The Journal of Infectious Diseases
MAJOR ARTICLE

Antibody Signatures Reflect Different Disease Pathologies in Patients With Schistosomiasis Due to *Schistosoma japonicum*

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Infection with *Schistosoma japonicum* causes high levels of pathology that is predominantly determined by the cellular and humoral response of the host. However, the specific antibody response that arises during the development of disease is largely undescribed in Asian schistosomiasis–endemic populations. A schistosome protein microarray was used to compare the antibody profiles of subjects with acute infection, with early or advanced disease associated with severe pathology, with chronic infection, and subjects exposed but stool negative for *S. japonicum* eggs to the antibody profiles of nonexposed controls. Twenty-five immunodominant antigens were identified, including vaccine candidates, tetraspanin-related proteins, transporter molecules, and unannotated proteins. Additionally, individuals with severe pathology had a limited specific antibody response, suggesting that individuals with mild disease may use a broad and strong antibody response, particularly against surface-exposed proteins, to control pathology and/or infection. Our study has identified specific antigens that can discriminate between *S. japonicum*–exposed groups with different pathologies and may also allow the host to control disease pathology and provide resistance to parasite infection.

Keywords. schistosomiasis; antibody; human disease; *Schistosoma japonicum*; protein microarray; immunology.

Schistosomiasis affects >250 million people in Africa, South America, and Asia and is a significant cause of morbidity and death [1]. Schistosomiasis has several clinical stages of disease [2, 3]. The pathology resulting from larval migration and egg deposition in the host causes acute, chronic, and advanced/hepatosplenic disease types [2, 4]. The Asian schistosome, *Schistosoma japonicum*, typically causes more-severe pathology than *Schistosoma mansoni* and *Schistosoma haematobium* [5, 6]. Acute Asian schistosomiasis occurs in naive hosts during the first infection and in subjects from schistosomiasis-endemic areas after reinfection [2, 4]. The nonspecific symptoms, such as fevers and eosinophilia, often resolve quickly, but chronic disease will follow unless the infection is cured, although acute disease in some individuals progresses directly to advanced disease [2, 4]. Continuous egg laying during infection and the associated pathology within the host characterize chronic disease. During long-term infection, granuloma formation around eggs lodged in the liver can, in some individuals, cause severe fibrosis that leads eventually to advanced schistosomiasis. Advanced schistosomiasis symptoms include portal hypertension, hepatosplenomegaly, and gastrointestinal varices; severe disease can result in mortality [4, 7]. Currently, determination of disease severity requires clinical assessment with specialized techniques, such as ultrasonography, liver biopsy, and radiography [2, 4].

Schistosomiasis pathology severity is determined by infection intensity and the immune response of the infected individual [4, 8]. In addition to genetic variation of immune response genes in the human population [2, 4, 7, 9], differences in pathology and resistance to reinfection have been associated with parasite-specific antibody responses [8–12]. However, the specificity and the differential reactivity of human schistosomiasis antibodies are still largely unknown. Studies using parasite extracts and limited sets of recombinant proteins [11, 13–17] have identified some antigens differentially recognized by human schistosomiasis disease groups. High-throughput screening techniques may lead to the discovery of more antigens. For example, a study using a microplate array of recombinant proteins has identified a promising *S. japonicum* diagnostic assay [18], and we have recently used a schistosome protein microarray to examine the antibody profiles of *S. mansoni*–exposed subjects in Brazil [19].

This study, using the same protein microarray [19] to interrogate Asian schistosomiasis, characterized the specific serum immunoglobulin G (IgG) reactivity of different clinical disease types. Analysis of sera from patients with acute infection

Received 17 March 2015; accepted 19 June 2015; published online 6 July 2015.
Presented in part: 13th International Congress of Parasitology, Mexico City, Mexico, August 2014 (abstract 1630).
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The Journal of Infectious Diseases® 2016;213:122–30

DOI: 10.1093/infdis/jiv356

(hereafter, the acute group), chronic infection (the chronic group), early advanced disease (the early advanced group), and late advanced disease (the late advanced group) and from subjects who were exposed to *S. japonicum* infectious water but were negative for eggs in stool specimens (the stool-negative [SN] group) from Dongting Lake, Hunan, China, revealed surprising results: despite high whole-parasite antibody responses in nearly all of the groups, only subjects with mild pathology or no symptoms (ie, subjects in the chronic and SN groups) had greater reactivity to microarray proteins and recognized a specific set of mostly surface antigens, compared with subjects with acute and advanced disease. Furthermore, we have discovered antigens capable of classifying disease groups based on IgG reactivity that may also shed light on human immunity and pathology to schistosomiasis japonica.

