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Changes in Serum Protein in Mice Infected with *Hymenolepis nana* Eggs

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ABSTRACT. We investigated the changes in serum protein contents of male ddY mice following primary and secondary infections with *Hymenolepis nana* eggs. There was a marked decrease in the total serum protein contents throughout the course of both primary and secondary infections. The reduction of total serum protein contents occurred as early as 1 day after primary and secondary infections and was particularly prominent after secondary infection. A quiet similar pattern of reduction was observed in the levels of serum albumin. The total globulin content increased immediately after primary infection, decreased to nearly the normal level at day 4, and again increased to a peak at day 7 after infection. In secondary infection, elevated total globulin contents decreased to lower than the normal level at day 2 and quickly increased thereafter. The alpha- and beta-globulin contents fluctuated after primary and secondary infections showing a pattern similar to that of total globulin contents. The levels of gamma-globulin increased with moderate fluctuation and reached a maximum level on day 7, showing a 13-fold increase in primary infection and 23-fold increase in secondary infection. The results clearly showed that the changes in the serum protein contents early in the infection process were important to understand the pathogenicity of *H. nana*.

Key words : electrophoresis — *Hymenolepis nana* — serum protein — hypergammaglobulinemia — hypoalbuminemia

It is well known that chronic infections of parasitic helminths cause a wide variety of clinico-pathological changes in man and animals. We have studied hematological and clinico-chemical changes of mice following primary and secondary infections with the dwarf tapeworm, *Hymenolepis nana*.[1,2] *H. nana* is a widely distributed and clinically important cestode parasite of man and murines and differs from almost all of the other cestodes in being able to complete its entire life cycle in a single host. The host is therefore consecutively exposed to the tissue invasive and migrating oncosphere larvae, developing cysticercoids which sequester only 4 days in the intestinal tissue, and adult tapeworms which live in the intestinal lumen. In addition, by virtue of its strong immunogenicity, repeated infections of *H. nana* give rise to a variety of immunological reactions and inflammation during the early phase of reinfection in mice.[3-9] It is likely, therefore, that the clinico-pathological syndromes

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at the early phase of primary and secondary infections may be of particular importance in understanding the pathogenicity of *H. nana*.

Changes in serum proteins of mice and rats infected with *H. nana* have been repeated by Noda,[9] Katiyar *et al.*[10] and Bhopale *et al.*[11] We have suggested that hypoproteinemia with prominent hypoalbuminemia may be an important feature of *H. nana* infection in mice.[2] The present study provides more detailed observations on the alteration of serum proteins with a special attention to the chronological changes during 14 days after primary and secondary infections. We used cellulose polyacetate membrane electrophoresis for fractionation of serum proteins, which is known to be a reliable method for monitoring changes in serum proteins during various pathologic processes.[12]

MATERIALS AND METHODS

Host animals and parasites : Healthy male mice of the ddY strain were used. They were 6 weeks old and weighed 29–35g at the beginning of the experiment. Weekly fecal examination by the egg floatation method revealed no accidental infection with *H. nana* in all mice. The mice were housed in plastic cages in groups of 10 and were given laboratory chow and tap water *ad libitum*. The maintenance of *H. nana* in ddY mice, collection of mature eggs, removal of eggshells before infection and method of oral infection have been described previously.[1]

Experimental design : Three groups of mice, the primary infection group, secondary infection group and control (uninfected) group, were examined. Fifty mice in the primary infection group were infected orally with 1000 eggs. Ten mice each were killed 1, 2, 4, 7 and 14 days after infection, and their blood samples were used for the determination of serum protein contents. Ten mice killed 4 days after infection were examined for cysticercoid infection by the method of Hunninen.[13] They had an average of 128 intestinal cysticercoids (range 8–341). The secondary infection group comprised 50 mice. They were initially exposed to 1000 eggs and were challenged with 2000 eggs 14 days after the primary infection. Blood samples were collected 1, 2, 4, 7 and 14 days after the secondary infection. The control group consisted of 10 mice which were not infected; they were killed between 6 and 8 weeks of birth. All mice were fasted for 12 hours prior to blood collection. Blood was collected from the postcaval vein of mice under light anesthesia with an intraperitoneal injection of sodium pentobarbital (25.6 mg/kg), and was transferred to a test tube. Blood samples were left to clot at room temperature for 2–3 hours. The sera were stored at −35°C until analysis. Blood collection was routinely carried out between 8 and 11 A.M. to minimize variations due to the circadian rhythm of the animals.

