Immunology, 1971, **20**, 451.

Reaginic Antibodies in Dogs Infected with *Echinococcus granulosus*

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(*Received 9th March* 1970)

Summary. Serum samples from twenty dogs infected with *Echinococcus granulosus* were tested for the presence of homocytotropic skin-sensitizing antibodies. Five of the twenty sera were positive in this test, while none of the sera from twenty normal dogs was positive. The antibody was thermolabile and susceptible to 2-mercaptoethanol reduction.

Reaginic antibodies to cestode antigens have not been described previously in dogs, though they are frequently associated with helminth infection in other animals and may play a role in acquired resistance.

INTRODUCTION

Reaginic antibodies have been described in dogs with naturally occurring hypersensitivity to plant pollens (Patterson, Chang and Pruzansky, 1963; Rockey and Schwartzman, 1967) and to antigens of *Toxocara canis* (Patterson, Roberts and Pruzansky, 1969), a common nematode parasite.

Turner, Dennis and Berberian (1935) studied the skin response of dogs infected with *Echinococcus granulosus* to the intradermal inoculation of hydatid cyst antigens and, although they concluded that the test had no diagnostic value, a few animals were shown to have immediate-type skin hypersensitivity. We have re-investigated this phenomenon and demonstrated the presence of a homocytotropic skin sensitizing antibody in the serum of some dogs having echinococcosis.

MATERIALS AND METHODS

Twenty dogs were selected at random from a group of approximately forty which had been experimentally infected with 20,000 scolices of *E. granulosus* 10 weeks previously. Direct skin tests were made on each animal by inoculating intradermally 0·1 ml of sheep hydatid fluid diluted with sterile phosphate buffered saline (PBS) to contain 195 μg protein per ml. Control inoculations of 0·1 ml of PBS were also made. Twenty normal dogs were similarly examined. Serum samples were obtained from both infected and non-infected animals, and the presence or absence of active infection with *E. granulosus* was confirmed at necropsy of each dog.

All the sera were tested in the PCA test using normal dogs as recipients. Each serum sample was examined in at least two recipients. The animals were tranquillized with barbiturates given orally, shaved along the lateral part of the thorax and inoculated intradermally with 0·2 ml quantities of the sera under test. 48–72 hours later an intravenous inoculation was given consisting of 1 per cent Evans Blue (0·5 ml/kg body weight) together with hydatid fluid (0·5 mg protein/kg body weight).

Positive sera were titrated using doubling dilutions in PBS to sensitize the skin of recipients. The effect of mercaptoethanol treatment on the reaginic antibody activity was determined using the method of Rockey and Schwartzman (1967).

Indirect haemagglutination and immunodiffusion tests were carried out on each serum sample. Formolized sheep red cells were prepared and sensitized with antigens of sheep hydatid cyst fluid using the method of Hirata and Brandriss (1968). Titrations were performed using the Cooke microtitre system (Cooke Engineering Co., Va, U.S.A.), beginning with a 1:8 dilution. Ouchterlony analysis was done on microscope slides using 1·25 per cent agar (Bacto-agar Difco) in veronal buffer, pH 8·2. Wells of 3 mm diameter were cut with the centres 7 mm apart. Lyophilized hydatid fluid was reconstituted and used as antigen in varying concentrations up to 50 mg/ml. Slides were washed in buffer, dried and stained with Amido Schwartz. Serum from several human cases of hydatidosis were used as positive controls in these tests.

RESULTS

The diameter of the skin reactions was measured between 30 and 60 minutes after inoculation. The average diameter of the papule produced in infected dogs was 14 mm with a range from 7 to 22 mm. In normal dogs the reactions were of 10 mm average with a range from 7 to 14 mm.

Those animals having the largest immediate skin reactions were also tested intradermally with a saline extract prepared from adult strobilate *E. granulosus* containing 2 mg protein per ml. Positive reactions again appeared but the intensity of the response was no greater than with larval cyst fluid.

Positive PCA reactions usually began to appear within 15 minutes of intravenous challenge with antigen, reaching a maximum between 30 and 60 minutes. Strongly reactive sera produced a solid blue stained area up to 45 mm in diameter (Fig. 1) though occasionally the central part remained white with only the periphery markedly blue. Sometimes tortuous blue lines extended downwards from the reaction, possibly following the direction of lymphatic vessels.

Five of the twenty sera from infected dogs gave positive reactions in this test. The highest titre obtained was 1:320. There was no clear association between the size of the immediate skin reaction and the presence of circulating reagins, although serum from the dog having the largest reaction was positive in the PCA test. None of the sera from uninfected animals was positive.

Intradermal inoculation of positive serum followed by intravenous challenge 14 days later produced positive reactions demonstrating persistence of the skin-fixed antibody, but no activity could be detected when challenge was delayed to 28 days. None of the sera produced reactions when intravenous administration of antigens followed 4 hours after intradermal sensitization with serum.

A number of dogs having immediate skin test responses to the intradermal inoculation of antigen were challenged intravenously with hydatid fluid. Within minutes the onset of classical signs of anaphylactic shock were apparent, including vomiting, diarrhoea and prostration with laboured breathing. Most animals recovered and were able to walk within 2 hours, although one animal died during the 21 hours following shock. The activity of the allergen(s) in hydatid fluid was not destroyed by boiling for 30 minutes and was not removed by dialysis of boiled fluid against PBS (100 volumes) for 24 hours.