MATERIALS AND METHODS

Study Subjects

All subjects provided written informed consent, and ethics committee approval was received from human ethics committees (QIMR Berghofer Medical Research Institute, Brisbane, Australia, and the Hunan Institute of Parasitic Diseases [HIPD], Yueyang, China). Screening of migrant fisherman populations around the Dongting Lake region, Hunan province, China, was performed; this population has a high prevalence of *S. japonicum* infection and, owing to water contact, substantial occupational exposure to *S. japonicum*, and served as the source of subjects in the chronic and SN groups (Supplementary Table 1). The 10 subjects in the chronic group were clinically asymptomatic but currently infected with schistosomes, and the 15 subjects in the SN group had an undetectable parasite burden despite exposure to potentially infectious water. The water exposure characteristics, number of previous schistosome infections and treatments, clinical history, pathologic characteristics of previous schistosomiasis episodes, and demographic characteristics were determined with a standardized questionnaire completed by subjects under the supervision of HIPD staff. Schistosome infection was diagnosed using serologic analysis and stool examination. Serum samples were checked for recent schistosome infection by evaluating immunoglobulin M (IgM) and IgG antibody reactivity to an *S. japonicum* egg extract, using an indirect hemagglutination assay [2, 20]. If the serum sample was *S. japonicum* antibody positive, a fresh stool specimen was evaluated, using the miracidium hatching test and by averaging the findings on 3 Kato-Katz thick smear slides [21]. A positive result of either stool test was considered indicative of a current infection, thereby reducing the overall false-negative rate [21, 22]. Ten subjects in the acute group, 15 in the early advanced group, and 15 in the late advanced group, as classified by Chinese Ministry of Health criteria [7, 20], were recruited from local hospitals in the study area. Late advanced subjects, like early advanced subjects, had an ultrasonography-based diagnosis of hepatosplenic pathology but also had biopsy-confirmed advanced liver fibrosis and were undergoing splenectomy to treat symptoms [7] (Supplementary Table 1). Ten uninfected adult subjects native to areas of nonendemicity in China and no known history of schistosomiasis were used as negative controls in this study. All infected subjects were given a single curative dose of praziquantel (40 mg/kg). Parasite-specific serum antibody levels for all 75 study subjects were measured by an enzyme-linked immunosorbent assay (ELISA) against soluble worm antigen (SWAP), soluble egg antigen (SEA), deglycosylated SWAP, and deglycosylated SEA. See the Supplementary Methods for further details.

Protein Microarray Printing, Probing, and Data Analysis

A schistosome protein microarray with 289 features (Supplementary Table 2) was manufactured and probed as previously described [19, 23–25]. The primary purpose of the protein microarray—vaccine and diagnostic antigen discovery—guided the selection of schistosome protein sequences and design of the protein microarray. Recombinant schistosome proteins expressed in bacteria and yeast, protein microarray control features, and cell-free *Escherichia coli*–expressed (RTS) schistosome proteins were printed robotically onto nitrocellulose slides. The protein microarrays were checked for quality, and serum samples were probed. Serum IgG binding was detected with fluorescently labeled antibodies and a microarray scanner. The raw protein microarray data (Supplementary Table 3) were corrected for local background. The data were then normalized and analyzed (Supplementary Table 4). Normalized data were inverse $\log_2$ retransformed for data visualization. The definition of seropositive, cross-reactive, seroreactive, and immunodominant features and detailed data analysis procedures are found in the Supplementary Methods.

RESULTS

Antibody Reactivity Against *S. japonicum* Extracts

Apart from SN subjects, whole-parasite IgG reactivity was significantly higher in schistosome-exposed groups, compared with the negative controls (Figure 1 and Supplementary Figure 1). The anti-SWAP and anti-SEA response (Figure 1) was generally high across the disease groups; acute and early advanced subjects had the highest average optical densities, with the absence of a current infection in the late advanced subjects possibly explaining the lower anti-SEA response. Deglycosylation caused a marked reduction in IgG reactivity against adult (Supplementary Figure 1*A* and *C*) and egg (Supplementary Figure 1*B* and *D*) extracts, particularly for the acute and advanced groups. Notably, the generally high antibody reactivity against deglycosylated SEA and glycosylated SWAP and SEA by the acute and advanced groups was not matched by the protein microarray data (Figure 2 and Supplementary Tables 2–4).

