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The presence of *Syphacia obvelata* in laboratory mice (BALB/c) – parasite antigens in immune response

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Summary

In conventional mice colonies, mouse pinworm, *Syphacia obvelata* is found very often. Several studies indicate that infection with this parasite can modulate the immune system of the host and can affect experimental final results. The aim of our study was to investigate the most immunogenic proteins of *S. obvelata* inducing both local and systemic immune response in naturally infected laboratory mice. Protein extracts of *S. obvelata* were analysed by Western blotting to examine their antigenic character. The antigens were probed with serum and mucosa of *S. obvelata* naturally infected mice. Surface and somatic antigens were recognized by serum and mucosal IgG, IgA and IgM antibodies. The most immunogenic and dominant proteins were observed. Proteins of Mw ~ 70, 65 and 48 kDa showed the most evident reaction with serum and mucosa antibodies of infected animals. Surface and somatic antigens of nematode *S. obvelata* eliciting immune response in laboratory mice may be useful in development of a diagnostic test which could be applied for the infection control prior the experiments.

Key words: *Syphacia obvelata*; pinworms; laboratory mice; immune response

Introduction

In biological and biomedical research, where obtaining of reliable and reproductible results is very important, the use of living laboratory animal models is still necessary. The environments of laboratory animals are standardised in order to reduce variation in results, especially in studies performed during the development and registration pharmaceutical products and chemicals. Still little is known about the effects of environmental changes on the biological variation in experimental results (Baumans, 1998) as well as laboratory mice are seldom investigated for autochthonous ecto and endoparasites prior their use in the experiments (Pinto *et al.*, 1994, 2001). The control or eradication of worm burdens in laboratory animals ensures the proper procedures in scientific research. In colonies of conventional mice, mouse pinworm, *Syphacia obvelata* is found very often (Pacoń & Piekarska, 2004). It has been reported that sensitivity and accuracy of results may be compromised when infections agents are present in the colonies (Gilioli *et al.*, 2000). Also, studies carried out by Sato *et al.* (1995) indicate that infection can modulate the immune system of the host and affect experimental final results (Pinto *et al.*, 1994; Bazzano *et al.*, 2002).

Syphacia obvelata is an intestinal nematode belonging to the family *Oxyuridae*, which comprise the largest group of helminth parasites of laboratory animals. This is a small, white, cylindrical worm found in the caecum and colon of mice, sporadically occurring also in rats and hamsters. The life cycle is simple and direct (Dick & Wright, 1973). The host becomes infected by ingesting eggs directly from the perianal region of an infected animal, or indirectly from contaminated water and food. Retroinfection may possibly occur (Taffs, 1976). According to research of Bazzano *et al.* (2002) the frequency of *S. obvelata* in laboratory animals ranged from 9 % to 74 % and the intensity of infection was from 13 to 67 specimens. In the present study carried out by us the prevalence of infection was as high as 66 % however the intensity ranged from 1 to 250 specimens. According to Taffs (1976) the prevalence of *S. obvelata* infection in laboratory animals may be a function of: age (the intensity decreases with an increasing age), sex (higher prevalence in male hamsters than females), strain and the host status. Our previous examinations of 176 laboratory mice (BALB/c strain) demonstrated that the prevalence of infection in males was 70 % (with the intensity of infection of 1 – 102) and in females was 52 % (with the intensity of infection of 1 – 34 individuals) – (Okulewicz & Perec, 2003).

During the infection the host is exposed to parasite-derived

antigens which can be recognized by the immune system. These are usually proteins, glycoproteins and carbohydrates which are located on the parasite surface or excretory/ secretory products. Some antigens elicit humoral and cellular immune responses both local and systemic (Wędrychowicz, 1997; Kołodyński & Okulewicz, 1998). Detection and recognition of nematode antigens increases with the development of specific immunodiagnostic methods and help to understand protective mechanisms of the hosts which are elicited during infection. The knowledge of these protective antigens becomes useful in development of diagnostic tests and production of medicaments and vaccines.

