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Immunoglobulins Associated With Passive Transfer of Resistance to *Taenia taeniaeformis* in the Mouse

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Summary. Mice were found to be protected against *Taenia taeniaeformis* infection by passive transfer of serum collected from donors 28 days after infection. The protective activity resided exclusively in the first fraction of 7S immunoglobulins eluting from DEAE–cellulose at pH 5·8 with 0·05 M phosphate buffer. This fraction contained 7Sγ1 and 7Sγ2 immunoglobulins but no detectable γA, γM or skin sensitizing activity. Fractions containing 7Sγ2 alone were ineffective in passive transfer.

INTRODUCTION

Leid and Williams (1974a) have recently shown that passive transfer of resistance to *Taenia taeniaeformis* infection in the rat can be achieved with immunoglobulins of the 7Sγ2a type. The biological characterization of antihapten antibodies of this nature had previously been described by Morse, Bloch and Austen (1968). They demonstrated short-term sensitization of rat skin for passive cutaneous anaphylaxis (PCA) and antigen-induced release of SRS-A from neutrophils and histamine from mast cells. However 7Sγ2a antibodies to *T. taeniaeformis* were inactive in PCA tests (Leid and Williams, 1974b), suggesting the possibility that subpopulations of this immunoglobulin with distinct biological functions might be stimulated by helminthic infections.

We have pursued the peculiar association of protective activity against *T. taeniaeformis* with physico-chemically distinct immunoglobulin types and report here on the localization of the protective capacity of infected mouse serum in an immunoglobulin fraction normally associated with short-term PCA and mast cell sensitization in the mouse (Revoltella and Ovary, 1969; Binaghi, 1971).

MATERIALS AND METHODS

Parasite

The strain of *T. taeniaeformis* used in these experiments was derived from gravid segments obtained from Mr C. E. Claggett in the Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland. The parasite was maintained as described by Leid and Williams (1974a).

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Experimental animals

Female 28-day-old mice and rats were obtained from Spartan Research Animals, Haslett, Michigan.

Antisera

Twenty-eight-day-old mice were infected orally with 400 eggs of *T. taeniaeformis* and 28 days later were exsanguinated by severing the major thoracic vessels under CO_2 anaesthesia. Serum was stored at $-20°$.

Fractionation of antiserum

Antiserum was centrifuged at 10,000 *g* for 10 minutes and 4-ml portions were dialysed overnight against 0·1 M Tris–HCl buffer pH 8·0, and applied to a Sephadex G-200 column ($2{\cdot}5 \times 100$ cm), equilibrated against the same buffer. The ascending portion of the first peak was taken and this fraction contained γM and one β-globulin arc demonstrable in immunoelectrophoresis. The descending portion of the first peak, together with the 7S peak, were further fractionated on DEAE–cellulose. Stepwise elution was performed using sodium phosphate buffers in the following sequence: 0·005 M, pH 7·8; 0·01 M, pH 7·8; 0·05 M, pH 5·8; 0·1 M, pH 5·8; and finally 2 M NaCl. All buffers were made 0·015 M in NaCl. The pooled fractions under each peak (Fig. 1) were concentrated using polyethylene glycol and were tested against rabbit anti-whole mouse serum and anti-IgM and IgA (Meloy Laboratories, Springfield, Virginia) in both immunoelectrophoresis and double diffusion in gel tests, following the methods described by Leid and Williams (1974a).

Passive transfer

Fractions 1–5 from DEAE chromatography and the ascending portion of the first peak from gel filtration were restored to the original serum volume with phosphate-buffered saline. Each fraction was tested for its capacity to confer protection against a challenge of 300 eggs of *T. taeniaeformis* in mice. Mice were killed 21 days later and the results were analysed by a modified Student's *t*-test. This experimental procedure was repeated using two further batches of serum harvested in a similar manner from other groups of mice.

Passive cutaneous anaphylaxis (PCA)

All fractions from DEAE–cellulose chromatography were tested for their ability to provoke PCA in sensitized rats and mice following a modification of the procedure described by Revoltella and Ovary (1969). Positive samples were heated to 56° for 1 hour, or reduced and alkylated using the method of Nussenzweig, Merryman and Benacerraf (1964) and retested.