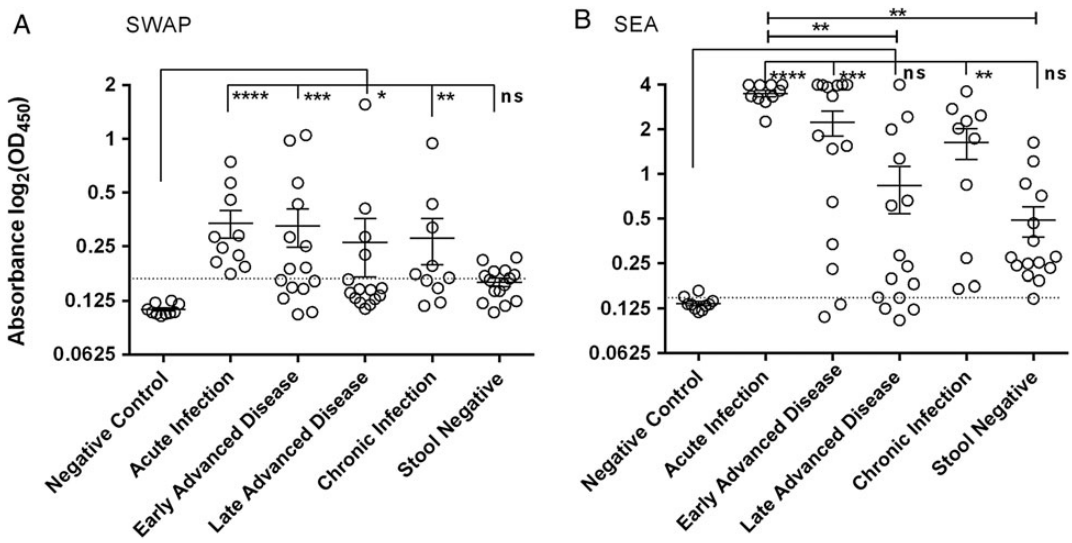

Figure 1. Human serum immunoglobulin G reactivity against parasites extracts. Soluble worm adult preparation (SWAP; *A*) and soluble egg antigen (SEA; *B*) reactivity were measured using an enzyme-linked immunosorbent assay and grouped by disease type. Positive reactivity (dotted line) is 2 SDs above the average of the negative control wells; error bars display the standard error of the mean. Statistical significance was measured using the Kruskal–Wallis test with the Dunn correction for multiple comparisons. ****$P < .0001$, ***$P < .001$, **$P < .01$, and *$P < .05$. Abbreviation: NS, not significant ($P > .05$).

Protein Microarray Antibody Profile

Numerous antigens on the protein microarray were recognized by sera from the study participants (Figure 2). Moreover, the consistently high control feature signal intensity (Figure 2*A* and Supplementary Table 3) and high SWAP and SEA reactivity (Figure 1) indicated that antibody integrity within the serum samples was high and suitable for protein microarray screening. The global signal intensity (ie, the average signal intensity for all protein microarray antigens within a serum sample), although not statistically different, was lowest for serum samples from the negative controls and acute subjects, compared with the other groups (Figure 2*B*). The global signal intensity correlated with ELISA reactivity to deglycosylated SWAP (Spearman $\rho = 0.411$; $P = 2.5 \times 10^{-4}$) but not to SWAP, SEA, or deglycosylated SEA (Spearman's $\rho = 0.104$, 0.131, and 0.164, respectively; $P > .05$ for all comparisons; $n = 75$).

From the 268 noncontrol features, 182 (67.9%; 33 in vivo recombinant proteins and 149 RTS proteins) were seropositive (Supplementary Table 3), based on a signal that was 3 SDs above the mean of the negative control features. However, in contrast to the high SWAP and SEA reactivity, the number of seropositive antigens was lower in the acute and late advanced subjects, compared with findings for the early advanced, SN, and chronic subjects, and were not statistically different from findings for the negative controls ($P > .05$, by analysis of variance; Figure 2*C*). The frequency of recognition followed a similar trend, particularly for a higher frequency of recognition values. The number of seropositive antigens shared by at least 20% and 50% of serum samples in a group (Figure 2*D*) was again lower in the negative control, acute, and late advanced groups, compared with the early advanced, chronic, and SN groups.

The magnitude of the antibody profile or number of seropositive antigens of schistosome-exposed individuals was independent of infection intensity and all other disease-related variables (Supplementary Table 5). Similar to previous protein microarray and serological schistosomiasis screening studies [16, 19, 26, 27], 20 cross-reactive proteins that were recognized by both schistosome-exposed and naive individuals were identified (Supplementary Figure 2 and Supplementary Table 6). These cross-reactive and nonspecific antigens give an indication of the background reactivity of individuals and can be eliminated as potential biomarkers for infection/disease.

Surface and Secreted Proteins and Vaccine Antigens Are Recognized by Sera From Subjects With Mild Disease Pathology

Seroreactive proteins were identified by excluding cross-reactive proteins from the seropositive antigens, selecting 122 differentially reactive proteins with significant *P* values between the human groups, and excluding 83 antigens with a frequency of recognition of <50% in at least 1 schistosome-exposed group. In total, 37 unique seroreactive antigens were identified (Figure 3 and Supplementary Table 7). Membrane and secretory proteins, as indicated by the presence of transmembrane domains and signal peptides (Supplementary Table 2), and cell surface ontologies or annotation (Supplementary Table 7) were prominent (27 proteins [73%]) among the seroreactive antigens. *S. japonicum* and *S. mansoni* candidate vaccines (Sj/Sm calpain/Smp80, SjGST-26, Sj/SmTSP2, and Sj/Sm29) were also seroreactive antigens.

