Response of Intestinal Mucosa to Trichinosis in immunized and non-immunized Albino Rat

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Abstract

The intestinal mucosa is reported to be greatly affected by infestation by *Trichinella spiralis*. The infestation by the worm is also controlled by intestinal tissue factors including cytokines and immune response. Vaccination with larval muscle antigen was reported to be of limited effect. However, vaccination with adult worm crude antigen may infer better protection. The aim of this work is to relate the histological, morphometric and immunological response of the intestinal mucosa of *T.spiralis* infested rat to worm burden, female fecundity and worm expulsion before and after immunization with crude antigen of the adult worm. This relationship is expected to give better evaluation of immunization by this antigen.

45 adult albino rats were allotted among three equal groups: control, *T.spiralis* infested and immunized infested group. Infection was done by 300 freshly isolated infective *T.spiralis* larvae. Five rats from each group were sacrificed at the end of the first, second and third week of infestation. The efficacy of immunization was assessed weekly by measuring adult worm burden and female fecundity, in relation to changes in the histological appearance, relative villus length, number of goblet, and mast cells and the amount of histamine in the intestine.

The results indicated that the worm burden was at its peak by the end of the first week after infestation. The mean protection value due to immunization in this parameter was 67%. Immunization resulted in a protection value relative to larval emergence of 38%. Maximum changes in the host tissue parameters were detected a week after that of worm burden. Measurement of the host intestinal response represented 78% change in villus/crypt ratio, 13.3% in number of goblet cells in the villus and 16.22 in the crypts, 22.5% in mast cell number and 16.8% in histamine content. The results indicate that immunization by crude antigen of the adult *T.spiralis* has a protective effect of the intestinal mucosa of the host due to inhibition of worm vitality and larval emergence. Mast cell production of histamine and goblet cell secretion of mucins could also play a role in protection.

Introduction

Trichinosis remains an important zoonotic food-born parasitic disease of world wide distribution (Murrell, 2001). *Trichinella spiralis* parasitizes many mammals including man. The pig is considered the main component of the epizootologic chain (Leiby et al., 1990).

Although in Egypt pork meat is not a common dish due to religious instructions, yet the risk of infection is still there, since sheep proved to be a source for human trichinosis (Rosa et al., 1992; Kim, 1993; and Theodoropoulos et al., 2000). Recently, *Trichonella* larvae have been also detected in ostriches (Piergilli-Foretti et al., 2001) and horses (Viveros et al., 2001). The presence of Trichinella in these animals followed the use of proteins of animal origin in breeding herbivorous animals, which is now a common practice in many countries (Pozio et al., 2001) including Egypt.

The relationship between parasites and hosts can be considered according to the concept that the host is an environment providing microhabitats for parasites to exploit (Rollinson and Anderson, 1985). Parasites divert resources from the host to their own use. So, the host is under strong selection pressure to stop, or at least, reduce this drain of his resources. One result of these pressures has been the evolution of
acquired immunity which can introduce changes in the environment of the parasite that are disadvantageous to it, and thus, help to restore the balance in favor of the host (Walkelin, 1993). There is a possible role of intestinal epithelial cells of the host in the protective immune response to *T*. *spiralis*. This role has been developed from observation of modified epithelial cell structure and function during a challenge infection. These changes included molecular and functional changes represented by reduced wheat germ lectin binding to the epithelial brush border membrane in infected rats (Bell, 1998), reduced hexose transport (Hessel et al., 1982), increased potential difference across epithelial tight junctions (Russell & Castro, 1985), and chloride ion secretion, which is linked to a change from fluid uptake to net fluid secretion by enterocytes (Bell, 1998). The functional changes in epithelial cells described in these studies have been linked to the immune system through immune serum transfer (Bell, 1998). Immunity seems to play a role in expulsion of the adult worms of *T*. *spiralis* by the host. Although, it seems to be an ambiguous multi factorial process, but involvement of certain antibodies and cytokines has been well recognized lately (Bell, 1998).

Although immunization has proven to be one of the most powerful weapons for prevention of infectious diseases, little prospect exists for the development of vaccines for parasitic infections (Gamble and Murrell, 1987). There is no doubt that the development of successful vaccination against human trichinosis is greatly needed. Prior infection with *T*. *spiralis* results in nearly complete immunity to challenge infection (Murrell, 1985). Antigens derived from different life cycle stages, likewise, can induce protective immunity (Gamble, 1985). However, it was found that the protection against challenge infection using antigens derived from the muscle of the larvae was partial (Murrell and Despomier, 1984 and Gamble, 1985).

