HETEROLOGOUS REACTIONS INVOLVING PARASITES, BLOOD GROUP ANTIBODIES AND TISSUE COMPONENTS

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Summary

The sera of many patients with malaria and filariasis, and also anti-A and B blood group sera, were found to react by immunofluorescence with the somatic musculature of nematodes, especially Ascaris, and human and rat muscle, especially the skeletal type. These reactions were attributed to a polysaccharide related to AB substance in adult nematodes and to raised AB antibodies in malaria.

Similar heterologous reactions were found to involve the integument of schistosome adult worms and the microfilariae of Loa loa, which were attributed to the incorporation of host AB blood group substances into the parasite. Other parasites and sera gave mainly negative results.

These heterologous reactions constitute a potential hazard in immunofluorescence tests, against which skeletal muscle provides a control. The antibodies concerned were not operative in complement fixation tests, but there was a relationship to anti-complementary activity which suggested the transient presence of a circulating antigen.

Introduction

Unexplained immunofluorescent reactions, involving Ascaris lumbricoides adult worm and the skeletal muscle in which it was wrapped, were observed during a study of the possible value of Ascaris as an antigen for an indirect immunofluorescent test (IFT). Robinson and Christian (1968) had used Ascaris as the antigen for the complement fixation test (CFT) in filariasis.

These reactions appeared to be of sufficient intensity and frequency to warrant a separate study.

Materials and methods

Sera. Sera were collected from patients in this hospital with proved active helminthic and protozoal infections. Control sera were collected from 350 patients without any known infections though they had visited the tropics; and from 120 patients in St. Pancras Hospital, London, who it was assumed were unlikely to have visited the tropics. Sera were normally stored at -70°C. unless they were used fresh. Low titre positive control sera in small aliquots were used to standardize all tests throughout the period of investigation. Anti-A and B blood group sera were obtained from a blood group reference centre. The potency of the anti-A serum was given as 1/512, and the anti-B as 1/128.

Antigens. Adult worms, larvae, microfilariae and protozoa were obtained wherever possible from patients or they were cultured; non-human species were obtained from the appropriate animals. Schistosoma mansoni cercariae and hydatid hooklets were obtained from Wellcome Reagents Ltd. Fresh adult worms were wrapped in rat skeletal muscle and frozen sections were cut before being stored at -70°C.; they showed no detectable loss of potency after 9 months. With A. lumbricoides (female) the cuticle and intestinal organs were removed. Malaria blood was centrifuged and the red cell pellet was washed twice in saline. Thin films were wrapped individually in tissue paper for storage at -70°C.

Rat heart, skeletal muscle, liver and kidney, and human thyroid and stomach from patients who came to necropsy within 6 hours of death, were stored in liquid nitrogen until required for sectioning. For absorption studies, tissues were sonicated in saline and a known volume of homogenate was used to standardize all tests throughout the period of investigation. Anti-A and B blood group sera were obtained from a blood group reference centre. The potency of the anti-A serum was given as 1/512, and the anti-B as 1/128.

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Rat heart, skeletal muscle, liver and kidney, and human thyroid and stomach from patients who came to necropsy within 6 hours of death, were stored in liquid nitrogen until required for sectioning. The rat tissues were tested by IFT in parallel with corresponding human tissues with comparable results. For absorption studies, tissues were sonicated in saline and a known volume of homogenate was incubated overnight at 4°C. with serum at 1/50; this was then centrifuged and tested by IFT.

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Immunofluorescent test. Sections of adult worms were fixed in acetone (10 minutes). Smears of protozoa and larvae were mostly fixed in methanol (10 minutes). Malaria films were unfixed. Unfixed tissue sections were washed thoroughly in phosphate buffered saline. Sera were used at a standard dilution of 1/32, including the anti-A and B sera.

IFT was performed by the standard sandwich technique using FITC conjugated antihuman globulin serum (Wellcome) at optimal dilution of 1/25. In a few cases antisera monospecific for the 3 main classes of immunoglobulin were employed. Evans blue counterstain (10 minutes) was normally used at 1/10,000 with all antigens except malaria, with which it was used at 1/1,000. Results were read with a Leitz ortholux microscope using an interference filter.

