Effects of Mannose on Pathogenesis of Acanthamoeba castellanii

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Abstract: Acanthamoeba spp. are single-celled protozoan organisms that are widely distributed in the environment. In this study, to understand functional roles of a mannose-binding protein (MBP), Acanthamoeba castellanii was treated with methyl-alpha-D-mannopyranoside (mannose), and adhesion and cytotoxicity of the amoeba were analyzed. In addition, to understand the association of MBP for amoeba phagocytosis, phagocytosis assay was analyzed using non-pathogenic bacterium, Escherichia coli K12. Amoebae treated with mannose for 20 cycles exhibited larger vacuoles occupying the most area of the amoebic cytoplasm in comparison with the control group amoebae and glucose-treated amoebae. Mannose-selected amoebae exhibited lower levels of binding to Chinese hamster ovary (CHO) cells. Exogenous mannose inhibited >50% inhibition of amoebae (control group) binding to CHO cells. Moreover, exogenous mannose inhibited amoebae (i.e., man-treated) binding to CHO cells by <15%. Mannose-selected amoebae exhibited significantly decreased cytotoxicity to CHO cells compared with the control group amoebae, 25.1% vs 92.1%. In phagocytic assay, mannose-selected amoebae exhibited significant decreases in bacterial uptake in comparison with the control group, 0.019% vs 0.03% (P < 0.05). Taken together, it is suggested that mannose-selected A. castellanii trophozoites should be severely damaged and do not well interact with a target cell via a lectin of MBP.

Key words: Acanthamoeba castellanii, mannose-binding protein, adhesion, cytotoxicity, Escherichia coli K12
The percentage of LDH release was calculated as follows: (LDH activity in control samples × 100) / total LDH activity in experimental sample. The supernatants were collected and examined for host cell cytotoxicity by measuring lactate dehydrogenase (LDH) activity at 492 nm. The percent LDH release was calculated as follows: No. of unbound amoebae / total number of amoebae × 100 = % unbound amoebae. The numbers of bound amoebae were deduced as follows: 100% - unbound amoebae = % bound amoebae. To determine the effects of exogenous saccharides on amoebae binding to the host cells, adhesion assays were performed in the presence of mannose (100 mM final conc.). Briefly, amoebae were pre-incubated with the saccharides for 1 hr at room temperature. Following this incubation, amoebae plus saccharides were transferred to CHO cell monolayers and adhesion assays performed as described above.

To determine the ability of Acanthamoeba to produce host cell death, cytotoxicity assay was performed as previously described [9]. Adhesion assay was done, and the plates were observed periodically for monolayer disruptions under a phase contrast microscope for up to 24 hr. Following this incubation, the supernatants were collected and examined for host cell cytotoxicity by measuring lactate dehydrogenase (LDH) activity at 492 nm. The percent LDH release was calculated as follows: (LDH activity in control samples × 100) / total LDH activity in experimental sample.
Fig. 1. Morphologic changes of *A. castellanii* trophozoites by adding mannose. Mannose was added to the amoebae for 24 hr, resulting in 1 cycle. At the end of 20 cycles, the following resultant amoebae were obtained. (A) control group, (B) glucose-treated amoebae, and (C) mannose-treated amoebae. Arrows indicate larger vacuoles in cytoplasm of mannose-selected amoebae. × 250.

Fig. 2. Adhesion and cytotoxicity assay of mannose-selected *A. castellanii* trophozoites to CHO cells. After mannose-selected amoebae (MS) were incubated with CHO cells for 1 hr at 37°C, adhesion assays were performed (A). Also, they were pre-incubated with exogenous saccharides for 1 hr and then added to CHO cells for 1 hr as mentioned in materials and methods. Cytotoxicity was measured post co-incubation of 24 hr (B). Results are representative of 3 independent experiments performed in triplicate. Asterisks indicate a significant difference, i.e., *P* < 0.05, using the paired t-test, one-tail distribution.

Fig. 3. Phagocytosis of mannose-selected *A. castellanii* trophozoites using non-invasive *E. coli* K12. To determine the role of a mannose-binding protein in mannose-selected amoebae (MS), phagocytosis assays were performed. (A) represents bacterial association with amoebae and (B) represents ratio of bacteria per amoeba. Results are representative of 3 independent experiments performed in triplicate. Asterisks indicate a significant difference, i.e., *P* < 0.05, using the paired t-test, one-tail distribution.

(i.e., man-treated) binding to CHO cells by <15% (Fig. 2A).

Next, to determine the ability of control group and mannose-selected amoebae to produce host cell death, cytotoxicity assays were performed. As described in Fig. 3, mannose-select-
ed amoebae exhibited significantly decreased cytotoxicity to CHO cells compared with the control group amoebae, 25.1% vs 92.1% (Fig. 2B) (P < 0.05; using the paired t-test, one-tail distribution). The cytotoxicity of control group and mannose-selected amoebae pre-incubated with mannose was 45.8% and 24.3%, respectively (Fig. 2B). Overall, these findings showed that mannose-selected amoebae showed severely affected cytotoxic functions.

To determine the phagocytic ability of the control group and mannose-selected amoebae, phagocytosis assays were performed using live *E. coli* K12. The experiment demonstrated that mannose-selected amoebae exhibited significant decreases in bacterial uptake compared to control group, 0.019% vs 0.03% (P < 0.05) (Fig. 3A). Interestingly, bacteria and amoebae ratio also decreased in mannose-selected amoebae compared with the control group amoebae (Fig. 3B).

The MBP of *A. castellanii* is thought to play a key role in the pathogenesis of the infection by mediating the adhesion of parasites to the host cells. The isolation by chromatography on mannose affinity gel and molecular cloning revealed that about 400 kDa proteins constituted multiple 130 kDa subunits. In addition, they also composed of 3.6 kb of the amoeba genome, and therefore, included the coding for a precursor protein of 833 amino acids [11]. Other MBP cloning was reported sequenced with 1,081 nucleotides coding for 194 amino acids from *A. castellanii* [12]. Through gas chromatography combined with mass spectrometry, the carbohydrate composition of cyst walls of *Acanthamoeba* revealed a high percentage of galactose and glucose and small amounts of mannose and xylose [13].

In this present study, we demonstrated that the pathogenic potential of *A. castellanii* is severely damaged by saturating MBP with mannose. Interestingly, a long time treatment of mannose to *A. castellanii* trophozoite forms induced larger vacuoles than control group and glucose-treated amoebae. It implies that exogenous mannose would change the composition of amoeba cytoplasm or act as a nutrient. MBP is located on extracellular matrices of *A. castellanii* and may play a role in contact as a manner of extracellular protein interactions. Thus, we employed adhesion and cytotoxicity assays to elucidate functional roles of MBP. Mannose-selected amoeba exhibited lower adhesion and cytotoxicity than the control group. Moreover, exogenous mannose was pre-incubated with amoeba for 1 hr and then added to CHO target cells. The results showed that the adhesion and cytotoxicity of control group and mannose-selected amoebae pre-incubated with exogenous mannose were more decreased as amoebae were not added with exogenous mannose. Using the bacteria *E. coli* K12 as a nutrient or prey, the phagocytic activity of mannose-selected amoeba was severely damaged. The data demonstrating the pathogenic potential of *A. castellanii* trophozoites are associated with the mannose-binding protein and provide the candidate of pathogenic target to further target-based therapy.

**ACKNOWLEDGMENT**

Funding for this paper was provided by Namseoul University.

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