

Leucocyte Variation, an Insight of Host Defenses During Hymenolepiasis and Restoration with Praziquantel

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Parvathi and Karemungikar: Leucocyte Variation and Host Defenses in Hymenolepiasis

Haematological studies in helminthiasis reveal drastic alterations in the white blood cells (leucocytes), and its various components like neutrophils, lymphocytes, monocytes and eosinophils. The use of proper anthelmintic

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agent, restores normalcy in the infected host. These variations during helminth infections reflect the host defense status in combating the parasitic attack. The present study involves the evaluation of these total and differential haematological alterations, induced in the laboratory mouse *Mus musculus*, infested with the intestinal helminth, *Hymenolepis nana* (dwarf tapeworm), and treated with the praziquantel, using an automatic Coulter Counter.

Key words: Coulter counter, *Hymenolepis nana*, histomorphology, *Mus musculus*, praziquantel, white blood cells

Blood is one of the most important specimen studied during parasitic infections and diseases in mice as experimental models. The total leucocyte (white blood cell) count (TLC) is an important diagnostic tool to assess the host immune status and resistance to disease or infection^[1,2]. The differential leucocyte count (DLC) is a significant parameter in the blood picture of an animal, especially during any kind of stress from disease, trauma, and infection. The leucocytes of most strains of mice mainly contain neutrophils, lymphocytes, monocytes and eosinophils, while basophils are almost absent^[3]. Most of the routine haematological tests described by Dacie^[4] for humans and by Schalm^[5] for larger animals are applicable to rats and mice, using electronic cell counters.

Hymenolepis nana, is a cosmopolitan intestinal cestode helminth of the warmer climates, whose entire life-cycle is completed in the bowel, so infection can persist for years if left untreated. This worldwide infection was previously common in the southeastern USA, and has been described in crowded environments and individuals confined to institutions. *H. nana* infections can grow worse over time because, unlike in most tapeworms, *H. nana* eggs can hatch and develop without ever leaving the definitive host. Praziquantel, the broad-spectrum anthelmintic, (2-cyclohexyl carbonyl-1,3,4,6,7,11b-hexahydro-2H-pyrazino(2,1-a)isoquinoline-4-one), showed one hundred percent cure with 25 mg/kg body weight dosage in various hosts^[6]. In the present investigation, these blood parameters were assayed using an automatic Coulter Counter.

Adult healthy male Swiss albino mice, *Mus musculus* about 4-5 weeks old, weighing 20-25 g were maintained under GLP conditions^[7], fed with the commercial pellet diet and water *ad libitum* in polypropylene shoe-box cages with sterile paddy husk bedding which was periodically changed. One-third of the total mice were kept as the uninfected control batch. Two-thirds mice were orally infected with about 100 viable eggs of *Hymenolepis nana* per

mouse, and maintained as the infected batch. These infected mice were thus maintained until the 16th day of infection to complete the life cycle of the parasite. On the 16th day, half of the infected mice were given a single oral dose of praziquantel, a 0.2 ml suspension at 25 mg/kg bodyweight of the host mice. This batch was maintained for 3 days as the treated batch. The control, infected and treated batches of mice were sacrificed at appropriate times. Blood was collected by the decapitation method^[8], anticoagulated with 1.5 mg/ml blood of the dipotassium salt of ethylene diamine tetra acetic acid (EDTA), the total and differential leucocyte counts analyzed using an automatic Blood Analyzer^[9,10], the Beckman Coulter HMX Haematology Analyzer with Autoloader which is a quantitative, automated haematology analyzer.

The Coulter VCS established WBC differential technology using three measurements: Individual cell volume (V); high frequency conductivity (C) and Laser-light scatter (S). This combination technology provides abundant cell by cell information that is translated by the instrument into conventional stained film leucocyte categories. The leucocyte counts assayed were, total leucocyte count (TLC number/cu.mm) and differential leucocyte count (DLC) which includes neutrophils (N), lymphocytes (L), eosinophils (E), monocytes (M), expressed in %.

The statistical values for each blood parameter of a batch is the mean of five individual observations, showing significance at probability $P < 0.05$, and non-significant (NS) at $P > 0.05$. According to applicability of the values, Parametric 't' test and non-parametric Mann-Whitney Rank Sum test were applied.

The WBC showed variations in the uninfected, *H. nana* infected and praziquantel treated host blood. The infected host blood registered a 24% decrease, the praziquantel treated, a 16% increase, over the control host blood for the TLC. In the DLC category, the neutrophils (N) showed a 10% drop, lymphocytes increased by 27%, while monocytes, 2%

and eosinophils, 4%, made their appearance, in the infected host blood. Basophils were not found.