RESULTS

A typical DEAE–cellulose elution profile for 7S mouse immunoglobulins is shown in Fig. 1. Two peaks were consistently eluted with the 0·05 M phosphate buffer, pH 5·8, and these were separated and identified as F3 and F4. These two fractions contained no detectable γA or γM, but immunoelectrophoretic analysis using rabbit anti-whole mouse serum showed that they contained distinct populations of 7Sγ1 and 7Sγ2 immunoglobulins (Fig. 2).

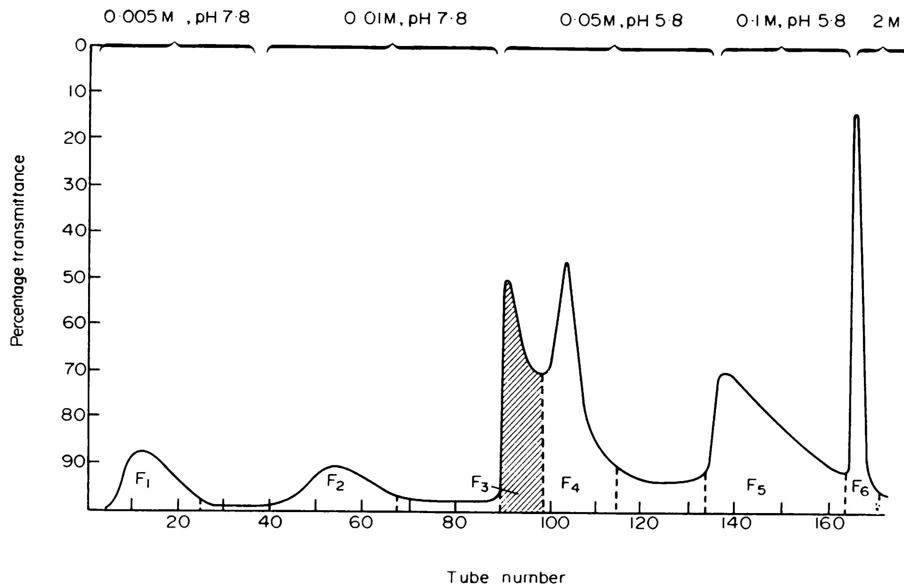

FIG. 1. DEAE–cellulose elution profile at 280 nm of 7S immunoglobulins from mice infected with *T. taeniaeformis*. Fractions F1–F5 were tested for protective capacity in passive transfer experiments and activity was localized in F3 (hatched area). PCA activity was restricted to F4.

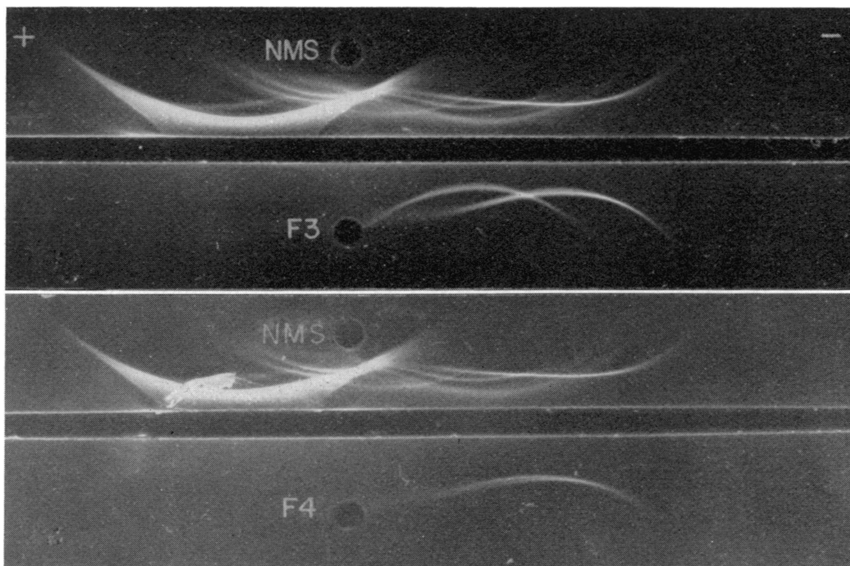

FIG. 2. Immunoelectrophoretic analysis of fractions of mouse 7S immunoglobulins eluted from DEAE–cellulose with 0·05 M phosphate buffer, pH 5·8. F3 and F4 were the first and second peaks, respectively, and the troughs were filled with rabbit anti-whole mouse serum.

The results obtained from a typical passive transfer experiment are shown in Table 1, where the average numbers of parasites developing in the livers in each group are recorded. Protective capacity was exclusively and consistently associated with F3, and there was no evidence of passive transfer with other fractions from DEAE chromatography or with the macroglobulin fraction from Sephadex G-200 gel filtration.

