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Circulating immune complexes in onchocerciasis

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SUMMARY

Circulating immune complexes were detected in sera of patients with both localized and generalized onchocerciasis by a ^{125}I-C1q binding assay but not by the IgG latex agglutination inhibition method. Gel filtration of sera demonstrated high molecular weight C1q-reactive material ($>2\times 10^6$ Daltons) which contained IgM but no IgG. Antibody titres to *Onchocerca volvulus* antigen were higher in patients with generalized disease than in those with the localized form. The lack of correlation between antibody titres and levels of immune complexes suggests that these immune complexes contain antigens other than those derived exclusively from the parasite. Although few of the symptoms of this disease are likely to be due to deposition of circulating immune complexes, the depression of delayed hypersensitivity reactions to the parasite found in patients with generalized onchocerciasis may be due to IgM immune complexes exerting an immunoregulatory role on T cell function.

INTRODUCTION

Onchocerciasis is a parasitic disease in which the adult filarial nematode worm *Onchocerca volvulus* becomes encapsulated in subcutaneous nodules and the larval forms or microfilariae invade the skin. The major clinical manifestations are the subcutaneous nodules (onchocercomata), skin lesions (onchodermatitis), eye lesions causing blindness and involvement of the lymphatic system resulting in elephantiasis (Meyers, Neafie & Connor, 1977; Gibson & Connor, 1978).

The cutaneous lesions differ widely in the generalized form of the disease. Most patients have some form of papular or pustular skin eruption, hyperpigmentation or depigmentation with atrophy of the skin, lymphadenopathy and lymphoedema; microfilariae are found readily in diagnostic skin biopsies. Despite similar diffuse microfilarial infiltration of the dermis, others have no clinical skin changes or itching.

Localized skin lesions, typically involving only one limb, occur in a very small percentage of cases. The affected area is hyperpigmented, thickened and intensely pruritic, but microfilariae are sparse in full thickness skin biopsies.

Specific antibodies of different immunoglobulin classes are produced in patients with both localized or generalized onchocerciasis (Bartlett, Bidwell & Voller, 1975; Somorin, Heiner & Ajugwo, 1977) but only those with the localized form have delayed hypersensitivity reactions to the parasite; this suggests that patients with generalized lesions have a defective cell-mediated immune response (Ngu & Blackett, 1976; Bartlett *et al.*, 1978; Ngu, 1978) but the mechanisms for this are unclear.

As well as having a direct inflammatory role, immune complexes may modulate the immune response. Hence, the demonstration of circulating immune complexes in onchocerciasis and other parasitic diseases (WHO, 1977) stimulated this study to see if different types of immune complexes play a role in the diverse clinical manifestations of the infection.

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We have analysed, therefore, the sera of patients with localized reactive type and generalized onchocerciasis for IgG and C1q binding immune complexes and antibodies to the parasite; we have studied also the size of the complexes in selected individuals.

MATERIALS AND METHODS

Patients. Two groups of patients from the villages around Yaounde (United Republic of Cameroon) were studied; twenty had the generalized form of the disease (fourteen females and six males, age range 16–50 years) and thirteen had the localized type disease (nine females and four males, age range 18–30 years). In the latter group, only one limb was involved predominantly, but in a few the adjacent part of the body was also affected; onchocercomata were not seen although many patients had regional lymphadenopathy.

Controls. Twenty-six healthy subjects from the same villages were studied for comparison (nine females and seventeen males, age range 20–54 years). None of these had previously had onchocerciasis or been treated for filariasis.

Clinical symptoms and signs. All patients and controls were examined physically for overt signs of onchocerciasis. Skin biopsies were taken with a corneo-scleral punch from the gluteal region, and, in patients with localized lesions, from the affected skin. The biopsies were then left in Hanks' balanced salt solution for a few hours and then examined microscopically for emergent microfilariae. The stools and blood from patients and controls were examined for parasites.

Sera. Blood samples were collected by venesection and allowed to clot at room temperature. Serum was separated soon after collection and stored frozen until analysis.

