Sex-Dependent Neutralizing Humoral Response to *Schistosoma mansoni* 28GST Antigen in Infected Human Populations

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The reduction of *Schistosoma* fecundity observed after experimental vaccination with the *Schistosoma mansoni* 28-kDa glutathione S-transferase (Sm28GST) antigen has been related to the inhibition of glutathione S-transferase (GST) enzymatic activity by specific antibody. The humoral immune response to the protective antigen Sm28GST and to the epitopes involved in the enzymatic site (amino acid [aa] sequences 10–43 and 190–211) was evaluated in infected individuals before chemotherapy treatment. The capacity of the serum samples to inhibit GST enzymatic activity was assessed. Specific IgG3 response was predominant in the male population with a low intensity of infection and was associated with maximal GST inhibition. In contrast, the neutralizing activity of serum samples from women with a low intensity of infection was correlated with high specific IgA response specifically directed toward the 190–211 epitope. These results strongly support the hypothesis that GST-neutralizing IgG3 and IgA isotypes are sex dependent. The relationship of this specific acquired immune response with the level of intensity of infection is discussed.

The 28-kDa glutathione S-transferase of *Schistosoma mansoni* (Sm28GST) has been selected by the World Health Organization (WHO) as a vaccine candidate against schistosomiasis [1]. Immunization of rodents [1, 2] and monkeys [3] with recombinant Sm28GST induces a significant decrease in tissue-egg deposition associated with a reduction of the pathology after experimental infection. The mechanism involved was elucidated partly when a close relationship was observed between the inhibition of Sm28GST enzymatic activity and a reduction in schistosome fecundity [2]. Indeed, inhibitory antibodies specific to the 10–43 or 190–211 amino acid (aa) sequences involved in the enzymatic site of Sm28GST have been shown to reduce tissue-egg number and egg viability in experimental models [2]. The relevance of these observations was highlighted when an association was discovered between the acquired immune response to Sm28GST and the resistance to reinfection after treatment, in infected human populations [4]. This resistance was associated with the inhibition of the enzymatic activity mediated by specific antibody responses.

All these studies were done to study immunity to infection in animals or immunity to reinfection after chemotherapy in human populations. However, recent work has demonstrated that treatment with praziquantel induces dramatic changes in specific antibody responses [5]. Therefore, the aim of our study was to evaluate acquired neutralizing humoral responses to Sm28GST and to its major epitopes involved in the enzymatic site, before chemotherapy, in infected humans.

Materials and Methods

**Studied population.** The studied population was drawn from the village of Guidakhar, located near the Senegal River (northern Senegal), in a recent focus (1988). No differences in prevalence or history of exposure were observed in relation to age or sex of...
individuals in this focus at the time of our study [6, 7]. The village is highly endemic for *S. mansoni* infection, with prevalence rates close to 100% for individuals 8–75 years of age. The diagnosis of *S. mansoni* infection was done by using the Kato technique [7], and the results were expressed as the geometric mean number of eggs per gram of feces (EPG), taken as the average of 5 separate examinations.

A cohort of 155 individuals positive for *S. mansoni*, consisting of 79 females and 76 males, was divided into 4 approximately equal groups (n = 39 ± 3) composed of similar numbers of males and females per group (males, n = 19 ± 2; females, n = 20 ± 1) and classified into age groups with a range of 12–15, 16–23, 24–34, and ≥35 years. Parasitologic results arranged according to sex indicated that the intensity of infection was significantly higher (P < .01) in males (mean EPG, 732; range, 12–6484) than in females (mean EPG, 180; range, 4–3976). For the immunological evaluation, the study sample subsequently was divided into 4 groups on the basis of sex and intensity of infection. The cutoff EPG values for low- and high-intensity infection were 732 for males and 180 for females, according to the geometric mean for both sexes and WHO recommendations (WHO Technical Report Series, no. 830).

All individuals found to be positive for *S. mansoni* were treated with praziquantel (40 mg/kg body weight) immediately after blood sampling. Pregnant or lactating women were excluded from the study, in accordance with WHO recommendations.

**Antigen preparation and synthetic peptides.** Recombinant Sm28GST was produced by expression of the corresponding cDNA clone in *Escherichia coli* (Transgène, Strasbourg, France), as described elsewhere [4]. Two different synthetic peptides derived from the primary structure of Sm28GST—that is, aa 10–43 and aa 190–211—were constructed according to methods described elsewhere [2].

