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HOMOCYTOTROPIC ANTIBODY IN SCHISTOSOMIASIS MANSONI

by

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Patients with schistosomiasis mansoni show several immunological responses such as: 1) reactions of the granulomatous type; 2) reactions mediated by IgG and IgM humoral antibodies; and 3) reactions mediated by homocytotropic or skin-sensitizing antibodies responsible for the immediate skin reaction to schistosome antigens.

The human homocytotropic antibody is responsible for immediate skin reactions and transfer of hypersensitivity in the Prausnitz-Küstner (P.K.) test, observed in allergic diseases provoked by several allergens, such as inhalants, foods, drugs and certain parasites.

So far, only one class of human homocytotropic antibody has been detected, but in several species two and even three types have been described (41).

Although the presence of antibodies demonstrated by the P.K. test has been reported in schistosomiasis mansoni, there is no information on their biological and immunochemical properties (2, 13, 32, 54), in contrast with several studies in animals (47, 49, 50).

The purpose of this paper is to study the antibody responsible of the P.K. test in schistosomiasis mansoni.

MATERIAL AND METHODS

1) *PRAUSNITZ-KÜSTNER (P.K.) TEST**Donors*

The sera were from patients in the Hospital Clinics of the Medical School, University of São Paulo and Federal University, Pernambuco (Recife), Brasil. All 35 donors presented schistosomiasis mansoni, confirmed by the finding of schistosoma eggs in the feces or by rectal biopsy. Donors with transmissible diseases, mainly hepatitis, were excluded.

The serum was separated, filtered in Seitz filters and kept frozen at -20°C . Sixty P.K. tests were made with untreated sera; 81 with sera heated at 56°C ; 17 with sera treated by 2-mercaptoethanol, and 12 using dilutions of sera or antigen. Ten questionable P.K. tests were repeated.

Effect of heating

1) In some experiments, sera were heated at 56°C for 2 hours (sera 6, 7, 9, 15, 18, 21).

2) In other experiments sera (1105 and 1135) were heated at 56°C for 30, 60, 120 and 240 minutes.

Reduction and alkylation of sera

Some sera were submitted to reduction and alkylation with 2-mercaptoethanol and iodoacetamide, following the technique described by *Mota & Perini* (34). Samples of these sera were compared with samples of the same untreated sera, and the P.K. tests were performed 24, 96, and 168 hours after the preparatory injection.

Recipients

Normal relatives of patients or volunteers with negative intracutaneous tests to schistosomin were used as recipients. Usually two different recipients were used.

Reading of the P.K. test

The usual standard P.K. technique was employed (48).

With some sera (1105 and 1135) several P.K. tests were made, in which schistosomin was injected after 1 hour, 3 hours, 6 hours, 24 hours, and 48 hours in skin sites previously treated with the donor serum. With serum 1256 the experiment was prolonged for 3, 5 and 7 days. For comparison similar experiments were made with patients' sera with positive P.K. test to an inhalant allergen (*Ricinus communis*) and food (fish).

A reading was made after 15 and 30 minutes, the size of the wheal and surrounding erythema was measured and these data were collected in translucent paper.

Schistosoma mansoni antigens (*Schistosomin*)

Schistosomin was prepared according to the technique described by *Pellegrino* (40). In the passive sensitization tests of monkey's ileum schistosomin was prepared in 0.85 per cent NaCl without preservatives.

Antibody titration

The titer of antibody in serum was expressed in two ways:

1. The greatest dilution of serum capable of producing a positive P.K. test, when two fold dilutions of 1/2 to 1/256 of sera and undiluted schistosomin were used.
2. The greatest dilution of schistosomin capable of producing a positive P.K. test, when two fold dilutions of 1/100 to 1/1600 of antigen and undiluted sera were used.

In the first titration sera 2, 6, 7 and 9 were used, in the second a pool of these sera was employed.

II) *P.C.A. IN MONKEYS*

The method of *Layton et al.* (28) was employed to perform the P.C.A. in monkeys.

