Revista da Sociedade Brasileira de Medicina Tropical
29(5):441-445, set-out, 1996.

ANTIBODY RESPONSE TO *SALMONELLA TYPHI* IN HUMAN SCHISTOSOMIASIS MANSONI

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Antibody response to Salmonella typhi *O and H antigens was evaluated in 24 individuals with either hepatointestinal or hepatosplenic schistosomiasis mansoni before and after typhoid vaccination, and compared with that of non-infected controls. Before vaccination,* Schistosoma-*infected patients showed a higher frequency of positive antibody to O antigen and the same frequency to H antigen when compared with that of healthy individuals. However, those with hepatosplenic schistosomiasis showed higher titres of antibody to H antigen than those with hepatointestinal disease or healthy individuals. Infected subjects, particularly those with hepatointestinal disease, showed a decreased response after typhoid vaccine. This diminished ability to mount an immune response towards typhoid antigens during schistosomiasis may interfere with the clearance of the bacteria from blood stream and, therefore, play a role in the prolonged survival of salmonella as observed in some patients with chronic salmonellosis associated with schistosomiasis.*

Key-words: Schistosomiasis mansoni. Salmonella typhi. *O and H antigens. Chronic salmonellosis associated with schistosomiasis.*

Activation of the immune system has been demonstrated during acute schistosomiasis[2,3,27] and, although modulation of both the cellular[15,27] and humoral[18,19] immune responses occur later during the chronic phase of the disease, high levels of anti-*Schistosoma mansoni* antibodies are still detectable. However, the ability of infected individuals to mount an immune response to unrelated antigens has not yet been clearly established.

It has been shown that some *Schistosoma*-infected individuals co-infected with salmonella produce less antibodies to both O and H antigens of this bacteria[20,23], and antibodies present in their sera show an impaired ability to inhibit *in vitro* salmonella growth[29]. However, serum from *Schistosoma*-infected hamsters can inhibit bacterial growth[13]. On the other hand, some human studies have indicated that schistosomiasis impairs the immune response to anti-salmonella vaccine[5], while others do not seem to support this conclusion[26].

An altered ability of *Schistosoma*-infected individuals to produce antibodies to unrelated antigens may result in a constraint to the elimination of some pathogens. For instance, it has been shown that antibodies help the clearance of salmonella from the blood stream[11]. Therefore, a deficient production of antibodies to salmonella in schistosomiasis may facilitate the development of the atypic form of salmonellosis which occurs in *Schistosoma*-infected individuals.

This work aimed at evaluating the antibody response to *Salmonella typhi* in *Schistosoma*-infected individuals before and after typhoid vaccine.

MATERIAL AND METHODS

The rules put forward by the Helsinki Declaration for experiments in human subjects[6,30] were strictly followed throughout this investigation.

Twenty six patients of both sexes, 13 to 43 years old (mean ± SD = 24 ± 10), mostly. Caucasians (75%) were studied. The diagnosis of schistosomiasis was established on clinical and parasitological grounds. Our study group

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Recebido para publicação em 05/10/95.

comprised 16 individuals with the hepatointestinal, and 10 with the hepatosplenic schistosomiasis, characterized according to the criteria defined by Prata[21]. Their clinical and nutritional status were satisfatory. Eggs of *Schistosoma mansoni* in faeces were quantified by the Kato-Katz method[8]. The control group comprised nine healthy individuals of both sexes, 20 to 50 years old (mean ± SD = 29 ± 9), 70% being Caucasians.

The *Salmonella typhi* vaccine, prepared with 10^8 formol-treated bacteria strain Ty2 by the Instituto Oswaldo Cruz, was intradermally administered in a single dose, as indicated by the manufacturer. Before vaccination and 30 days after serum antibodies to O and H antigens were detected using a Widal agglutination test[29] provided by Laborclin. Titres > 20 were considered as positive.

The results were statistically analised by the Chi-square test and Fisher's exact test, at a level of 5% of significance ($p < 0.05$).

RESULTS

Antibodies to O antigen of *Salmonella typhi* were detected in 3 out of the 26 *Schistosoma*-infected patients (11.5%) before the application of typhoid vaccine and in none of the normal controls (Table 1). In the positive individuals, the antibodies titres were 40, 80 and 160, and they presented 96, 672 and 984 eggs of *Schistosoma mansoni* per gram of faeces, respectively. The frequency of pre-vaccination positive titres to H antigen was the same in infected and healthy individuals (Table 1). However, the highest titres of antibody to H antigen were found in the hepatosplenic patients and no difference was detected between the hepatointestinal and control groups (Table 2).

Table 1 - Frequency of pre-vaccination positivity of antibody to Salmonella typhi *O and H antigens in hepatointestinal (HI) and hepatosplenic (HS)* Schistosoma mansoni*-infected patients and healthy individuals.*

Group	Antibodies to			
	O antigen		H antigen	
	nº	%	nº	%
Normal	0/9	0.0	4/9	44.4
HI	1/16	6.3	8/16	50.0
HS	2/10	20.0	5/10	50.0

$p > 0.05$ for both antigens by Chi-square test (3 x 2 table) and Fisher's exact test (2 x 2 table: HI x Normal, HS x Normal, HI x HS).