The aim of our study was to investigate the most immunogenic proteins of *S. obvelata* inducing both local and systemic immune response in naturally infected BALB/c mice.

Materials and Methods

Host and parasite

120 laboratory mice (*Mus musculus*) of BALB/c strain, 2 – 4 months old, both sexes with natural infection of *S. obvelata*, were used during the experiment. They came from the conventional colony of Institute of Genetics and Microbiology. Adults *S. obvelata* were harvested from the colon and caecum after sacrifice of the mice. All animal procedures were approved by the University of Wroclaw and Local Committee of Ethics.

Serum and mucosa collection

During postmortem, serum and intestinal mucosa samples were obtained for Western blot analysis (20 samples) – (De Vos *et al*., 1992). Uninfected mice provided controls. Collected serum and mucosa samples were individually stored at -40°C.

Isolation of surface proteins of S. obvelata

Collected nematodes were purified in PBS (pH 7.4) for 6 – 9 h and then washed 10 times in 0.15 M NaCl. The worms (at a concentration of $9x10^2$ adults per ml) were next suspended in 0.05 M carbonate/bicarbonate buffer (pH 8.2) and incubated with 0.3 mg/ml of sulfosuccinimidyl-2-(biotinamido)ethyl-1,3ditiopropioniate (NHS-SS-biotin – Sigma) for 1 h at room temperature in the dark. Biotinylation was terminated by washing extensively in 0.15 M NaCl. Then pinworms were homogenised and extracted in Tris-buffered-saline (TBS, 0.15 M NaCl in 10 mM Tris, pH 7.4) containing protease inhibitors (0.2 M Tris, 0.15 M NaCl, 0.02 M EDTA, 13.5 µM TLCK, 7.0 µM TPCK, 0.5 µM PMSF and 2 µM of antypain) in an ice-water bath. The homogenate was extracted overnight at 4ºC then centrifuged at 16000 g, 30 min, 4ºC – TBS extract (AP1 fraction). The pellet was resuspended in 500 µl TBS containing 1 % SDS and boiled for 5 min. The mixture was than recentrifuged for 30 min at 16 000 g, the supernatant was removed (AP2 fraction) and the pellet was resuspended in 500 µl TBS with 5 % 2-mercaptoethanol to obtain AP3 fraction (Keith *et al*., 1990; Wędrychowicz *et al.*, 1994). The extracts were frozen at -20°C.

Isolation of somatic proteins of S. obvelata

After 3 washings in Tris-HCl buffer (0.05 M, pH 8.0) containing 0.2 M NaCl and 1.0 % Triton X-100, adult worms at a concentration of $5x10^2$/ml were homogenized in a glass homogenizer on ice (AS fraction). Insoluble fragments were removed by centrifugation (25 000 g, 4°C) and the clear supernatant fraction was frozen at -20°C (De Graaf *et al.*, 1996).

Gel electrophoresis

Solubized surface and somatic extracts (at a concentration of 20 – 30 µg/ml) were analyzed on SDS – polyacrylamide gels comprised of a 6 % stack and 12 % resolving gel using the discontinuous buffer system of Laemmli (1970).

Western blot analysis

Western blotting was carried out following established protocols of Towbin *et al.* (1979). Polypeptides were transferred onto nitrocellulose membranes using 25 mM Tris – HCl (pH 8.3), 192 mM glycine, 20 % methanol. The blotting was carried out for 3 h at 60 V, 0.22 A. The membrane was blocked with 3 % BSA in PBS for 12 h in 4ºC. After rinsing briefly in 0.3 % milk – TBS the membrane was incubated with test and control serum - at 1:50 dilution and abomasal mucosa - at 1:20 dilution in blocking buffer, for 1h at room temperature and then washed 4x over a period of 10 min each time. The membrane was then incubated with HRP – conjugated anti – mice IgG (at 1:500 dilution) and anti – mice IgA and IgM (at 1:200 dilution) for serum and anti – mice IgG (at 1:500 dilution) and anti – mice IgA (at 1:100 dilution) for mucosa (Sigma). The membrane was incubated for 1 h at room temperature and washed 4 times for 10 min in 0.3 % milk – TBS. After washing the blots were developed and bound antibodies were visualized by adding 4-chloro-1-naftol in 10 % methanol in TBS and 0.03 % H_2O_2. The reaction was stopped in distilled water.