**Glutathione S-transferase (GST) activity–inhibition assay.** For inhibition of GST reactions, 10 μL of Sm28GST solution (4 μg/mL in 50 mM potassium phosphate at pH 6.5) was incubated with 20 μL of human immune serum for 1 h at 37°C in Immulon 3 Plates (Nunc, Roskilde, Denmark). The enzymatic reaction was done by using 1-chloro-2,4-dinitrobenzene (Sigma, St. Louis, MO) substrate, as described elsewhere [4]. Similar enzyme-inhibition tests were performed with appropriate controls (either no serum and/or enzyme). The individual percentage inhibition of Sm28GST activity was calculated by comparison with negative control samples (from 30 uninfected whites) of Sm28GST incubated in the same amount of uninfected human serum.

**Human antibody (Ab) levels (ELISA).** Sm28GST protein (10 μg/mL) or synthetic peptides corresponding to the 10–43 or 190–211 aa sequences of the Sm28GST enzyme (5 μg/mL) were coated on 96-well plates (Nunc, Roskilde, Denmark) overnight at 4°C. After saturation in PBS buffer containing 1% (w/v) bovine serum albumin (Sigma), individual serum samples were incubated overnight at 4°C at a 1 : 1000 dilution for IgH-L detection, at a 1 : 10 dilution for IgE and IgG4 detection, or at a 1 : 25 dilution for IgG1, IgG2, IgG3, and IgA detection. Corresponding biotinylated monoclonal Abs to human Ig isotypes (Southern Biotechnology Associates, Birmingham, AL) were incubated at a 1 : 1000 dilution (2 h at 37°C). Peroxidase-conjugated streptavidin (Amer sham, Les Ulis, France) was then added (1 : 2000; 1 h at 37°C). Colorimetric development was done as described elsewhere [3]. Identical ELISAs were performed on serum samples collected from 30 uninfected white individuals. Results are expressed as the change in optical density (OD) values according to the following formula: ∆OD = ODx - ODn, where ODx represents individual OD values of infected patients and ODn is the arithmetic mean of individual OD values for the 30 control individuals (IgG ODn, 0.22; IgA ODn, 0.24; IgG1 to IgG4 ODn < 0.2).

**Statistical analysis.** All data were analyzed with STATVIEW software (Abacus Concepts, Berkeley, CA). The Mann-Whitney U test was used to compare means between groups. The correlation between antibody levels and Sm28GST inhibition was analyzed by using Kendall’s rank correlation coefficient and subsequently was

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**Table 1.** Specific humoral immune response to *Schistosoma mansoni* 28-kDa glutathione S-transferase (Sm28GST) and correlation with inhibition of enzymatic activity.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High intensity of infection (EPG &gt;732)</td>
<td>Low intensity of infection (EPG &lt;732)</td>
</tr>
<tr>
<td>n</td>
<td>40</td>
<td>36</td>
</tr>
<tr>
<td>Age, mean (range)</td>
<td>26.6 (12–66)</td>
<td>31.8 (12–72)</td>
</tr>
<tr>
<td>EPG, mean (range)</td>
<td>2216 (844–6848)</td>
<td>214 (12–704)</td>
</tr>
<tr>
<td>Anti-Sm28GST antibody a, b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ig (H+L)</td>
<td>1.03 ± 0.60</td>
<td>1.07 ± 0.66</td>
</tr>
<tr>
<td>IgG3</td>
<td>0.54 ± 0.45</td>
<td>0.83 ± 0.43</td>
</tr>
<tr>
<td>IgA</td>
<td>0.15 ± 0.15</td>
<td>0.16 ± 0.18</td>
</tr>
<tr>
<td>Sm28GST inhibition c, d</td>
<td>8.4 ± 13.1</td>
<td>23.9 ± 21.1</td>
</tr>
</tbody>
</table>

Correlation (P) with inhibition of Sm28GST activity d

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG3</td>
<td>0.071 (NS)</td>
<td>0.236 (0.0396)</td>
</tr>
<tr>
<td>IgA</td>
<td>-0.454 (NS)</td>
<td>-0.009 (NS)</td>
</tr>
</tbody>
</table>

**NOTE.** EPG, geometric mean of number of eggs per gram of feces; NS, not significant.

- P value between groups with high or low intensity of infection (Mann-Whitney U test); significant for P < .05.
- Specific IgH+L, IgG3, and IgA antibody levels were expressed as the mean of individual optical density (OD ± SEM).
- Arithmetic mean of inhibitory activity of individual serum (%).
- Data are Kendall rank r values. P values were determined by Kendall rank correlation and are indicated in parentheses for each group; significant for P < .05.
controlled by multiple-regression analysis. Differences and correlations were considered significant at \( P < .05 \).

