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Hymenolepis diminuta infections in congenitally athymic (nude) mice: worm kinetics and intestinal histopathology

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Summary. Congenitally athymic (*nu/nu*) mice and their thymus-bearing (+/*nu*) littermates were used to study the effect of a tapeworm, *Hymenolepis diminuta*, infection, particularly the worm kinetics and histopathology of the small intestine. Groups of *nu/nu* and +/*nu* mice were infected once with 5 cysticercoids and examined for 20 days post infection.

Worms were expelled both in *nu/nu* and in +/*nu* mice, albeit earlier in the latter animals. In both groups specific antibodies could be detected. The antibody titre was highest in the +/*nu* mice, which also formed more pyroninophilic, including plasma cells. The number of eosinophils increased significantly in the infected *nu/nu* mice, but not in the +/*nu* mice. A significant increase in mast cells and globule leucocyte formation was observed in the infected +/*nu* mice, but none of these cells were found in *nu/nu* mice which also expelled the worms. No changes in the villus/crypt ratio in the jejunum were observed. The mitotic index of the epithelial crypt cells in the jejunum increased in the infected *nu/nu* mice reaching a peak at day 16 post infection.

After re-infection *nu/nu* mice were not able to expel worms earlier than after primary infection. In passive immunization experiments with serum from both infected *nu/nu* and +/*nu* mice no conclusive evidence was obtained for a role of serum antibodies in host protection. It was concluded that host protection to the tapeworm, *H. diminuta* was dependent on the number of worms and worms could be expelled in the absence of functional T-cells. The hypothesis was put forward that the functional antigens are related to the scolex region and not to the total worm mass.

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INTRODUCTION

Hopkins, Subramanian & Stallard (1972a, b) demonstrated immunity in the normal mouse to infections with the tapeworm *Hymenolepis diminuta* resulting in destrobilation and expulsion of the worms. For intestinal nematodes a relationship between immune expulsion and intestinal histopathology has been described, e.g. for *Nippostrongylus brasiliensis* (reviewed by Ogilvie & Jones, 1971) and for *Trichinella spiralis* (reviewed by Larsh & Race, 1975). A relationship between cytological reactions and immunity has also been demonstrated in intestinal tapeworms but only in chickens infected with *Raillietina cesticillus* (Gray, 1973, 1976).

For this reason we studied the histological reactions in the gut of normal mice infected with *H. diminuta*. In order to obtain more information on the immune mechanism it was decided to do

concomitant tests in congenitally athymic (nude) mice. In the nude mouse T-cell dependent immunity is impaired (Rygaard, 1973). Therefore, if the immune reaction against parasites is T-cell dependent a different reaction could be expected in these mice. This assumption was later confirmed by Jacobson & Reed (1974b) for *N. brasiliensis* using a mouse adapted strain, and by Ruitenberg & Steerenberg (1974) for *T. spiralis*. In both infections the expulsion of adult worms was impaired in the nude mice in contrast to thymus-bearing littermates.

In the present work primary and secondary *H. diminuta* infections of thymus-bearing mice, heterozygous for the *nu* gene (+/*nu*) and nude (*nu*/*nu*) mice were studied using 5 cysticercoids as the infection dose, and the small intestine was examined during the primary infection for plasma cells, mast cells, globule leucocytes and eosinophils. Furthermore, the formation of antibodies was studied and their possible protective role examined by passive transfer of serum from infected +/*nu* and *nu*/*nu* mice to nude mice.

Preliminary results of this investigation have been presented as abstracts (Andreassen, Hindsbo & Ruitenberg, 1975, 1976).

MATERIALS AND METHODS

Mice

Male SPF B_{10}LP nude (*nu*/*nu*) mice, 6 weeks of age, were obtained from the Central Laboratory for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands, where the mice were maintained by continual back-crossing with strain B_{10}LP. Comparisons were made with age-matched mice, heterozygous for the nude gene (+/*nu*).

After an acclimatization period of 2 weeks, the animals were used for the experiments. Animals were maintained under conventional conditions as described by Hesselberg & Andreassen (1975).

Parasite and parasitological methods

The strain of *Hymenolepis diminuta* used, the maintenance and infections were as described by Hesselberg & Andreassen (1975).