Complement fixation test. The CFT technique was that of ROITT and DONIACH (1958), who describe also the method of extracting tissues. The tissue extracts showed no significant anti-complementary activity. Sera were tested at 1/10 against tissue antigens.

Results

The IFT results, which are summarized in the table, comprise (a) reactions between homologous antigens and antibodies, (b) group specific reactions between members of a group that are known to be antigenically related and (c) heterologous reactions between apparently unrelated antigens and antibodies. The homologous and group specific reactions, which serve merely as positive controls, are shown in brackets. The numbers of sera and the amounts of the various antigens that were available varied considerably; with the anti-A and B sera there was only 1 of each. When the number of tests of any sort exceeded 15 (maximum 350) the result is recorded as the percentage of positives. In the remainder there were less than 10 tests of a sort, and with these one or more positives is recorded as plus, all negatives as minus.

Results were recorded as positive on the basis of bright green fluorescence at a standard serum dilution of 1/32. In some but not all cases reactions were taken to titre, and it was then found that the heterologous sera did not generally give such a high titre as the homologous or group specific sera, though the reactions were significantly stronger than those given by normal control sera which were all negative at 1/32 except for 3 out of 120 against Ascaris. Furthermore, the reactions in many cases were not extinguished by a relatively strong concentration of Evans blue (1/400).

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Serum</th>
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<tr>
<td></td>
<td>Non-tropical patients</td>
</tr>
<tr>
<td>Ascaris, adult</td>
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<tr>
<td>Toxascaris, adult</td>
<td>—</td>
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<tr>
<td>Litomosoides, adult</td>
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<tr>
<td>S. mansoni, adult</td>
<td>—</td>
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<tr>
<td>S. haematobium, adult</td>
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<tr>
<td>Malaria</td>
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<tr>
<td>Skeletal muscle</td>
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<td>Smooth muscle</td>
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<td>Heart muscle</td>
<td>0</td>
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<tr>
<td>Thyroid stroma</td>
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<td>Liver</td>
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<td>Kidney</td>
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These heterologous reactions may be considered as follows:

1. Over half the malaria sera tested, comprising infection with all the 4 species of *Plasmodium*, reacted with the somatic musculature of adult *Ascaris* worms and with human and rat skeletal muscle. The fluorescence with *Ascaris* muscle involved mainly sarcolemma, though there was also an irregular sub-sarcolemmal reaction (Figs. 1 and 2).
2. Anti-A and anti-B blood group sera gave an exactly similar pattern of fluorescence; and they reacted also with malaria parasites.

3. Malaria and anti-A and B sera gave less frequent reactions with heart muscle, smooth muscle and a component of the stroma of the thyroid gland. Some of these reactions were definite; others were less marked and difficult to read. Liver and kidney did not react significantly.

4. The sera of patients with filariasis, strongyloidiasis and schistosomiasis gave reaction patterns similar to that of malaria, though the incidence of positives was somewhat lower. Filaria sera invariably gave a weak but significant fluorescence with malaria parasites.

5. Only limited amounts of *Toxascaris leonina* and *Litomosoides* adult worm antigen were available, but the results suggested that cross-reactions with the somatic musculature similar to those of *Ascaris* might be widespread among the nematodes. However, these antigens were less sensitive than *Ascaris*. 

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**Fig. 1.** Fluorescence of somatic muscle of *Ascaris* (right) and rat skeletal muscle (left) with serum of a malaria patient.

**Fig. 2.** Fluorescence of rat skeletal muscle with serum of a malaria patient. Sarcolemma green; sarcoplasm red or orange.
6. *Schistosoma haematobium* adult worm gave some cross-reactions in a limited number of tests. The fluorescence was confined to the integument.

