Antigenic study of *Myxobolus rotundus* (Myxozoa: Myxosporea) using monoclonal antibodies

Y S Lu¹, M Li², Y S Wu² and J G Wang¹

¹ Laboratory of Fish Diseases, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei, P. R. China
² The First Military Medical University, Guangzhou, Guangdong, P. R. China

**Keywords:** *Myxobolus rotundus*, MAbs, PAbs, IFAT, antigen localization.

*Myxobolus rotundus* Nemeczek, 1911 is one of the most important myxosporans infecting crucian carp, *Carassius auratus auratus* (L.), in China (Wu & Wang 2000). Although in most cases mortalities rarely result from infection, the market value of infected fish is reduced because of the large, visible cysts present on the head, fin and body surface of the fish (Fig. 1). Monoclonal antibodies (MAbs) are a valuable tool for the recognition of myxozoan surface antigens (Saulnier & de Kinkelin 1996). Several MAbs against myxozoans have been reported (Bartholomew, Rohovec & Fryer 1989; Adams, Richards & Marin de Mateo 1992), but MAbs have not been raised against species of the genus *Myxobolus*. This paper describes the use of MAbs in an antigenic study of *M. rotundus*.

Spores of *M. rotundus* were collected from mature cysts on the surface of crucian carp from a fish farm in Wuhan City, Hubei Province, China. They were purified using a pepsin–trypsin digestion (PTD) method (Markiw & Wolf 1974) and sonicated. Soluble antigens were obtained after centrifugation at 10 000 g for 10 min and used to immunise Balb/c mice following the procedure described by Herbert (1978). Before mice spleens were isolated for fusion, sera were collected and used as polyclonal antibodies (PAbs). The MAbs were produced following the method of Campbell (1984). Antibodies specific to *M. rotundus* were screened by an enzyme-linked immunosorbent assay (ELISA) with soluble antigen (1 μg mL⁻¹) used for coating the microtitration plates (Ploy Sorp, Nunc, Denmark).

Trophozoites, spores and cyst capsules of *M. rotundus* were separated by a sucrose density gradient centrifugation as described by Wu & Wang (2000) and the cross-reactivity of antibodies (PAbs and MAbs) to trophozoites, spores, cyst capsules of *M. rotundus*, *M. rotundus* infected fish gills, *M. guangiaoensis* Wu and Wang, *M. magna* Wu and Li, *M. sinensis* Nie and Li, *Thelohanellus rohitae* Southwell and Prasad, *T. wuhanensis* Xiao and Chen and *Henneguya dogieli* Achmerov were studied by ELISA as described above. Antigens recognized by MAbs were determined by Western-blotting following the method described by Sambrook, Fritsch & Maniatis (1989), in which the concentration of soluble antigens used for electrophoresis was 0.2 mg mL⁻¹ and the dilution of antibodies (MAbs and PAbs) was 1 : 2000. The location of antibody-conjugated antigen on the parasite was determined using an indirect fluorescent antibody technique (IFAT) as reported by Kawamura (1977).

Two hybridomas (1A9 and 2D12) produced antibodies which reacted positively with *M. rotundus*. Cross-reactivity studies showed MAb 1A9 reacted specifically with *M. rotundus* spores, while MAb 2D12 reacted with both the trophozoites and spores of *M. rotundus*. The PAbs cross-reacted with trophozoites and cyst capsules of *M. rotundus* as well.
as *M. rotundus*-infected fish gills, *M. guanqiaoensis* and *T. rohitae* (Table 1). In Western-blot analysis, both of the MAbs recognized antigens from *M. rotundus* spores. The MAb 1A9 recognized a protein with a molecular weight (MW) of 103.6 kDa, while MAb 2D12 recognized a protein of 91.4 kDa (Fig. 2). Using IFAT, with PAbs, fluorescence was found to be densely concentrated on the posterior and the periphery of the *M. rotundus* spore, but was also detected on the trophozoite and cyst capsules (Fig. 3a–c). The MAb 1A9-conjugated antigens were mainly concentrated on two areas at the posterior of the spore, indicating common antigens on the two valves (Fig. 3d). However, MAb 2D12-conjugated antigens were localized on the anterior end of the spore; this MAb also reacted with trophozoites of *M. rotundus*, with fluorescence strongly concentrated in the endoplasm (Fig. 3e,f).

