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Circulating immune complexes in experimental filariasis

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SUMMARY

Circulating immune complexes have been investigated in jirds *(Meriones unguiculatus)* infected with the filarial nematode *Brugia pahangi*. Two-month-old male jirds were inoculated with seventy-five *B. pahangi* infective larvae into the left groin. At 8 months post-infection, sera of individual animals from a group of seventeen infecteds and seventeen age-matched controls were analysed for immune complexes by (1) a solid-phase C1q binding assay (C1q-SP) and (2) precipitation with 3·5% polyethylene glycol followed by binding of ^{125}I-labelled rabbit anti-jird Ig antiserum (PEG). A significant increase in the level of circulating immune complexes was shown in the infected group as compared with the controls for both assays, with a P value $=0{\cdot}005$ for PEG and $P=0{\cdot}001$ for C1q-SP. Using the mean of the control group ± 2 s.d. as the upper limit of the normal range, 24% of the infected group had elevated immune complex levels by the PEG assay, and 41% were elevated in the C1q-SP assay. A high degree of variability was noted in the levels of immune complexes among individual animals in the infected group by each test. No correlation between immune complex levels and numbers of circulating microfilariae was found in either assay.

INTRODUCTION

The successful survival of both host and parasite in filariasis is of great immunologic interest. Recent investigations have indicated that the persistence of the parasite may be due to an induced immunodepression as evidenced by diminished cellular and humoral responses (Weiss, 1978; Portaro, Kowalski & Ash, 1977; Dalesandro & Klei, 1976; Ottesen, Weller & Heck, 1977).

The presence of abundant antigen and antibody throughout the course of this disease led us to suspect a role for immune complexes in mediating this immunodepression. A number of experimental systems have shown that circulating immune complexes can provoke a variety of immunological effects including inhibition of T and B lymphocyte functions and interference with antigen presentation on the surface of macrophages (WHO Scientific Group, 1977). In addition, circulating and deposited immune complexes have been demonstrated in other parasitic diseases such as malaria, trypanosomiasis, schistosomiasis and onchocerciasis (Lambert & Houba, 1974; WHO Collaborative Study, 1978).

This study was designed to determine if circulating immune complexes were present in our model of filariasis and to what extent. Our experimental model consists of the Mongolian jird, *Meriones unguiculatus,* infected with *Brugia pahangi,* a lymphatic-dwelling filarial parasite of cats

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and dogs which closely resembles the human filarial parasites, *Wuchereria bancrofti* and *Brugia malayi,* in life cycle and morphology. Circulating immune complexes were measured by both a solid-phase C1q-binding assay and polyethylene glycol precipitation, followed by radiolabelling of jird Ig.

MATERIALS AND METHODS

Animals, parasites and sera. Seventeen male jirds of the inbred strain MON-Tum (strain 532) (Tumblebrook Farms Incorporated, West Brookfield, Massachusetts) were each infected with seventy-five *Brugia pahangi* L3s by the method of Ash & Riley (1970). At 8 months post-infection, individual eye bleedings were done on these animals and on seventeen age- and sex-matched controls. Sera were stored at −70°C until assayed. Microfilarial levels in the infected animals were determined by the Knott technique at the time that blood was drawn.

Reagents. Jird Ig was prepared from whole jird sera by a 33% ammonium sulphate precipitation.

Jird immune complexes for use as standards were prepared by mixing jird anti-BSA antisera with BSA in a quantitative precipitin test to determine the amount of specific antibody present. Complexes precipitated at equivalence were then washed with PBS and resuspended in five-fold antigen excess, incubated at 37°C for 60 min and 4°C for 18 hr, then centrifuged at 1,500 *g* for 15 min. Soluble complexes remained in the supernate and their presence was verified by precipitation with C1q in an immunodiffusion assay.

Rabbit anti-jird Ig antisera were prepared by immunizing rabbits with jird Ig. Purified antibody was prepared by adsorbing the antisera onto a cyanogen bromide-activated Sepharose 4B immunosorbent (Hudson & Hay, 1976) coupled to jird Ig, and specific antibody was eluted with 0·1 M glycine–HCl buffer, pH 2·8. Radiolabelling of purified antibody with ^{125}I was performed by the chloramine T method (McConahey & Dixon, 1966). Labelled protein was stored at −70°C.