7. *S. mansoni* and *S. haematobium* adult worms frequently demonstrated a speckled fluorescence in the integument. This is not classed as a heterologous reaction as it occurred also with normal sera; it is not recorded in the table.

8. The following parasitic antigens (not recorded in the table) gave homologous or group reactions but no heterologous reactions among the sera under consideration: *Entamoeba histolytica* trophozoites, *Leishmania donovani* pro-mastigotes, microfilariae of *Acanthocheilonema perstans*, infective stage larvae of *Brugia pahangi*, first stage larvae of *Dracunculus medinensis*, filariform larvae of *Strongyloides stercoralis*, second and third stage larvae of *Toxocara canis*, cercariae of *S. mansoni* and hydatid hooklets. The microfilariae of *Loa loa* reacted with anti-A blood group serum only.

9. Sera from patients with the following conditions (not recorded in the table) were not found to give heterologous reactions with the antigens under consideration: amoebiasis, giardiasis, leishmaniasis, trypanosomiasis, toxoplasmosis, ascariasis, hookworm, toxocariasis, trichiniasis, cysticercosis and hydatid cyst.

10. Of a non-selected series of sera that had proved to be anti-complementary during the course of CFT's for filariasis and schistosomiasis, 40 or 50% proved to react by IFT with the somatic musculature of nematodes and with skeletal muscle.

Cross absorption. Incubation of serum with skeletal muscle removed almost all the antibody against *Ascaris*. Smooth muscle, heart and liver partly inactivated the reaction against *Ascaris* and skeletal muscle. Kidney had no effect.

Antigen. Extraction of nematodes and tissues with chloroform and methanol abolished their IF activity. According to Gray (1965) this suggests that the antigens were glycolipid complexes.

Antibody. Nearly all the IF activity against nematodes and tissues was present in the IgG component.

Complement fixation tests. In a brief, tentative study it was found that CFT results did not correlate with IFT, using rabbit tissue antigens. Kidney gave a positive CFT against malaria, filaria and schistosoma sera; thyroid and liver less so; skeletal and smooth muscle were negative at 1/10. Thus the CF antibodies present in our sera were not significantly different from those found by Curtain et al. (1965) and Curtain and Simons (1972) in people in New Guinea.

Discussion

It is known that some pathogenic bacteria may acquire heterophile antigenic properties of the Forssman type by contact with host tissues; or that they may share antigenic determinants with blood group A antigen or with tissue components (see Wilson and Miles, 1964). It is also known that tissue antibodies make their appearance in some acute or chronic infections (Gajdusek, 1958; Hackett et al., 1960); and that they are more common in some tropical populations than in Europeans (Curtain et al., 1965; Curtain and Simons, 1972). Rizetto and Doniach (1973) suggest that such antibodies may represent an anamnestic response to an infection.

In parasitic infections, Smithers and his co-workers have produced evidence that the incorporation into the integument of schistosomulae of host antigens related to A and B blood group substances (not Forssman antigen) may have a protective immunological effect by masking the parasite (Smithers et al., 1969; Smithers, 1972). Hogarth-Scott (1968) found in normal human sera an antibody against the cuticle of nematodes. This probably explains the non-specific fluorescence which caused us to disregard nematode cuticle. Oliver-Gonzalez (1944) found that the antigenicity of a polysaccharide extracted from *A. suum* (Campbell, 1936) resembled the A and B specific substances of human blood; and that it neutralized human a and b agglutinins and Forssman antibody. He obtained a similar polysaccharide from many other helminths including *Schistosoma* (Oliver-Gonzalez and Torregrosa, 1944; Oliver-Gonzalez, 1946a, 1946b). A and B antibodies are known to be increased in malaria (Oliver-Gonzalez, 1944; Kano et al., 1968).
The principal finding of the present study, in brief, was that the sera of malaria and filaria patients, and anti-A and B blood group sera, interact by immuno-fluorescence with the somatic musculature of *Ascaris* and with human and rat skeletal muscle. In less pronounced form these reactions involved also other nematodes and other forms of host muscle. Muscle is not one of the tissues that were found by Holborow et al. (1960) to react immunologically with A and B antibodies. The tissue reactions reported here, which included a component of thyroid stroma, were all somewhat non-specific. The antibodies concerned may be akin to those in experimental infections which Thewani Ali and Oakley (1967) found to belong to 5 groups shared between 6 organs. The various heterologous reactions with which we are dealing are regarded as significant, however, in that they are seldom produced by normal sera, though they are often weaker than homologous reactions.