Immune complex detection. The IgG latex agglutination inhibition method (Levinsky & Soothill, 1977) and the radiolabelled C1q binding assay (^{125}I C1q BA) (Zubler *et al.*, 1976) were used to detect circulating antigen–antibody complexes in sera. Results were expressed as percentage (%) inhibition of IgG latex agglutination and the amount of heat-aggregated (63°C, 30 min) human IgG bound by a constant amount of radiolabelled C1q (μg/ml).

Some serum samples in which complexes were detected by ^{125}I-C1q BA, were separated on a calibrated Sepharose CL 6B column. All fractions were tested for presence of complexes by the ^{125}I-C1q BA, and results expressed as c.p.m. of ^{125}I-C1q bound after subtraction of background counts.

Enzyme-linked immunosorbent assay (ELISA) for antibody detection. Onchocerca volvulus antigen was prepared from live adult worms (Ngu, 1978); a solution containing 1 mg/ml of this antigen was incubated in polystyrene microhaemagglutination plates at 37°C for 6 hr. After washing with phosphate-buffered saline/Tween, serum diluted 1:500 in the same buffer were added overnight at 4°C and the plates washed as before. Any bound antibody was detected by an alkaline phosphatase-labelled anti-immunoglobulin, to which the appropriate substrate was added 3 hr later; after 15 min the reaction was stopped with 3 M NaOH and the absorbance of the mixture at 405 nm determined spectrophotometrically.

RESULTS

Clinical

The microfilarial counts per skin snip in the generalized form ranged from 5 to 175, and in the localized reactive type from 1 to 7. None was found in the twenty-three control subjects who agreed to have skin biopsies taken.

The parasitological profile was similar in both patient and control groups. In the patient population we found *Ascaris lumbricoides* in thirteen subjects, *Loa loa* in two, *Trichuris trichiura* in fourteen, *Necator americanus* in four, *Dipetalonema perstans* in one and *Plasmodium falciparum* in six. In control subjects the findings were: *A. lumbricoides* in fourteen, *Loa loa* in one, *T. trichiura* in three, *N. americanus* in four, *D. perstans* in eight and *P. falciparum* in two. Five patients and one control had no parasites, whereas more than one parasite species was found in ten patients and five controls.

Circulating immune complexes

The results of the IgG latex agglutination inhibition and ^{125}I-C1q BA are shown in Fig. 1. The African controls had higher mean levels of IgG complexes than European controls tested previously ($17{\cdot}8 \pm 7{\cdot}5$ versus $8{\cdot}9 \pm 4{\cdot}4$), although C1q binding results were similar. No difference between patients with localized or generalized disease and healthy controls was detected by the agglutination inhibition method. In contrast, clear differences in the three groups were found using the ^{125}I-C1q BA, where patients with the localized and generalized form of the disease had higher mean levels of immune complexes ($69{\cdot}4 \pm 52{\cdot}5$ and $97{\cdot}4 \pm 54{\cdot}9$ respectively) as compared to the control group ($15{\cdot}8 \pm 6{\cdot}2$; $P < 0{\cdot}001$).

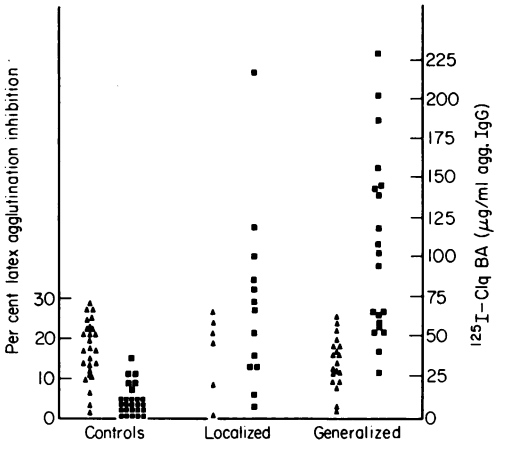

FIG. 1. IgG immune complexes (detected by IgG latex agglutination inhibition: ▲) and C1q binding complexes (detected by ^{125}I-C1q binding in polyethylene glycol method: ■) in patients with localized and generalized onchocerciasis and in control subjects from the same region of the United Republic of Cameroon. Results expressed as percentage inhibition for IgG complexes and as μg/ml of heat-aggregated IgG for C1q binding complexes.