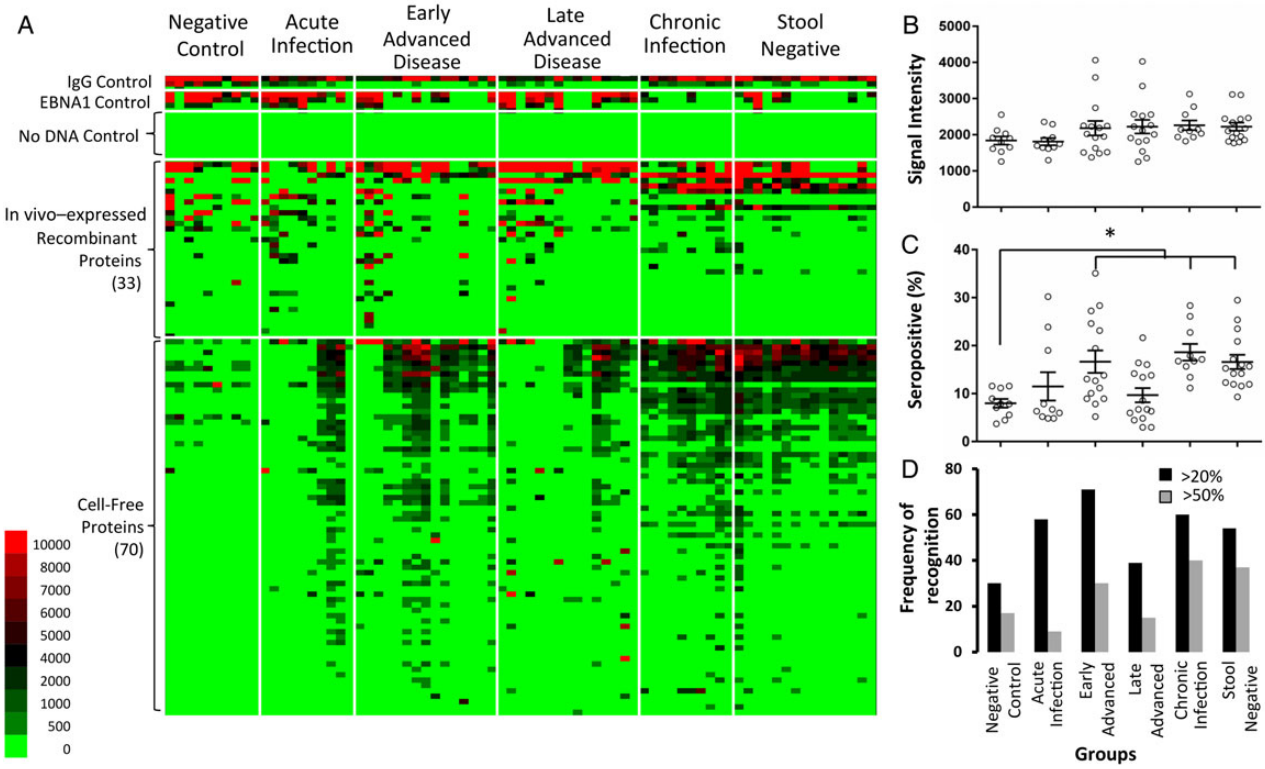

Figure 2. Immunoglobulin G (IgG) antibody reactivity of schistosome-infected and noninfected human samples. *A*, Heat map of serum samples from negative controls from an area where *Schistosoma japonicum* is not endemic (n = 10), subjects with acute infection (n = 10), subjects with early advanced disease (n = 15), subjects with late advanced disease (n = 15), subjects with chronic infection (n = 10), subjects with negative stool specimens (n = 15) probed on the schistosome protein microarray. The protein microarray features were ordered by decreasing average signal intensity, with the colors corresponding to the reactivity (legend); nonseropositive features, proteins without any positive signal intensity, were excluded (except for no DNA controls). Dilutions of Epstein-Barr nuclear antigen 1 (EBNA1 Control) and mixed species IgG (IgG Control) were used as positive controls; cell-free extract without plasmid DNA (No DNA Control) were negative controls. For displaying purposes only controls, in vivo–expressed recombinant proteins and the 70 most reactive cell-free proteins are presented. *B*, Dot plot of average group signal intensity with medians and 95% confidence intervals. *C*, Dot plot of average percentage of seropositive antigens per group. **P* < .05, by analysis of variance and the Tukey honest significant differences test, for groups with findings significantly different from those of negative controls. *D*, Bar plot of the number of antigens recognized by at least 20% or 50% of samples in each group (>20% or >50% frequency of recognition).

Much like the global protein microarray results, reaction against the seroreactive antigens was weaker among subjects in the negative control (mean signal intensity magnitude [±SEM], 1665 ± 92; frequency of recognition, 19%), acute (2204 ± 107; 34%), early advanced (2711 ± 250; 53%), and late advanced (2136 ± 158; 29%) was weaker, compared with findings among subjects in the SN (5275 ± 850; 77%) and chronic (5115 ± 528; 77%) groups. However, the majority of seroreactive antigens (26 [70%]) were only significantly recognized by the sera from the chronic and SN groups; in contrast, only 4 of the remaining antigens—Sj29 (AY814537) and hypothetical proteins (AY811479, AY809620, and AY808751)—were not recognized by sera from these 2 groups (Figure 3 and Supplementary Table 7). Moreover, the antibody reactivities for the chronic and SN groups were nearly identical and, on average, could not be differentiated from one another by using the identified antigens.

