particular allele from parents to affected offspring. The phenotype under study (infection level) depends on several covariates; some of these covariates could be confounder for the effect of others and their effect on the phenotype must be tested simultaneously (multivariate analysis). The multivariate method used here is logistic regression that specifies a regression relationship between the probability of an individual to develop a high infection level and various covariates, as follows:

$P(M^+/X_1,X_2,\ldots X_p) = [1 + exp\{-(\alpha + \Sigma\beta_i X_i)\}]^{-1}$, where $P(M^+/X_1,X_2,\ldots X_p)$ is the probability of being affected knowing X_1 to X_p covariates; α and β are constants and estimated in the analysis. The analysis tests whether β_i is significantly different from zero. Note that $exp\{\beta_i\}$ is the odds ratio associated with the covariate X_i, which measures the strength of the association between ξ and the phenotype, taking into account (adjusted to) the other covariates. With the stepwise procedure one can select the covariates significantly ($p < 0.05$) associated with the risk of being affected. The statistical SPSS software (version 10.0) was used for this analysis. Statistical data presented in this manuscript were not corrected for multiple testing. However, if we are applying these corrections, the main association stays significant.

Nuclear extract preparation

Nuclear extracts were prepared from PHA-stimulated fresh human peripheral blood T cells as described previously for 2 days, washed under acid conditions and cultured in 10% RPMI 1640 for 1 day without PHA before stimulation (24). Then cells were either left unstimulated or stimulated with IL-4 (100 ng/ml) or stimulated with 10 ng/ml PMA + 1 μg/ml ionomycin (I) for 30 min. Cell pellets were resuspended in lysis buffer (50 mM KCl, 25 mM HEPES, pH 7.8, 0.5% Nonidet P-40, 1 mM PMSF, 10 μg/ml leupeptin, 20 μg/ml aprotinin, 100 mM DTT), and subsequently incubated on ice for 5 min. Nuclei were collected by centrifugation at 2000 rpm, the supernatant was harvested as the cytoplasmic protein fraction. Nuclei were washed in buffer A without Nonidet P-40 and harvested at 2000 rpm. Nuclear pellets were resuspended in extraction buffer (500 mM KCl, 25 mM HEPES, pH 7.8, 10% glycerol, 1 mM PMSF, 10 μg/ml leupeptin, 20 μg/ml aprotinin, 100 mM DTT), frozen in dry ice, thawed slowly on ice, and finally centrifuged at 14000 rpm for 10 min. The supernatant was harvested and nuclear proteins quantified with the bicinchoninic acid protein assay reagent (Pierce).

EMSA

Complementary single-stranded oligonucleotides were commercially synthesized to span ~10 bp on either side of the variant nucleotide as follows: IL13-591, 5′-agacctggaa(a/g)tctgaacttt-3′.

Complementary strands were annealed by combining 2 μg of each oligonucleotide and 6 μl of 10× annealing buffer (500 mM Tris, 100 mM $MgCl_2$ and 50 mM DTT) in a 60-μl reaction, placing them in a boiling water bath for 5 min, and allowing them to cool to room temperature. Then, 2 μl of the double-stranded oligonucleotide probes were labeled with [^{32}P]dCTP. The DNA-protein binding reaction was conducted in a 20-μl reaction mixture consisting of 7 μg of nuclear protein extract from each cell condition, 1 μl of 1 mg of poly(dI-dC) (Sigma-Aldrich), 4 μl of 5× binding buffer (60 mM HEPES, 7.5 mM $MgCl_2$, 300 mM KCl, 1 mM ethylenediamine-tetraacetic acid, 2.5 mM DTT, 50% glycerol, and [4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride]) and 1.5×10^4 cpm of ^{32}P-labeled oligonucleotide probe (25). The DNA-protein binding reaction was incubated at room temperature for 20 min, then loaded on a 6% non-denaturing polyacrylamide gel and run for 2 h at 140 V.

