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The Influence of the Nematode *Syphacia oblevata* on Adjuvant Arthritis in the Rat

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Summary. The effect of infestation with the nematode *Syphacia oblevata* on adjuvant arthritis was studied in the rat. Animals with an established infestation with *Syphacia* were found to have a reduced incidence of arthritis after injection of Freund's complete adjuvant. Infested animals developing adjuvant arthritis were found to suffer from a less severe form of the disease than animals in which infestation had been eliminated with piperazine before immunization.

INTRODUCTION

It has been known for many years that the injection of Freund's complete adjuvant (FCA) may induce arthritis in the rat (Pearson and Wood, 1959). Strain differences in the incidence of the disease have also been reported (Zidek & Perlik, 1971). In the course of experiments in which rats from our inbred PVG/C rat colony were injected with ovalbumin in FCA we were pleased to observe no signs of adjuvant disease. The colony was found to be heavily infested with the common nematode parasite of rodents, *Syphacia oblevata*. After successful treatment of the infestation with piperazine we were surprised to observe a high incidence of adjuvant arthritis in animals immunized in a manner identical to that used previously. Here we report observations on this phenomenon.

MATERIALS AND METHODS

Animals

Inbred black-hooded PVG/C rats were used throughout the study, the colony being maintained by brother–sister matings from a stock originally obtained from the M.R.C. Animal Centre, Carshalton, Surrey.

Syphacia oblevata infestation was confirmed by examination of the caecal contents. Random samples of piperazine-treated animals were found to be worm-free up to 3 months later.

Piperazine treatment was performed using 'Antepar' Elixir (Borroughs Wellcome) added to the water supply, at a dose equivalent to 3 g of piperazine per litre, for 14 days.

Immunization

Immunization was performed at 11–14 weeks of age with 0·05 ml of FCA containing

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ovalbumin at each of three sites on the same day. The sites injected were both hind foot pads and an intradermal site on the midline over the sternum.

The FCA was produced by the emulsification of equal volumes of ovalbumin solution in physiological saline with incomplete adjuvant (Difco Laboratories) to which had been added human strain *Mycobacterium tuberculosis* at a concentration of 5 mg/ml. The ovalbumin doses employed varied between 0·375 and 15 μg per rat.

Arthritis

Except where otherwise stated animals were examined regularly after the 7th day post-immunization for the presence of arthritis. The majority of animals were observed until 28 days post-immunization; in those animals observed for up to 8 weeks, arthritis was never observed to occur *de novo* after 28 days. When present, the arthritis was scored on an arbitrary 4 point scale: 1+, minimal disease in which 1 joint only was involved; 2+, involvement of more than one joint but without marked swelling or erythema; 3+, involvement of both uninjected limbs with marked periarticular swelling, and 4+ as grade 3 but in addition severe general debility and muscle wasting.

RESULTS

INCIDENCE OF ARTHRITIS

Seventy-eight animals (twenty-nine male, forty-nine female) were immunized before piperazine treatment of the colony. Sixty-nine (thirty-five male, thirty-four female) piperazine treated worm-free animals and seventy-six (thirty-eight male, thirty-eight female) animals born after spontaneous re-establishment of *Syphacia oblevata* infestation in the colony were similarly immunized. The total incidence of arthritis observed in the three groups is shown in Table 1.

TABLE 1

TOTAL INCIDENCE OF ARTHRITIS IN RATS IMMUNIZED WITH FCA

Group	Male	Female	Male and female
A* Worm-infested	0/29	0/49	0/78
B† Worm-free	30/35 (86%)	8/34 (23%)	38/69 (55%)
C‡ Worm-infested	11/38 (29%)	7/38 (18%)	18/76 (24%)

* Before piperazine treatment of colony.
† Piperazine-treated.
‡ After re-establishment of *Syphacia oblevata*.