Results

Total Ig immune response to Sm28GST and inhibition of enzymatic activity. No significant difference in total Ig humoral response to Sm28GST (Ig[H+L]) was observed in relation to the intensity of infection or to sex (table 1). In contrast, the capacity of serum samples to neutralize Sm28GST enzymatic activity was significantly different according to intensity of infection, both in males and in females. Indeed, the inhibition of Sm28GST activity in the groups with a low intensity of infection was higher that in the groups with a high intensity of infection (\( P < .05 \)).

Sex-dependent variability of IgG3 and IgA responses to Sm28GST. Levels of specific IgG1, IgG2, IgG4, and IgE were similar between groups displaying high- and low-intensity infections (data not shown). However, significant differences in the levels of specific IgG3 and IgA were observed (table 1). Although no significant difference in the level of specific IgG3 was observed between the 2 groups of females, IgG3 antibody levels were significantly different in the 2 groups of males, with higher levels in the group with a low-intensity infection. In this latter group, a positive and significant correlation between the specific IgG3 response and the percentage of GST inhibition was observed (table 1). Data were analyzed by multiple regression with the inhibition of Sm28GST activity as the dependent variable. The relationship between the IgG3 response to Sm28GST and the percentage of GST inhibition remained statistically significant in the male group with low infection (\( P = .015 \)).

Although the specific IgA responses were similar in both groups of males, IgA antibody levels in females were significantly different between both groups of intensity of infection (table 1). A statistical analysis of the results from this group highlighted a significant positive correlation between the specific IgA response and GST inhibition (\( P < .05 \)), and this relationship remained significant after multiple-regression analysis (\( P = .024 \)).

Sex-dependent variability in responses to epitopes involved in the GST enzymatic site. Isotypic responses to aa 10–43 and 190–211 peptides involved in the GST enzymatic site were studied according to sex (table 2). In males showing a low intensity of infection, only the mean IgG3 response against the 1043 epitope was stronger than that observed in the high-intensity infection group. However, this finding and the correlation with Sm28GST inhibition were not statistically significant (table 2). No differences were observed in the levels of IgA response to both epitopes in the male population.

In contrast, the mean IgA Ab level specific to the 190–211 peptide was significantly higher in the group of females with low EPG. In this group, IgA response to the 190–211 aa sequence was correlated positively with Sm28GST inhibition (\( P < .01 \); table 2). These parameters remained statistically correlated after multiple-regression analysis with the inhibition of Sm28GST activity as the dependent variable and all immune responses to epitopes as the independent variable (\( P = .040 \)).

Discussion

The pathology of schistosomiasis is induced by the deposition of eggs in tissues and is related to the fecundity of the female parasite. Consequently, scientists involved in the development

| Variable | Males | | | Females | | |
|----------|-------|---|---|-------|---|
|          | High intensity of infection (EPG >732) | Low intensity of infection (EPG <732) | High intensity of infection (EPG >180) | Low intensity of infection (EPG <180) | |
| IgG3\[a\] | 10–43 peptide | 0.53 ± 0.71 | 1.08 ± 1.01 | 0.37 ± 0.45 | 0.35 ± 0.34 |
|          | 190–211 peptide | 0.33 ± 0.41 | 0.41 ± 0.31 | 0.39 ± 0.46 | 0.42 ± 0.58 |
| IgA | 10–43 peptide | 0.91 ± 0.78 | 1.01 ± 0.67 | 0.53 ± 0.32 | 0.70 ± 0.43 |
|          | 190–211 peptide | 0.57 ± 0.64 | 0.61 ± 0.59 | 0.32 ± 0.25 | 0.71 ± 0.43 |

NOTE. EPG, geometric mean of number of eggs per gram of feces; NS, not significant.

\[a\] Specific IgG3 and IgA antibody levels to peptides were expressed as the mean of individual optical density (\( \Delta OD \pm SEM \)).

\[b\] Significantly higher (\( P < .009 \)) than group with high intensity of infection (Mann-Whitney \( U \)-test).

\[c\] Data are Kendall rank \( r \) values. \( P \) values were determined by Kendall rank correlation and are indicated in parentheses for each group; significant for \( P < .05 \).
of a vaccine should consider not only immune responses leading
to the destruction of the parasite, but also those that induce a
reduction in its fecundity [1]. In numerous animal experiments,
immunization with Schistosoma 28GST antigen induced a
marked antifecundity immunity and, therefore, an antipatho-
logic effect that is associated strongly with an antibody-mediated
inhibition of 28GST activity [1, 8].

