WIADOMOŚCI PARAZYTOLOGICZNE
T. XXXII, NR 3: 1986
PROC. OF ICT NO XIX

CIRCULATING IMMUNE COMPLEXES IN MICE INFECTED WITH *TRICHINELLA SPIRALIS*

ZOFIA GOLIŃSKA, BARBARA MACHNICKA and ALICJA RYSIŃSKA

Military Institute of Hygiene and Epidemiology, Warszawa
Institute of Parasitology of the Polish Academy of Sciences, Warszawa

Introduction

Immune complexes (ICs) are formed when antigens interact with relevant antibodies which represent a normal component of the immune response leading to removal of antigen from host body. Besides their physiological function ICs can induce pathological changes when deposited in different tissues or influence the protective immunological mechanism (Theofilopoulos and Dixon, 1980; Contreras et al., 1982).

During infection with long-living parasites, favourable conditions are created for forming ICs, i.e. continuous release of soluble antigens capable of evoking a specific antibody response. ICs appear either in the circulation and/or at tissue sites. The presence of ICs has been detected during infection with some protozoa such as plasmodia, toxoplasmes and metazoa such as schistosomes, filarial worms, cestodes and trichinae (Houba, 1975; Geniteau et al., 1977; Contreras et al., 1982; Cottrell and Sturrock, 1982; Steward et al., 1982; Pini et al., 1983; Siegel and Remington, 1983).

The immunobiology of *Trichinella spiralis* characterized by the rapid growth of adult parasites in the intestine, the production of larvae which after migration settle in the fibres of striated muscles are connected with intensive metabolism of the parasites and the release of both excretions and secretions which stimulate production of antibodies. Thus, formation of circulating ICs during *T. spiralis* infection might be expected.

The present study examines the dynamics of ICs in CFW strains of mice, experimentally infected with *Trichinella spiralis*.

Material and methods

Hosts and parasites. Male, inbred CFW mice (from State Institute of Hygiene breeding) between 2 and 3 months of age at the beginning of the experiments were used. Their weight, was 22-25 g.

They were fed with commercial pellets, allowed to drink ad libitum and maintained under conventional conditions. Mice were infected with *T. spiralis* larvae obtained from muscle tissue by pepsin-HCl digestion. The mice were divided into three groups: in the first animals were infected with 200 larvae of *T. spiralis*; the second mice were infected with 400 larvae; the third group consisted of uninfected, control animals. Three sets of experiments were performed. The examinations were carried out from 2 to 13 weeks after infection.

ICs determination. The level of ICs in individual mouse serum was determined according to Digeon as modified by Grzybowski et al. (1980). Blood was collected from plexus orbitalis. The sera were separated by centrifugation at room temperature. Immune complexes were examined on the same day sera were collected. Each serum was mixed in equal portion (50 µl) with 6% PEG (Polyethylene glycol 6000, LOBO, Austria) in borate buffer (0.1 M, pH 4) — final concentration of 3%. The mixture was incubated for 18 h at 4°C and then centrifuged at 10 000 rpm for 20 min. at 0°C. The supernatant was decanted and the precipitates were washed twice with cold 3% PEG solution. The pellets were dissolved in 50 µl of 0.1 N sodium hydroxide, and the quantity of precipitated proteins was measured by means of an especially adapted method for protein estimation in paper electrophoresis. 5 µl of each sample was deposited on Whatman nr 1 paper discs, which were allowed to dry for 10 min. at room temp. and then at 120°C for 10 min. Proteins were stained for 2 h in a 0.1% solution of bromophenol blue in absolute ethanol containing 10% $HgCl_2$. Next the discs were destained for 3 h by means of 0.5% acetic acid. After that, they were dried at room temp. and stain compounds were eluted with 5% Na_2CO_3 in 50% ethanol for 30 min. Absorbance of the eluates was measured at 600 nm. Bovine serum albumin was used as a protein standard.

The results were considered positive when they showed higher values over the mean values of control animals increased by two standard deviations.

Preparation of anti-ICs sera. Two male rabbits of race White from Popielno aged 6 months, and weighing approximately 3.5 kg each, were injected in week intervals for 2 months. The animals were inoculated with ICs precipitated with PEG consisting of more then 3 mg/ml protein. Each injection consisted of 0.1 ml of ICs in PEG emulsified with 0.1 ml Freund adjuvant. The first, third and fifth injections which were administered directly to popliteal lymph nodes included complete Freund adjuvant. The remaining immunizations were given subcutaneously with incomplete Freund adjuvant.

Goat anti-mouse globuline serum was a product of Gibco Laboratories (Grand Island).

The rabbit anti-serum against ICs as well as goat anti-mouse globulin were examined by double diffusion in 0.9% agarose gel in PBS pH 7.2 against a soluble antigen from *T. spiralis* larvae containing 1.2 mg/ml protein.