Monkeys: *Saimiris sciureus sciureus* (L) and *Cebus negritus* (L) were used.

P.C.A. technique: The skin of the monkeys was previously shaved with an electrical hair clipper. Then a 0.10 ml of the mixture of sera positive to P.K. reactions (2, 5, 7 and 9) was injected intracutaneously. These injections were given in two to four different sites in 1:1 and 1:4 dilutions.

Schistosomin was injected intravenously after 24 hours in a dose of 0.10 ml together with 2 ml of a 1 per cent solution of Evans blue. In other experiments, schistosomin was injected intracutaneously in the eyelid 24 hours after the serum, preceded or not of an intravenous injection of Evan's blue at 1 per cent 15 minutes before.

Positive reactions consisted in the appearance within a few minutes of a blue circumscribed area in the sensitized spot. When the direct intradermal injection of antigen was used the positive reactions were smaller in size, similar to a P.K. test.

III) *PASSIVE SENSITIZATION OF MONKEY ILEUM*

The method of *Arbesman et al.* (3) was employed using apparently normal monkeys, *Alouatta sp.*

After verifying that schistosomin did not produce contraction in the non sensitized muscle, 0.20 ml of schistosomin in 1/1, 1/5, 1/10 and 1/50 dilutions were added, and contractions were recorded in a chymograph.

IV) QUANTITATION OF IMMUNOGLOBULIN E (IgE)

ROWE's technique (46) was used with the following reagents:

- 1) Sheep antihuman IgE, provided by Pharmacia Fine Chemicals, Uppsala, Sweden.
- 2) Rabbit gammaglobulin labelled with ^{131}I and containing antibodies to sheep IgG, provided by the Disciplina de Imunologia da Escola Paulista de de Medicina, São Paulo, Brasil.
- 3) A reference preparation for IgE (Pharmacia Fine Chemicals, Uppsala, Sweden), containing 10,000 ng/ml.
- 4) Kodak koridex X-ray film, 9×12 cm, developed in Kodak DX80 developer.

RESULTS

The results are summarized in Tables 1, 2, 3 and 4.

I) PRAUSNITZ-KÜSTNER TEST (P.K.)

a. *Positive reactions*

The P.K. test was positive in 28 out of 35 sera from donors with schistosomiasis. The intensity of reactions was identical with 20 sera in both when 2 recipients were used. A difference in reactivity was observed in the recipients with the sera 2, 7, 13, 14 and 1256. With the sera 14 and 1256 one of the recipients presented a definite positive reaction and the other showed a negative reaction.

Thirty-one sera were from patients with the hepatosplenic form, and 4 others with the intestinal form of schistosomiasis. The intensity of positive reactions ranged from wheals and erythema, significantly larger than those reactions provoked only by serum or schistosomin (at least 5 mm more), up to wheals larger than 5 mm and erythema larger than 10 mm (Table 1).

Negative reactions occurred with sera 3, 4, 10, 11, 16, 19 and 22, from patients with hepato-splenic forms, except for no. 3 who had the intestinal form. Five of these sera were from children or young adults. All positive reactions occurred with sera from patients over 21 years of age.

TABLE 1.
Positive P.K. Tests in *Schistosomiasis mansoni*.

Sera (numbers)	Intensity of the reactions			Skin reaction	Total
	wheal (mm)	pseudo- pods	erythema (mm)		
1, 3, 17, 28, 31	5	no	10 to 15	1 +	5
2, 12, 15, 18, 20, 21, 27, 29, 33	5 to 10	no	15 to 20	2 +	9
6, 8, 9, 13, 14, 30, 1105	10 to 15	yes	20 to 30	3 +	7
7, 23, 24, 25, 26, 32, 1256	15 to 20	yes	over 30	4 +	7

b. Effect of heating

1) Sera heated at 56° C for 2 hours lost their property to produce P.K. reaction (sera 6, 7, 9, 15, 18 and 21).