C1q was prepared from human time-expired plasma by the method of Agnello, Winchester & Kunkel (1970) and stored at −70°C for no longer than 2 months.

Solid-phase C1q assay. A modification of the method of Hay, Nineham & Roitt (1976) was developed for our system. Polyvinyl chloride microtitre plates (220–25, Cooke Laboratory Products, Alexandria, Virginia) were coated with 300 μl of C1q at 5 μg/ml in PBS by incubation at 4°C for 20 hr. After one wash with PBS, 300 μl of 1% HSA in PBS was added to each well and incubated for 2 hr at room temperature. After three washes with PBS the plates were ready for use in the assay. Test serum, 50 μl, was incubated with 100 μl 0·2 M EDTA, pH 7·5, at 37°C for 30 min and 4°C for 30 min, to inactivate complement. EDTA-treated sera, 20 μl, was then added to each test well and the wells were topped up with PBS. Each test was performed in duplicate. The plates were incubated at 37°C for 60 min and at 4°C for 20 hr and then washed with PBS three times. Bound complexes were then labelled with 300 μl of ^{125}I-rabbit anti-jird Ig antiserum at 10 μg/ml in 1% HSA and incubated for 60 min at 37 and 4°C for 30 min. Plates were then washed three times with PBS and seven times under tap water, wells cut apart and counted in a gamma counter. Results were reported as ng anti-Ig bound/well.

PEG precipitation assay. A modification of the method of Creighton, Lambert & Miescher (1973) was used in our system. One hundred microlitres each of test sera, known positive and known negative sera, were added to 100 μl cold 7% PEG (polyethylene glycol, mol. wt 6,000, in PBS) in glass tubes and vortexed immediately to give a 3·5% solution. Each test was performed in duplicate. Sera were then incubated at 4°C for 18 hr and then centrifuged at 2,500 *g* for 15 min. The supernate was decanted and the precipitate was washed with 500 μl cold 4% PEG, centrifuged again as above, and resuspended in 100 μl of PBS. Soluble immune complexes were then labelled by the addition of 100 μl of ^{125}I-rabbit anti-jird Ig antiserum in a concentration of 10 μg/ml. Tubes were incubated at 37°C for 60 min and at 4°C for 30 min. Two hundred microlitres of 8% PEG was then added to each tube and the tubes were incubated for 18 hr at 4°C. Centrifugation and washing of the precipitate was performed as above and the radioactivity associated with the precipitate was determined by counting in a gamma counter. Results are reported as per cent ^{125}I-rabbit anti-jird Ig antisera precipitated.

Statistical analysis. Differences between the control and infected groups were calculated by both Student's *t*-test and a χ^2 test. For the χ^2 test, the proportion of individual infected animals which fell beyond the 90th percentile of the control group was calculated and compared with the proportion of individual control animals (10%) falling beyond this percentile (WHO, 1978). Spearman rank correlation was used to analyse the relationship between the two assays and their individual correlations to microfilariae levels.

RESULTS

PEG precipitation assay

Sera from seventeen infected animals and their age- and sex-matched controls were individually analysed in this assay, since pooling of infected sera could conceivably result in the formation of immune complexes between some animals in antibody excess and others in antigen excess. Fig. 1 shows the individual levels of these sera as reported in per cent ^{125}I-anti-Ig ppt. The upper limit of normality has been taken as 2 standard deviations above the mean of the uninfected animals. The concentration of immune complexes was significantly elevated in the infected group (mean $\pm$ s.d.: $39{\cdot}7 \pm 3{\cdot}7\%$ ^{125}I-anti-Ig ppt) as compared with the control group ($36{\cdot}2 \pm 3{\cdot}4\%$ ^{125}I-anti-Ig ppt) by Student's *t*-test ($P < 0{\cdot}005$) and by the χ^2 test ($P < 0{\cdot}005$).

C1q-SP assay

The same sera used in the PEG precipitation assay were again individually analysed in the C1q-SP assay. Fig. 2 shows the individual levels of these sera as reported in ng anti-Ig bound/well. The upper limit of normality has been defined as 2 standard deviations above the mean of the uninfected animals. The infected group (mean $\pm$ s.d.; $76{\cdot}5 \pm 15{\cdot}9$ ng anti-Ig bound/well) demonstrates a significantly raised level of immune complexes compared with the control group ($60{\cdot}2 \pm 12{\cdot}0$ ng anti-Ig bound/well) by both Student's *t*-test ($P < 0{\cdot}001$) and by the χ^2 test ($P < 0{\cdot}005$).