The aim of this work was to study the interaction between the extent of successful infection and the structure and activity of intestinal mucosa of rats infected by *T*. *spiralis* worm alone or after immunization by crude antigen of the adult worm.

**Material And Methods**

**Animals:**

Forty five parasite free male inbred albino rats, 8-10 weeks old were divided into 3 equal groups: Control (C), parasite infected (P), and pre-immunized parasite infected (PI) group.

**Parasite:**

*T*. *spiralis* larvae were primarily obtained from infected pig diaphragm slaughtered at El-basateen abattoir, Cairo. They were maintained in the laboratory by serial passages in albino rats. Isolation of larvae from tissues was done according to Wassom et al., (1983).

**Antigen**

Crude adult worm antigen (AA) was prepared according to the method of Goyal and Wakelin (1993). 7 day-old adult worms were collected from experimentally infected rats. The protein content of the worm homogenate was determined according to the method of Lowery et al. (1951). Homogenates were stored in 1ml vials stored at -20ºC till use.

**Protocol of Immunization and Infection**

Rats of the immunized-infected group (PI) were immunized by two subcutaneous injections of 100µg AA emulsified in complete Freund’s adjuvant (CFA, Sigma). The injections were one week apart. Rats of group (C) and (P) were injected in the same way by antigen free solution of CFA in phosphate buffer system (PBS). One week after the last injection, each animal from groups (P) and (PI) was infected with 300 freshly isolated infective larvae of *T*. *spiralis* (Goyal and Wakelin, 1993).

**Evaluation of the Efficacy of Immunization**

Five rats from each group were sacrificed after ether anaesthesia by the end of the first, second and third week after parasite
infection. The small intestine was dissected out free of mesentry and subjected to the following examinations:

A. Adult worm burden was performed according to the method described by Gamble (1985).

B. Female worm fecundity was performed by *in vitro* release of larvae by female worms (Goyal and Wakelin, 1993). Fifty adult female worms were recovered on day 7 post-infection from 5 rats of each of the (P) and (PI) groups. The fifty worms of each group were incubated in universal tube containing 10 ml Hank’s culture medium containing 10% fetal calf serum. The tubes then incubated for 4 hours at 37°C. The contents were filtered through two layers of gauze. The filtrate was transferred to centrifuge tubes. Few drops of formalin were added to each tube. The filtered culture was centrifuged for 10 mins at 1000rpm. The supernatant was decanted and the pellet was re-suspended in 1 ml of PBS. The newly emerged larvae were counted in 10 aliquots of 10µl. The counts were expressed as (larvae/female/24hrs).

C. The percent protection was calculated in the immunized group according to Wang and Bell (1987).

**Histological Studies**

**Histological preparations**

A jejunal segment was dissected from the rats of each group, approximately 6 cm long. The segment was divided into 3 pieces each.

The first piece was opened, washed and fixed in Carnoy fluid for 24hrs. It was processed for paraffin embedding. 5µm sections were stained with toluidine blue for demonstration of mast cells.

The second piece was fixed in buffered formol. It was then processed prepared for paraffin embedding. Serial 5µm sections were mounted on clean slides. Slide with one section was stained by hematoxylin and eosin (Hx&E) for general structure, and the next was stained by alcian blue – PAS (AB-PAS) stain (Mowry, 1958) for demonstration of goblet cells and characterization of the type of mucin.

**Quantitative Histology**

Using Hx&E stained sections, 10 well oriented villi and crypts per rat were subjected to measurement of villus length and crypt depth using a calibrated micrometer mounted on Leitz microscope.

The alternative sections stained by AB-PAS were used to count the goblet cells in well oriented villi and crypts. The number of goblet cells per constant length using a Leitz microscope and eye piece graticule was recorded for 10 villi and crypts on each slide. Parts of the sections with well oriented villi and crypts with intact muscularis mucosa were chosen for counting goblet cells. Ten slides representing different animals of each group were counted.

Ten sections from the opened piece of jejunum stained with toluidine blue were used to count the number of mast cells per 10 constant areas from each slide. A Leitz microscope and eye piece graticule was used.

**Histamine Content**

The third piece of jejunum was used to estimate the amount of histamine in µg/gm of intestinal tissue. The assay was performed according to the method of Lorenz et al.(1977) using the modified fluorometric assay.

**Statistical Analysis**

Data was statistically analyzed and graphically represented using Microsoft excel.

**Results**

**Effect of Parasite on Intestinal Villi and Crypts**

**Histology and Histochemistry**

In Hematoxylin and eosin stained sections, the intestinal mucosa of the control shows long finger like villi projecting in the lumen and crypts in between (Plate I: 1).
After one week of infection with the parasite, the villi became short with blunt tips (Plate I: 2). The crypts appear relatively short and the muscle layer appears thickened. In parts of the sections, the worms appear invading the villi causing damage to the epithelial layer (Plate I, 2-insert). These changes are more prominent by the second week post infection (Plate I: 3).