Visualization Analyses of Seroreactive Antigens

Hierarchical clustering and principal component analysis (PCA) reinforced the strong and broad reactivity found in sera from the chronic and SN groups, compared with sera from the acute, early advanced, and late advanced groups. Unbiased hierarchical clustering (Figure 4*A*) using the seroreactive antigens clearly separated all of the chronic and SN subjects (cluster III) from acute, early advanced, and late advanced subjects in cluster I, for whom findings were moderately reactive, and from acute subjects, late advanced subjects, and negative controls in cluster II, for whom findings were weakly reactive. PCA was used as a visualization tool to discriminate human samples on the basis of reactivity to the seroreactive antigens (Figure 4*B*) and supported the hierarchical clustering analysis findings. The elevated magnitude and broad serum antibody response of the chronic and SN subjects (cluster III) clearly separated this grouping from the acute and advanced subjects with moderate reactivity (cluster I), and both clusters were distinct from the negative controls and subjects with weakly reactive findings (cluster II). The primary and secondary principal components described 69.7% and 10.1%, respectively, of the variability within the samples.

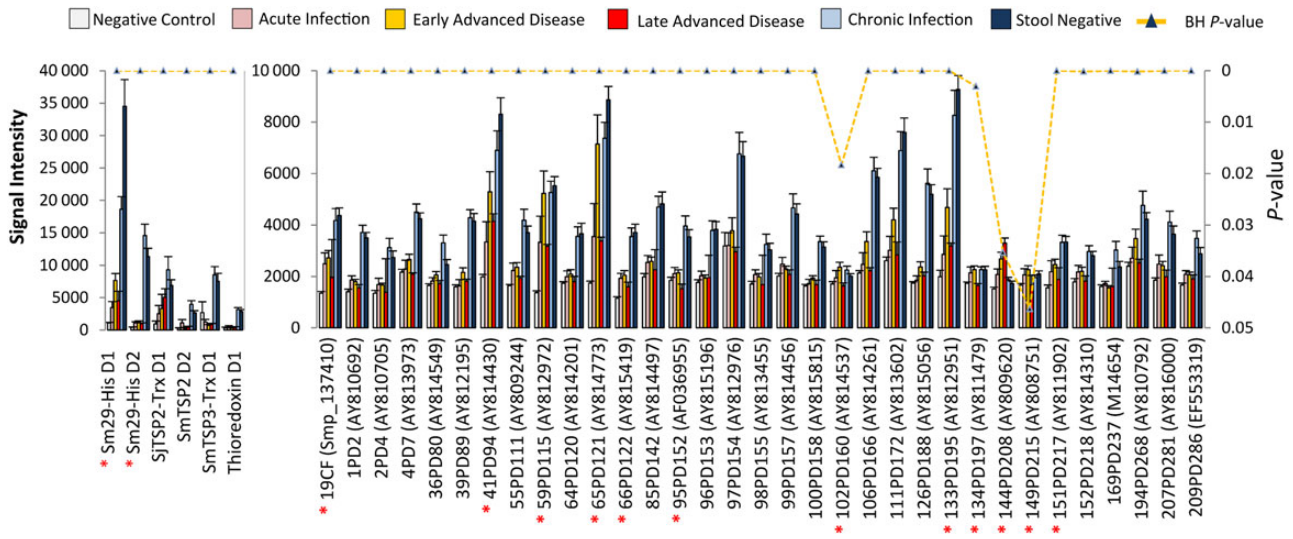

Figure 3. Seroreactive antigens in schistosome-exposed individuals and negative controls. Shown are proteins (n = 39) recognized by at least 50% of individuals within a schistosome-exposed group and with significant Benjamini and Holmberg *P* values between groups. The groups are negative controls (10), subjects with acute infection (10), subjects with early advanced disease (15), subjects with late advanced disease (15), subjects with chronic infection (10), and subjects with negative stool specimens (15). For all antigens except those marked with an asterisk, the significant group averages are for subjects with chronic disease and those with negative stool specimens. The signal intensity is represented as a column, with error bars representing the standard error of the mean. *P* values are displayed as a line graph. Note the change of scale for the first 6 antigens.

