

Immunocytochemical Detection of Recombinant Biomphalysin on *Schistosoma mansoni* Sporocysts

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[Abstract] Schistosomiasis, or bilharzia, is a tropical disease caused by worms of the genus *Schistosoma* which infect about 200 million people. The life cycle of the parasite requires *Biomphalaria*, a specific genus of freshwater snails, as intermediate. Using an interactome approach employing *B. glabrata* plasma and *S. mansoni* primary sporocyst extracts, we identified a new cytolytic protein called Biomphalysin that displays similarities to members of the β -PFT superfamily known to form channels in targeted membranes. To investigate its mechanism of action, we produced a recombinant protein flanked by an N-terminal 6 histidine tag. Then, we investigated the ability of Biomphalysin to interact with the sporocyst tegument. This optimized protocol describes an immunocytochemical procedure to detect histidine tagged recombinant protein on the sporocyst tegumental membrane.

Materials and Reagents

1. Paraformaldehyde (PAF) (Sigma-Aldrich, catalog number: 158127)
2. PBS (Sigma-Aldrich, catalog number: P4417)
3. BSA (Sigma-Aldrich, catalog number: A3803)
4. poly-D-Lysine-coated slides (culture slides) (BD Biosciences, Falcon®, catalog number: 354632)
5. Mouse anti-HisG monoclonal antibody (Life Technologies, catalog number: R940-25)
6. Alexa Fluor 594 goat anti-mouse IgG (Life Technologies, catalog number: A 110005)
7. Dako fluorescent mounting medium (Dako, catalog number: S3023)
8. Primary sporocysts of *S. mansoni* (Brazilian strain) used for immunocytochemical experiments were obtained by transferring miracidia to Chernin's balanced salt solution (CBSS) and maintaining at 26 °C under normoxic conditions for 24 h (Yoshino and Laursen, 1995). Then, 100 primary sporocysts were incubated for 1 h with 30 nM of recombinant Biomphalysin protein (Galiner *et al.*, 2013). As negative control, 100 sporocysts were used without having been treated with recombinant biomphalysin.

9. N terminal His(6)-tagged biophalysin was expressed *in vitro* using the Rapid Translation System (RTS 500 Wheat Germ CECF Kit) (5 PRIME, catalog number. 2402500)
10. 4% PAF (see Recipes)
11. PBS/3% BSA (see Recipes)
12. PBS/1% BSA (see Recipes)
13. Anti- HisG antibody 1:500 (see Recipes)
14. Anti-mouse IgG 1:1,000 (see Recipes)

Equipment

1. Coverslip 24 x 60 mm (VWR International, catalog number: 631-1575)
2. BD BioCoat™ Poly-D-Lysine 8-well CultureSlides (Becton, Dickinson and Company, catalog number: 354632)
3. Eppendorf centrifuge (Eppendorf , model: 5810R)
4. Swinging agitator (Fisher scientific, model: 10758995)
5. Fluorescence confocal laser-scanning microscope (ZEISS, model: LSM 700)

Procedure

1. Collect 100 sporocysts and transfer to culture slide coated with poly-D-Lysine.
2. Centrifuge culture slide at 800 x g for 2 min.
3. Aspirate supernatant and fix sporocysts with 200 µl 4% PAF during 1 hour at room temperature.
4. Centrifuge culture slide at 800 x g for 2 min and aspirate supernatant.
5. Wash twice with 200 µl PBS and repeat step 4.
6. Add 200 µl PBS/3% BSA and incubate for 2 h at room temperature without agitation.
7. Centrifuge culture slide at 800 x g for 2 min and aspirate supernatant.
8. Add 200 µl anti-His antibody diluted at 1:500 in PBS and incubate 1.5 h at room temperature with a shaking speed of approximately 12 oscillations per minute.
9. Wash three times with 200 µl PBS during 5 min with a shaking speed of 12 oscillations per minute and between wash, centrifuge culture slide.
10. Incubate sporocysts with 200 µl Alexa Flour 594-conjugated anti-mouse IgG diluted 1:1,000 in PBS/1% BSA for 45 min at room temperature and protect slides from light with a shaking speed of 12 oscillations per minute.
11. Repeat step 9.
12. Place 2 drops of Dako fluorescent mounting medium on slide and cover with a coverslip.

13. Leave mounted slide overnight at 4 °C and protect slides from light before observation.

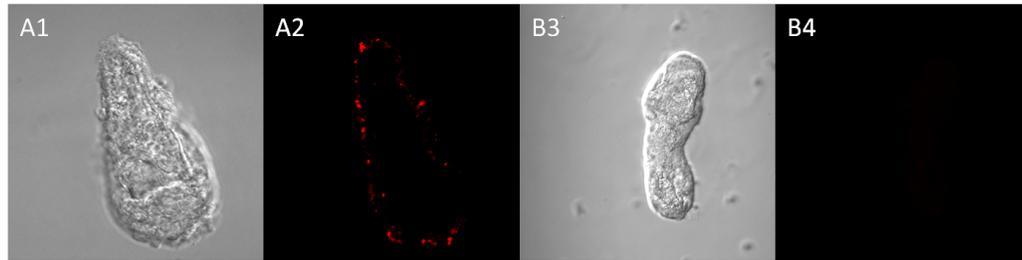


Figure 1. Immunolocalization of recombinant Biomphalysin on *S. mansoni* sporocyst. Sporocysts were treated or not with recombinant Biomphalysin (A : positive and B ; negative control, respectively) and immunostained using anti-His primary IgG and Alexa Fluor 594-conjugated secondary antibodies. Images 1 and 3 were taken under Nomarski light microscopy; images 2 and 4 under using a fluorescence confocal laser-scanning microscope (Zeiss LSM 700).

Recipes

1. 4% PAF
Dissolve 4 mg PAF in 100 ml PBS (heat to 60 °C for 1 h with stirring)
The cooled solution can be filtered and stored at -20 °C
2. PBS/3% BSA
0.3 mg BSA in 10 ml PBS
3. PBS/1% BSA
0.1 mg BSA in 10 ml PBS
4. Anti- His antibody 1:500
0.5 µl anti-his antibody in 250 µl PBS only
5. Anti-mouse IgG 1:1,000
0.5 µl anti-mouse IgG in 250 µl PBS/1%BSA

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