Experimental design

Exp. 1. *Primary infection.* Thirty *nu*/*nu* and 30 +/*nu* mice 8-weeks-old were each infected with 5 cysticercoids of *H. diminuta* from beetles infected 15–18 days previously. Five mice of each group were killed at day 3, 7, 10, 13, 16 and 20 post infection, between 9.20 and 11.45 h. Non-infected control mice were killed at day 0.

Exp. 2. *Secondary infection.* Eleven *nu*/*nu* and 11 +/*nu* mice (8-weeks-old) were primarily infected with 5 cysticercoids of the same batch and on the same day as in Exp. 1. Each mouse was re-infected at day 21 with 5 cysticercoids from beetles infected 20–22 days previously and killed in groups of 5 and 6 animals at days 28 and 34, respectively, i.e. 7 and 13 days after re-infection. Uninfected control mice were killed at day 34. Autopsies were performed between 8.50 and 11.45 h.

Exp. 3. *Serum transfer.* Because an expulsion of worms was found during a primary infection of nude mice (Exp. 1) and it is known (Manning, Reed & Jutila, 1972) that T-independent humoral immunity occurs in nude mice, it was thought worthwhile trying passive immunization with antiserum from both infected +/*nu* and *nu*/*nu* mice of nude mice (see Preparation of serum).

Two experiments were set up:

Exp. 3a. In order to simulate a secondary infection, antiserum was administered at the same day as a primary infection with 10 cysticercoids from beetles infected 52–56 days previously. Autopsy was performed 7 days later just prior to the start of the expulsion of a 5 worms primary infection from the nude mice.

Exp. 3*b*. In order to study the effect of antiserum treatment on a primary infection of 2 worms, a number which is normally not expelled from nude mice (Andreassen, unpublishedres ults), antiserum was administered on day 7 post infection. The cysticercoids employed were obtained from beetles infected 42–49 days previously. Autopsy was again performed 7 days after treatment (14 days post infection). Experiments 3a and 3b were conducted according to the following plan:

Experiment	3a	3b	Treatment (1 ml by intraperitoneal route)
Group	1	6	0·85% NaCl
Group	2	7	Antiserum from infected +/*nu* mice
Group	3	8	Serum from uninfected +/*nu* mice
Group	4	9	Antiserum from infected *nu*/*nu* mice
Group	5	10	Serum from uninfected *nu*/*nu* mice

10 mice per group.

Autopsy and measurements

Exp. 1 *and* 2. The mice were killed by CO_2 and the peritoneal and thoracic cavity were opened. The intestine was removed and pinned at the anterior end to a long wax tray filled with 0·85% NaCl, stretched under a tension of 5 g using a wire spring and then fixed at the posterior end. The intestine was divided into sections by means of a marked rubber band which could be stretched to the individual lengths of the intestines. Five sections were used: (1) 0–17%; (2) 17–30%; (3) 30–43%; (4) 43–56%; and (5) 56–100% from the pylorus. Section 1 consisted mainly of duodenum, sections 2–4 of jejunum and section 5 of ileum. In Exp. 1 each of the sections 2–4, where most of the worms are found, was cut longitudinally and rolled up from the posterior end with the mucosa outwards on a wooden stick to form a so called Swiss-roll (Reilly & Kirsner, 1965) and then transferred to the various fixatives (see histological techniques; histology was only done in Exp. 1). Prior to preparing the Swiss-rolls of sections 2–4 worms visible to the naked eye were recovered. Caecum, colon and rectum were removed to examine the possible presence of other intestinal helminths. Sections 1 and 5, caecum, colon and rectum were placed in Petri-dishes containing Hanks's saline solution, they were shaken vigorously and the dish was examined for worms under a stereomicroscope (× 12 magnification). The number of worms per mouse was calculated from the actual number of scolices found except in one mouse where it was calculated on the basis of the worm tails.

All experimental animals were free of cestodes other than *H. diminuta* at autopsy, but one pinworm was found in one of the infected nude mice on day 13, 16, 28 and 34 post infection. Two, 4 and 13 pinworms were found in 3 control nude mice killed on day 34. The few pinworms found were thought not to influence the results presented.

Exp. 3a. The mice were killed by ether and the small intestine removed and handled as in Exp. 1 and 2 except that it was divided in to 8 sections: 0–5, 5–10, 10–20, 20–30, 30–40, 40–50, 50–75 and 75–100% from the pylorus. The intestinal sections were processed as the sections 1 and 5 in Exp. 1 and 2. The total length and dry weight of the worms from each mouse were measured as described by Hesselberg & Andreassen (1975).