Detection of glycoproteins with Con A

After the transfer the blots were blocked in 0.1 % Tween 20 in PBS at room temperature for 1 h. Next, the membrane was incubated with biotinylated Con A (10 µl/ml PBS, pH 7.4) for 1 h at room temperature and then the blots were washed 4 x 5 min in PBS and incubated with HRP (20 µl/ml PBS) for 1 h at room temperature. After washing four times with PBS, the membrane was developed with 4-chloro-1-naftol as a substrate. The reaction was stopped in distilled water (Schallig *et al.*, 1994).

Results

Electrophoresis of surface proteins in the fraction AP1 revealed 22 proteins with Mw's ranged from 126 kDa to 27 kDa. In the fraction AP2 we identified 14 antigens ranged from 126 to 30 kDa and the fraction AP3 showed 11 antigens ranged from 124 to 49 kDa. Detection of glyco-

Table 1. Molecular weights of fractions of antigens recognized by serum Igs of infected mice

Extraction buffer	MW of protein fractions recognized by IgG	MW of protein fractions recognized by IgA	MW of protein fractions recognized by IgM
TBS	~102, 95, 89, 70, 65, 60, 58, 50, 48, 39, 35, 32	~117, 95, 84, 70, 50, 39, 35	~117, 115, 110, 65, 60, 48, 39, 35
SDS	~110, 106, 91, 75, 70, 61, 55, 46	~119, 110, 70, 57	~110, 106, 91, 75, 70, 55
2-Me	~108, 91, 75, 70, 58, 52	~117, 97	~91, 58, 49
TBS + NaCl + Triton X-100	~ 104, 87, 72, 66, 65, 63, 54, 53, 50, 47, 41, 37, 32, 31	~ 87, 79, 72, 69, 67, 66, 65, 44	~ 112, 80, 79, 69, 65, 63, 47, 44, 41, 37

MW – molecular weight [kDa]; TBS – Tris buffered saline, SDS – SDS containing buffer; 2-Me – 2-mercaptoethanol containing buffer

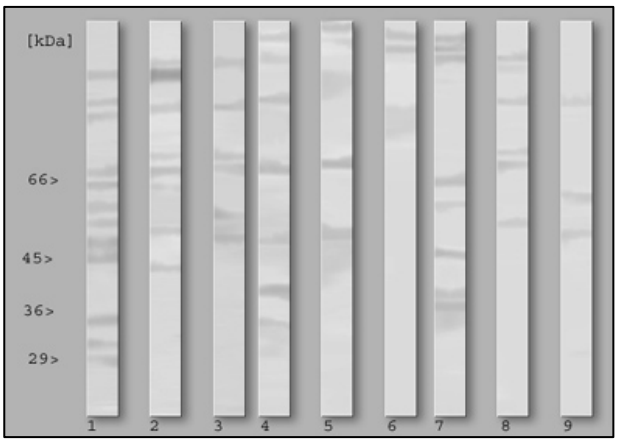

Fig.1. Western blots of *S. obvelata* surface extracts showing the polypeptides recognized by serum antibodies. Tracks: 1, 2, 3 – AP1, AP2, AP3 extracts recognized by serum IgG; 4, 5, 6 – AP1, AP2, AP3 extracts recognized by serum IgA; 7, 8, 9 – AP1, AP2, AP3 extracts recognized by serum IgM

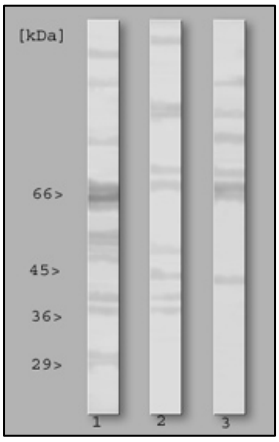

Fig.2. Western blots of *S. obvelata* somatic extract showing the polypeptides recognized by serum antibodies. Tracks: 1 – proteins recognized by serum IgG; 2 – proteins recognized by serum IgM; 3 – proteins recognized by serum IgA