Although the differences between the pre-piperazine treatment group and the subsequent groups are statistically highly significant, we do not consider this a valid comparison as the former group was not methodically examined for arthritis in the same manner as the two subsequent groups. Although minor degrees of arthritis could have been overlooked it is highly unlikely that more marked degrees would not have been noted. Further analyses were therefore restricted to the latter two groups.

Between the worm-free group and the infested group the differences in the incidence of arthritis are statistically significant both for the whole groups and the males alone

(χ^2 test, $P<0{\cdot}001$). However, the incidence in the female worm-free group is not significantly different from that in either the male or the female worm-infested animals.

A similar pattern is observed when the incidence of arthritis at a fixed time after immunization is considered (Table 2).

TABLE 2

INCIDENCE OF ARTHRITIS ON THE 21ST DAY POST IMMUNIZATION WITH FCA

	Male	Female	Male and female
Worm-free	21/30 (70%)	5/29 (17%)	26/59 (42%)
Worm-infested	9/38 (24%)	7/38 (18%)	16/76 (21%)
P*	<0·001	n.s.	<0·001

* P value derived by χ^2 test (n.s. = not significant, comparison of worm-free and worm-infested rats).

There was no significant difference within groups of comparable worm status in the incidence of arthritis between animals immunized with 0·375, 1·5 or 15 μg of ovalbumin.

SEVERITY OF ARTHRITIS

Animals with severe arthritis usually had evidence of disease over a number of weeks whilst those considered to have minimal disease frequently had involvement of one interphalangeal or metacarpophalangeal joint for a few days only, usually in the 3rd week. Worm-free animals with arthritis more frequently had disease judged to be severe or moderate (3+ or 4+) compared to the worm-infested animals (sixteen out of thirty-eight compared to two out of eighteen, $P<0{\cdot}001$). Worm-infested animals developing arthritis were more likely to have minimal disease (eleven out of eighteen compared to five out of thirty-eight, $P<0{\cdot}001$).

The mean of the arthritis scores for the worm-free and worm-infested groups as a whole is shown in Table 3. Statistical analysis (Student's t-test) shows the differences between these groups and between the males alone to be highly significant ($P<0{\cdot}001$).

The mean scores of the animals developing arthritis is shown in Table 4. The scores in the worm-free animals being higher than in worm-infested animals developing arthritis.

TABLE 3

ARTHRITIS SCORES OF WHOLE GROUPS (THE MEANS OF MAXIMAL VALUE OBTAINED FOR EACH ANIMAL)

	Male	Female	Male and female
Worm-free	2·23 ± 1·22	0·34 ± 0·87	1·36 ± 1·35
Worm-infested	0·51 ± 0·97	0·21 ± 0·47	0·37 ± 0·78
P*	<0·001	n.s.	<0·001

* Student's t-test.

TABLE 4

ARTHRITIS SCORES OF ANIMALS DEVELOPING ARTHRITIS (MEANS OF MAXIMAL VALUE OBTAINED FOR EACH ANIMAL)

	Male	Female	Male and female
Worm-free	2·60 ± 0·86	1·88 ± 0·99	2·45 ± 0·92
Worm-infested	1·82 ± 0·98	1·143 ± 0·38	1·56 ± 0·86
P*	< 0·02	n.s.	< 0·01

* Student's t-test.

DISCUSSION

Suppression of adjuvant arthritis has been reported in rats infected with a strain of *Plasmodium berghei* (Greenwood, Voller and Herrick, 1970). Here we report the probable suppressive effect of *Syphacia oblevata* infestation on the incidence and the severity of adjuvant arthritis in rats.

The reduction in both the incidence and severity of arthritis in infested males is statistically highly significant, there was no significant difference in the incidence of arthritis in infested females, and although there was a reduction in the severity of the disease the difference in female arthritic scores was not statistically significant. The differences in the observed incidence of arthritis in the two worm-infested groups may have been due to the methodology employed in the earlier experiments, or due to the much higher level of infestation within the colony at that time. It is unlikely that our observations are directly the result of piperazine treatment, as in unpublished experiments we have shown no increase in the incidence or severity of adjuvant arthritis in rats injected with FCA alone and pretreated with piperazine for 1 month at a dosage inadequate to clear *Syphacia oblevata* infestation.