Exp. 3b. The mice were killed by ether and the small intestine removed and handled as in Exp. 1 and 2 except that the intestine was slit open throughout its length and the scolex position of each worm was marked with a pin and the distance from pylorus measured. In the mice where both worms were not found the whole intestine was processed as the sections 1 and 5 in Exp. 1 and 2. The individual worm length and dry weight were measured as described by Hesselberg & Andreassen (1975).

Histological techniques

Conventional techniques. For general morphology tissues (section 4) were fixed in buffered formalin diluted 1 : 10, paraplast sections were stained with haematoxylin and eosin (H & E). For specific staining of pyroninophilic (i.e. immunocompetent) cells, including plasma cells, tissues (section 2) were fixed in 90 parts Zenker's fluid, 5 parts commercial formalin (=40% formaldehyde) and 5 parts 2% trichloroacetic acid (ZFT). Paraplast sections were stained with methyl green-pyronine (M.P.). For specific staining of intestinal mast cells and globule leucocytes tissues (section 3) were fixed in a fixative containing 0·8% formalin and 4% acetic acid and were stained with toluidine blue (Ruitenberg & Elgersma, 1976). For specific staining of eosinophils formalin-fixed, paraplast-embedded sections were stained with Giemsa.

Evaluation of the histological response. For the examination of possible histopathological changes in the morphology of the mucosa of the jejunum two criteria were used:

(1) Villus/crypt ratio by measuring the length of 10 villus/crypt units per animal by means of a Visopan (Zeiss).

(2) Mitotic index in the crypts of Lieberkühn by counting a total of 200–300 nuclei in approximately 10 crypts per animal. The number of mitoses was expressed per 100 epithelial crypt cells.

The numbers of pyroninophilic cells, mast cells, globule leucocytes and eosinophils were counted per 20 villus/crypt units and expressed per villus/crypt unit. A villus/crypt unit represents a portion of gut mucosa, lying between two gland crypts and the lamina propria of the villus above (Jarrett, Jarrett, Miller & Urquhart, 1968).

Serological techniques

Sera were tested for the presence of specific anti-

bodies to *H. diminuta* with indirect immunofluorescence (IF). For this purpose *H. diminuta* cysticercoids were collected from beetles in general infected 2–3 weeks previously and washed in phosphate buffered saline (PBS) (pH 7.2; 0·01 M).

The cysticercoids were then placed on glass slides coated with egg albumin. By immersing the slides in liquid nitrogen (temperature −190°) the cysticercoids were quick-frozen (Ruitenberg & van der Sleen, 1972). Slides were stored at −20°. Upon use the slides were fixed in acetone at −20° for 10 min. After drying the slides at 37° for 30 min, they were subjected to the usual staining procedure (Ruitenberg, Kampelmacher & Berkvens, 1968). Serum samples were examined in two-fold serial dilutions in PBS. Sera from uninfected mice and PBS were used as controls. Fluorescein-conjugated rabbit-anti-mouse immunoglobulin (Nordic Pharmaceuticals and Diagnostics, Tilburg, The Netherlands) was used as conjugate. The conjugate did not react with cysticercoids of *H. diminuta*. Only fluorescence of the surface of the cysticercoid was regarded as specific. The highest serum dilution giving definite fluorescence was taken as the end-point titre.

Preparation of serum for passive immunization

Fifty +/*nu* and 50 *nu*/*nu* mice were infected with 10 *H. diminuta* each at days 0 and 14 and killed at day 28 by CO_2. After opening the thoracic cavity blood was collected from the heart, allowed to clot for 1–2 h at room temperature and overnight at 4° before centrifugation. Pooled serum from each group was stored 10 weeks at −20°. Normal serum from uninfected +/*nu* and *nu*/*nu* mice was stored lyophilized until use.

Statistical methods

The statistical test used was the Wilcoxon rank sum test both for the parasitological data and the cell counts (Remington & Schork, 1970).