Results

Selection of study subjects for the genetic analysis

This study was conducted on subjects with high infection levels and their parents. Occasionally, the genotype of a missing parent was inferred from two siblings. Because infection intensities and prevalence were shown to be lower in Ségué than in Boul, the infection intensity (120 eggs/ml urine) defining the 10% most infected subjects in Ségué was used as general cut-off value in both villages to define the high infection subjects who were included in the analysis. Fig. 1 shows that this cut-off was met by 16.4% of Boul inhabitants. Fig. 1 also confirms that the highest infections were observed in Boul: 0.8% and 2.1% of inhabitants of Ségué and Boul, respectively, excreted >960 eggs. All subjects with infection levels >120 eggs/20 ml urine (three to six independent experiments) were included in the study if their parents had given blood. When there was more than one child excreting >120 eggs/20 ml in a nuclear family, the child with the highest infection was selected.

The levels of CAA in serum (up to 5 ng/ml) were described as strongly correlated with egg excretion in schistosome infection (19, 26). CAA titration was performed on both villages. All subjects with CAA concentrations over 1854 pg/ml were included in the study. This value is defining the 10% highest concentration of CAA in Ségué and was used as the general cut-off value in both villages. The analysis includes 10% of Ségué subjects and 15% Boul subjects.

The search for polymorphisms in the promoters of the IL4, IL5, and IL13 genes was performed on 989, 502, and 1876 bp for IL4, IL5, and IL13, respectively, because these regions contain transcriptional regulatory elements.

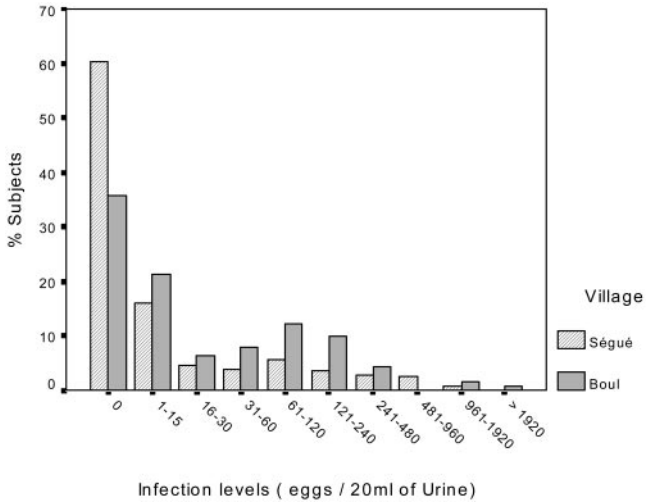

FIGURE 1. Description of infection intensities and prevalence in Ségué and Boul villages.

IL4 SNP analysis

Three sets of primers were used to detect polymorphisms within the IL4 gene. The second and third primer sets, that are overlapping, detected the same polymorphism (data not shown). PCR products from several subjects were sequenced in both directions (data not shown). Sequence alignments provided evidence that these two primer sets detected the IL4-590C/T polymorphism previously described (27). The first and fourth sets of primer did not detect additional mutation.

IL5 SNP analysis

No polymorphism has yet been reported in the IL5 gene. Two sets of primers were used to analyze the promoter (Fig. 2*A*). The second set of primers detected one mutation by SSCP analysis. The PCR products, obtained with this set of primers, revealed different patterns in subjects 1028 or 1029 suggesting the existence of one polymorphism (Fig. 2*B*). PCR products from these subjects were sequenced on both strands. Chromatograms and sequence alignments showed a G to A transition at position −202 from the transcription start (Figs. 2, *C* and *D*). Subject 1028 is heterozygous (G/A) whereas subject 1029 is homozygous (G/G) at this position.

IL13 SNP analysis

Eight sets of primer were necessary to study the IL13 promoter (Fig. 2*A*). PCR products, obtained with the third set of primers, from subject 945 showed different patterns compared with the patterns from subject 965 (Fig. 2*E*). The sequence of the PCR products showed an A to G transition at position −1258 from the transcription start (Fig. 2, *F* and *G*). This transition destroys a *Bse*RI restriction site. The fourth set of primers also detected a polymorphism corresponding to the previously described IL13-1055 polymorphism (data not shown) that has also been referred to as IL13-1111. Finally, PCR products, obtained with the sixth primer set, from subjects 559 and 554, identified an A to G transition at the position −591 from the transcription start (Fig. 2, *H–J*) that creates a *Drd*I restriction site.

