

RRST-Biochemistry

Mast Cell Responses in Female Somatic Antigen Non- Sensitized and Sensitized Albino Rats During Experimental Ascariasis

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Article Info	Abstract
Article History Received : 16-03-2011 Revised : 27-04-2011 Accepted : 28-04-2011	Mast cells remain one of the most enigmatic cells in the body and they play important role in cellular immunity. Ascariasis is a global disease and its play a major role in the etiology of childhood malnutrition. The recent estimates reveal that 1.4 billion individuals are infected and thousands of deaths per year occur due to intestinal obstruction, mass dysentery syndrome or severe iron deficiency anemia. In present study mast cells were studied in female somatic antigen immunized and subsequent challenge infections. Four groups each containing 4 albino rats were used: i) Control ii) Infected iii) Female Somatic antigen immunized and iv) Female somatic antigen immunized and challenge infection. 0.25 ml female somatic antigen with complete adjuvant was injected intra muscularly in thighs of albino rats. The rats sacrificed on 21st day of post challenge infection. Albino rat's small intestines were examined histological for mast cells. Count of mast cells was made randomly from the muscularis to the mucosal surface in 20 fields. The results were expressed as the number of mast cells per 20 microscopic fields. In present studies the numbers of mast cells were increased during (low and high doses) experimental ascariasis. Biogenic substances released from mast cells would increase the permeability of the mucosa to antibodies and would have an effect on the expulsion of the parasites.
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©ScholarJournals, SSR	Key Words: Mast cells, Female Somatic Antigen, <i>Ascaris lumbricoides</i>

Introduction

Mast cells remain one of the most enigmatic cells in the body and they play important role in cellular immunity. *Ascaris lumbricoides* is a remarkably infectious and persistent parasite that infects a quarter of the world's population [1, 2]. The recent estimates reveal that 1.4 billion individuals are infected and thousands of deaths per year occur due to intestinal obstruction, mass dysentery syndrome or severe iron deficiency anaemia [3]. Ascariasis is a global disease and its play a major role in the etiology of childhood malnutrition [4]. Mast cells secrete significant amounts of numerous proinflammatory mediators which contribute to a number of chronic inflammatory conditions, including stress mediated intestinal ulceration, rheumatoid arthritis, interstitial cystitis, scleroderma and Crohn's disease [5-8]. Mastocytosis is intimately associated with helminth infections particularly that live in the intestinal tract [9]. In present study mast cell have been studied in female somatic antigen immunized rats and subsequent challenge infections.

Materials and Methods

Parasitic adult *Ascaris lumbricoides* were obtained from the patient suffering from ascariasis. The eggs of *A.lumbricoides* were cultured [10]. The embryonated eggs were counted by dilution methods. Experiments were done using *Ascaris lumbricoides*-Albino rat experimental model.

For the preparation of female somatic antigen, female *A. lumbricoides* were taken in homogenizing tube and added 10 ml phosphate buffer solution and homogenized it with the help of homogenizer. The homogenate was centrifuged for 10 minutes at 3000 rpm and supernatant liquid retained. This supernatant liquid was used as female somatic antigen.

Immunizing dose

0.2 ml Mandate monolate, 1.8 liquid paraffin, 1ml antigen and 1ml NaCl mixed and was injected through insulin syringe.

Experimental Design

According to the experimental protocol the albino rats were divided into four groups -A, B, C and D. Groups Band D were further divided into B₁ and B₂ and D₁ and D₂ respectively. Group A served as control group B as infected. Group B was further divided into the group B₁ and B₂. Groups B₁ and B₂ were infected with 500 and 2000 infected eggs respectively. Group C consisted of female somatic antigen immunized albino rats. Group D consisted of female somatic antigen and subsequently given challenge infection. This group comprised the albino rats immunized with female somatic antigen and after 15 days of immunization, rats were challenged with 500 and 2000 embryonated eggs of *A. lumbricoides*. This group was further divided into the following groups. Group D₁ immunized with the female somatic antigen and challenged

with 500 embryonated eggs of *A. lumbricoides*. Group D₂: Immunized with the female somatic antigen and challenged with 2000 embryonated eggs of *A. lumbricoides*.