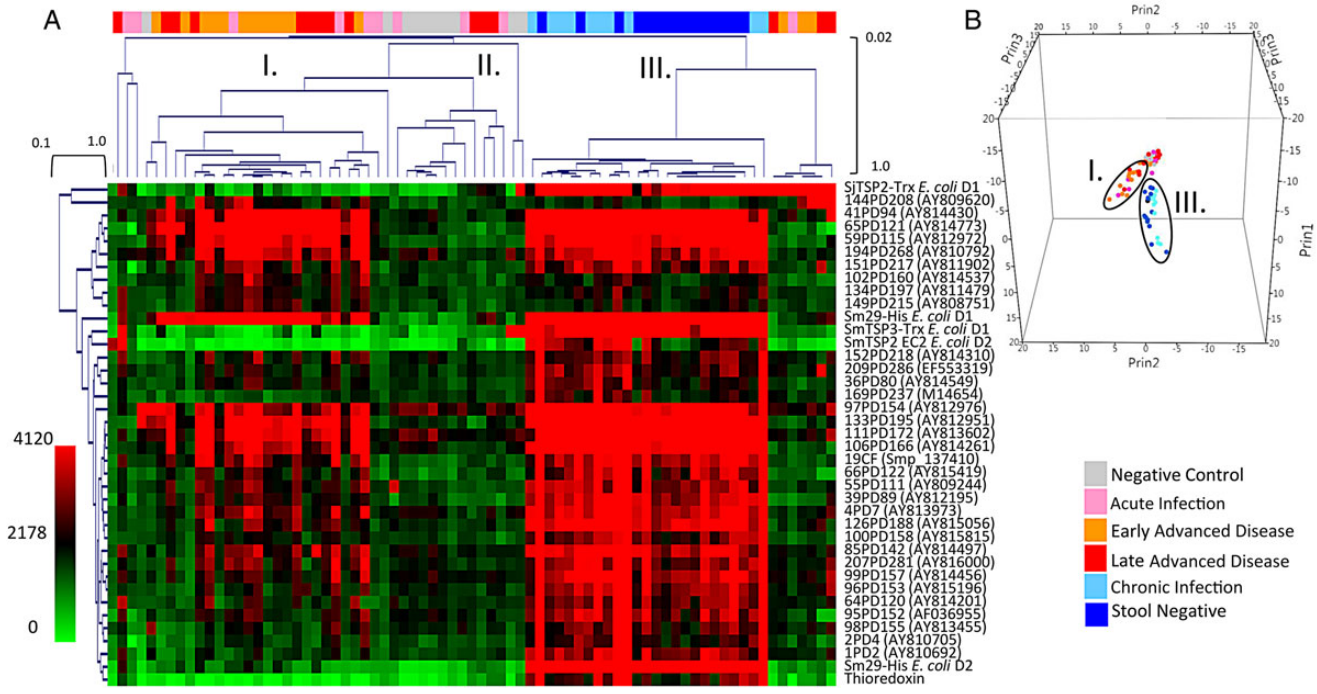

Figure 4. Clustering of subjects with chronic infection and those with negative stool specimens from remaining samples, using seroreactive antigens. *A*, Two-way hierarchical cluster diagram of the 39 seroreactive protein microarray features (rows) and 75 human serum samples (columns). Seroreactive antigens were recognized by at least 50% of any schistosome-exposed groups with significant Benjamini and Holmberg *P* values between groups. *B*, Principal component analysis 3-dimensional plot of seroreactive antigens from human samples. Samples group into similar clusters (marked) discovered from hierarchical clustering (Figure 4*A*). The major clusters are labeled as follows: high-reactivity samples from subjects with advanced disease and those with acute infection (cluster I) and subjects with chronic infection and subjects with negative stool specimens (cluster III). The remaining negative controls and low-reactivity samples that compose cluster II are not labeled.

Table 1. Serodiagnostic Antigens for *Schistosoma japonicum*–Exposed Individuals

Accession Number	Details	Disease Group Diagnostic
AAC98911[a]	Sm29, vaccine investigated in pre-clinical trial	All
ABQ44513[a,b]	Sj tetraspanin (TSP) 2, vaccine investigated in pre-clinical trial	Ch/SN
AAN17276[a,b]	SmTSP-2, vaccine investigated in pre-clinical trial	Ch/SN
CCD59145[a,b]	SmTSP3, 25 kDa protein	Ch/SN
Smp_137410	Smp80/Sm calpain A, vaccine investigated in pre-clinical trial	Ch/SN, Ac, EA
AY810692[b]	Glucose transport	Ch/SN
AY814549[b]	SPARC precursor	Ch/SN
AY812195[b]	Extracellular superoxide dismutase	Ch/SN
AY814430	Sj calpain A, vaccine investigated in pre-clinical trial	Ch/SN, EA
AY809244[b]	Hypothetical; DEA-box helicase	Ch/SN
AY812972	Hypothetical	Ch/SN, EA
AY814201[b]	Hypothetical; gcn related	Ch/SN
AY814773	Hypothetical	Ch/SN, EA
AY815419	Hypothetical	Ch/SN, Ac, EA
AY814497[b]	Hypothetical	Ch/SN
AF036955	SjTSP3	Ch/SN, EA
AY815196[b]	SjTSP26	Ch/SN
AY813455[b]	SjTSP5	Ch/SN
AY814456[b]	SjTSP11	Ch/SN
AY815815[b]	SjTSP6	Ch/SN
AY815056[b]	sim. XP 392549; MARVEL transmembrane protein	Ch/SN
AY812951	Mastin precursor, peptidase	Ch/SN, EA
AY811902	Syntenin	Ch/SN, EA
AY816000[b]	Cytochrome b561	Ch/SN
EF553319[b]	SjTSP2, vaccine investigated in pre-clinical trial	Ch/SN

[a] In vivo–expressed recombinant protein.

[b] Proteins diagnostic for subjects with chronic infection (Ch) and those who had negative stool specimens (SN) only.

Subjects with acute infection (Ac) and those with early advanced disease (EA).