RESULTS

Parasitological data

Primary infections. The number of mice infected and the percentage recovery of worms in Exp. 1 are shown in Table 1, from which it can be seen that nude mice also expel their worms, but more slowly than normal mice. At day 10 all nude mice were 100% infected while on day 20 no worms were found. The three infected nude mice at day 13 harboured 5, 4 and 5 worms and the two infected nude mice at day 16 had 5 and 4 worms respectively, indicating that when the worms are expelled from the individual mouse they are all expelled at about the same time. Destrobilated worms were not found in the sections of the small intestine examined (1 and 5). From Table 2 it can be seen that the worms are migrating anteriorly in the small intestine of the nude mice from day 7 to day 10 but posteriorly during expulsion after day 10, as may be judged from the high recovery in the duodenum at day 10 and in the ileum at days 13 and 16.

Secondary infections. Due to the removal of worm-containing sections the recovery of worms on day 7

Table 1. Primary infection of *Hymenolepis diminuta* in mice

Days post infection	No. +/*nu* + *nu*/*nu* mice infected with 5 cysticercoids day 0	No. +/*nu* mice infected	No. *nu*/*nu* mice infected	% Recovery of worms from +/*nu* mice	% Recovery of worms from *nu*/*nu* mice
7*	5+5	4 or 5	4 or 5	>48	>52
10	5+5	0	5	0	100
13	5+5	0	3	0	56
16	5+5	0	2	0	36
20	5+5	0	0	0	0

* Because of the small size of the worms and the technique used some worms were not found, so the data from day 7 are minimum values.

Table 2. Primary infection of *nu/nu* mice with 5 *Hymenolepis diminuta*. Mean percentage recovery of worms per mouse in sections of the small intestine, showing the migration of the worms during the infection

Days post infection	% from pylorus		
	0–17 Duodenum	17–56 Jejunum	56–100 Ileum
7	4	>48*	0
10	80	12	8
13	12	8	36
16	12	4	20
20	0	0	0

* The figure is a minimum because of the small size of the worms and the technique used.

could not be evaluated. On day 13 after the secondary infection in +/*nu* mice no worms were found. Three of 6 nude mice with a secondary infection harboured on day 13 post infection a total of 40% of the administered larvae. Neither the recovery nor the position of the worms were different from those observed in the primary infected nude mice at day 13 post infection (Table 1).

Histological data

During the *H. diminuta* infection no significant changes in villus/crypt ratio or in the mitotic index were observed in the +/*nu* or in the *nu/nu* mice.

The mean number ±s.d. of pyroninophilic cells per villus/crypt unit in non-infected *nu/nu* mice was much lower than that in non-infected +/*nu* mice (Table 3). After a single infection with 5 cysticercoids in *nu/nu* mice a sudden and significant increase in number of pyroninophilic cells was observed at day 16 post infection ($P<0.01$), whereas in +/*nu* mice a gradual increase in number of pyroninophilic cells was observed being significant from day 7 post infection (except day 10). Although no reliable distinction between various pyroninophilic cells can be made with the M.P. staining method, the majority of the pyroninophilic cells showed the morphological characteristics of plasma cells.

After a single infection with 5 cysticercoids in *nu/nu* mice a gradual increase in number of eosinophils was observed during the observation period (Table 4) with the following P values vs day 0 (day 10 and 13, $P<0.05$; day 16, $P<0.01$). In the +/*nu* mice the eosinophil number varied between a minimum on day 3 and a maximum on day 10 with no significant difference between individual days.

The data presented in Table 5 indicate that intestinal mast cells and globule leucocytes were

Table 3. Effect of *H. diminuta* infection (5 cysticercoids) on numbers of pyroninophilic cells in the jejunum of male *nu/nu* and +/*nu* mice at various days post infection

Day	Pyroninophilic cells*	
	nu/nu	+/*nu*
0	3·5 ± 1·1	17·6 ± 2·3
3	3·8 ± 1·4	18·3 ± 3·4
7	5·2 ± 1·5	20·9 ± 1·3†
10	3·7 ± 2·2	21·6 ± 3·0
13	5·1 ± 1·3	23·4 ± 3·8†
16	10·5 ± 2·1‡	23·4 ± 5·8†
20	3·5 ± 2·0	23·0 ± 4·8†

* Mean number of cells (±s.d.) of 20 villus/crypt units per animal; 5 animals per day (expressed per villus/crypt unit).
† Wilcoxon test: day post infection vs day 0. $P<0.05$.
‡ Wilcoxon test: day post infection vs day 0. $P<0.01$.