Distribution of the polymorphisms in the study population

To these five polymorphisms was added the polymorphism IL13 Arg130Gln which has been associated with increased serum IgE levels in three different populations with asthma (22). The frequencies of these various polymorphisms, in the parents, are shown in Table II. Statistical analysis showed that these polymorphisms were in Hardy-Weinberg equilibrium ($\chi^2 < 5.99$).

Family-based studies to test for an association between polymorphisms and high infection levels

The family-based association test was used to analyze the transmission of alleles from the heterozygous parents to high infection children (23). The most frequent alleles, in the study sample, were IL4-590T, IL5-202C, IL13-1258A, IL13-1055C, IL13-591A, and IL13 Arg130Gln G. In an additive model, polymorphism IL13-1055C/T showed a trend for an association. Indeed, 29 children, born to heterozygous parents, had the IL13-1055C allele, as compared with the expected number ($n = 23$) based on the hypothesis of no association ($Z = 1.94$ and $p = 0.05$). For the polymorphism

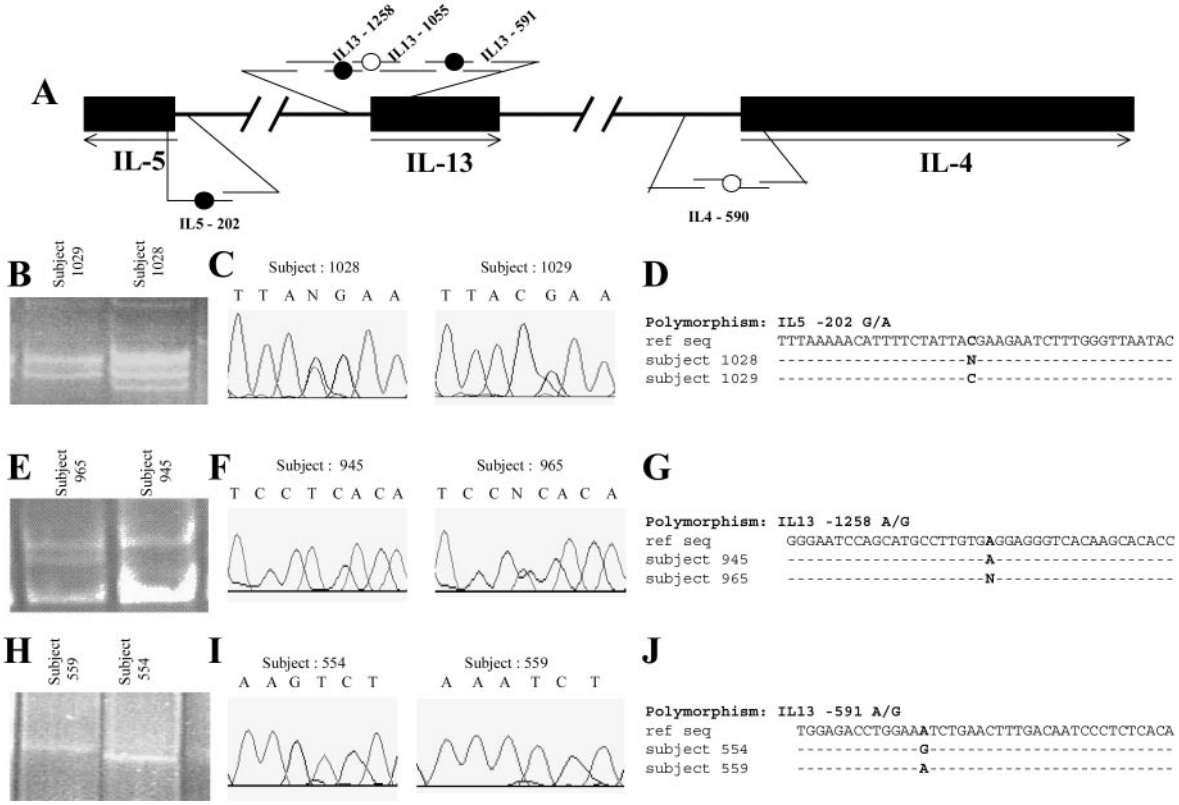

FIGURE 2. Analysis and characterization of new polymorphisms in IL5 and IL13 genes. The search for polymorphisms in the promoter of the IL4, IL5, and IL13 genes was performed by SSCP. It allows us to identify five polymorphisms located in the promoters of these genes (*A*). Three of these polymorphisms were not described previously (IL5-202C/T, IL13-1258A/G and IL13-591A/G). SSCP patterns are described respectively in *B*, *E*, and *H*. PCR products obtained from several patients were sequenced on both strands and chromatograms are provided in *C*, *F*, and *I*. In the IL5 gene, a G to A transition at position −202 from the transcription start was identified (*D*). In IL13 genes, two polymorphisms located in the promoter induce, respectively, A to G transition at position −1258 (*G*) and −591(*J*) from the transcription start.

Table II. *Frequencies of the polymorphisms in the population of Ségué/Boul*[a]

Polymorphisms		Frequencies on Parents (%)	Hardy-Weinberg Equilibrium Test χ^2 (p Value)
IL4-590	T/T	24 (58.5)	
	T/C	16 (39.0)	0.79 (0.67)
	C/C	1 (2.5)	
IL5-202	C/C	41 (97.6)	
	C/T	1 (2.4)	0.01 (0.99)
	T/T	0 (0)	
IL13-1258	A/A	24 (60.0)	