2) Table 2 sums up the results obtained with P.K. tests, in which sera were heated for 30, 60, 120 and 240 minutes. Schistosomin was injected after 1, 3, 6, 24 and 48 hours.

Sera 1105 and 1135, heated at 56° C at the different times above mentioned, lost their reactivity after 4 hours, and the intensity of reactions was smaller after 2 hours. The intensity of reactions decreased after 2 hours heating at 56° C. A slight difference in the intensity of reactions with schistosomin was observed comparing to the results of two other positive sera with antibodies against *Ricinus communis* and fish (*Gadus morrhua*).

Positive P.K. test to schistosomin could be observed with intervals that ranged from 1 hour to 7 days. When the readings were made after 3, 6, 24 and 48 hours a slight difference in the intensity of reactions was observed, a larger diameter

TABLE 2.

Comparison of P.K. Tests among Sera from 1) Patients with Schistosomiasis and 2) Patients Allergic to Fish and Castor Bean, according to: 1) Sensitization Period; 2) Effect of Heating (56° C) in Different Periods of Time; 3) Characteristics of P.K. Reaction, from 1 Hour up to 48 Hours.

Effect of heating (56° C) in different periods of time (minutes)	Fish (0.02 ml)		Castor bean (0.02 ml)		Schistosomin (0.02 ml)	
	papule (mm)	erythema (mm)	papule (mm)	erythema (mm)	papule (mm)	erythema (mm)
t = 1 hour	SENSITIZATION PERIOD					
0	12	35×40	20	40×50	21	35×40
30	11	30×30	13	25×30	19	30×35
60	5	15×18	12	22×32	20	25×25
120	—	—	—	—	15	—
240	—	—	—	—	16	—
t = 3 hours	SENSITIZATION PERIOD					
0	9	28×35	12	40×45	11	20×25
30	5	15×20	11	35×35	7	15×15
60	—	8×8	8	30×33	10	20×25
120	—	5×5	5	12×12	5	11×12
240	—	4×4	—	8×8	—	—
t = 6 hours	SENSITIZATION PERIOD					
0	17	45×50	16	40×40	10	30×35
30	—	30×20	12	30×30	10	20×25
60	—	10×8	11	30×30	8	15×15
120	—	—	11	—	6	15×15
240	—	—	3	—	—	—
t = 24 hours	SENSITIZATION PERIOD					
0	20	45×55	17	45×50	11	35×40
30	13	30×45	15	35×40	9	25×30
60	6	12×15	12	30×30	7	12×12
120	—	5×5	9	20×25	6	10×15
240	—	5×5	6	—	—	—
t = 48 hours	SENSITIZATION PERIOD					
0	18	40×35	21	35×45	9	30×35
30	5	15×12	13	30×40	9	20×30
60	2	—	10	30×40	8	15×15
120	2	—	7	20×30	—	—
240	6	—	—	—	—	—

TABLE 3.

Comparison of P.K. Tests between Sera treated with Mercaptoethanol and non-treated Sera.

Sensitization period (hours)	Treated with mercaptoethanol (numbers)	Schistosomiasis sera		
		Diameter of P.K. reaction (mm)	Not treated	Diameter of P.K. reaction (mm)
24	23+25	30×40	23+25	50×50
96	23+25	45×50		
24	24+26	20×45	24+26	50×50
168	24+26	30×40	24+26	40×70
24	23+26	15×40		
168	23+26	negative		
24	1256	30×40	1256	40×40
72			1256	35×40
168			1256	40×50

being observed after 24 hours. After 3, 5 and 7 days the reactions continued with similar intensity.

c. *Antibody titration by the P.K. test*

In the 4 sera selected for titration, responsible for strongly positive P.K. test, it was observed that the greatest dilution of the sera capable of producing a P.K. reaction was the following: serum 6—positive up to a 1/4 dilution; sera 2 and 9—positive up to a 1/8 dilution; and serum 7—positive up to a 1/128 dilution.