Tab.2 Molecular weights of surface antigens recognized by mucosal Igs of infected mice

Extraction buffer	MW of protein fractions recognized by IgG	MW of protein fractions recognized by IgA
TBS	~117, 84, 70, 65, 58, 50, 48, 44, 39	~84, 65, 58, 50, 48, 39
SDS	~91, 70, 61, 52, 48	~52, 48
2-Me	~75, 52, 49	~82, 75, 49

MW – molecular weight [kDa]; TBS – Tris buffered saline, SDS- SDS containing buffer; 2-Me – 2-mercaptoethanol containing buffer

proteins using Con A revealed in TBS extract – 15 bands, SDS extract – 12 and finally in 2-Me extract – 5 bands. SDS-electrophoresis of AS extract showed 23 protein bands with molecular weights ranged from 90 to 27 kDa as well as 15 bands of glycoproteins were detected.

The biotinylated surface proteins were electrophoresed, blotted and probed with serum and mucosa samples of infected and control mice. Numerous surface proteins of adult worms were recognized by antibodies of naturally infected mice (Tab.1, Fig.1). Out of 22 polypeptides found in TBS extract 12 were recognized by the serum IgG, 7 by IgA and 8 polypeptides by IgM. Next fraction of SDS extract resolved 8 bands with IgG, 4 with IgA and 6 with IgM. Within 2-Me fraction the reaction was observed with 4 bands for IgG, 2 for IgA and 3 bands for IgM. Western blot of AS extract showed reactions of 14 protein bands with serum IgG, 8 bands with IgA and 10 with IgM class (Tab.1, Fig. 2).

Mucosal IgG recognized 9 bands in AP1, 5 bands in AP2 and 3 bands in AP3 protein fraction bands. Mucosal IgA revealed reactions with 6 antigens of AP1 fraction, 2 of AP2 and 3 antigens of AP3 fraction respectively (Tab. 2, Fig. 3). In all tested serum and mucosa samples, immune response against surface and somatic antigens of *S. obvelata* was observed however fraction AP1 developed stronger reactions than fractions AP2 and AP3. Serum and mucosa samples obtained from control mice didn't recognize any protein extracts.

Humoral immune response was elicited against all fractions of proteins both AS and AP. Surface protein of 70 kDa and somatic protein of 65 kDa were recognized by serum IgG, IgA and IgM classes. Moreover proteins of 70 kDa and 48 kDa were recognized by mucosal antibodies.

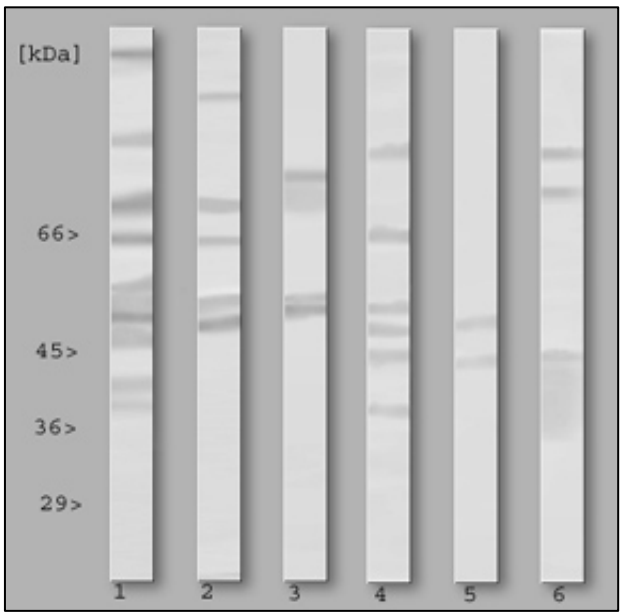

Fig.3. Western blots of *S. obvelata* surface extracts showing the polypeptides recognized by abomasal mucosa antibodies. Tracks: 1, 2, 3 – AP1, AP2, AP3 extracts recognized by mucosal IgG; 4, 5, 6 – AP1, AP2, AP3 extracts recognized by mucosal IgA