	A/G	14 (35.0)	0 (1)
	G/G	12 (5.0)	
IL13-1055	C/C	14 (27.0)	
	C/T	32 (61.5)	3.53 (0.17)
	T/T	6 (11.5)	
IL13-591	A/A	30 (56.6)	
	A/G	20 (37.7)	0.02 (0.99)
	G/G	3 (5.7)	
IL13 Arg130Gln	G/G	28 (57.1)	
	G/A	18 (36.7)	0.002 (0.99)
	A/A	3 (6.2)	

[a] The genotype distributions of the six polymorphisms, on the parents, did not deviate from the Hardy-Weinberg equilibrium.

IL13-591A/G, 21 children had received the IL13-591A allele, which is significantly different from the number ($n = 15.8$) predicted based on the hypothesis of no association ($Z = 2.44$ and $p = 0.01$) (Table III). In a recessive model, for the polymorphism IL13-591A/G, 9 children, born to heterozygous parents, had the IL13-591A allele, which is different ($Z = 2.94$ and $p = 0.003$) from the expected number ($n = 4.3$) based on the hypothesis of no association (Table III). These results suggest that alleles IL13-1055C and IL13-591A are associated with an increased risk of infection.

Multivariate analysis between IL13 polymorphisms and high infection levels

The results described above suggest that alleles IL13-1055C and IL13-591A are associated with an increased risk of infection. A significant linkage disequilibrium was found between these two polymorphisms ($p < 10^{-5}$). To test this observation further, we genotyped the whole study population for the IL13-1055C/T and IL13-591A/G polymorphisms. We tested for an association between subject genotypes and infection levels by linear regression (stepwise procedure) including all variables that are known to affect infection by schistosomes in that population (age, gender, and village of origin). This analysis showed that age ($p < 10^{-3}$), gender ($p < 10^{-3}$), village ($p < 10^{-3}$), and IL13-1055C/T genotype ($p = 0.04$) were all significantly associated with infection in the whole population (and IL13-591A/G was excluded). This result was more significant when the analysis was limited to the village where exposure and infection levels were the highest (Boul) (genotype, $p = 0.002$). IL13-1055C/C and IL13-1055C/T were associated with the highest infection levels, whereas IL13-1055T/T was associated with the lowest infection levels. This result is illustrated in Fig. 3 which shows the average egg excretion for the three genotypes in the whole study population.