Although higher levels of C1q binding complexes were found more frequently in patients with generalized onchocerciasis, the difference between the two groups of patients was not statistically significant.

Antibodies to Onchocerca volvulus antigen

The antibody titres tested by ELISA ranged from 0·50–0·68 (0·59±0·05) in the control group, to 0·67–1·50 (0·91±0·24) in localized onchocerciasis and 0·79–1·90 (1·42±0·31) in the generalized disease (Fig. 2). There were highly significant differences in the antibody levels of patients and normal disease-free subjects ($P<0·001$). Moreover, higher titres of specific antibodies were found in patients with generalized onchocerciasis compared with those with the localized form ($P<0·001$).

There was no significant correlation between the levels of C1q binding complexes and the antibody titres of the patients (Spearman–Rank correlation analysis: $r = 0·307$, P = n.s.).

Rheumatoid factor

None of the sera contained rheumatoid factor as tested by the slide latex agglutination technique.

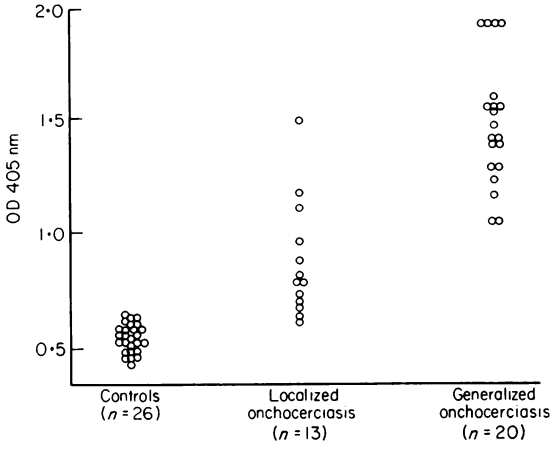

FIG. 2. Antibodies to *Onchocerca volvulus* in control subjects and in patients with localized and generalized onchocerciasis detected by solid-phase ELISA technique using alkaline phosphatase-labelled anti-immunoglobulin. Results expressed as optical density units.

Size of immune complexes

Results of three sera separated on a calibrated Sepharose CL 6B column are shown in Fig. 3. Both in the generalized and in the localized form of disease the C1q binding complexes were detected in the size range from 1·5 to 4×10^6 Daltons molecular weight (mol. wt). Small molecular weight C1q reactants were detected also in all samples. Pooled fractions from the high molecular weight material reacting with C1q were shown to contain IgM but no IgG by double immunodiffusion in agar, after a concentration of 100-fold. These fractions eluted in a molecular weight range greater than that of native IgM (Fig. 3).

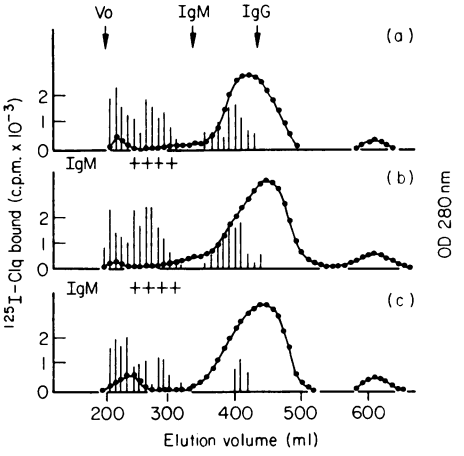

FIG. 3. Gel filtration as a calibrated Sepharose CL 6B column of sera from one patient with generalized disease (a) and two patients with localized onchocerciasis (b & c). All fractions were tested for immune complexes by ^{125}I-C1q binding. Elution positions of void volume (4×10^6 Daltons), IgM ($9{\cdot}7 \times 10^5$ Daltons) and IgG ($1{\cdot}56 \times 10^5$ Daltons) are shown. All the sera showed C1q binding material in the 1·5–4·10^6 Dalton range as well as some binding to low molecular weight fractions (monomer–dimer IgG region). High molecular weight fractions when concentrated showed IgM by double diffusion in agar but no IgG, in an elution position of greater molecular weight than native IgM.