The greatest schistosomin dilution capable of producing a P.K. reaction when a mixture of the 4 selected sera was used was 1/800.

d. *Reduction and alkylation of sera*

The reduction and alkylation with 2-mercaptoethanol and iodoacetamide did not eliminate the positivity of the P.K. test, 24, 96 and 168 hours after the preparatory injection, although the intensity of reactions was smaller than that ob-

served with untreated sera (Table 3). Two experiments with P.K. tests were made 168 hours after the preparatory injection. One of the reactions was negative and the other was positive, although of smaller intensensity than the one observed after 24 hours.

P.K. positive sera, after undergoing reduction and alkylation and at the same time being heated at 56° C for 2 hours showed a negative result when the P.K. test was made 24 hours later.

II) P.C.A. IN MONKEYS

A positive P.C.A. test in the monkey *Cebus negritus* was observed when mixtures of sera 2, 5, 7 and 9 were injected by intradermal route in the eyelid, and 24 hours later, schistosomin was injected at the same site without previous injection of Evans blue. The reaction consisted of an erythematous papule with pseudopods. Edema developed 10 minutes later. When the serum was injected intracutaneously in the eyelid or abdomen and 24 hours later the schistosomin and the Evans blue were injected intravenously, the reactions obtained were scattered and generally produced a bluish color all over the skin.

III) PASSIVE SENSITIZATION TEST OF THE MONKEY ILEUM

Only in two out of 6 monkeys in which the test was made, the response was considered positive. This consisted of the contraction of the muscle in contact with the antigen, and no response of the same after continuous additions of schistosomin.

The results were negative when the ileum was incubated with the same sera, but heated at 56° C for 4 hours. Additions of histamine phosphate were used to find out whether the preparation responded normally.

TABLE 4.

Relationship between IgE Levels and P.K. Reactions. Presence of Another Parasitosis.

Sera numbers	Levels of IgE (ng/ml)	P.K. reaction	Parasites in the stool
23	2450	4+	Ancylostoma duodenale
24	3150	3+	—
25	2500	1+	Ancylostoma duodenale and Strongyloides stercoralis
26	2450	1+	Ancylostoma duodenale and Ascaris lumbricoides
27	2350	2+	—
28	300	1+	Trichuris trichiura
29	2000	2+	Trichuris trichiura
30	2450	3+	Trichuris trichiura
31	500	1+	Ancylostoma duodenale
32	2300	4+	Ancylostoma duodenale
33	900	2+	—

IV) IMMUNOGLOBULINE (IgE)

Eight out of eleven sera in which IgE was measured showed increased concentrations (Table 4). There was a correlation between the intensity of P.K. reaction and the IgE concentration in the serum, inasmuch as the P.K. reaction was weakly positive in the two sera with 300 and 500 ng/ml of IgE, while in sera with more than 2,000 ng/ml IgE the P.K. test was strongly positive. In 8 patients another intestinal parasitosis was found. In two patients the fecal examinations were negative, but they had previously been treated for parasitosis. In only one of the patients the fecal examination was negative without a previous history of intestinal parasitosis.

DISCUSSION

The P.K. test was positive in schistosomiasis mansoni in 80 per cent of the sera; the same percentage was found by Stanworth (51) on studying this test in sera from patients

allergic to the most varied allergens. Seventy per cent of the sera from human patients with schistosomiasis gave positive P.C.A. reactions in monkeys (47). It was worth pointing out that among the 7 sera with negative P.K., 5 were from patients under 21 years of age.

Differences of reactivity to the same schistosomiasis serum sample were observed, since the same serum produced highly positive P.K. reactions in some recipients, and others did not respond.

With some restrictions, the P.K. test may be used for titration of the reaginic activity (1). Titres of antibodies found in schistosomiasis *mansoni* were comparatively low (1/128 for the serum, and 1/800 for the antigen) when compared with highly powerful sera of some patients allergic to castor beans and pollens.