Multivariate analysis between polymorphisms and high CAA concentrations

The effect of the IL13-1055 genotype could be due to an effect on egg excretion and/or on the schistosome larvae, the elimination of which is associated with strong IgE and eosinophil immune responses (12–15). To determine which of these hypotheses is correct, we quantified circulating worm Ags (CAA), which are an indicator of worm load, in the blood of all villagers and looked for an association with the IL13-1055 genotypes. The IL13-1055T/T genotype was associated with lower CAA levels in blood than the other two genotypes (Fig. 4). To further test this result, we performed a multivariate analysis, including other variables known to affect CAA levels. In the whole population, using a linear regression analysis, we found a trend ($p = 0.1$) toward an association between IL13-1055 genotypes and CAA levels when taking into account age ($p < 10^{-3}$) and the village of origin ($p < 10^{-3}$) (the other variables were excluded). When the study was limited to Boul village, the significance of the association increased ($p = 0.02$). Thus, IL13-1055 genotypes are also associated with worm

Table III. *Analysis of association between IL4, IL5, and IL13 polymorphisms and infection levels in subjects with schistosomiasis* (S. haematobium[a])

Polymorphisms	Allele Frequency[b]	Number of Informative Family[c]	Risk Allele	*S* Statistic		*Z* Score[d]	*p* Value
				Observed[e]	Expected[f]		
Additive model							
IL4-590T/C	0.80	11/33	T	14	16	−1.16	0.25
IL5-202C/T	0.99	1/21	C	N/A	N/A	N/A	N/A
IL13-1258A/G	0.76	13/33	A	18	16.3	0.93	0.35
IL13-1055C/T	0.57	23/33	C	29	23	1.94	0.05
IL13-591A/G	0.73	14/33	A	21	15.83	2.44	0.01
IL13 Arg130gln	0.74	11/35	G	13	13	0	1
Recessive model							
IL4-590T/C	0.80	11/33	T	3	5.25	−1.37	0.17
IL5-202C/T	0.98	1/21	C	N/A	N/A	N/A	N/A
IL13-1258A/G	0.76	10/33	A	5	4.8	0.11	0.92
IL13-1055C/T	0.57	16/33	C	9	6.14	1.52	0.13
IL13-591A/G	0.73	11/33	A	9	4.33	2.97	0.003
IL13 Arg130Gln	0.74	5/35	G	4	3	1.06	0.28

[a] Thirty-five nuclear families were include in this transmission disequilibrium test analysis. It includes 15 complete trios (father mother and the affected child) and 20 uncomplete trios (10 (one parent + the affected child and one unaffected sibling), 2 (one parent + the affected child and two unaffected siblings), 1 (one parent + the affected child three unaffected siblings), 7 (one parent + the affected child).

[b] Calculated from the genotype of parents.

[c] With at least one heterozygous parent.

[d] $Z = (S - E(S))/Var(S)$, where S is the test statistic (i.e. observed), $E(S)$ is the expected value according to the null hypothesis (H0), and $Var(S)$ is the variance of the statistic test according to H0.

[e] Number of transmitted allele.

[f] Based on the hypothesis of no linkage disequilibrium.

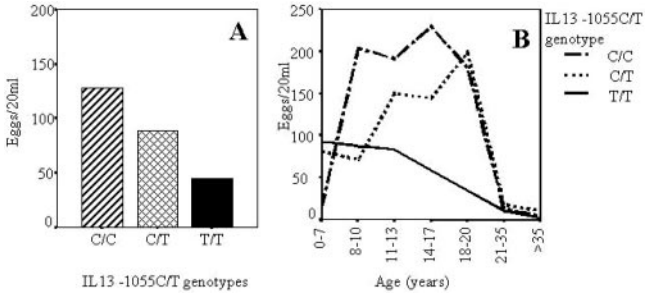

FIGURE 3. IL13-1055T is associated with protection against infection in the whole population. Infection levels were quantified by counting eggs in urine as in Table I. IL13-1055 genotypes of $n = 462$ subjects were typed by primer extension. Average mean egg excretion is shown for the subjects with the different IL13-1055 genotypes (*A*). Infection levels by IL13-1055 genotypes in the different class of ages. Age classes were defined as to include the same number of subjects (*B*).

load, with IL13-1055T/T subjects having the lowest worm load. The fact that this association was weaker than that with eggs suggests that IL13 may be involved in protective immunity against both larvae and eggs.