Discussion

Many papers present the data on the parasites of farm and domestic animals, very important from the economical point of view. On the other hand, still little is known about naturally found parasites of laboratory animals e.g. pinworms, and their influence on experimental results. Pinworms commonly infecting laboratory mice include *Syphacia obvelata* and *Aspiculuris tetraptera*. It has been reported (Jacobson & Reed, 1974) that athymic (nu/nu) mice have increased susceptibility to pinworm infection. The prevalence of infection remains high even in well-managed animal colonies. While parasites are usually nonpathogenic, rectal prolapse, intussuspection, faecal impaction, rough hair coat and poor weight gain have been reported (McNair & Timmons, 1977). There are a few reports documenting the effects of pinworms on research. Pinworms infection affecting mice physiologic functions have influenced experimental results. A significant reduction of activity of mice infected with *S. obvelata* was observed in behavioral studies (McNair & Timmons, 1977). Wagner *et al.* (1988) reported clear growth differences between pinworm-free and pinworm-infected rats. Experimentally infected with *S. muris* animals grew slower than uninfected ones. It was reported that in laboratory rats infected with *S. muris*, despite of absence of apparent histopathological changes, intestinal transport of water and electrolytes is significantly decreased due to pinworm infection (Lübcke *et al.*, 1992).

In pinworm infection as in many other helminthiases immunity is mostly humoral. Infection with *S. obvelata* induces a proliferation of T- and B-lymphocytes in spleen and lymph nodes and occasional germinal center formation (Beattie *et al.*, 1981). For the first time investigations by Sato *et al.* (1995) have demonstrated *Syphacia*-specific antibodies (IgG) against *S. obvelata* somatic antigen in experimentally pinworm-infected mice (AKR/J strain).

In this study we demonstrate IgG, IgA and IgM antibody isotypes against somatic and surface antigens during the course of natural infection with *Syphacia obvelata* in laboratory mice (BALB/c strain). Proteins were extracted and then analyzed on SDS-polyacrylamide gels. Polypeptides labelled with Con-A were also found in all extracts of adults of *S. obvelata*. We examined the local and systemic immune response against these antigens. The results of the examination of serum samples showed stronger immune response characterized by numerous immunogenic protein bands than with the mucosal antibodies, although intestinal mucosa is a habitat for *S. obvelata*. Surface polypeptides of AP1, AP2 and AP3 fractions with Mw's ranging from 119 to 32 kDa detected by serum IgG, IgM and IgA were involved in eliciting systemic humoral immune response. Mucosal antibodies recognized less surface antigens with the molecular weights ranging from 117 to 39 kDa. In all tested serum and intestinal mucosa samples stronger antigenic reactions were observed among polypeptides of AP1 fraction than among polypeptides of AP2 and AP3 or AS fraction. Sato *et al.* (1995) observed on day 26 PI a dominant band of 65 kDa in somatic extract. This protein was observed in surface fractions reacting with IgG and IgM classes and somatic fraction reacting with all examined isotypes of antibodies. Studies of Brett (1983) and Sato *et al*. (1995) found out that pinworm infection could activate host immune system by antibody production to nonparasitic antigenic stimulus.

Extraction of surface and somatic antigens of nematode *S. obvelata* and eliciting immune response in mice may have an application for the control of the infection in laboratory mice since many papers, e.g. by Shibahara (1999), Gilioli *et al.*(2000) or Pacoń and Piekarska (2004), inform about parasites in laboratory animal houses. Studies should be focused on the proteins of both extracts which show reactions with examined classes of antibodies and then identification of protective proteins would have an application value. In our paper, proteins of Mw ~ 70, 65 and 48 kDa showed evident reaction with serum and mucosa antibodies of infected animals.

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