Immunodominant Antigens as Potential Disease Biomarkers

The predictive value of the seroreactive antigens as disease biomarkers was measured using areas under the curve (AUCs) from the receiver operating characteristic curve (ROC; Supplementary Table 8). The chronic and SN groups were combined for AUC analysis. To select immunodominant antigens, proteins were excluded when recognized by >40% of the negative controls, when not recognized by ≥60% of subjects in at least 1 schistosome-exposed group, or when the AUC was <0.9. This resulted in 25 immunodominant antigens (Table 1) recognized by specific serum antibodies in the *S. japonicum*–endemic population that we considered as potential biomarkers of pathology/disease types. Most of the immunodominant antigens (16 of 25) were only diagnostic for chronic and SN subjects and had high AUCs and frequency of recognition values against either the negative controls or all of the remaining groups combined (Table 1 and Supplementary Table 7). Consequently, these 16 antigens, detected only by the chronic and SN serum samples, appear to be excellent for discriminating schistosome-exposed individuals with mild pathology or, potentially, resistance against severe pathology. The remainder, depending on the antigen, are potential biomarkers for schistosome exposure, early advanced and acute disease, and mild pathology (chronic and SN groups).

The *S. mansoni* tegumental protein Sm29 (AAC98911) had high AUCs and frequency of recognition values and was detected by all of the schistosome-exposed groups. Besides Sm29, early advanced subjects produced specific antibodies against 8 other antigens, including Sj calpain A, the *S. mansoni* homologue and vaccine antigen Sm calpain A/Smp80 [28], 3 hypothetical proteins, tetraspanin 3 [SjTSP3], mastin-like protease, and syntenin (Table 1). Acute samples recognized only 2 of these 8 antigens strongly: Sm calpain A/Smp80 and hypothetical AY815419. The former is highly transcribed in *S. mansoni* cercariae and the latter is one of only 3 immunodominant antigens (including Sj calpain A) detected proteomically in *S. japonicum* cercariae (Supplementary Table 2). Accordingly, 3 antigens (Sm29, hypothetical AY815419, and Sm calpain A) may be effective for diagnosing current or previous schistosome infection, with the possible exception of infection in late advanced subjects.

Similar to the seroreactive antigens, a majority of the immunodominant antigens (18 proteins [72%]) were surface or secreted proteins (Supplementary Tables 2 and 7). While antibodies against individual surface-located tetraspanins [29, 30] have been commonly found in sera of individuals from areas of endemicity, other tetraspanins have not been studied. The immunodominant proteins included 6 RTS tetraspanins that shared very high sequence homology, excluding SjTSP26 (Supplementary Table 7). Additionally, a syntenin (AY811902), a protein that has multiple functions, including interaction with tetraspanins, particularly TSP2 [31], and was partially protective in an *S. mansoni* vaccine [32], was also in the immunodominant protein set. Other surface-exposed proteins included a glucose-transporter, proteases (calpain and mastin), the extracellular Cu^{2+}-Zn^{2+} superoxide dismutase (AY812195) [33], and several proteins with limited annotations. The tegumental iron transporter Sj cytochrome b561 (AY816000) [34], fibrosis factor SPARC precursor/osteonectin (AY814549), and MARVEL transmembrane protein (AY815056) were also immunodominant antigens (Table 1).

DISCUSSION

To better understand the human antibody response following exposure to schistosomes, sera from Chinese subjects in an *S. japonicum*–endemic area were used to probe a customized schistosome protein microarray. Unlike our previous study comparing chronically infected individuals to putatively resistant individuals for vaccine discovery [19], this work identified

specific antibody responses by using subjects with well-defined clinical diagnoses (ie, acute, early advanced, late advanced, and chronic disease), as well as uninfected controls and SN migrant fishers exposed to *S. japonicum* [14, 21] (Supplementary Table 1). This study has several main findings. First, despite generally high levels of whole-parasite serum antibodies, subjects with mild or no pathology (ie, those in the chronic and SN groups) recognized a discrete set of antigens, whereas subjects with more-severe pathology (ie, those in the acute and advanced groups) did not have this antibody profile. Second, antigens capable of discriminating different disease types were identified. Third, pathology appeared to be related to antibody recognition of defined and typically surface-exposed parasite proteins.

The similarity between the SN and chronic subjects was apparent in the protein microarray data and specifically in their reactivity to the seroreactive antigens. Data visualization further emphasized this point. However, it is uncertain whether these findings indicate that the SN subjects had undiagnosed chronic infection or whether they were uninfected but had a similar antibody profile to that of chronic subjects. The population survey used to identify the chronic and SN subjects was cross-sectional, and previous schistosomiasis diagnoses were unavailable. However, subjects were checked using indirect hemagglutination serologic analysis, Kato-Katz thick smears, and the miracidial hatching test at the time of blood collection, thereby reducing the rate of false-negative findings [21, 22]. Therefore, SN subjects were presumed to be uninfected although they had previous schistosome infections, had repeated contact with schistosome-infected water, and were not treated with praziquantel for at least 2 years prior to sample collection (Supplementary Table 1).