The IL13-591A/G polymorphism does not modify the binding of nuclear factors

We examined whether IL13-591A/G SNP modified putative transcriptional regulatory motifs (⟨www.gene-regulation.com/pub/programs/alibaba2/index.html⟩). A sequence motif (tggagaacctg gaaatctgaactttgacaa) incorporating the SNP at position −591 is predicted to bind the transcription factor Oct-1 (motif sequence: ATGCAAAT). The A to G transition identified at the position −591 from the transcriptional start site alters this predicted binding motif. It raises the possibility that this polymorphism results in differential DNA-protein interactions, resulting in altered regulation of IL-13 production. EMSA, using nuclear extracts from PHA-activated human peripheral T cells that were stimulated with IL-4 or PMA + ionomycin, was performed to determine whether DNA protein interactions were altered by the polymorphisms at either site. The IL13-591G allele formed only one DNA protein complex as the IL13-591G allele. The complex does not depend on the kind of stimulation applied (Fig. 5). Competition with cold probes or unspecific probe (SP-1: ggggaggcgtggcctgggcggactggg gagtggcga) does not alter this complex formation. Under these stimulation conditions, however, no difference in DNA-protein interaction was observed. Thus, we have no evidence that the polymorphism IL13-591A/G may modify the transcription level of the IL13 gene.

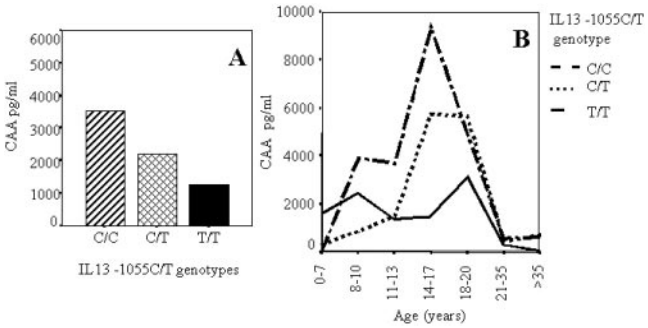

FIGURE 4. IL13-1055T is associated with lower concentrations of circulating anodic Ags (CAA) in serum. CAA blood concentration is a measure of worm load. Titration was conducted in serum; the cut-off value of the assay was 30 pg/ml (specificity of 98%). *A*, CAA levels according to IL13-1055C/T genotypes. *B*, Infection for each of the IL13-1055 genotypes in the different age classes.

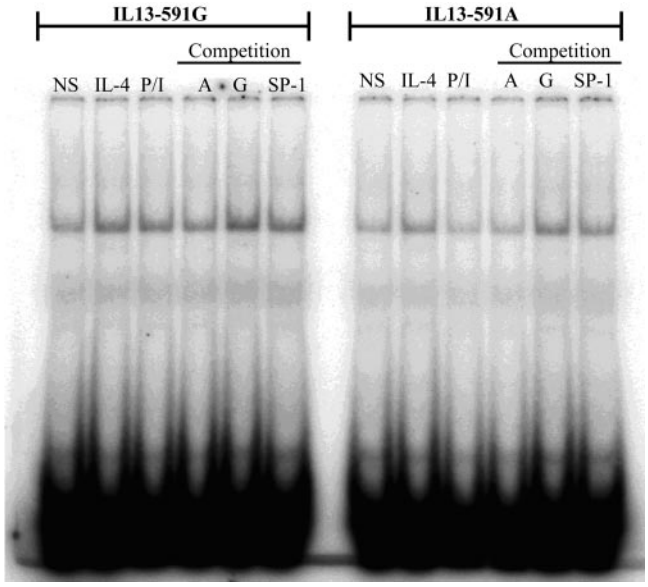

FIGURE 5. EMSA analysis of the IL13-591A/G polymorphism. This analysis compared DNA-nuclear protein interactions with oligonucleotides from either the common IL13 promoter (IL13-591A) or the variant (IL13-591G). Common and variant promoter formed the same complex with nuclear extracts from stimulated (PMA/I or IL4) or unstimulated (NS) human peripheral T cells. Competitions with cold probes or unspecific probes do not inhibit the complex formation that indicate that this complex is specific to the tested region.

