Abstract

Fasciola’s excretory-secretory material comprises chiefly cathepsin B and cathepsin L. These cysteine proteases are proposed as major mediators of parasitism, and are considered targets for vaccination. In order to assess the vaccine efficacy of these enzymes, single and multivalent recombinant protein vaccinations of adult-stage *F. hepatica* cathepsin L5, metacercarial-stage *F. gigantica* cathepsin L1g and juvenile-stage *F. hepatica* cathepsin B were analysed in rats against *F. hepatica* challenge infection. The protective efficacy of anti-fluke vaccines was evaluated in terms of parasitological parameters (recovered fluke burden, fluke body size and wet weight) and pathological changes (liver damage score) in rats. The rats vaccinated with recombinant proteins were shown to have significantly fewer and smaller flukes than the control rats. A maximum protection of 83% was seen in the group vaccinated with a combination of cathepsin B and cathepsin L5.

Liver fluke vaccines: Vaccination Against Fasciolosis by a Multivalent Vaccine of Recombinant Stage-Specific Antigens

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1. Introduction

*Fasciola hepatica* and *F. gigantica* cause liver fluke disease in ruminants and humans. This emerging human pathogen is highly prevalent in South American countries, Northern Iran and Egypt (1). These two major parasite species are prevalent in temperate and tropical regions respectively, which means that infection can occur worldwide. Though anti-parasitic drug treatment is effective against disease, emerging drug resistant strains leads to the need for the development of a viable vaccine. Although much research into vaccination against the parasite has been undertaken, no commercial vaccine is as yet available.

Cysteine proteases are common virulence mediators of parasites, and are produced by all stages of the fluke life cycle. The mediate biological functions including excystment, tissue invasion and immune evasion (2). Adult fluke cathepsin L and Newly Excysted Juvenile (NEJ) cathepsin B are the prominent proteolytic enzymes of their respective ES materials. *F. hepatica* cathepsin L5 (3, 4), cathepsin B (5–8) and *F. gigantica* cathepsin L1 (9) are promising targets for vaccines against *Fasciola* infection and are evaluated here.

In order to assess cysteine protein vaccine potential, recombinant protein expression is usually desirable. The yeast expression system has been a useful tool for the functional expression of cathepsin L1 and L2 (10, 11), cathepsin L5 and cathepsin B (5, 7). Here we evaluate the vaccine efficacy of the multivalent vaccines using yeast-expressed recombinant protein proteases derived from juvenile and adult-stage liver fluke in the rat fasciolosis model. Some of the data presented here was recently published by Jayaraj and colleagues (12).

2. Recombinant vaccine production, vaccination and challenge infection

The expression and purification of cathepsin B, cathepsin L5 and cathepsin L1g from *S. cerevisiae* BJ 3505 cells were preceded according to Law et al. (2003) and Smooker et al. (2000) (4, 7). The expressed proteins were purified by
Ni-NTA affinity chromatography and eluted fractions were used for vaccine formulations (13). Sprague Dawley male rats were used in the vaccination experiment for testing yeast expressed protein vaccines, and they were aged six weeks at the time of vaccination and 12 weeks at the time of parasite challenge. Seven to nine rats were used per vaccination group and the trial was assessed by the Monash University animal ethics committee, Australia. Rats were vaccinated with 20 µg of recombinant proteins in 200 µL 0.9% saline adjuvented with 1 mg/mL Quil A. Control rats received Quil A in 0.9% saline only. The rats were vaccinated thrice at two weekly intervals via the intraperitoneal route. For the parasite challenge, metacercarial cysts (infected stage) from laboratory Lymnae tomentosa snail cultures (Macarthur Agricultural Institute, Menangle, NSW, Australia) were used. At week 12 (2 weeks after the third vaccination) rats were infected orally using a gavage needle with 25 metacercarias in 1 mL 0.4% carboxy methyl cellulose (Sigma).

3. Necropsy and analysis of pathological and parasitological parameters

Vaccinated rats were humanely sacrificed at week 20 (eight weeks after infection), and their livers removed. Pathological lesions of livers were observed by the naked eye and liver damage determined (blind scoring). The severity and the intensity of lesions was quantitatively scored from 0 to 5 (14). Live adult flukes inside the lumen of bile duct were dissected out by using forceps and the whole liver was dissected, incubated in saline at 37°C for 1 hour and filtered through a 200 µm mesh sieve for detection of flukes. The influence of recombinant vaccines on the fluke recovery, body size and wet weight are important factors of protection (15-17). The body lengths from head to tail and body width of each worm from all vaccinated and control rat groups were measured using a dissection microscope with a millimetre scale (18, 19).

4. Results and Discussion

The vaccinated rats were sacrificed 8 weeks after challenge infection and the extracted livers assessed for the damage scores (Table 1A). In the control rat group, four rat livers had the high score of 4 (mean 3.3), indicative of damage (Figure 1). All groups containing either cathepsin B or L5 have a significantly reduced damage score compared to the control group, with the divalent B/L5 group having the least mean damage, at 0.9. The mean liver fluke burden from the vaccinated rats is also given in Table 1A. In rats vaccinated with any combination of recombinant proteins, recovered numbers of liver flukes are significantly lower than in control rats. Recovered liver fluke length and width, total mass of recovered flukes and liver damage are the major factors often used to indicate the protection against the severity of parasite infection (17, 18). The liver fluke body length, width (shown in Figure 1) and wet weights are reduced in the vaccinated groups (Table 1B). These results show that vaccination with the multivalent cathepsin B/L5 leads to less liver damage, lower fluke numbers and the lowest mean wet weight compared to control rats.