Results

The dynamics of circulating ICs in the sera of CFW mice infected with 200 and 400 *T. spiralis* larvae is shown in Fig. 1. During the first 4 weeks of infection the level of ICs was the same as in control animals. A considerable elevation in the level of ICs was noted at weeks 5 (73 and 75% respectively) and 6 (55 and 50% respectively) after infection. At week 7 the levels of ICs were in the range of normal values. Further observations, until week 13 after infection showed, results which could be considered positive only on weeks 8, 9, and 10.

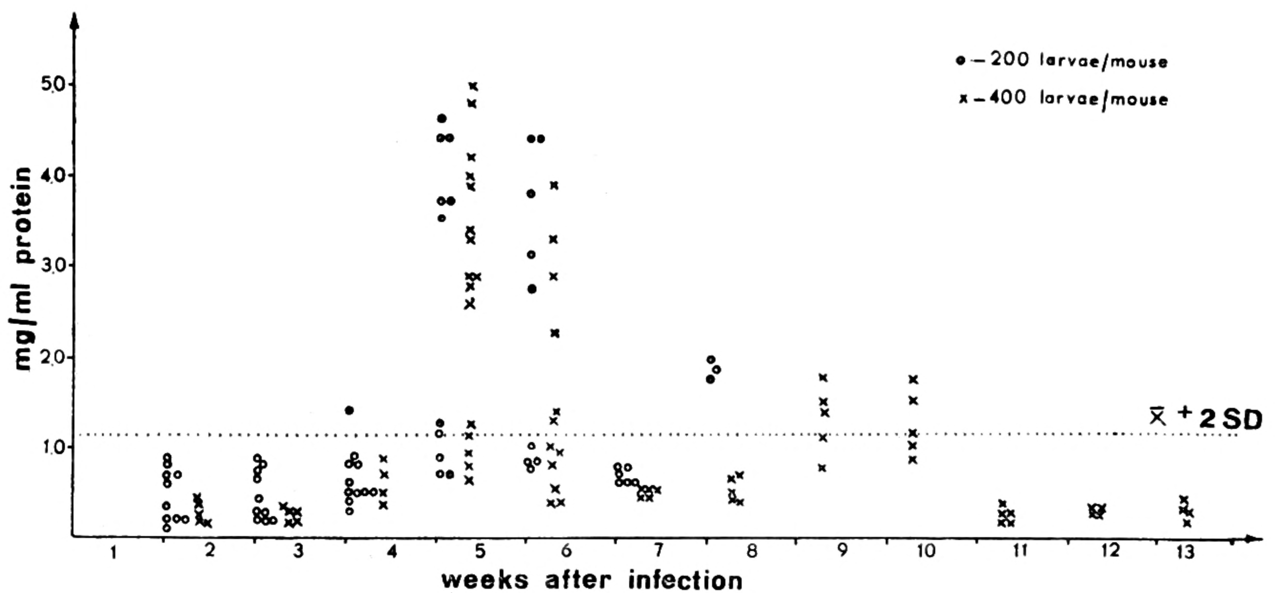

Fig. 1. Circulating immune complexes (CIC) during the course of Trichinella spiralis infection in CFW mice by the PEG precipitation test. The dotted line shows the control level above which the results are considered as positive ones

Double diffusion in agarose gels of rabbit anti-ICs serum against *T. spiralis* larval antigen, normal CFW mouse serum and ICs showed the lines of identity between ICs and normal mouse serum as well as one line which was common for parasite antigen and ICs (Fig. 2). Goat anti-mouse globulin did not reacted with *T. spiralis* larval antigen.

Discussion

The technique applied for the detection of circulating ICs in the blood serum, PEG precipitation required the acceptance as the criterion of positive results the values which exceeded twofold standard deviation

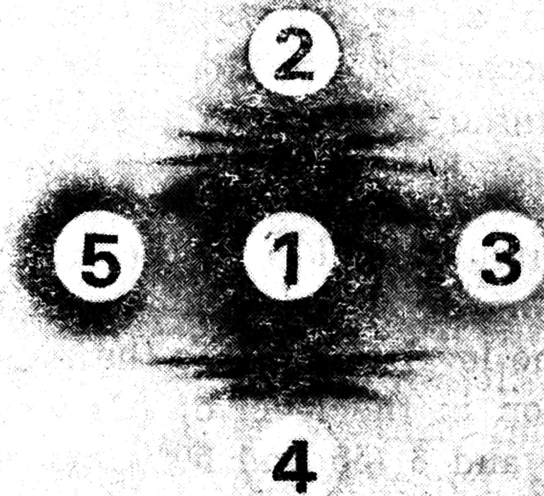

Fig. 2. Double immune-diffusion. *1* — rabbit anti-ICs serum, *2* — CFW mice serum, *3* — *T. spiralis* larval antigen, *4* — ICs, *5* — *T. spiralis* larval antigen diluted 1 : 20

of values which were obtained with sera of uninfected mice of the same strain. It seemed reasonable as lately the ICs has been stated in healthy population of man as well as in conventional animals without any clinical symptoms (Contreras et al., 1982; Slausen and Lewis, 1979).