Determination of total serum protein contents : The total serum protein was determined by the biuret method using commercially available kits (Wako Pure Chem. Co., Ltd.). All the reagents for electrophoresis were purchased from Wako Pure Chemicals. The serum from each mouse was fractionated by electrophoresis on cellulose polyacetate membrane utilizing a twenty-sample applicator (Jookoo. Co., Ltd.) at 0.8 mA per 1 cm/membrane for 40 minutes in 0.06M sodium veronal buffer, pH 8.6. After drying, the strips were stained

with 0.5% Ponceau 3R for the detection of protein zones. The protein patterns were examined by a scanning densitometer (Jookoo. Co., Ltd.) after clearing the cellulose polyacetate membranes in decahydronaphthalene. Cellulose polyacetate electrophoresis could separate albumin, alpha-1-, alpha-2-, beta- and gamma-globulins. The absolute values of albumin, alpha-1-, alpha-2-, beta- and gamma-globulins were calculated from the percentage of each protein fraction. The total globulin content was determined by subtracting the value of albumin from that of total serum protein. The statistical significance of differences between control and experimental groups was examined by Student's *t*-test.

RESULTS

The absolute values of fractionated serum proteins are shown in Figs. 1 and 2, and the relative values are presented in Table 1.

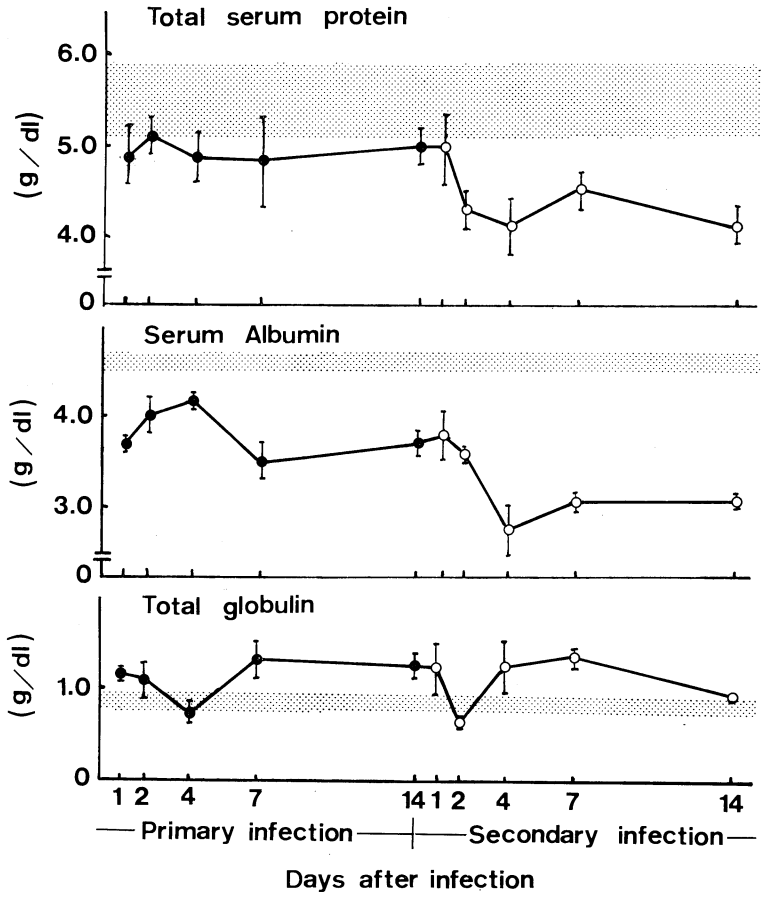

Fig. 1. Changes in total serum protein, serum albumin and total globulin in male ddY mice infected with *H. nana* eggs. (mean ± S. D) Dotted areas show normal ranges.