Table 4. Effect of *H. diminuta* infection (5 cysticercoids) on numbers of eosinophilic cells in the jejunum of male *nu/nu* and +/*nu* mice at various days post infection

Day	Eosinophilic cells*	
	nu/nu	+/*nu*
0	2·0 ± 1·2	5·0 ± 2·2
3	3·0 ± 1·9	3·6 ± 0·5
7	3·7 ± 1·0	4·8 ± 2·3
10	4·5 ± 1·4†	7·4 ± 3·4
13	5·4 ± 2·5†	6·7 ± 1·9
16	5·0 ± 0·8‡	4·7 ± 0·9
20	6·1 ± 3·8	3·7 ± 1·0

* Mean number of cells (±s.d.) of 20 villus/crypt units per animal; 5 animals per day (expressed per villus/crypt unit).
† Wilcoxon test: day post infection vs day 0. $P<0.05$.
‡ Wilcoxon test: day post infection vs day 0. $P<0.01$.

Table 5. Effect of a *H. diminuta* infection (5 cysticercoids) on numbers of intestinal mast cells and globule leucocytes in the jejunum of male *nu/nu* and +/*nu* mice at various days post infection

	nu/nu		+/*nu*	
Day	mast cells*	glob. leuc.*	mast cells*	glob. leuc.*
0	0	0	0	0
3	0	0	0·4 ± 0·9	0
7	0	0	4·4 ± 5·0	5·8 ± 3·1†
10	0	0	11·8 ± 6·9†	96·2 ± 31·6†
13	0	0	6·6 ± 4·0†	73·2 ± 33·2†
16	0	0	3·4 ± 1·7†	34·8 ± 20·8†
20	0	0	3·4 ± 1·7†	20·2 ± 14·3†

* Mean number of cells (±s.d.) of 20 villus/crypt units per animal; 5 animals per day (expressed per villus/crypt unit).

† Wilcoxon test: day post infection vs day 0. $P < 0{\cdot}01$.

absent from the non-infected *nu/nu* and +/*nu* mice. In a previous study (Ruitenberg & Elgersma, 1976) few mast cells were found in non-infected +/*nu* mice and negligible numbers in *nu/nu* mice. However, in that study 300 villus/crypt units were examined versus 20 in the present study. After a primary infection, mast cells were observed in the infected +/*nu* mice from day 3 onwards (peak at day 10 post infection), whereas no mast cells were found in the infected *nu/nu* mice during the experimental period. Globule leucocytes were observed in the infected +/*nu* mice from day 7 post infection onwards. In the infected *nu/nu* mice no globule leucocytes were seen.

Serological data

IF titres were determined in pools of sera of 5 animals per day post infection. In *nu/nu* mice only very low titres (1 : 2) were observed on days 7, 13 and 16. In +/*nu* mice, higher titres were observed (>1 : 8 at day 13 and 1 : 4 on day 16 and 20). The sera from non-infected animals yielded negative results, indicating that specific antibodies to *H. diminuta* were detected in the infected animals. From these observations it may be concluded that nude mice can produce antibodies to *H. diminuta* albeit to a lower extent than +/*nu* mice. This suggests that *H. diminuta* antigens induce both T-dependent and T-independent immune responses in the mouse.

In the sera from animals infected twice (Exp. 2) IF-titres of the same order were found only in the +/*nu* mice.

The sera from secondary infected mice used for passive immunization showed no IF titre in the *nu/nu* mice and a 1 : 2 titre in the +/*nu* mice, which in both groups were lower than from primarily infected mice.

Serum transfer experiments

In Exp. 3a (the 10-worm infection; treatment on day of infection) the mean percentage recovery in the 5 groups ranged from 89 to 96%, the mean position of the worms from 20·3 to 24·6% from the pylorus, the mean worm length per mouse from 36 to 47 mm and the mean worm dry weight per mouse from 0·25 to 0·40 mg. No significant difference was seen between the groups.

