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

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Abstract

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 anaemia. 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.

Key Words: Mast cells, Female Somatic Antigen, Ascaris lumbricoides

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 Cohns disease [5-8]. Mastocytosis is intimately associated with helminthes 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 and190 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>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Infected group</th>
<th>Immunized with female somatic antigen</th>
<th>Immunized with female somatic antigen challenge infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21 day</td>
<td>21 day PI</td>
<td>21 day PI</td>
<td>15day PIM + 21 day PCI</td>
</tr>
<tr>
<td>Number of Mast Cells</td>
<td>500</td>
<td>2000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.E ±</td>
<td>1.1547</td>
<td>± 0.5774</td>
<td>± 0.5774</td>
<td>± 1.1547</td>
</tr>
<tr>
<td>PIM: Post Immunization; PCI: Post Challenged Infection; PI: Post Infection.</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**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 (500and 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].

**References**