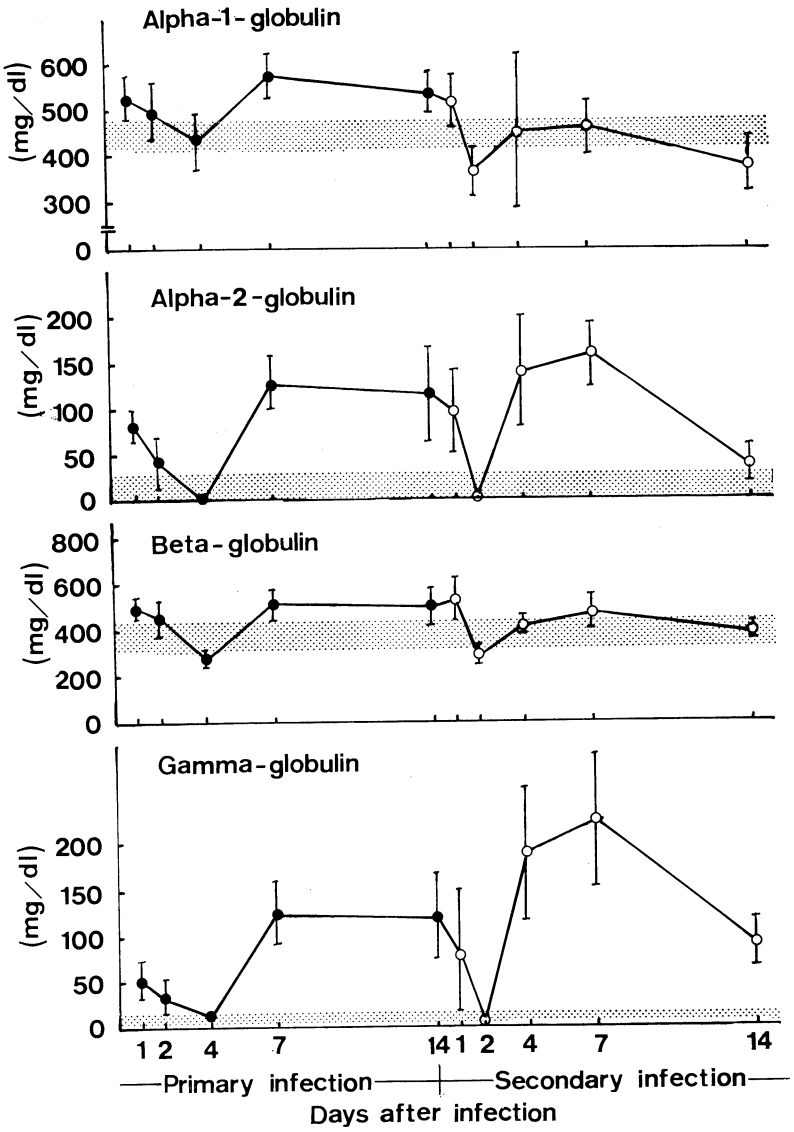

Fig. 2. Changes in serum protein components in male ddY mice infected with *H. nana* eggs. (mean ± S. D) Dotted areas show normal ranges.

There was a marked decrease in the total serum protein contents throughout the course of both primary and secondary infections. The reduction of total serum protein contents occurred as early as 1 day after primary and secondary infections and was particularly prominent after secondary infection. A quiet similar pattern of reduction was observed in the levels of serum albumin. The absolute values of serum albumin contents at day 14 of infection were only 3.2–3.7 g/dl in the primary infection group and 2.9 g/dl in the secondary infection group.

The total globulin content increased immediately after primary infection, declined to nearly the normal level at day 4, and again increased to a peak

TABLE 1. Percentages of serum protein components of male ddY mice infected with *H. nana* eggs. (mean ± S.D)

	Days after infection	Percentage of serum protein components						A/G ratio
		albumin	globulin					
			total	alpha-1	alpha-2	beta	gamma	
Uninfected control		84.3 ± 2.1	15.7 ± 2.1	8.0 ± 0.6	0.2 ± 0.3	6.7 ± 1.1	0.2 ± 0.1	5.51 ±0.84
Primary infection	1	76.8 ± 1.8	23.2 ± 1.8	10.6 ± 1.0	1.7 ± 0.3	10.0 ± 0.9	1.0 ± 0.4	3.34 ±0.34
	2	79.4 ± 4.0	20.6 ± 4.0	9.7 ± 1.2	0.7 ± 0.6	9.0 ± 1.6	0.5 ± 0.4	4.05 ±0.98
	4	85.2* ± 1.9	14.8* ± 1.9	8.8 ± 1.2	0	5.8* ± 0.8	0.2* ± 0.1	5.90* ±0.91
	7	71.9 ± 4.1	28.1 ± 4.1	11.8 ± 1.0	2.6 ± 0.6	10.7 ± 1.4	2.6 ± 0.7	2.65 ±0.65
	14	74.7 ± 3.0	25.3 ± 3.0	10.8 ± 0.9	2.3 ± 1.0	9.9 ± 1.7	2.4 ± 0.9	3.00 ±0.69
Secondary infection	1	75.4 ± 5.6	24.6 ± 5.6	10.4 ± 1.1	1.9 ± 0.9	10.7 ± 1.9	1.6 ± 1.3	3.03 ±0.69
	2	84.4* ± 1.9	15.6* ± 1.9	8.5 ± 1.2	0	6.7* ± 0.9	0.1* ± 0.1	5.32* ±0.59
	4	69.2 ± 7.1	30.8 ± 7.1	11.1 ± 4.1	3.4 ± 1.5	10.4 ± 1.0	4.7 ± 1.8	2.22 ±0.56
	7	69.1 ± 2.4	30.9 ± 2.4	10.3 ± 1.2	3.5 ± 0.8	10.6 ± 1.6	5.0 ± 1.6	2.21 ±0.22
	14	76.8 ± 1.6	23.2 ± 1.6	9.2 ± 1.5	0.8 ± 0.5	9.5 ± 0.8	2.3 ± 0.7	3.34 ±0.30

* Differences between the values of control and experimental groups are not statistically significant.