Discussion

Schistosomes enter their host via the skin and after a few hours migrate through the vascular and lymphatic system and develop into adult worms in mesenteric veins and bladder plexus. While migrating, young worms pass through the lungs several times. Schistosomes are vulnerable to immune attack when in the skin and lungs (28, 29). Repeated antigenic challenge through the skin by molecules released from schistosomula biases the immune response to Th2. This response, in particular eosinophils and IgE, has been associated with protection against reinfection (12–15).

IL-13 could play a unique role in skin and lung immunity against schistosome. First, IL-13 is critical for the entry and survival of eosinophils in the skin and in the lungs, as shown in various pathological conditions (30–33). IL-13 increases VCAM1 expression on endothelial cells (34), which enhances eosinophil adherence via β_1 integrin. IL-13 also increases CD69 expression on eosinophils (35) and the production of GM-CSF by epithelial cells (36). Both of these actions increase eosinophil survival. Second, IL-13 may play a unique role in the induction of the Th2 response by the skin route (37). Indeed, IL-13, more than IL-4, has been shown to be required for the priming of a Th2 response by soluble persistent Ags in the skin. In addition to this skin priming effect, IL-13 exerts positive actions during the induction of Th2 responses (38–41).

IL-13 may also play a role in egg destruction by eosinophils in the intestine and bladder. IL-13 was also shown to play an important role in mouse gut immunity to *Nippostrongylus brasiliensis* (42) by stimulating secretion of mucus by goblet cells and possibly by its effects on eosinophils. Hence, it appears that IL-13 plays a unique role in Th2-mediated immunity in skin and gut. Research

on asthma has also indicated that IL-13 plays unique effects in Th2 immunity in the lungs (32, 33).

Urinary disease is caused by schistosome egg and worm Ags. Fibrosis of ureters is an important factor in hydronephrosis. Reducing the number of eggs should prevent fibrosis and disease. This, however, does not account for the effects of IL-13 on eosinophils, which contribute to inflammation and tissue damage, and hence to extracellular matrix protein deposition. Furthermore, IL-13 has strong profibrogenic effects as documented in *S. mansoni*-infected mice (43) in which IL-13 was shown to increase collagen deposition in the liver (44). Therefore, on the one hand, IL-13 may protect against infection and therefore reduce the risk of disease and, on the other hand, IL-13 may contribute to disease by increasing eosinophil infiltration and promoting fibrosis. However, we did not detect an association between kidney hydronephrosis or bladder wall thickening and IL13-1055C/T or IL13-591A/G (data not shown). The effects of IL-13 on anti-infection but not on anti-disease immunity are consistent with our previous finding that infection and hepatic fibrosis are subject to separate genetic control in humans with chronic *S. mansoni* infections. One major gene on chromosome 5q31-q33 controls infection (6, 7). Another major locus controls periportal fibrosis and maps close to IFNGR1 on 6q23 (45). Protection against infection is linked to a Th2 immune response, whereas protection against disease is increased by IFN-γ.

We conclude that protection against schistosomes is increased by the IL13-1055T/T genotype, which also aggravates asthma (46). Finally, we would like to advance the following hypothesis that will need confirmation. Helminth infections have long been suspected to have selected for alleles that predispose to asthma and atopy. It is unlikely that this selective effect is related to enhanced resistance to urinary tract disease because we did not find an association between urinary tract disease and either of the IL13-1055 alleles. However, eggs and the eosinophil-infiltrated egg granuloma cause considerable disease of the genital tract in young women: up to 75% of women living in areas in which *S. haematobium* is endemic suffer from lesions of the uterus, cervix, vagina, or vulva. Female genital schistosomiasis is associated with infertility (47), abortion, extrauterine pregnancy (48), and spread of the agents responsible for sexually transmitted diseases (49). Epithelial lesions, tumors, and ulcers are associated with the presence of *S. haematobium* eggs in the lower genital tract (50). These pathological disorders have significant effects on divorce: husbands of women with female genital schistosomiasis are more likely to have children with other women (51). These effects of *S. haematobium* eggs on female reproductive capacity may select for IL13 genotypes that reduce egg load in genital tissues but aggravate the risk of asthma (47–51).

Disclosures

The authors have no financial conflict of interest.

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