The reaginic antibody activity was destroyed in all positive sera by heating to 56° for 30 minutes. Treatment with 2-mercaptoethanol resulted in complete loss of activity. Lyophilization of positive sera was found not to reduce skin sensitizing ability. Thus far attempts further to characterize the antibody using Sephadex G-200 columns and sucrose gradient ultracentrifugation (according to the methods of Rockey and Schwartzman, 1967) have not been successful. A satisfactory separation of the major immunoglobulin

FIG. 1. Passive cutaneous anaphylactic reaction in recipient animal inoculated intradermally with sera from dogs infected with *E. granulosus*. Intravenous challenge with antigen and Evans blue followed 48 hours later. The arrows mark the blue stained areas of positive reactions.

classes has been achieved but it has not been possible to demonstrate reaginic activity in any of the fractions, presumably due to either loss or dilution of activity during the purification procedures. Further work on this aspect is continuing.

None of the dog sera reacted in either haemagglutination or immunodiffusion tests. Titres of up to 1 : 32,768 were observed with the human sera and many heavy bands were seen in Ouchterlony plates. No bands appeared with the dog sera, which were also used concentrated three-fold with varying antigen concentrations to no effect.

DISCUSSION

Human and animal infection with *E. granulosus* occurs in most countries throughout the world and in certain areas has become a public health and economic problem of prime

importance (Schantz and Schwabe, 1969). The domestic dog plays a central role in the transmission of this disease, yet immunological responses in canine echinococcosis have been little studied.

Gemmell (1962) presented evidence suggesting that a degree of acquired resistance developed in dogs after repeated infection, manifested by a slower rate of growth of parasites in immune animals. The mechanism by which this effect is produced is not

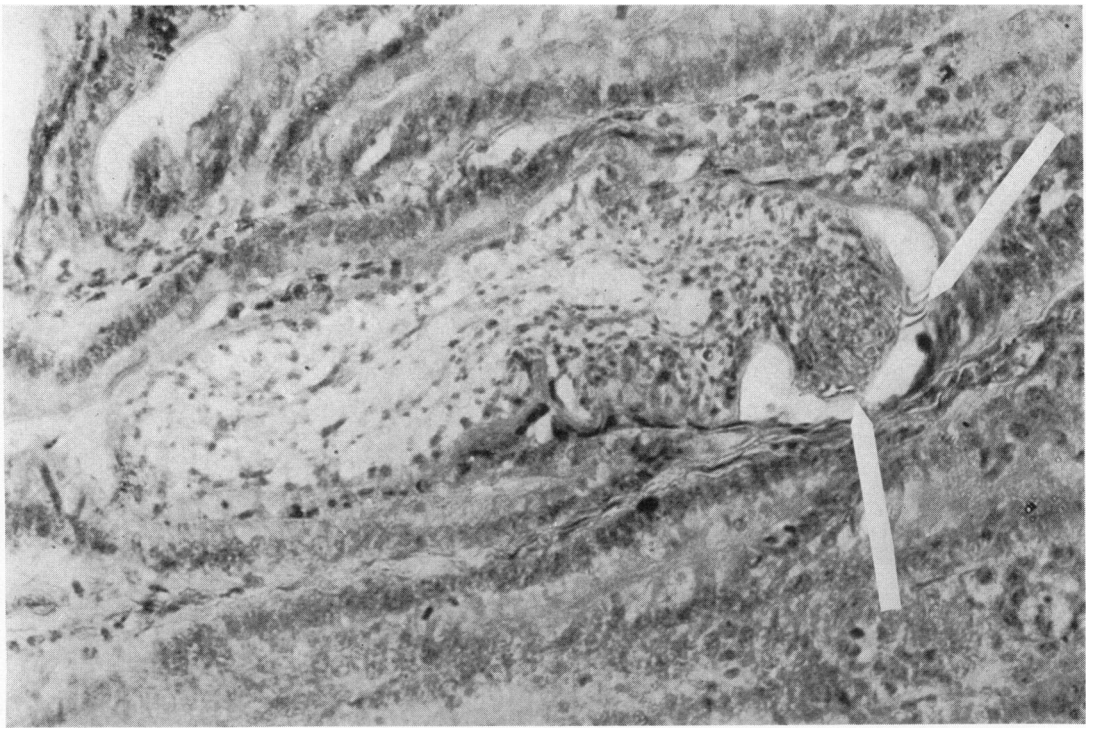

FIG. 2. Histological section from duodenum of dog 72 hours after experimental infection with *E. granulosus* showing relationship between the parasite and host mucosa. Arrows indicate hooks on the scolex of the worm. The epithelium lining the crypt has become flattened and squamous in this area. (H & E, ×600.)

known although circulating antibodies to parasite antigens have been detected in some infected animals by means of bentonite flocculation test (Chordi, Gonzalez-Castro and Tormo, 1962). Our experiments indicate that a thermo-labile homocytotropic skin-sensitizing antibody can also be demonstrated in the serum of some dogs during the course of infection with *E. granulosus*.

Unlike *Toxocara canis*, which undergoes a complex tissue migratory phase of development in the dog, *E. granulosus* lives in the lumen of the intestine of this host. The relationship between the scolex of the parasite and the mucosa of the host is very intimate, however (Fig. 2) and Smyth and Smyth (1968) have suggested that the worm may be regarded as a tissue parasite if the mucosal surface close to the scolex breaks down. This has been shown to occur sometimes during the early days of infection when the scolex is found deep in the crypts of Lieberkühn (Smyth and Smyth, 1968). Immunological responses may be

stimulated at this time although in preliminary experiments (unpublished observations) circulating reagins were not detected in infected dogs until the 7th week of infection. No response has been detected so far using the indirect haemagglutination or immunodiffusion tests.

Reaginic antibodies are commonly associated with helminth infections and may, in some cases, be related to acquired resistance (Ogilvie, 1964). Further work is required in order to determine if the antibody observed here plays any role in the development of immunity to *E. granulosus* in the dog.

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