Our findings demonstrate that low or no pathology in exposed individuals is accompanied by serum IgG recognition of a defined set of mostly surface and secreted proteins. Indeed, among these immunodominant antigens, which were mostly reactive with chronic and SN antiserum, were numerous proteins with signal peptides, transmembrane domains, and surface membrane gene ontologies (Table 1 and Supplementary Tables 2 and 7). In particular, multiple *S. japonicum* tetraspanins and the tetraspanin-associated Sj syntenin [31] were recognized predominantly by sera from the chronic and SN subjects. The detection of multiple tetraspanins could be caused by antibodies cross-reactive against shared tetraspanin epitopes [35] or by specific antibodies against a tetraspanin web consisting of tetraspanins and associated molecules in the parasite tegument [29, 31, 36]. Other tegument or surface-associated immunodominant proteins included *S. mansoni* vaccine candidates about to commence human trials (calpain/Smp80 and TSP-2) [28, 37]; Sm29, another leading vaccine candidate [38, 39]; and transmembrane proteins (glucose transporter, MARVEL, and cytochrome b561 related).

In addition to identifying antigens recognized among subjects with different schistosomiasis pathologies, our findings also provide clues about the development of pathology and mechanisms of resistance to reinfection in human populations. The link between parasite-specific antibodies with pathology and resistance is well studied. Differing antibody responses have been associated with schistosomiasis pathology and resistance in Hunan and other parts of China, in the Philippines, in Brazil, and in Africa [9, 11, 12, 14, 29, 40, 41]. Specifically, antibodies directed against tegument proteins are believed to be necessary for the development of resistance [29, 39, 41]. A number of hypotheses exist to explain the development of human schistosome immunity. Individuals may become resistant to more-intense or more-severe infection by immunological exposure to antigens following repeated parasite death (via drug treatment or prolonged exposure), thereby allowing recognition of a set of so-called threshold antigens [12, 42], recognition of natural population variants of individual parasite antigens [43–45], or so-called B-cell epitope spread from adult-stage antigens to vulnerable larval-stage antigens [41]. Finally, it has been proposed that the high antiglycan responses apparent in the acute and advanced groups are a smokescreen that diverts the immune system from more-protective responses against protein antigens [46–48].

Our findings lend weight to some of these hypotheses. We have shown that recognition of immunodominant antigens, consisting largely of surface proteins, is associated with mild pathology. It is unclear whether immune recognition of these immunodominant antigens causes or is the consequence of the mild pathology in these subjects. For instance, host genetics, an important correlate of pathology [2, 4, 7], may promote an immune response favorable to parasite elimination and therefore expose the host to more schistosome antigens. Nonetheless, immune recognition of threshold antigens is believed to have antifecundity effects that will directly reduce the severity of pathology in the host [42]. Potentially, the identified immunodominant antigens and, presumably, other unidentified proteins may contribute to the development of infection with mild pathology. In contrast, high total parasite IgG responses by the subjects with more-severe pathology in this study show that lack of recognition of the immunodominant antigens is not the consequence of a suppressed antiparasite antibody response. While many of the specific antigens recognized by the subjects with more-severe pathology are unknown, egg proteins and smokescreen glycan antigens, as suggested by our ELISA results, may be prominent [46–48].

Pending further investigation, the diagnostic potential of the identified immunodominant antigens could be significant. Compared with current methods of assessing pathology, typically based on clinical examination and ultrasonography [2, 4], a preliminary serological assay using defined antigen-specific antibodies would be an important advance and may inform

medical treatment requirements. Although some of the immunodominant antigens have been investigated in *S. japonicum*–endemic populations [17, 29, 38], the majority are untested biomarkers that could be used to differentiate disease groups. Additionally, considering the importance of the IgG subclasses, IgM, and immunoglobulin E in human serological studies [19], future research should examine the reactivity of these antibody classes against the identified immunodominant antigens and other protein microarray antigens.

However, while our results using well-defined clinical groups and rigorous data analyses are quite clear, these findings should be confirmed by further field studies in *S. japonicum*– and *S. mansoni*–endemic areas. Larger subject groups and prospective sampling will determine help whether, for example, antibody recognition of the immunodominant antigens is associated with or, more speculatively, is predictive of schistosomiasis pathology. In addition, only a subset of potentially high-value protein targets was used for the current protein microarray, and no glycans and few cytoplasmic and egg-stage antigens were screened. Future studies with a more complete schistosome proteome may reveal other antigens that are recognized by different disease types.

In conclusion, screening of samples from individuals living in an area of *S. japonicum* endemicity with the schistosome protein microarray has demonstrated significant differences in the antibody profiles for sera from individuals in the acute, advanced, chronic, and SN groups. A set of 25 antigens, most of which are surface-located proteins, including several *S. mansoni* and *S. japonicum* tetraspanins, as well as previously described vaccine candidates, were identified. Subjects in the acute and advanced disease groups had a high total antiparasite antibody response but a reduced antibody response against protein microarray antigens, compared with subjects in the chronic and SN groups. This suggests that subjects with mild pathology use a broad and strong antibody response, particularly against surface-exposed proteins, to control pathology and/or infection. These findings may allow serological differentiation of schistosomiasis phenotypes and have further elucidated the human antibody response during schistosome infection.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

Acknowledgments. We thank Drs Andreas Hoffman, Asiah Osman, Wenbao Zhang, and Jun Li, for providing recombinant *S. mansoni* and *S. japonicum* proteins.

Financial Support. This work was supported by the National Health and Medical Research Council Australia (grants APP1037304 and APP1002245, senior principal research fellowship to D. P. M., and principal research fellowships to A. L. and D. L. D.).

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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