Immunizing schedule

Female somatic antigens of 0.25 ml per albino rat were injected intramuscularly in thighs of experimental host as per experimental design. The rats were autopsied after 21st day of post infection was given after 15 days of immunization and the rats were autopsied after 21st day of post challenge infection.

Counting of Mast cells

Albino rat's small intestines were examined histological for mast cells. The small intestines were removed from albino rats at necropsy. Five centimeter segment was cut. The segment of control, infected and treated albino rat's tissue were kept in 10% formalin then stored at 70% ethanol, dehydrated in ethanol, embedded in paraffin wax and sectioned at 5µm. The sections were stained with chrysoidin for 10 minutes. The number of mast cells was enumerated using ax10 eye piece containing a grate with 1mm² areas and a x40 objective lens. Count of cells was made randomly from the muscularis to the

mucosal surface in 20 fields [11]. The results were expressed as the numbers of cells per 20 microscopic fields.

Statistical Analysis

The data obtained during counting of mast cells were analyzed by two way ANOVA method.

Results

Mast cells constitute one of the most important components of cellular immune responses. In control group of albino rats mast cells were observed to be 100 per 20 microscopic fields throughout the small intestines on the twenty first day. The number of mast cells was observed to be group B₁ were 105 per 20 microscopic fields and group B₂ were 111 per 20 microscopic fields on the twenty first day of post infection. The number of mast cells were 117 per 20 microscopic fields of group C on the twenty first day of post immunization. The number of mast cells were 148 and 190 per 20 microscopic fields of group D₁ and D₂ respectively on the twenty first day of immunization and post challenge infection. The results are depicted in Table 1.

Table 1: Number of Mast cells in albino rats immunized with female somatic antigen and challenged with low and high doses of *A. lumbricoides*

Parameter	Control	Infected group		Immunized with female somatic antigen	Immunized with female somatic antigen challenge infection	
	21 day	21 day PI	21 day PI	21 day PIM	15day PIM + 21 day PCI	15day PIM + 21 day PCI
		500	2000		500	2000
Number of Mast Cells	100	105	111	117	148	190
S.E	± 1.1547	± 0.5774	± 0.5774	± 0.5774	± 1.1547	± 4.6188

PIM: Post Immunization; PCI: Post Challenged Infection; PI: Post Infection.

Discussion

In the present experiment, the number of mast cells was increased during (low and high dose) experimental ascariasis. Albino rats immunized with female somatic antigen and challenged with different dose (500 and 2000) embryonated eggs of *A. lumbricoides* infection reveals significant increase of mast cells. Intestinal mastocytosis is observed in certain intestinal helminthes infections and these rapidly generated mast cells in the intestinal mucosa are thought to play an important role in the mucosal defense against intestinal parasites [12]. Mast cells have an important role in worm expulsion [13, 14]. Biogenic substances released from mast cells would increase the permeability of the mucosa to antibodies and would have an effect on the expulsion of the parasites. The classical function of mast cells is as end stage effector cells releasing proteases and inflammatory proteins such as degranulation, their granules also contain preformed cytokines i.e. IL-4 and IL-13 [15]. Both mast cells and eosinophils possess cell surface receptors for homocytotropic antibody that provide a mechanism for association with

antibody specific for parasite antigens that can trigger appropriate effector mechanisms against infecting parasites. Marked worm expulsion was associated with the increased mucosal mast cell number [16, 17]. Several recent studies imply functional role for mast cell proteases (MCP) in the expulsion of some gastro intestinal helminthes [18, 19]. Mucosal mast cells (MMC) are responsible for protection against helminthes infection [20, 21].

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