Although the pathological mechanisms of adjuvant arthritis are imperfectly understood, it is considered to be an immunologically mediated disease and it may be adoptively transferred by thoracic duct lymphocytes (Whitehouse, Whitehouse and Pearson, 1969). Humoral mechanisms are probably involved in its pathogenesis however, as it may occur in neonatally thymectomized animals (Isakovic and Waksman, 1965), and disease activity is suppressed by cobra venom factor (Kouronakis, Nelson and Kapusta, 1973). A number of factors have been described as suppressing induction of the disease and these include immunosuppression with ALS (Curry and Ziff, 1968) and with drugs (Ward, Cloud, Krawitt and Jones, 1964). The incorporation of large doses of BSA in the immunizing adjuvant has been shown to inhibit development of the disease (Isakovic and Waksman 1965), and our results could be due to antigenic competition between parasite antigen and the arthritogenic antigen. Infections may also produce alterations in reticuloendothelial activity and induce interferon production, both of which have been shown to effect the development of adjuvant disease (Vacher, Deraedt and Benzoni, 1973; Kapusta and Mendelson, 1969). Our findings would be compatible with an immunosuppressive effect of *Syphacia oblevata*, a non-invasive nematode normally confined to the gastrointestinal tract.

In rats in which a reaginic response has been induced by injection of ovalbumin and adjuvant a marked potentiation of the reagin response follows nematode or trematode

infection (Orr and Blair, 1969; Jarrett, 1972). It appears that an interval of about 10 days between antigen injection and infection is necessary for this effect. In contrast, infection with *Toxoplasma* in mice is followed by depletion of cortical thymocytes and of thymus dependant areas of lymph node and spleen. In addition there is a reduced humoral response to other subsequently presented antigens (Huldt, Gard and Olovson, 1973). Mice infected with *Plasmodium berghei* and Maloney leukaemia virus simultaneously have an increased incidence of leukaemia and a reduced production of mercaptoethanol-resistant antibody (Bomford and Wedderburn, 1973). Suppression of the humoral response to SRBC and enhanced retention of skin allografts have been reported in mice previously infected with the nematode *Trichinella spiralis* (Faubert and Tanner, 1971; Svet-Moldavsky, Shaghiyan, Chernyakhouskaya, Mkheidze, Litouchenko, Ozeretskovskaya and Kadaghidze, 1970). A reduced number of rosette-forming cells has been demonstrated in the spleens of mice immunized with SRBC 30 days after infection with *T. spiralis*, and at the same time the bone marrow of such animals has a much reduced capacity to reconstitute irradiated, thymectomized animals (Faubert and Tanner, 1974). Thus the effect produced by parasites on the immune response to other antigens appears to be dependent on the time relationship between infection and immunization. A controlling role for T cells in IgE production has previously been suggested (Okumura and Tada, 1971), antithymocyte serum given to rats 5 or 8 days after immunization causing marked potentiation of the reagin response (Okumura, Tada and Ochiai, 1974). In parasitic infection impaired allograft rejection, reduced IgG production and IgE potentiation might well result from the effects of impaired T-cell function acting at different stages in the immune response. During induction of the immune response impaired T-cell–B-cell co-operation may result in a reduced humoral response. Interference at a later stage of the same immune response with the T-cell suppressor function may be responsible for potentiation of the IgE response.

Syphacia oblevata is an extremely common parasite of laboratory rodents including 'specific pathogen-free' strains, and we have found it to be endemic in a number of colonies. It is normally considered non-pathogenic and is difficult to irradicate permanently from a colony. We consider our finding may be relevant to some reported interstrain differences in the incidence of adjuvant arthritis.

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