DISCUSSION

We have demonstrated that high levels of C1q binding immune complexes occur in the sera of patients with both localized and generalized onchocerciasis. These complexes did not contain IgG since we failed to demonstrate them by an IgG Fc receptor-based technique; furthermore, the high molecular weight C1q binding material following gel chromatography of sera contained IgM but no IgG. These results are in keeping with the WHO collaborative study (Lambert *et al.*, 1978) comparing eighteen methods of immune complex detection in which the complement-based techniques detected circulating immune complexes more frequently in tropical parasitic diseases than did the Fc receptor ones.

The striking differences between the results of the C1q binding assay and the IgG latex agglutination inhibition method could be explained in terms of a predominant IgM response to the antigens involved. Such an IgM response may be due to the antigenic variation with modulation of surface antigens which occurs as a mechanism of parasite adaptation (Bloom, 1979). Indeed, the polyclonal B cell activation which occurs in mice infected with *Trypanosoma brucei* also leads almost exclusively to IgM antibody production to a variety of antigens, including anti-DNA, anti-syngeneic red blood cells, anti-thymocyte antibodies and rheumatoid factors (Houba, Brown & Allison, 1969; Klein *et al.*, 1970; Hudson *et al.*, 1976; Kobayakawa *et al.*, 1979). It is likely that some of the immune complexes found in our patients contain antigens not derived from the parasite, since, although antibodies of different immunoglobulin classes to *Onchocerca volvulus* may be demonstrated in sera of infected patients (Bartlett *et al.*, 1975) there was no correlation between the specific antibodies found and the C1q binding immune complexes.

IgM rheumatoid factors were not found in the patients' sera but we did not test for other autoantibodies.

The mere demonstration of circulating immune complexes does not necessarily imply that they are responsible for any of the features of the disease. Although the pathology of the skin lesions, which resemble an Arthus reaction, suggests that there may be local formation of immune complexes, (Bartlett *et al.*, 1978; Ngu, 1978), it is less clear what role the circulating immune complexes play. Clinical signs suggestive of immune complex damage such as erythematous cutaneous lesions, fever, myalgia and arthralgias occur only in these patients following treatment with diethylcarbamazine or Suramin; patients with particularly heavy infestations may also get arthritis, proteinuria and shock following treatment. Such symptoms developing soon after the beginning of therapy are probably due to a sudden release of large quantities of surface antigens from the dying parasites and may result in the formation of a qualitatively different type of immune complex, such as those associated with renal lesions in other diseases (Levinsky & Soothill, 1979). Since all patients in this study were tested before treatment was started and none of them had symptoms of renal or joint disease, it is probable that the circulating C1q binding complexes do not have a direct inflammatory role in the disease, but exert an indirect modulating influence on the immune system.

The polyclonal B cell proliferation which occurs in *Trypanosoma brucei*-infected mice is paralleled by a decrease of both T and B cell functions, both *in vitro* and *in vivo* (Hudson *et al.*, 1976; Askonas *et al.*, 1979) and this effect is due to active suppression (Jayawardena & Waksman, 1977). Recently, antigen-specific T cell suppression has been demonstrated in the generalized form of the disease, in contrast to the localized-reactive type where no such suppression was observed (Ngu, 1978). It is possible that this antigen-specific cellular immunosuppression is due to modulation of T lymphocytes by suppressor cell activation after interaction with the Fc portion of the complexed immunoglobulin or the antigenic determinants on the complexes. Indeed, both IgG and IgM complexes can activate T cells *in vitro* to develop suppressor activity (Hayward *et al.*, 1978).

This still does not explain the differences observed clinically in our patients, nor the localization of the disease to a particular area. Further studies on host responses in onchocerciasis are needed to establish the relevance of the circulating immune complexes in this disease and whether these complexes do indeed affect the host's cell-mediated responses to the parasite.

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REFERENCES

Askonas, B.A., Corsini, A.C., Clayton, C.E. & Ogilvie, B.M. (1979) Functional depletion of T and B memory cells and other lymphoid cell subpopulations during trypanosomiasis. *Immunology*, **36**, 313.