Comparison of the PEG precipitation assay and the C1q-SP assay

Using the mean $\pm$ 2 s.d. as the upper limit of the normal range, 24% of the infected group had elevated immune complex levels by the PEG assay and 41% were elevated in the C1q-SP assay. Of the thirty-four sera obtained from infected and control animals, thirty (88%) were found to be either postive (four cases) or negative (twenty-six cases) in both assays. Rank correlation analysis showed a significant correlation between the two assays ($r = 0{\cdot}356$, $P < 0{\cdot}025$).

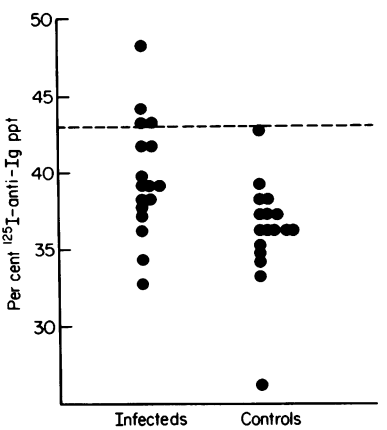

Fig. 1. Immune complexes in *B. pahangi*-infected jirds as detected by the PEG precipitation assay. The limits of normality are indicated by the dotted line at 2 s.d. above the mean of the control group.

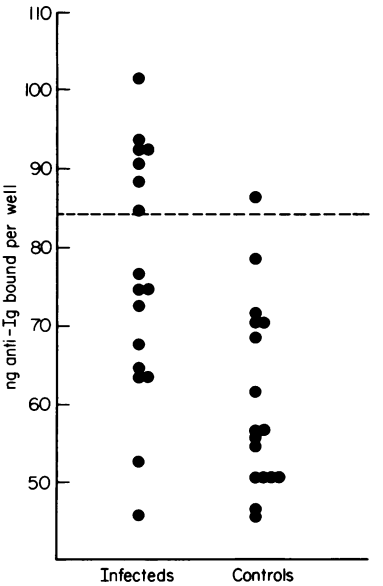

Fig. 2. Immune complexes in *B. pahangi*-infected jirds as detected by the C1q-SP assay. The limits of normality are indicated by the dotted line at 2 s.d. above the mean of the control group.

Correlation of both assays with microfilaraemia

Microfilarial counts from peripheral blood were obtained from infected animals at the same time that sera were drawn for the assays. No correlation was shown between these levels and either of the two assays by the rank correlation test.

DISCUSSION

This study has demonstrated the presence of circulating immune complexes in jirds with experimental filarial infections by two assays—the polyethylene glycol precipitation assay and the C1q solid-phase assay. The advantage of studying infected sera by two methods is that each assay depends on different physical or biological properties for the detection of the complexes. PEG precipitation, in conjunction with labelled rabbit anti-jird Ig-specific antibody, depends on solubility changes related to high molecular weight and on the presence of jird Ig in the complex. C1q-SP assay depends on the ability of the complex to bind to complement and the presence of jird Ig in the complex. Statistically significant differences between control and infected groups in both assays doubly confirm our findings.

The discrepancy in the percentage of infecteds found positive for immune complexes by the PEG precipitation assay (24%) and the C1q-SP assay (41%) can be explained by the particular technical limits of each assay. PEG precipitation preferentially detects somewhat larger complexes in antigen excess and is not known to be as senstive an assay as the C1q-SP. Although C1q-SP is more sensitive, it preferentially detects smaller complexes in antigen excess which must be capable of binding C1q. A single serum sample containing different sized complexes composed of antigens of varying densities of antigenic determinants, some capable of fixing complement and some incapable, could give quite different results in these two assays. However, when Spearman rank correlation was performed over the entire sample of infecteds and controls, a reasonable correlation between the two assays was seen ($P \leq 0{\cdot}05$).