Haematological pictures reflect the immune response of the host to infection. The present investigation revealed some important pathological aspects of the helminth infection in the host. Depleted level of TLC, Leucopenia, in the blood of infected mice could be due to severe loss of cellular components of the blood because of haemophagy (Table 1). This could be due to result of a series of changes in the immunological setup of the mouse under parasitic stress^[11-13]. Wright (1960) suggested that a decrease in WBC count could be because of autolysis caused due to hydrolytic enzymes leaked out of cells under parasitism, which could also indicate either haemorrhage^[14] or the increase in the haemopoietic machinery of the host to cope up the rising infection.

Differential leucocytes such as lymphocytes and neutrophils are produced by the erythrocytic tissue. The decrease in the phagocytic neutrophil count, Neutropenia, in the infected host indicates the tendency of losing defense mechanism during parasitism due to the lysis of these cells by the enzymes of the parasite (Table 2).

Lymphocytes, the immunocompetent cells, are responsible for the immune response of the host. The present increase in the lymphocyte count, Lymphocytosis, reflects the host's immune response to overcome parasitic stress, after the phagocytic neutrophils failed in checking the invading parasites (Table 2). This is in accordance with the findings of Fetterer *et al.*^[15] who found an increase in the immunity to *H. nana* infection by transfer from spleen cells. Lymphocytes are essential for the repair mechanism.

There is also an increase in the eosinophil count in infected host blood (Table 2). Eosinophilia is a hallmark of helminthiasis^[16]. The eosinophilia observed in the present study is in accordance with that observed by Furukawa *et al.*^[17] in the peripheral blood of mice due to Hymenolepiasis.

The increase in monocytes, Monocytosis, too, is an immunological response of the infected host to combat hymenolepiasis (Table 2). The Kupffer cells of liver are believed to be the source of monocytes. This is substantiated with the increase in the Kupffer cells noted in infected liver in some studies. It could be possible that some toxic metabolites may be transported from intestine to liver, resulting in these changes^[18-20].

TABLE 1: TOTAL LEUCOCYTE COUNT (TLC) (/CU.MM) OF MUS MUSCULUS DURING HYMENOLEPIS NANA INFECTION AND TREATMENT WITH ANTHELMINTIC PRAZIQUANTEL

Parameter	Batch	Mean±SD	't' value	% Change
Total leucocytes	Control	3700±1.225	-	
	Infected	2800±1.658	*	-(24.324)
	Treated	4300±1.581	*	+(16.216)

The values are mean of five individual observations. ±SD indicates standard deviation. *Indicates statistical significance, at $P < 0.05$; (NS) indicates non-significant at $P > 0.05$. (*) non-parametric Mann-Whitney rank sum test applied, for others "t" test applied.

TABLE 2: DIFFERENTIAL LEUCOCYTE COUNT (DLC) (%) OF MUS MUSCULUS DURING HYMENOLEPIS NANA INFECTION AND TREATMENT WITH ANTHELMINTIC PRAZIQUANTEL

Parameter	Batch	Mean±SD	't' value	% Change
Neutrophils (N)	Control	67±0.860	-	-
	Infected	60±0.707	*	-(10.448)
	Treated	72±0.707	*	+(7.463)
Lymphocytes (L)(*)	Control	30±0.707	-	-
	Infected	38±0.707	*	+(26.667)
	Treated	28±0.707	*	-(6.667)
Monocytes(M)(*)	Control	-	-	-
	Infected	2±0.707	*	-
	Treated	-	-	(NS)
Eosinophils(E)(*)	Control	-	-	-
	Infected	4±0.707	*	-
	Treated	-	-	(NS)

The values are mean of five individual observations. ±SD indicates standard deviation. Figures in parenthesis are percent change of infected and treated batches over control resp. *Indicates statistical significance, at $P < 0.05$; (NS) indicates non-significant at $p > 0.05$. (*) non-parametric Mann-Whitney rank sum test applied, for others "t" test applied.

Thus the variations in white blood parameters of the infected host in this study, leucopenia, neutropenia, lymphocytosis, eosinophilia and monocytosis, indicate the various defense mechanisms adopted by the host to combat the *H. nana* infection. The values of all the parameters in the treated batches of host blood are nearer to the uninfected control host blood value, which clearly proves the efficacy of the anthelmintic praziquantel as a single oral dose regimen in restoring normal haematological profile in the infected host. All of these results are tabulated.

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