In Exp. 3b (the 2-worm infection; treatment on day 7 post infection) the mean percentage recovery in the 5 groups ranged from 95 to 100% and the mean position of the worms from 9·1 to 17·6% from the pylorus without any significant difference between the groups. The mean worm length per mouse was significantly larger ($P < 0{\cdot}01$) in the saline treated group (no. 6) (445 ± 118 mm) than in group 7, treated with serum from previously infected +/*nu* mice (268 ± 109 mm). However, since group 8, treated with normal serum from uninfected +/*nu* mice had a mean of 309 ± 114 mm which is not significantly different ($P > 0{\cdot}10$) from group 7 the first significance is invalidated in respect of specific antibody effect. This emphasizes the importance of normal serum controls in this type of experiment. Those groups (10 and 9) treated with normal and immune sera from *nu/nu* mice had a mean worm length per mouse of 346 ± 186 mm and 383 ± 106 mm respectively which were not significantly different ($P > 0{\cdot}10$) from group 6 (the saline-treated group). The figures of the mean worm dry weight per mouse were: group 6: 23·38 ± 7·14, group 7: 9·27 ± 5·18, group 8: 10·73 ± 6·99, group 9: 15·59 ± 14·27 and group 10: 15·20 ± 8·62 mg showing again that resistance to *H. diminuta* could not be transferred passively by immune serum.

DISCUSSION

An important finding described in the present paper

is the expulsion of the tapeworm *H. diminuta* from the intestine of nude mice. This is in contrast to the results described by Jacobson & Reed (1974a & b) and Ruitenberg & Steerenberg (1974) that nude mice were unable to expel intestinal nematodes. Since this finding was in contrast to what was expected, nude mice from the same commercial source were tested for the possible presence of functional T-cells by performing phytohaemagglutinin (PHA) stimulation and skin transplantation studies. The data obtained were compatible with the concept that the nude mice used in the present study did indeed not possess functional T-cells (Kreeftenberg & Ruitenberg, unpublished results). Furthermore, the nude mice were able to mount a humoral immune response to the T-independent antigen pneumococcal polysaccharide type III (S III) (Ruitenberg & Buys, unpublished results). This is consistent with published evidence (Manning *et al.*, 1972).

Finally, lymphocytes from spleens of nude mice collected at day 16 after infection with 5 cysticercoids of *H. diminuta* did not show a PHA, concanavalin A or lipopolysaccharide responsiveness different from that of spleen cells from non-infected nudes of the same age (Kreeftenberg, unpublished results).

The mean survival time—defined by Befus (1975), as the first day on which $\geq 50\%$ of the worms administered to a group of mice had been destrobilated or lost—was in this experiment between 14 and 16 days in the nude mice, but 7 to 10 days in the $+/nu$ mice. The mean survival time for *H. diminuta* in mice has been shown to be host strain-dependent ranging from 9 to 16 days (Hopkins *et al.*, 1972a; Befus, 1975), host age-dependent, i.e. shorter survival time in older mice (Befus & Featherston, 1974) and also dependent on the infection dose, the higher the dose the shorter the mean survival time (Befus, 1975). However, expulsion of *H. diminuta* from nude mice does not occur at all infection doses.

Bland (1976) showed that nude mice infected with one *H. diminuta* were not able to expel this worm—at least up to day 33—this unresponsiveness being similar to that in adult thymectomized irradiated mice. This is in agreement with our own studies (Andreassen, Hindsbo & Vineberg, unpublished results), since we observed that 1 or 2 worms are not expelled from nude mice—at least until day 136 post infection—reaching maturity and having normal apolysis from day 18. Furthermore, we found that in infections, with 10 and 20 worms destrobilation and expulsion had started already at day 10 and in some cases as early as day 8 in nude mice.

Whether the expulsion of the primary infection of 5 *H. diminuta* in nude mice is an immunological response is not shown in the present investigation, it is however rendered probable by the suppression of the expulsion by cortisone treatment (Andreassen, Hindsbo & Vienberg, unpublished results). Transplantation of thymus glands or thymus cells to nude mice (Isaak, Jacobson & Reed, 1975) results in the expulsion of a 3-worm infection as in thymus-bearing littermates showing that expulsion of *H. diminuta* from mice is enhanced by thymus competence, supporting the concept that expulsion has an immunological basis (Hopkins *et al.*, 1972b).

Hopkins *et al.* (1972a) and Befus (1975), using normal mice, found a host-mediated secondary response. In our secondary infected nude mice no lower recovery was recorded. More extensive experiments are needed, however, to show if an anamnestic response on recovery and/or growth of *H. diminuta* in nude mice can occur in other circumstances than those of the present study.