Table 2 - Frequency of high titres of antibody to salmonella H antigen in hepatointestinal (HI) and hepatosplenic (HS) Schistosoma mansoni*-infected patients and healthy individuals before typhoid vaccine.*

Group	Titre > 80	
	nº	%
Normal	1/4	25
HI	2/8	25
HS	4/5	80

$p > 0.05$ by Fisher's exact test (2 x 2 table: HI x Normal, HS x Normal, HI x HS).

Thirty days after vaccination, 20.8% of *Schistosoma*-infected patients and 55.5% of normal controls presented antibodies to salmonella O antigen. Within the group of infected individuals a difference in the frequency of positivity was found between the hepatointestinal (12.5%), hepatosplenic (37.5%) and normal (55.5%) subjects (Table 3). An antibody response to H antigen was also less frequently found in the hepatointestinal (50%) than in hepatosplenic (75%) or normal individuals (100%) ($p<0.05$) (Table 3).

Table 3 - Frequency of antibody response to Salmonella typhi *O and H antigen in hepatointestinal (HI) and hepatosplenic (HS) patients and healthy individuals after typhoid vaccination.*

Group	Antibody response to			
	O antigen		H antigen	
	nº	%	nº	%
Normal	5/9	55.5	9/9	100
HI	2/16	12.5*	8/16	50**
HS	3/8	37.5	6/8	75

$p = 0.07$ for O antigen by Chi-square test (3 x 2 table)

$p = 0.03$ for H antigen by Chi-square test (3 x 2 table)

* $p = 0.058$ for O antigen by Fisher's exact test (2 x 2 table: HI x Normal)

** $p = 0.02$ for H antigen by Fisher's exact test (2 x 2 table: HI x normal)

DISCUSSION

Before vaccination, 11.5% of *Schistosoma*-infected patients showed positive antibody response to salmonella O antigen as compared to no response of healthy controls. However, the basal frequency of reactivity to salmonella H antigen in our infected group was similar to that of the controls. This same frequency of antibodies to salmonella was previously shown by Shikanai-Yasuda et al[25] in hepatosplenic patients and by Milhomen and Suassuna[14] in healthy individuals.

The antibody to salmonella O antigen, found before vaccination of *Schistosoma*-infected patients, is an IgM[22,26] and its presence

indicates recent infection by this bacteria[1,4,10]. However, since our patients had neither a history nor any clinical evidence of overt salmonellosis, it is possible that anti-O agglutinins were originated from subclinical salmonella bacteremias. Our finding of increased levels of anti-H antibodies in subjects with hepatosplenic schistosomiasis supports this hypothesis. Another possibility which cannot be ruled out is the occurrence of cross antigens between salmonella and schistosome[7].

In our study, schistosome-infected patients, particularly those with the hepatointestinal disease, showed an inadequate antibody response to salmonella O and H antigens after a single dose of typhoid vaccine. The immune response of hepatosplenic subjects appears to be less impaired, as previously shown after three doses of the vaccine[26] in those with this form of the disease. The finding that supressor T lymphocytes ($CD8^+$) are increased in hepatointestinal patients and decreased in those with hepatosplenic schistosomiasis[28], may help understanding these results. Alternatively, the better antibody response of hepatosplenic individuals may be due to previous contact with salmonella, made easier by the porto-systemic shunts found in these patients. The increased pre-vaccination levels of anti-salmonella antibodies in this group favors this possibility.

It has been recognized that both the functions of T lymphocytes[15,17] and macrophages[16] are altered in *Schistosoma*-infected patients. Our data demonstrate that the ability to produce antibodies to unrelated antigens is also impaired in schistosomiasis. The fact that increasing the antigenic stimulus may induce antibody production in infected subjects[26] may be interpreted as antigenic competition[12] between schistosome[27] and salmonella[9] antigens present in the serum of these individuals.

The decreased ability of schistosome-infected individuals to mount an adequate immune response to salmonella may very well contribute to the establishment and prolonged survival of this bacteria in subjects suffering from schistosomiasis.

RESUMO

A resposta de anticorpos para os antígenos O e H da Salmonella typhi *foi avaliada em 24 indivíduos com esquistossomose hepatointestinal ou hepatoesplênica antes e após vacinação antitifoídica, e comparada com a resposta de indivíduos controles normais. Antes da vacinação, pacientes esquistossomóticos mostraram uma maior frequência de anticorpos positivos para o antígeno O e a mesma frequência de anticorpos positivos para o antígeno H quando comparada com aquela de indivíduos controles normais. Porém, aqueles com esquistossomose hepatoesplênica mostraram títulos maiores de anticorpos para o antígeno H do que aqueles com a forma hepatointestinal da doença ou os indivíduos controles normais. Pacientes esquistossomóticos, particularmente aqueles com a forma hepatointestinal, mostraram uma menor resposta após a vacinação antitifoídica. Esta menor capacidade para apresentar uma resposta imune para antígenos de salmonela pode interferir com a retirada da bactéria da corrente sanguínea e, portanto, contribuir como um dos determinantes de sua prolongada sobrevivência como observada em pacientes com salmonelose septicêmica prolongada associada à esquistossomose.*

Palavras-chaves: Esquistosomose mansoni. Salmonella typhi, *Antígenos O e H. Salmonelose septicêmica prolongada associada à esquistossomose.*

ACKNOWLEDGEMENTS

We thanks Mr. Rodolfo Gomes de Oliveira and Mr. Joaquim Cândido de Lima by their help in the field studies.

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