In sections of the infected immunized group, the deteriorative changes appear less prominent than in the non-immunized group after one and two weeks of infection. (Plate I: 4, 5).

In sections stained by alcian blue-PAS, acidic as well as neutral mucin secreting goblet cells appear distributed along the villi and crypts of the control group (Plate II: 6). The intensity of the goblet cells is clearly high in non-immunized group (Plate II: 7) and moderately high in immunized group (Plate II: 8).

In toluidine blue stained sections, mast cells are less in control (Plate III: 9) than in treated groups (Plate III: 10).

Plate I: Jejunal epithelium of 1) Control, 2) One week after worm infection: The insert represents the invasion of the epithelium by the adult worms., 3) Two weeks after infection, 4) Immunized infected group one week post infection, 5) Immunized infected two weeks post infection.

(HX, E, X100)
Plate II: Goblet cells in the villi and crypts of 6) Control, 7) infected animals two weeks post infection, 8) Immunized infected animals two weeks post infection. (PAS, alcian blue X100)

Plate III: Mast cells at two weeks post infection 9) non-immunized infected group 10) immunized-infected animals (Toluidine blue X400)

Quantitative Evaluation:

Worm Burden

The change in worm burden after infection in non-immunized and immunized groups over the period of the experiment was used as a criterion for parasite infection. The worm burden at zero point was considered zero.

In figure 1-4, the worm burden has reached its beak value after one week. It started to decline over the next two weeks as the worms invade the mucosa. In the immunized group, there was 67% decrease in worm burden compared with the non-immunized group (table 1, figure 6).

Female worm Fecundity

The in vitro evaluation of the female worm fecundity indicated a 38% decrease in the number of larvae emerging from adults isolated from immunized compared to those isolated from non-immunized rats (table 1).

Villus/crypt Ratio:

In non-immunized group, there was a decrease in the mean ratio of the length of the villus relative to crypt length (figure 1). The least value was obtained on the second week after infection; after which a relative increase was obtained. The rate of the decrease in this ratio was 78% less after immunization.
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The number of Goblet cells
Infection with *T. spiralis*, resulted in an increase in the number of goblet cells per unit area of the villar surface (table 2). The maximum value was obtained on the second week, one week after the maximum worm burden (figure 2). In immunized group the increase in the number of goblet cells was 13.3% more than in the non-immunized group (table 1).

The trend in the change in the number of goblet cells in the crypts was similar to that of the villi (table 2, figure 3). However, the rate of increase after two weeks was more in crypts than villi. The percent increase in the number of crypt goblet cells after immunization was 16.22%.
The number of Mast cells

The number of mast cells per unit area in the lamina propria of the jejunum of infected rats was proportional to the increase in the worm period. The peak however was obtained after two weeks of infection (table2, figure 4). In immunized rats the value of mast cell number was higher than in non-immunized. The increase at the peak value was 22.5%.
The Histamine content
The histamine content was directly proportional to the mast cell number (figure 5).

As in mast cell number, immunization resulted in an increase in histamine (table 2). The increase, however, was slightly less than that of mast cell number (table 1) suggesting an increase in cell proliferation not accompanied by similar increase in histamine secretion.

Table (1): Percent protection due to immunization of *T. spiralis* infected rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>worm burden</th>
<th>Larval emergence</th>
<th>Villus/Crypt ratio</th>
<th>Villus goblet cell count</th>
<th>Crypt goblet cell count</th>
<th>Mast cell count</th>
<th>Histamine content</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of non immunized on 2nd wpi</td>
<td>67</td>
<td>38</td>
<td>78</td>
<td>13.3</td>
<td>16.22</td>
<td>22.5</td>
<td>16.8</td>
</tr>
</tbody>
</table>

Table (2): Mean numbers of goblet cells, mast cell /10 vcu of jejunal villi and Crypts and intestinal histamine in studied group