The MAb 1A9 reacted specifically with *M. rotundus* spores, suggesting that some of the antigens of *M. rotundus* are stage-specific and the antigen profile of the parasite may change throughout its life history. Bartholomew *et al.* (1989) reported changes in the antigenic profile of *Ceratomyxa shasta* over its life cycle. On the other hand, MAb 2D12 reacted with both the spores and trophozoites of *M. rotundus*, indicating that there was some antigenic relationship between the different stages of the parasite as also reported in *Myxosoma cerebralis* (Markiw & Wolf 1978). The fact that neither of the MAbs reacted with other myxosporeans shows that some antigens of *M. rotundus* are species-specific, which was also found in a study using MAbs against *Tetracapsula bryosalmonae* (PKX) (Morris, Adams, Feist, McGeorge & Richards 2000). Pauley (1974) suggested that the cross-reactivity of a specific antiparasite antibody with normal tissue antigens might indicate that the parasite mimics these antigens in order to evade a host immune reaction. The reaction of PAbs against *M. rotundus* with both cyst capsules and host tissues suggests the presence of such common antigens between parasite and host. Although the valves of the *M. rotundus* spore are developed from two valvogenic cells differentiated asynchronously from a single primary cell (Wu & Wang 2001), fluorescence was found to be strongly concentrated on two symmetric points of the valves as tested by MAb 1A9 and 2D12 with IFAT. Possibly, therefore, there are symmetric

### Table 1

Cross-reactivity analysis of MAbs and PAbs against *Myxobolus rotundus*.

<table>
<thead>
<tr>
<th></th>
<th>PAbs</th>
<th>MAb 1A9</th>
<th>MAb 2D12</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Myxobolus rotundus</em> spores</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>M. rotundus</em> trophozoites</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Cyst capsule of <em>M. rotundus</em></td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>M. rotundus</em>-infected gill tissue</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>M. guanqiaoensis</em></td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>M. magna</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>M. sinensis</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Thelohanellus rohitae</em></td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>T. wuhanensis</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Henneguya dogieli</em></td>
<td>–</td>
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Reactions are designated as positive (+) or negative (−). 

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Figure 1  Crucian carp, *Carassius auratus auratus*, infected by *Myxobolus rotundus*, showing cysts on the head.

Figure 2  Western-blot analysis showing the antigens recognized by monoclonal and polyclonal antibodies against *Myxobolus rotundus*. Lane representations are: (M) MW markers, with weight in kDa at both sides of the gel; (1) polyclonal antibodies; (2) MAb 1A9, showing MW of conjugated antigen, 103.6 kDa; (3) MAb 2D12, showing MW of the recognized antigen, 91.4 kDa.
surface antigen epitopes on the two valves of the *M. rotundus* spore.

As suggested by Adams *et al.* (1992), MAbs are valuable tools for the investigation of the parasite’s surface antigens and to study its life cycle. The MAbs 1A9 and 2D12 can be utilized as probes to study the surface antigens of *M. rotundus* spores. Moreover, MAb 2D12 might be a useful tool in the diagnosis of early infections of *M. rotundus*, as it reacts with trophozoites.

**Acknowledgements**

We thank many colleagues for their technical assistance, and Drs P. Nie and G. T. Wang for their advice and comments on the manuscript. This research was financially supported by the Project for Knowledge Innovation of the Chinese Academy of Sciences (No. 1999051 and No. KSCX2-1-04).

**References**


Pauley G.B. (1974) Fish sporozoa: extraction of antigens from *Myxosoma cerebralis* spores which mimic tissue.

**Figure 3** Indirect fluorescent antibody tests with monoclonal and polyclonal antibodies against *M. rotundus*. (a) Localization of the antigens on *M. rotundus* spore probed with PAbs (bar = 7 µm); (b) reaction of PAbs with the trophozoite of *M. rotundus* (bar = 10 µm); (c) reaction of PAbs with the cyst capsule of *M. rotundus* (bar = 15 µm); (d) incubation of MAb 1A9 with *M. rotundus* spore, IFAT indicates that MAb 1A9 binds to symmetric antigens (arrowed) on the two valves of the spore (bar = 7 µm); (e) concentration of fluorescence on the endoplasm (arrowed) of the trophozoite probed by MAb 2D12 (bar = 6 µm); (f) MAb 2D12 conjugated symmetric antigens (arrowed) on *M. rotundus* spore (bar = 8 µm).


Received: 26 July 2001
Accepted: 20 December 2001