The close correspondence of the reaction patterns given by nematode and mammalian muscle on the one hand, and by anti-AB blood group sera and the sera of malaria and filaria patients on the other, suggests that the reactions are due to closely related if not identical antibodies and antigenic components. The antibody is predominantly IgG in type and the antigen, according to solubility tests, appeared to be a glycolipid complex, which is perhaps not far removed from the polysaccharide of Oliver-Gonzalez.

The situation with regard to other parasites involved in heterologous reactions is a little different. The speckled fluorescence of the integument of adult schistosomes with normal and with anti-A and B sera was distinct from the homologous reactions with sera of schistosome patients, which were both solid and more brilliant. The speckling did not correspond to any morphological feature of the worm, and the response was not always equal with A and B sera. Thus the *S. mansoni* worm tested reacted with all sera except one which was blood group AB (i.e. with no A or B antibodies), whereas the *S. haematobium* worm tested reacted only with anti-B serum; the fluorescence in this case was non-speckled. Similarly the *Loa loa* microfilariae tested fluoresced with anti-A but not anti-B serum. The patient from whom the microfilariae were obtained was of blood group A. Blood group discrepancies of this sort were never detected with the nematode adult worm and filaria reactions. Thus it would appear that the fluorescence of the schistosomes probably represents incorporation of A or B blood group substances into the integument of the worm, as suggested by Smithers, and that a similar phenomenon may occur with the microfilariae of *Loa loa*. Whether the latter is of any immunological significance is not clear.

It is suggested, therefore, that there are 4 possible mechanisms for the AB heterologous reactions: (1) adult nematodes and some other worms contain a polysaccharide related to AB substance; (2) this substance induces antibody formation; (3) AB antibodies in malaria patients are raised and react with this substance; and (4) AB blood group substances of the host are incorporated into the blood forms of *Schistosoma* and probably *Loa loa*.

The antigenic relationship through blood group substance of filaria and malaria is interesting. Endomyocardial fibrosis has been found to be associated with a high frequency of both filaria antibody (Ive et al., 1967) and malaria as well as tissue antibodies (Shaper et al., 1967; Shaper, 1972). These might possibly be connected in some way with the acid mucopolysaccharide which Connor et al. (1968) have demonstrated in the endocardium in endomyocardial fibrosis.

The most important practical consideration is that the AB muscle antibodies are a potential source of cross-reactions in immuno-fluorescent diagnostic tests using nematode adult worms, of the sort encountered by Duxbury and Sadun (1967). The other antigens referred to may present a similar risk. The present results go far to explain the greater specificity of larval as compared to adult nematode antigens which is often noted. In view of the comments of Oliver-Gonzalez it is perhaps surprising that more attention has not been given to this serological hazard. Skeletal muscle appears to provide a useful control antigen.

Whether these heterologous reactions may be a complicating factor in other serological tests is not clear, but they shed light on a previously unexplained problem with CFT's. We had noticed that in the CFT for schistosomiasis, filariasis and possibly other conditions, patients with anti-complementary sera prove to have the parasitic infection more frequently than would be expected. It is now found that such sera frequently contain AB-muscle antibody. It is suggested that release of Oliver-Gonzalez polysaccharide from the parasite, due to its death or otherwise, would result in neutralization of anti-AB and Forssman antibody, and the consequent failure of lysis of sheep red cells would be interpreted as anti-complementary activity. It is inferred instead that it might be anti-haemolytic activity due to a transient circulating antigen, presumably present in the form of an immune complex; however, this might itself be a source of complement consumption.
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