In our nude mice a distinct forward migration was observed in 5-worm infections (Table 2) and the worms were expelled later than in the $+/nu$ mice. In normal mice infected with one worm a similar anterior migration was observed by Hopkins *et al.* (1972a), Befus & Featherston (1974) and Befus (1975) but in a 6-worm infection, which was rejected more quickly, no forward migration was seen (Befus, 1975). Unpublished results by Andreassen, Hindsbo & Vienberg showed no forward migration in nude mice infected with 20 *H. diminuta*, which were destrobilated and expelled more quickly than a 5-worm infection. These observations taken together point to a possible inverse relationship between forward migration and time of expulsion, so that forward migration only occurs in mice with none or with a relatively slow expulsion, but not in mice with a quicker expulsion.

From the histological studies in the intestinal tract the lack of changes in villus/crypt ratio and in the mitotic index suggests that *H. diminuta* does not exert a significant pathogenic effect on the mouse intestine.

Specific antibodies are produced in both infected $+/nu$ and nu/nu mice as shown by the IF titres and supported by the production of pyroninophilic cells, including plasma cells. This indicates that antigenic

information was passed to the immune system in spite of the absence of light microscopically visible structural changes in the epithelial lining. Whether or not antibodies to *H. diminuta*, which are also produced in rats (Harris & Turton, 1973) and man (Turton, Williamson & Harris, 1975), play any role in the expulsion is not known, and our negative results from the passive transfer experiments do not exclude such a possibility.

In the present study a statistically significant increase in tissue eosinophils was observed in the infected *nu/nu* but not in the infected +/*nu* mice. This is a surprising finding since in nematode infection, tissue and blood eosinophilia have been described as thymus-dependent phenomena (Basten & Beeson, 1970; Ruitenberg, Elgersma, Kruizinga & Leenstra, in press). At present we have no explanation for this finding. Other authors published conflicting results with regard to tissue or blood eosinophilia in cestode infections. Gray (1973, 1976) did not find an increase in eosinophils in chickens primarily infected with *R. cesticillus*. Turton *et al.* (1975) described blood eosinophilia in man during a *H. diminuta* infection indicating that this tapeworm infection can induce an eosinophilic response.

The number of intestinal mast cells and globule leucocytes increased during the infection in +/*nu* mice but not in *nu/nu* mice. This is in agreement with other studies in which the effect of *T. spiralis* on the production of mast cells and globule leucocytes was examined (Ruitenberg & Elgersma, 1976). The most probable explanation is that in the formation of intestinal mast cells, the thymus plays an essential role. The findings in the +/*nu* mice are in agreement with studies in nematode infections where immune expulsion and degranulation of mast cells in one way or another are connected events (Miller, 1971; Ruitenberg, Teppema, Kruizinga & Elgersma, 1975), but it is in contrast to the studies of Gray (1973, 1976) who found an increase in mast cell number but not in globule leucocytes during the expulsion of an infection with *R. cesticillus*, a cestode in chickens. In this investigation it is, however shown, that the expulsion of *H. diminuta* can occur in nude mice without contribution of mast cells.

The protective immunity to *H. diminuta* in the mouse seems to be dose-dependent, since in normal mice the expulsion of a 6-worm infection occurs earlier than a 1-worm infection (Befus, 1975) and in *nu/nu* mice a 5-worm infection is expelled, although later than in the +/*nu*, whereas a 2-worm infection is not expelled. If the nude mice used indeed lack T-cell activity, as suggested by the previously mentioned results by Kreeftenberg (unpublished), the host response can be divided into a thymus-dependent and a thymus-independent one, if not the response can be explained on T-cell activity alone.

In a study on *Hymenolepis citelli* infections in mice Hopkins & Stallard (1974) suggested the worm biomass or more likely the total worm surface area constitutes the immune threshold above which the rejection response is initiated.

Since in *nu/nu* mice 5 worms are expelled at a stage when the total worm biomass is lower than the total worm biomass reached by a 2-worm infection which is not terminated (Andreassen, Hindsbo & Vienberg, unpublished results), we want to put forward the hypothesis that the functional antigens are related to the scolex region and not to the total worm biomass or surface area, i.e. the more scolices the more antigen and the earlier initiation of the host rejection response.

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