It is evident from the scattergram figures for both assays that a considerable amount of individual variability in complex levels exists in both the infected and the control groups. This variability is understandable in the group subjected to a dynamic infection which has had 8 months

to equilibrate. Although given the same number of infective larvae at the onset and kept under identical conditions, the common measurement of infection, the microfilarial level, in individuals in our infected group fluctuated widely (between 2 and 250 microfilariae/200 μl of blood). It is not surprising that the immune complex levels would also fluctuate. Unfortunately, Spearman rank correlation showed no relationship between these levels. Immune complex formation is a normal immune response and its presence can be expected at some low level even in the control animals. The variation in these levels could be attributed to individual animal responses to the sort of everyday immune assaults to which all individuals are subjected. Other studies on immune complex levels in experimental infections (Santoro *et al.*, 1978) have relied upon pooling of serum samples for both control and infected values. We felt that pooling of samples would result in a loss of information on two counts: (1) In a dynamic parasitic infection followed for several months, it is possible that some animals may be in antigen excess and some in antibody excess. Pooling would create new complexes or, at the very least, throw the native immune complexes into an unnatural imbalance. (2) The whole problem of the individual responses of animals and in-group variation could not be observed.

Although the differences between infecteds and controls were significant, the actual complex levels in the infecteds were not high. We did not expect to find very high levels since the nephritis, vasculitis and decreased complement levels usually associated with generalized immune complex disease (WHO Scientific Group, 1977) have not been seen in our experimental model. Localization of the immune complex appears to depend on the ability to bind C3b, the size of the complex (larger complexes being more readily localized than smaller ones), and the ratio of antigen to antibody (complexes at equivalence are most damaging) (Scherzer & Ward, 1978). It may be that the complexes found in our system do not fit these criteria. However, intravascular thrombosis and inflammation of pulmonary arteries have been observed in jird filarial infections and might be attributed to the action of immune complexes (Vincent, Frommes & Ash, 1976).

One possible role for immune complexes in filariasis may be as immunoregulators of the host's immune response. Immune complexes have been shown to mediate a variety of immunosuppressive effects such as modulation of the activation and effector functions of T and B lymphocytes, and presentation of antigen on the surface of macrophages (WHO Scientific Group, 1977). Filarial worms can survive in their host for many years, and it may be that immunosuppression mediated by immune complexes contributes to their persistence.

A functional role for immune complexes in filariasis has been implicated in several recent findings. An inverse relationship between levels of microfilaraemia and antibody titre to microfilariae has been seen by several investigators (Ponnudurai *et al.*, 1974; Wong & Guest, 1969). This phenomenon could be due either to the lack of production of specific antibody, or to an inability to detect antibody because it is absorbed by circulating microfilariae or combines with their soluble products to form immune complexes. Microfilarial cytoplasm was found to be capable of absorbing antibody provided the sera used were not from the host patient but from patients with homologous infections (Ridgley & Hedge, 1977). In studies of adherence of peripheral blood leucocytes to microfilariae, Subrahmanyam *et al.* (1978) found that sera from infected patients without circulating microfilariae caused an intense adhesion while no such adhesions were seen with sera from microfilaria carriers or normal subjects. In studies on *Dipetalonema viteae* infections, peritoneal exudate cells from hamsters were also found to adhere to microfilariae only in the presence of 19S antibody fractions from hamsters that had suppressed or were about to suppress their microfilaraemia (Tanner & Weiss, 1978). Haque *et al.* (1978) found that the release of *D. viteae* microfilariae from female worms is inhibited by a factor present in the sera of infected animals, and that the level of this suppressive factor falls during microfilaraemia. This absence of adhesion-promoting activity and microfilaria-suppressing ability has been attributed both to a lack of specific antibody production or to the possible presence of 'blocking factors' in infected sera. In addition, Weiss (1978) found that sera of hamsters chronically infected with *D. viteae* caused marked inhibition of transformation to filarial antigen by sensitized lymphocytes. This inhibition could also be mediated, either directly or indirectly, by antigen–antibody complexes in the circulation. Our findings may offer a possible explanation for some of these recent observations; however, the possibility that immune complexes are an epiphenomenon should not be overlooked.

A study of the immune response in filariasis is made more difficult by the dynamics of the

infection, the complexity of the antigens involved, and the variations in response among individual animals. The true importance of the role of immune complexes in this disease awaits the determination of the composition of the complex, specification of relative antigen or antibody excess in the system, and elucidation of a critical *in vivo* role.

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