### Table 1. A. The experimental design of the vaccine trial and analysis of mean liver damage scores and mean Fluke burden in infected rats.

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>Rats/group</th>
<th>Antigen dose</th>
<th>Liver damage</th>
<th>Fluke Burden</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control</td>
<td>8</td>
<td>0 µg</td>
<td>3.3 ± 1.0</td>
<td>4.38 ± 0.74</td>
</tr>
<tr>
<td>2 Cat B</td>
<td>8</td>
<td>20 µg</td>
<td>1.3 ± 0.8b</td>
<td>1.75 ± 1.04c</td>
</tr>
<tr>
<td>3 Cat L5</td>
<td>8</td>
<td>20 µg</td>
<td>1.3 ± 1.1a</td>
<td>2.13 ± 0.35c</td>
</tr>
<tr>
<td>4 Cat L1g</td>
<td>9</td>
<td>20 µg</td>
<td>1.9 ± 1.1</td>
<td>2.22 ± 1.3a</td>
</tr>
<tr>
<td>5 Cat B/L5</td>
<td>8</td>
<td>10 µg of each antigen</td>
<td>0.9 ± 1.0b</td>
<td>0.75 ± 0.46c</td>
</tr>
<tr>
<td>6 Cat B/L1g</td>
<td>7</td>
<td>10 µg of each antigen</td>
<td>1.9± 1.0</td>
<td>1.71 ± 0.95c</td>
</tr>
<tr>
<td>7 Cat L5/L1g</td>
<td>8</td>
<td>10 µg of each antigen</td>
<td>1.6± 1.1a</td>
<td>1.75 ± 1.16c</td>
</tr>
<tr>
<td>8 Cat B/L5/L1g</td>
<td>7</td>
<td>6.6 µg of each antigen</td>
<td>1.9± 1.3</td>
<td>1.85 ± 1.35c</td>
</tr>
</tbody>
</table>
B. Body size and mean wet weight of recovered liver flukes at necropsy.

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>Mean body length (mm)</th>
<th>Mean body Width (mm)</th>
<th>Mean wet weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control</td>
<td>23.6 ± 3.41</td>
<td>10.48 ± 3.49</td>
<td>120.6 ± 31.1</td>
</tr>
<tr>
<td>2 Cat B</td>
<td>7.86 ± 2.72</td>
<td>4.33 ± 1.58</td>
<td>44.06 ± 27.8</td>
</tr>
<tr>
<td>3 Cat L5</td>
<td>10.58 ± 2.15</td>
<td>4.82 ± 1.5</td>
<td>50.00 ± 17.2</td>
</tr>
<tr>
<td>4 Cat L1g</td>
<td>12.15 ± 3.18</td>
<td>5.5 ± 1.19</td>
<td>68.65 ± 29.7</td>
</tr>
<tr>
<td>5 Cat B/L5</td>
<td>4.00 ± 2.5</td>
<td>2.25 ± 1.38</td>
<td>28.87 ± 18.7</td>
</tr>
<tr>
<td>6 Cat B/L1g</td>
<td>9.53 ± 3.5</td>
<td>5.61 ± 2.02</td>
<td>45.06 ± 21.6</td>
</tr>
<tr>
<td>7 Cat L5/L1g</td>
<td>11.00 ± 5.16</td>
<td>5.4 ± 2.24</td>
<td>57.93 ± 24.8</td>
</tr>
<tr>
<td>8 Cat B/L5/L1g</td>
<td>11.07 ± 4.15</td>
<td>5.53 ± 1.8</td>
<td>58.15 ± 32.9</td>
</tr>
</tbody>
</table>

This result indicates liver fluke development was retarded in the vaccinated groups. It may be that a fluke vaccine does not need to induce sterile immunity, but reduce the pathology to a level that is tolerated by the animal. A reduction in fluke burdens will also reduce the numbers of eggs passed by infected animals, reducing pasture contamination. Our study indicates that juvenile stage-specific recombinant proteins (B, L1g) are able to mediate immunity to liver fluke infection, but that protection is greatest when a juvenile stage protease (cathepsin B) is used in concert with an adult stage protease, L5. The greatest protection (83%) was afforded by a vaccine with both these cathepsins, and two rats in this group did not have any detectable liver flukes. This indicates that multivalent vaccination is probably optimal for this large, multicellular parasite, and this may be a general feature of such vaccine strategies.

In conclusion, the protective immunity elicited by recombinant protein vaccination appears to evoke effector and memory responses against infection. We have shown that such vaccination induces strong B cell responses (12), although the precise effector mechanisms leading to fluke control have not been elucidated. That a cocktail of juvenile and adult stage Fasciola recombinant proteins induced the better protective immunity than individual protein alone indicates that stage specific, multivalent recombinant vaccines against parasites may be feasible.

Figure 1: Analysis of liver damage and F. hepatica parasite size in control and vaccinated rats at necropsy.

Two representative livers are shown here: a liver from a control rat (first photograph), showing enlarged lobes with necrotic lesions (liver damage score 4); Photograph two, a Cathepsin B vaccinated rat liver showing a healthy, pliable and soft glassy appearance with minimal damage (liver damage score 0.5). The right hand panel shows a representative large size fluke from a control rat and a smaller fluke from a protein vaccinated.
5. References