7 days after infection. During secondary infection, elevated total globulin contents decreased to lower than the normal level at day 2 and quickly increased thereafter. The alpha- and beta-globulin contents fluctuated after primary and secondary infections showing a pattern similar to that of total globulin contents. Changes in the levels of these serum proteins were minimum, although differences between control and experimental values were statistically significant at day 7 and 14 of infection. The concentration of gamma-globulin in normal ddY mice was 0.01±0.005 g/dl. After infection, the levels of gamma-globulin increased with moderate fluctuation and reached a maximum level on day 7, showing a 13-fold increase in primary infection and 23-fold increase in secondary infection.

DISCUSSION

We have reported previously that infections by *H. nana* eggs affect the host animals in many ways and cause a variety of clinico-pathological changes. It appears that malnutrition or malabsorption due probably to the histological damage to the small intestine and the nutritional requirements of the worms are related to the occurrence of slight anemia and changes in serum protein, serum albumin, blood urea nitrogen and other clinico-chemical parameters in

mice.[1,2] Infections by *H. nana* also responsible for prominent leukocytosis with secondary peripheral eosinophilia.[1]

The present studies confirmed the changes in serum proteins of mice during early phases of primary and secondary infections with *H. nana* eggs. The most remarkable changes in serum proteins were the decrease in albumin and increase in total globulins. These results are consistent with the reports of Noda,[9] Katiyar *et al.*[10] and Bhopale *et al.*[11] In addition, we confirmed that the alterations in these biochemical parameters at very early phase of infection is important to understand the pathogenicity of *H. nana*, since most of the changes are initiated as early as 1 or 2 days after infection.

Although hypoproteinemia and hypoalbuminemia were observed in both primary and secondary infections during the course of the experiment, the decrease in concentrations of both total serum protein and albumin were more prominent after secondary infection, suggesting that repeated infection by *H. nana* is important in giving rise to a more advanced state of illness. Supporting evidence was reported by Friedberg *et al.*[6], Miyazato *et al.*[7] and Furukawa *et al.*[8], who showed that the upper small intestine of mice was severely damaged after secondary infection. Kramer *et al.*[14] reported that the excretion of albumin from intestinal mucosa in rats began as early as 1 minute after *H. nana* infection. It is also possible that a competitive nutritional requirement of the worms, especially that of adult worms that persist in the intestinal lumen for a long period of time, may be responsible for the prolonged hypoalbuminemia.

Alpha-1- and alpha-2-globulins showed a little fluctuation after primary and secondary infections. Seward *et al.*[15] reported that the decrease in the serum protein content was accompanied with a rise in the alpha-2-globulin content in man. However, these changes were minimum in the present study, and we consider them to have no physiological significance.

Katiyar *et al.*[10] reported a temporary rise in the beta-fraction in rats infected with *H. nana*. They suggested that the increase in the beta-fraction may be caused by toxic metabolic products of the developing parasite. This hypothesis has never been given further confirmation. In our experiment, the beta-fraction of the serum protein did not change during the course of the experiment.

The contents of gamma-globulin attained maximum values on day 7 of both primary and secondary infections, showing 13- to 23-fold increases above the control value, respectively. Hypergammaglobulinemia has been reported to occur in various larval cestode infections.[16–19] Our results also indicate that hypergammaglobulinemia is a feature of *H. nana* infection in mice. This phenomenon is apparently related to the rapid onset of acquired resistance of mice to *H. nana*. The immune mechanisms operative in *H. nana* infection is not fully understood, but it has been shown that gamma-globulin in infected mice contains antibodies[9] which have a protective action.[20]

The very low level of a gamma-globulin in normal ddY mice seems to be interesting and may be a subject of further study. For instance, C57BL/6J and Charles River CD-1 strains of mice are known to have 0.40 ± 0.10 and 0.37 ± 0.013 g/dl gamma-globulin in the sera,[21,22] while normal ddY mice used in the present study had only 0.01 ± 0.015 g/dl gamma-globulin.

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