Our study evaluated the humoral immune response to
Sm28GST in human S. mansoni infection before chemotherapy
treatment. The total anti-Sm28GST Ig response did not vary
with the sex of the infected individual or with the intensity of
infection, whereas inhibition of Sm28GST activity was signif-
ically higher, for both sexes, in the groups displaying low-
intensity infections, compared with that of groups displaying
high-intensity infections. Analysis of the relationship between
the inhibition of Sm28GST activity and the specific isotype
profile demonstrated the highly probable neutralizing function
of IgG3 and IgA and their variations with the sex of individuals
and the intensity of infection. The IgG3 response to Sm28GST
was correlated with GST inhibition in males with a low intensity
of infection. In contrast, a correlation between IgA response
and 28GST inhibition was recorded in females from the group
with a low intensity of infection. In human populations infected
by S. mansoni, the antibody-dependent neutralizing effect on
the GST enzymatic properties has been correlated previously
to the reduction of egg output and egg viability [4]. In our
study, the major responses in both cases were associated with
infection of low intensity, suggesting a possible protective role
of neutralizing IgG3- and IgA-acquired responses.

In addition, this immune variability is not the only mecha-
nism that is dependent on the sex of the infected patient, be-
cause the recognition of crucial epitopes also seems to be spe-
cific. Whereas the IgG3 isotype directed to total Sm28GST
protein seems to play a key role in GST neutralization in males,
we were unable to show a significant correlation between these
epitopic IgG3 responses and Sm28GST inhibition. This global
neutralizing IgG3 response could be involved in the recognition
of other epitopes involved in the enzymatic activity of the GST.
The identification of such epitopes is under investigation by
using peptide-mapping strategies. In contrast, in females a
higher IgA response to the 190–211 epitope, which was cor-
related significantly with GST inhibition, was observed in the
group with a low intensity of infection. This last result strength-
ens the role of the IgA isotype in 28GST inhibition [4] and
suggests that the major recognition of the 190–211 epitope by
IgA could be critical in terms of a parasite antifecundity effect
in the human female population. Taken together, these results
suggest a differential isotypic specificity to epitopes involved in
GST enzymatic activity between males and females.

Several physiological events could explain the observed sex-
dependent differences in the quality of the immune response.
Indeed, it is well known that sex can have a direct effect on
immunity in humans [9] and that sex steroids could have a
direct effect on cytokine production during parasitic infection
[10]. In addition, testosterone has been shown to induce the
secretion of interferon-γ [11], a cytokine closely associated to
the production of IgG3 [12]. In contrast, estrogen can increase
transforming growth factor–β and interleukin-10 secretion [13,
14], which thus could have an effect on the production of IgA
[15]. Taken together, these results suggest a strong influence
of sex steroid hormones on the sex-dependent immune response
observed in our study.

History of S. mansoni exposure, genetic background, or other
individual habits or events such as pregnancy cannot be ex-
cluded to explain the different immunity observed between
males and females in our study. Nevertheless, the sex-dependent
specific immunity observed could play a key role in the differ-
ence in resistance to infection and subsequent development of
the pathology, which is often evoked according to the sex of
the infected individual [9].

Acknowledgments

We sincerely thank the chief and the entire population of Guidakhar
village, who participated in this study. We also thank Abdoulaye Ly,
the Senegalese Ministry of Health, and the Senegalese Ethical Com-
mittee for their support. We also extend special thanks to Monique
Marguerite, Modou Diagne, Aliou Thiam, Denis To Van, and Edith
Bassene at the laboratory of Sor and to Bocar Daff, Jean-Pierre Piau,
Seydou Tine, Sohibou Guindo, and Seydou Sow at Richard-Toll for
their participation in this study. We are grateful to Christophe Decem
and Jean-Pierre Domnierz for organizing the study and to Serge Roche
for his contribution to the medical examinations. We also wish to thank
Dr. André Tartar of Chimie des Biomolécules, Institut Pasteur de Lille,
for kindly providing Sm28GST-derived synthetic peptides and Anne-
Marie Schacht and Jan de Bont for their helpful advice.

References

1. Capron A. Schistosomiasis: forty years' war on the worm. Parasitol Today
glutathione S-transferase and immunity against parasite fecundity and
egg viability: role of the amino- and carboxyl-terminal domains. J Im-
munol 1993; 150:940–9.
3. Boulanger D, Reid GDF, Sturrock RF, et al. Immunization of mice and
baboons with the recombinant Sm28GST affects both worm viability and
fecundity after experimental infection with Schistosoma mansoni. Parasite
4. Gryzch JM, Grezel D, Xu CB, et al. IgA antibodies to a protective antigen
opment of acquired immune responses to Schistosoma haematobium in-
infection in a recently exposed community in northern Senegal. Am J
7. Marguerite M, Gallisost MC, Diagne M. Cellular immune responses of a
Senegalese community recently exposed to Schistosoma mansoni: corre-
lations of infection level with age and inflammatory cytokine production