TABLE 1

PROTECTIVE CAPACITY OF ANTISERUM AND IMMUNOGLOBULIN FRACTIONS ISOLATED BY COLUMN CHROMATOGRAPHY IN RECIPIENT MICE CHALLENGED BY MOUTH WITH 300 EGGS OF *Taenia taeniaeformis*

Protein fraction transferred*	Number of mice	Mean number of larvae ± s.d.†	S.e. of mean	*P* value
Normal mouse serum	6	75·5 ± 19·3	7·9	
Immune mouse serum	6	0·5 ± 0·83	0·34	< 0·001
19S fraction of antiserum	6	76·0 ± 41·6	16·98	n.s.
F1-0·005 M DEAE–cellulose eluate	6	80·3 ± 46·78	19·1	n.s.
F2-0·01 M DEAE–cellulose eluate	6	68·8 ± 34·86	14·23	n.s.
F3-0·05 M (peak 1) DEAE–cellulose eluate	6	3·3 ± 4·5	1·83	< 0·001
F4-0·05 M (peak 2) DEAE–cellulose eluate	6	64·67 ± 30·5	12·45	n.s.
F5-0·1 M DEAE–cellulose eluate	6	74·0 ± 21·74	8·88	n.s.

n.s. = Not significant.

* By intraperitoneal injection.

† Average number of larvae developing in the livers of each group.

Fractions 1–6 were tested for the ability to produce both homologous and heterologous PCA reactions in mice and rats, respectively. Latent periods of 2 hours and 72 hours were allowed prior to challenge. PCA activity was detected only in F4 (second peak 0·05 M eluate). This serum activity was shown in homologous and heterologous systems after both 2 hours and 72 hours sensitization periods, but was destroyed by heating to 56° for 60 minutes and by reduction and alkylation with 2-mercaptoethanol and iodoacetamide.

DISCUSSION

In our experiments the distribution of protective activity against *T. taeniaeformis* in immune mouse serum appears to correspond most closely to that of the 7Sγ1 immunoglobulins. Although the protective fraction, F3, contained both 7Sγ1 and 7Sγ2 immunoglobulins, fractions F1 and F2, which eluted earlier from DEAE–cellulose, contained only slow-moving 7Sγ2 and showed no protective activity. It is possible, of course, that a physico-chemically distinct population of 7Sγ2 antibodies, which does not elute prior to the step involving 0·05 M phosphate buffer, was responsible for the transfer of immunity. However, the abrupt appearance of both 7Sγ1 and protective capacity in F3 is remarkably coincidental and is the basis for our tentative conclusion that antibodies of the 7Sγ1 type are most likely to be responsible for the passive resistance we observed. Further work will be required in order to consolidate this position.

Clearly, protective antibodies were not of the γA or γM type, nor had they demonstrable skin-sensitizing activity. The latter characteristic must be considered in relation to the recent findings of Revoltella and Ovary (1969). They were able to distinguish two skin-sensitizing antibodies in the sera of mice immunized against DNP, one of which was

detectable after a short latent period of 2 hours and the other at 72 hours. These two antibodies, 7Sγ1 and reagin, were shown to have very similar physico-chemical properties but were separated under conditions of anion-exchange chromatography similar to those used in our experiment. The PCA activity which we detected in F4 was heat-labile, sensitive to reduction and alkylation, and active in rat skin and therefore corresponds to the reagin of Revoltella and Ovary (1969) and other workers (Mota, Sadun and Gore, 1969; Bach and Brashler, 1973). The fact that PCA reactions could be provoked after only 2 hours of sensitization with reagin is in agreement with the observations of Stechschulte, Orange and Austen (1970).

Peculiarly, however, we were unable to demonstrate short-term homologous PCA reactions with F3 even though protective antibodies, probably 7Sγ1 in type, were present in this fraction. This situation is reminiscent of the observations of Leid and Williams (1974a) on 7Sγ2a antibodies in rat serum, and lends support to the suggestion that some populations of antigenically identifiable immunoglobulins have biological functions in helminth infections which are quite distinct from those described for antibodies showing antihapten activity. Nevertheless, the tentative association of protective activity with 7Sγ1 in the mouse and 7Sγ2a in the rat indicates that these two antibody types serve some analogous function, although it does not appear to involve the mast cell.

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