When compared with two other sera with reagins to castor beans and fish the reaginic antibody of schistosomiasis showed one of the fundamental characteristics of other homocytotropic antibodies as far as thermolability is concerned. It was found that after heating for 2 hours, the P.K. capacity of the sera gradually decreased until a total disappearance of reactions after heating at 56° C for 3 or 4 hours.

Another characteristic of the reaginic antibody lies in its inactivity after reduction and alkylation (29, 43) but this did not occur, except partially, with some schistosomiasis sera. Indeed, some sera undergoing this treatment for 3 hours continued provoking P.K. reactions after 24, 48, and 168 hours. According to previous observations, the skin-sensitizing activity of the IgE antibody decreases very much after reduction in 0.1 M 2-mercaptoethanol for one hour, followed by alkylation with iodoacetamide, although 0.01 M 2-mercaptoethanol does not impair the skin-sensitizing activity (18). These authors' further studies on the effect of reduction-alkylation of IgE antibodies indicate that the binding activity of the antibodies to the antigen decreased approximately one third by the reduction with 0.1 M 2-mercaptoethanol followed by alkylation. It was also found that the sensitization

with IgE antibody treated with 2-mercaptoethanol followed by alkylation did not remain on the skin for a long time. Apparently 2 different portions of the molecule in the IgE antibody had been affected namely, the sites of the antibody combination and those of fixation in the tissue (35).

The passive transfer of human reagins is obtained in some very close primate species. All the developed primates are sensitized; whereas, in those which are underdeveloped the transfer experiments are more difficult to obtain (24, 26, 27, 28). Probably for this reason the passive sensitization of monkeys' ileum with schistosomiasis reaginic serum was not obtained in all animals. P.C.A. reaction with schistosomiasis serum was obtained when a similar P.K. technique was used. When the antigen and the dye were injected intravenously the reactions were not typical of P.C.A., being extensive and diffusely blue.

The activity of homocytotropic antibody is related to immunoglobulin E (IgE) (5, 16, 17, 20). In patients with schistosomiasis, the skin-sensitizing activity of the reaginic serum is neutralized by the specific antiserum against IgE (47). The IgE purified fraction of a reaginic serum could produce P.K. reaction in a 1/80,000 dilution (17, 19).

In human schistosomiasis *mansoni* high IgE values in the serum were usually found, and a correlation between the IgE titre and intensity of P.K. reaction was observed. The presence of another intestinal parasitosis in 8 patients could also contribute to the high levels of IgE. The intensity of the reactions should be related to the IgE concentration in the serum, as they are not produced by antibodies belonging to other immunoglobulins (18).

The average concentration of IgE in the serum of normal individuals is approximately 250 ng/ml (6). In human schistosomiasis *mansoni* values up to 3,150 ng/ml were found. These results agree with *Kojima et al.* (25), who found high levels of IgE in 60 cases of schistosomiasis *japonica*. High IgE concentrations are found in the serum of patients with parasitic diseases (8, 22). High serum levels of IgE have

been observed in patients with *Ascaris* (22), intestinal capillariasis with IgE values above 15,000ng/ml (44), visceral larva migrans (15) and trichinosis (45). Other parasitosis (Ancylostomiasis, paragonomiasis and fascioliasis) were also followed by high IgE levels (5, 25, 50). An increase of IgE concentration in the serum was confirmed in the study of visceral larva migrans, where a perfect correlation between homocytotropic antibody titre and high IgE levels was observed (15).

In a preliminary study of patients with asthma and rhinitis, high serum IgE levels were found with an average of 1191 ng/ml compared with a normal average of 250 ng/ml. Further studies of asthma and atopic dermatitis in the child and in the adult showed remarkable increases of IgE in the serum (5, 6).