<table>
<thead>
<tr>
<th>Groups</th>
<th>w.p.i</th>
<th>goblet cell/villus</th>
<th>Goblet cell/ crypt</th>
<th>Mast cells 10vcu</th>
<th>Histamine content ug/ gm tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>19.5+1.6</td>
<td>11.2+1.1</td>
<td>109+23</td>
<td>2.89+1.23</td>
</tr>
<tr>
<td>non-immunized</td>
<td>1</td>
<td>36+03*</td>
<td>22+1.2*</td>
<td>218+36*</td>
<td>6.06+0.03*</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>49.3+3.7*</td>
<td>612+17*</td>
<td>612+17*</td>
<td>11.32+3.1*</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>22.3+1.8</td>
<td>336+28*</td>
<td>336+28*</td>
<td>7.1+1.6*</td>
</tr>
<tr>
<td>Immunized</td>
<td>1</td>
<td>42.6+0.1*</td>
<td>26+0.8*</td>
<td>380+18*</td>
<td>7.81+2.1*</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>56.8+0.18*</td>
<td>37+1.8*</td>
<td>789+20*</td>
<td>13.61+2.4*</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>24.1+0.03</td>
<td>29+0.2*</td>
<td>493+0.01*</td>
<td>8.92+0.06*</td>
</tr>
</tbody>
</table>

P<5.05-0.01 in GII and III compared by control using chi-square test
Discussion

Trichinosis can be fatal in the case of infection with high number of worms (Macleod, 1984). Trichinosis became of interest to Egyptian physicians after the recent discovery of *Trichinella spiralis* infection of lamb meat (Rosa et al., 1992; Kim, 1993; and Theodoropoulos et al., 2000).

The maximum number of worms were recorded in the intestinal mucosa one week post infection. This time coincides with the time needed for the development of sexually mature adults (Campbell et al.,1999). Immunization with 2 successive subcutaneous injection of100µg crude adult worm antigen I week apart followed by challenge infection by 300 infective *T*.spiralis larvae resulted in a significant reduction in worm burden compared to the infected non-immunized. The protection was 67%. In addition, 38% protection resulted from the effect on female worm fecundity with complete worm expulsion by the end of 3 rd w.p.i. Similar results have been published by Ortega-Pierres et al.(1986) and Darwish et al.(1996).

The interpretation of the reduction in adult worm burden draws a clear conclusion that the immune status created by prior immunization enhances the overall protective immunity of the host against *T*.spiralis infection. This protection could be through several mechanisms including limitation of fecundity of female worms as indicated in the present work, or elimination of circulating newborn larvae but clearly not through rapid expulsion of adult worm. (Murrell,1985). The antifecundity effect may be due to the gut immune response induced by the injected antigens (Murrell,1985). Inflammatory events in the intestinal tissues (Darwish et al.,1996), and attack of the male organ, being cuticular in nature, by antibodies elicited by injected antigen will interfere with fertilization of the female with subsequent affection on fecundity (Ortega-Pierres et al.,1986).

The morphological and morphom- etric measures of the intestinal mucosa of the studied groups showed that, in non-immunized rats, a significant decrease in villus/crypt ratio is observed and reached its peak by the end of the 1st w.p.i. Gross inflammatory changes in the intestine could be involved in the shortening of the villi of infected group. Similar changes were also reported by Uber et al. (1980), Alizadeh&Wakelin (1982) and Sayed El-Ahl et al.(2001).

Expulsion of the adult worms of *T*.spiralis by the host seems to be an ambiguous multi factorial process but involvement of certain antibodies and cytokines is well recognized lately (Bell,1998). In addition, there seems to be a possible role of epithelial cells in the protective immune response to *T*.spiralis manifested by observation of modified epithelial cell structure and function during a challenge infection. These changes included reduced wheat germ lectin binding to the epithelial brush border membrane in infected rats (Bell,1998), reduced hexose transport (Hessel et al., 1982), increased potential difference across epithelial tight junctions (Russell&Castro,1985), and chloride ion secretion, which is linked to a change from fluid uptake to net fluid secretion by enterocytes (Bell,1998). The functional changes in epithelial cells described in these studies have been linked to the immune system through immune serum transfer (Bell,1998).

The number of goblet cells of infected rats progressively increased to reach a peak value by the end of the first week. Similar observations were reported by Friend et al.(1996) and Miller (1996).The increase in goblet cells mean number was accompanied by an increase in acid mucin. These quantitative and qualitative changes facilitated the expulsion of worms completely by the 3rd. w.p.i in immunized group. Koninkx et al. (1988) attributed the increase of acid mucin to the increase in the number of sulfomucin-containing goblet cells. Khan et al. (1995) reported that intestinal mucus contributes to the immune-mediated prevention of establishment of parasites.