Bartlett, A., Bidwell, D.E. & Voller, A. (1975) Preliminary studies on the application of enzyme immunoassay in the detection of antibodies in Onchocerciasis. *Tropenmed. Parasitol.* **26**, 370.

Bartlett, A., Turk, J., Ngu, J., Mackenzie, C.D., Fugsang, H. & Anderson, J. (1978) Variation in delayed hypersensitivity in Onchocerciasis. *Trans. R. Soc. Trop. Med. Hyg.* **72**, 372.

Bloom, B.R. (1979) Games parasites play: how parasites evade immune surveillance. *Nature*, **279**, 21.

Gibson, D.W. & Connor, D.H. (1978) Onchocercal lymphadenitis: clinicopathological study of 34 patients. *Trans. R. Soc. Trop. Med. Hyg.* **72**, 137.

Hayward, A.R., Layward, L., Lydyard, P.M., Moretta, L., Dagg, M. & Lawton, A.R. (1978) Fc-receptor heterogeneity of human suppressor T cells. *J. Immunol.* **121**, 1.

Houba, V., Brown, K.N. & Allison, A.C. (1969) Heterophile antibodies, M-antiglobulins and immunoglobulins in experimental trypanosomiasis. *Clin. exp. Immunol.* **4**, 113.

Hudson, K.M., Byner, C., Freeman, J. & Terry, R.J. (1976) Immunodepression, high IgM levels and evasion of the immune response in murine trypanosomiasis. *Nature*, **264**, 256.

Jayawardena, A.N. & Waksman, B.H. (1977) Suppressor cells in experimental trypanosomiasis. *Nature*, **265**, 539.

Klein, F., Mattern, P. & Van Der Korman Bosch, H.J. (1970) Experimental induction of rheumatoid factor-like substances in animal trypanosomiasis. *Clin. exp. Immunol.* **7**, 851.

Kobayakawa, T., Lous, J., Izui, S. & Lambert, P.H. (1979) Autoimmune response to DNA, red blood cells and thymocyte antigens in association with polyclonal antibody synthesis during experimental African trypanosomiasis. *J. Immunol.* **122**, 296.

Lambert, P.H., Dixon, F.J., Zubler, R.H., Agnello, V., Cambiaso, C., Casali, P., Clarke, J. Cowdery, J.S., McDuffie, F.C., Hay, F.C., Maclennan, I.C.M., Masson, P., Müller-Eberhard, H.J., Penttinen, K., Smith, M., Tappeiner, G., Theofilopoulos, A.N. & Verroust, P. (1978) A WHO collaborative study for evaluation of eighteen methods for detecting immune complexes in serum. *J. clin. Lab. Immunol.* **1**, 1.

Levinsky, R.J. & Soothill, J.F. (1977) A test for antigen–antibody complexes in human sera using IgM of rabbit

antisera to human immunoglobulins. *Clin. exp. Immunol.* **29**, 428.

LEVINSKY, R.J. & SOOTHILL, J.F. (1979) The heterogeneity of immune complexes in disease. In *26th Colloquim Protides of Biological Fluids* (ed. by H. Peeters), p. 243. Pergamon Press, Oxford and New York.

MEYERS, W.M., NEAFIE, R.C. & CONNOR, D.H. (1977) Onchocerciasis: invasion of deep organs by *Onchocerca volvulus*. *Am. J. Trop. Med. Hyg.* **26**, 650.

NGU, J.L. (1978) Immunological studies on Onchocerciasis. *Acta Trop.* **35**, 269.

NGU, J.L. & BLACKETT, K. (1976) Immunological studies in Onchocerciasis in Cameroon. *Trop. Geogr. Med.* **28**, 111.

SOMORIN, A.O., HEINER, D.C. & AJUGWO, R.E. (1977) Immunoglobulin E in Nigerian Onchocerciasis. *Am. J. Trop. Med. Hyg.* **26**, 872.

WHO REPORT (1977) The role of immune complexes in disease. *WHO Tech. Rep. Ser.* 606.

ZUBLER, R.H., LANGE, G., LAMBERT, P.H. & MIESCHER, P.A. (1976) Detection of immune complexes in unheated sera by a modified ^{125}I-C1q binding test. *J. Immunol.* **116**, 232.