The results reported here demonstrated that infection with *T. spiralis* in CFW mice induce, in a high percentage of animals sera, appearance of ICs. The formation of its depends on the nature of the antibodies and antigens involved as well as their relative concentration (Theofilopoulos and Dixon, 1980). Although soon after infection the developing parasites produce an abundance of soluble antigens and evoke antibodies, the intensive growth of larvae in the muscles seems to be necessary for formation of circulating ICs. It can not be ruled out that the absence of ICs early after infection with *T. spiralis* is related with a low affinity of antibodies, which changes with the duration of infection (Houba, 1975). The results of our examinations pointed out the presence of ICs in CFW mice at the fifth week after infection. It is in accordance with observations of Geniteau et al. (1977) on patients with clinical trichinosis; ICs could be detected using the ^{125}J-labelled C1q binding test as late as 30-40 days after infection. Similar observations have been made in experiments performed on animals infected with *Schisostoma mansoni*; in mice, ICs were noted 40-50 days after infection (Santoro et al.,

1979) and 40-70 days after infection (Bout et al., 1977), in baboons on week 4-6 after infection (Cottrell and Sturrock, 1982; Takai, 1983) has been pointed out the presence of ICs in the sera of rats infected with *Angiostrongylus cantonensis* with maximum 40-70 days after infection. From the above quoted observations, which are similar to our findings, it can be concluded that a long and continuous supply of soluble antigens conditions the formation of circulating ICs. Because it is not unlikely that circulating ICs are formed with metabolic antigens of parasite, it is proper to remind the observations of Kent (1963) on rabbits, which proved that antibodies to metabolic antigens can be detected only at 26 days or later after infection with *T. spiralis.*

The results of our examinations did not show that infective dose could influence the level or period post infection in which ICs appeared. Similarly, Steward et al. (1982) examing the sera of patients infected with *Onchocerca volvulus* did not state any correlation between filarial load and the level of ICs.

In the first half on this century a number of papers describing kidney pathology after infection with *T. spiralis* were published (Gould, 1970). Later, Schoenfeld and Edie (1967), described the case of glomerulonephritis after infection with *T. spiralis* suggesting ICs aetiology. The study on the mice experimentally infected with *T. pseudospiralis* described by Żeromski et al. (1978) proved that glomerulonephritis of mesangial and membranoproliferative type took place from 14 days to 6 months after infection. In these examinations were evidenced deposits of antigen-antibody complexes by the immunofluorescent studies using fluorescein isothiocyanate labelled rabbit immune globulin against *T. spiralis,* rabbit immune globulin against mouse immunoglobulins and rabbit immune globulin against mouse IgG in glomeruli of the kidneys. It was pointed out too, that the complexes contained the heterologous complement. It is likely that during the infection with *T. spiralis* or *T. pseudospiralis,* different parasitic antigens can lead to formation of different types of ICs of dissimilar size and affinity to the tissues. ICs stated in kidneys could not be identical to those found in circulation. From the examinations performed by Machnicka (1963) it can be concluded that ICs formed during infection with *T. spiralis* appear in excess of antigen, probably are very small, do not bind complement and do not exerting phlogogenic effect (Theofilopoulos and Dixon, 1980).

The attempt to demonstrate the presence of antibodies to larval *T. spiralis* antigen in sera of the rabbits immunized with ICs appeared difficult. In the majority of agarose plates, a weak precipitin line which formed between rabbit anti-ICs serum and *T. spiralis* larval antigens dissapeared in acidic medium, i.e. during staining in 1% amidoblack

dissolved in 7% acetic acid. Before staining the plates had to be fixed with 96% ethanol.

Except for pathogenic influence which can be exterted by ICs during infection with *T. spiralis,* suppression of humoral and cellular immune response to the own and unrelated antigens was observed (Faubert and Tanner, 1974; Jones et al., 1976, Svet-Moldavsky et al., 1970). Moreover, in successive stages of infection different mechanisms of suppression due to ICs as blocade of antigen receptors on T and B lymphocytes, through antigen masking, activation of suppressor cells, etc., can operate (Flaubert, 1982).