Two properties observed in the homocytotropic antibody in human schistosomiasis deserve special attention, since they have not been mentioned in the studies on homocytotropic antibodies: 1) Positive P.K. reaction one hour after the injection of donor's serum, contrary to the conventional time period of at least 6 hours. However, this also has occurred with serum of patients allergic to castor beans and fish; 2) resistance of antibody to reduction and alkylation by 2-mercaptoethanol and iodoacetamide. Some sera thus treated still produced a P.K. reaction 24-168 hours later, although with a smaller intensity than those not treated.

The homocytotropic antibody of human schistosomiasis behaved like the thermostable antibody of the guinea pig: the early capacity of inducing a passive sensitization in the skin and the reactivity only partially diminished by the 2-mercaptoethanol treatment. On the other hand, it displayed properties as a thermolabile antibody, since it lost its capacity to provoke P.K. when heated at 56° C for 2-4 hours, and since the intensity of reactions proportionally decreased with the time of heating.

The aforementioned characteristics could suggest the existence of two subclasses of homocytotropic antibody in schisto-

schistosomiasis mansoni, both of the reaginic type, destroyed by heat and reacting in the skin over one week; only one of them probably being resistant to 2-mercaptoethanol. Nevertheless, more information will be necessary in the future in order to clarify this subject. In this context, *Girard's* findings (11) of two types of reaginic antibodies in the serum of a patient allergic to penicillin should be pointed out. A similar heterogeneity in patients allergic to pollens was mentioned (19).

Sera from different individuals may require different periods of heating for the destruction of their reaginic activity (10, 42), suggesting some chemical heterogeneity. The existence of subclasses of IgE with a reaginic activity which have not yet been described or other homocytotropic immunoglobulins still undefined is also suggested (1).

No rigid analogy between the human and the animal system is to be expected, but it would be tempting to assume the existence of more than one type of human homocytotropic antibody as occurs, for example, in rabbits, guinea pigs and rat. In these animals, two classes of homocytotropic antibodies have been demonstrated (36, 52). Three different homocytotropic antibodies have been demonstrated in the guinea pig (41). One type of homocytotropic antibody in rabbits belongs to a class of immunoglobulin similar to IgE (57). Homocytotropic antibodies in sheep apparently belong to this immunoglobulin (23) as well as reagins occurring in experimental schistosomiasis in monkeys (9).

The human schistosomiasis is an excellent model for a better understanding of the function of the human homocytotropic antibody. Very little is known about the function of this antibody in schistosomiasis. One might assume that this antibody could have some importance in the protective function, as was suggested in experimental animals by Zvaifler (56).

It is surprising that homocytotropic antibodies in human schistosomiasis do not provoke clinical manifestations of the allergic type as expected. The natural mechanisms that block the reaginic activity of these antibodies in vivo constitute a fascinating line of research.

SUMMARY

One hundred and eighty P.K. tests, in different experimental conditions were made, using sera of 35 patients with schistosomiasis mansoni. Sera from 80 per cent of the patients with schistosomiasis transferred passively reaginic antibodies by means of the P.K. test.

The antibody responsible for the P.K. reaction in schistosomiasis mansoni showed characteristics similar to the homocytotropic antibody described in allergic diseases, namely:

1. It is destroyed after 2 hours of heating at 56° C.
2. It remains in the recipient tissues at least for 168 hours.
3. It is passively transferred to other primates.
4. It presents a diminished activity when treated with 2-mercaptoethanol and iodoacetamide.
5. Immunoglobulin E (IgE) was found in high concentrations in 9 out of 11 sera showing a correlation with the intensity of the P.K. reaction.

Some properties not described in human homocytotropic antibodies were observed: a) The ability to produce P.K. reactions one hour after the serum injection, and b) some sera treated with 2-mercaptoethanol lost only part of their activity and remained in the tissues for at least 168 hours.

Based on these findings, a possible heterogeneity of homocytotropic antibodies in schistosomiasis mansoni was discussed. Schistosomiasis mansoni can be used as a practical model for the study of the human homocytotropic antibody.

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