A significant increase in the mean number of mast cells was recorded over the three weeks of the experiment which indic-
ates the precise role played by these cells in worm expulsion. The effect of immunization was manifested in the form of enhancement of mastocytosis. Recently interpretations of mucosal mast cell involvement in worm expulsion were necessarily based upon quantitative data from histological preparations of intestinal tissue. Although such an approach allows correlations to be made between the kinetics of infection and changes in the number of mucosal mast cells present, yet it does not measure mucosal mast cell function directly. In the present study, mucosal mast cell activity was quantified by measuring histamine content in the intestinal tissues. In the gastrointestinal tract and at the cellular level, histamine is found largely in mast cells, mast cell like histaminocytes and in acid secreting glands in the stomach (Range & Dale, 1991). Release of cytokines has been proposed as a mechanism in immune response in trichinosis. Ridinger et al. (1996) first characterized the cytokine profile in immunized mice including IL-2 and IL-3 which could induce mastocytosis and enhanced eosinophilia. Bell (1998) reported that IL-3 is the most important cytokine that can influence mast cell numbers.

In conclusion, The histological and morphometric data support the parasitological data that immunization with worm antigen ameliorates the danger of trichinosis.

References
Response of Intestinal Mucosa to Trichinosis

الأستجابة المعوية للترايكينوزس في الفأر الأبيض المحصن والغير المحصن

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لقد وجدت هناك علاقة بين الغشاء المبطن للامعاء الدقيق والإصابة بذبء الترايكينيلاسيبريليز. فيما يتأثر هذا الغشاء بتواجد الديدان وغزها به أثناء وضع نباتاتها. لأن الديدان تتأثر بمعالجه هذا الغشاء من جزيئات خلوية (ساتوكاينز) ونشط مناعي. وقد وجد أن التحصين محفزات للأجسام المضادة (الإنتيجن) مستخلصة من عضلات البرق наз غير معدية في مقاومة هذه الدودة. غير أنه من المتوقع أن يكون للتخصين مستخلص كلي للدودة البالغة أكثر تأثيراً ضد الإصابة بهذه الدودة. يعد هذا البحث دراسة العلاقة بين التثبيت الهستولوجي والتغيرات في مدى الاستجابة المناعية والقياسات المورفولوجية لانخفاض الإعفاء (حالة الإصابة بالترايكينيلا وحدها أو الإصابة بعد التحصين) مستخلص الديدان البالغ (من ناحية). وبين التكاثر العددي والقدرة على انجاب البرق наз من ناحية أخرى في كل من الحيوانات المصابة معالياً بالديدان، ومتلكه المستخلص الديدان البالغ قبل اصابتها. حيث من المتوقع أن تؤدي مثل هذه الدراسة إلى تقييم أفضل لتآثر التحصين بهذا المستخلص.

استخدم في هذا البحث 45 جزءًا أميّق بلف فستم إلى ثلاث مجموعات: المجموعة الضابطة المجموعة المصابة العملية بالترايكينيلا سيريز. و المجموعة المصابة قبل إصابتها بالديدان. ولقد تم ذبح خمس قناع من كل مجموعة عند نهاية كل من الأسبوعين الأول والثاني و الثالث بعد الإصابة. وقد تم تقدير تأثير التحصين أسبوعياً بقياس التواجد العددي للديدان البالغة والقدرة على انجاب البرق наз وذلك بالقياسات في الشكل الهستولوجي والطويل البنائي للخلايا بالنسبة لعدد ليبرزين وعدد الخلايا الكاسحة والخلايا المنكسة وكذلك كمية الهيستامين في النسيج المعوي.

وقد بينت النتائج أن التواجد العددي للديدان البالغة وصل إلى قمة في نهاية الأسبوع الأول بعد الإصابة حيث كانت نسبة الوفيات في الفئران المصابة 76% كما كانت الوفيات بقياس القدرة على انجاب البرق наз 38% بعد التحصين. وقد أظهرت القياسات على مساحة الأمعاء أن استجابة الأنسجة للأصابة قد وصلت إلى مسافة في نهاية الأسبوع الثاني - أي بعد أسبوع من القاء التواجد العددي للديدان البالغة. و.setResult النتائج أن الاصابة بالموضوع في الفئران المنكسة في الطول البنائي للخلايا كانت 78% بينما كان في عدد الخلايا الكاسحة فتفاخص 13.3% وفي عدد ليبرزين 16.22% وكانت النتيجة بالنسبة للخلايا المنكسة 22.5% بينما كانت في كمية الهيستامين 16.8%.

وقد ردا النتائج على أن التحصين بالمستخلص الكلي لديدان الترايكينيلا سيريز البالغ تعزى وقارة لانخفاض الإصابة في الحيوانات المصابة نتيجة للتآثر على نمو الديدان وتآثر البرق наз وقد يكون لافراز المخاط المحصور من الخلايا الكاسحة والهيستامين من الخلايا المنكسة أثر في هذه الوفيات.