Otrzymano: 20 VI 1985

Authors' address:
Kozielska 4, 01-163 Warsaw

LITERATURE

1. Bout, O., Santoro, F., Carlier, Y., Bina, J., Capron, A.: Circulating immune complexes in schistosomiasis. — *Immunology,* 33, 17-22, 1977.
2. Contreras, C. E., Orezco, A., Sanchez, P., Ortega, G., Bianco, N. E.: Physiological aspects of circulating immune complexes in the normal population. — *Clin. Exp. Immunol.*, 48, 693-699, 1982.
3. Cottrell, B., Sturrock, R. F.: The modulation of specific and nonspecific cell-mediated immunity in baboons (*Papio anubis*), injected with *Schistosoma mansoni.* — *Immunology,* 5, 341 - 348, 1982.
4. Faubert, G. M.: The reversal of the immunodepression phenomenon in trichinellosis and its effect on the life cycle of the parasite. — *Parasite Immunol.,* 4, 13-20, 1982.
5. Faubert, G. M., Tanner, C. E.: The suppression of sheep rosette-forming cell and the inability of mouse bone marrow cells to reconstitute competence after infection with the nematode *Trichinella spiralis.* — *Immunology,* 27, 501-505, 1974.
6. Geniteau, M., Vorroust, P. J., Smith, M. D., Morel-Mareger, L., Saimet, G., Couland, J. P.: Circulating immune complexes in six patients with trichinellosis. — *Clin. Exp. Immunol.,* 30, 141-144, 1977.
7. Gould, S. E.: Trichinellosis in man and animals. Ch. C. Thomas Publishers, Springfield, Illinois, U.S.A., 1970.
8. Grzybowski, J., Kuszczak, B., Rysińska, A., Kubica, J., Przystupa, J., Kwiek, S., Bukowska, B.: Comparative investigations of in vitro and in vivo scalding of mouse skin. — *Arch. Immunol. Ther. Exp.,* 28, 253-264, 1980.
9. Houba, V.: Immunopathology of nephropathies associated with malaria. — *Bull. Wld. Hlth. Org.,* 52, 199-207, 1975.
10. Jones, J. F., Crandall, C. A., Crandall, R. B.: T-dependent suppression of the primary antibody response to sheep erythrocytes in mice infected with *Trichinella spiralis.* — *Cell. Immunol.,* 27, 102-110, 1976.
11. Kent, N. H.: Fractionation, isolation and definition of antigens from para-

sitic helminths. In: Immunodiagnosis of helminthic infections. — *Amer. J. Hyg.* (Monogr. Ser.), 22, 30-46, 1963.

12. Machnicka, B.: The occurrence of antigen in the blood and urine of rabbits in the course of experimental infection with *Trichinella spiralis.* — *Wiad. Parazytol.*, 9, 465-475, 1963.
13. Pini, C., Pastore, R., Valesini, G.: Circulating immune complexes in sera of patients infected with *Echinococcus granulosus.* — *Clin. Exp. Immunol.*, 51, 572-578, 1983.
14. Santoro, F., Vandemeulebroucke, B., Capron, A.: *Schistosoma mansoni*: circulating antigens and immune complexes in infected mice. — *Exp. Parasitol.*, 47, 392-402, 1979.
15. Schoenfeld, M. R., Edis, G. T.: Trichinellosis and glomerulonephritis. — *Arch. Pathol.*, 84, 625-626, 1967.
16. Siegel, J. P., Remington, J. S.: Circulating immune complexes in toxoplasmosis: detection and clinical correlates. — *Clin. Exp. Immunol.*, 52, 157-163, 1983.
17. Slausen, D. O., Lewis, R. M.: The comparative pathology of glomerulonephritis in animals. — *Vet. Pathol.*, 16, 135-164, 1979.
18. Steward, M. W., Sisley, B., Mackenzie, C. D., El Sheikh, H.: Circulating antigen-antibody complexes in onchocerciasis. — *Clin. Exp. Immunol.*, 48, 17-24, 1982.
19. Soet-Moldavsky, G. J., Shadhijan, G. S., Chernyakhowskaya, J., Mikheidze, D. M., Litovchenko, T. A., Ozeretskovskaya, N. N., Kadaghidze, Z. G.: Inhibition of skin allograft rejection in *Trichinella* infected mice. — *Transplantation*, 9, 69-70, 1970.
20. Takai, A.: Immunopathological studies of rats infected with *Angiostrongylus cantonensis.* I. Circulating immune complexes and deposition of the immune complexes on the tissues. — *Acta med. et biol.*, 30, 105-123, 1983.
21. Theofilopoulos, A., Dixon, F. J.: Immune complexes in human diseases. — *Amer. J. Pathol.*, 100, 531-559, 1980.
22. Żeromski, J., Jarczewska, K., Górny, M.: Evidence for the presence of antigen-antibody complexes in the course of experimental infection with *Trichinella pseudospiralis.* — Trichinellosis. Eds Ch. Kim and Z. S. Pawłowski, 303-312, New England, Univ. Press, 1978.