

## Protective Effects of a Monoclonal Antibody to a Mannose-Binding Protein of *Acanthamoeba culbertsoni*

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*Acanthamoeba culbertsoni* is the causative agent of granulomatous amoebic encephalitis (GAE), a condition that predominantly occurs in immunocompromised individuals and which is typically fatal. A mannose-binding protein (MBP) among lectins was shown to have strong *A. castellanii* pathogenic potential when correlated with major virulence proteins. In this study, protective effects were analyzed using the monoclonal antibody to *A. culbertsoni* MBP by quantification and were also compared with other free-living amoebae. For the amoebial cytotoxicity to the target cell, amoeba trophozoites were incubated with Chinese hamster ovary (CHO) cells. For the protective effects of antibodies, amoebae were pre-incubated with them for 4 h and then added to the target cells. After 24 h, the supernatants were collected and examined for host cell cytotoxicity by measuring lactate dehydrogenase (LDH) release. The cytotoxicity of *A. culbertsoni* to the CHO cells showed about 87.4%. When the monoclonal antibody was pre-incubated with *A. culbertsoni*, the amoebial cytotoxicity was remarkably decreased as shown at LDH release (1.858 absorbance), which was represented with about 49.9%. Taken together, it suggested that the monoclonal antibody against MBP be important to inhibit the cytotoxicity of *A. culbertsoni* trophozoites to the target cell. The antibody will be applied into an *in vivo* functional analysis, which would help to develop therapeutics.

**Key Words:** *Acanthamoeba culbertsoni*, Mannose-binding protein, Monoclonal antibody, Cytotoxicity

*Acanthamoeba culbertsoni* is the causative agent of granulomatous amoebic encephalitis (GAE), a condition that predominantly occurs in immunocompromised individuals and which is typically fatal (Schuster and Visvesvara, 2004; Walochnik et al., 2008).

*Acanthamoeba* has been of interests due to (i) produce serious human infections associated with a rise in the number of immunocompromised patients and contact lense wearers, (ii) its potential role in ecosystems and (iii) its ability to act as a host/reservoir for microbial pathogens (Khan, 2006).

Lectins are well known to be important to induce cytotoxicity in target cells by *A. castellanii* causing amoebic keratitis (AK). Among them, a mannose-binding protein (MBP) was shown to have strong *A. castellanii* pathogenic potential when correlated with major virulence proteins (Garate et al., 2006; Panjwani, 2010; Kim et al., 2012). The MBP was cloned and revealed to possess about 130 kDa-molecular weight (MW). However, in *A. culbertsoni*, it is not well reported about the MW and functional roles yet. Recently, we reported the production of the monoclonal antibody to *A.*

Received: November 1, 2018 / Accepted: December 6, 2018

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**Table 1. Protective effects of DG11 to the cytotoxicity of *A. culbertsoni* to CHO cells.** *A. culbertsoni* was pre-incubated with DG11 of a monoclonal antibody for 4 h and then LDH release was measured. Lysed CHO; CHO cells treated with a lysis buffer for 30 min. Lysed *A. culbertsoni*; *A. culbertsoni* treated with a lysis buffer of Triton X-100 for 30 min at 37 °C., SD; standard deviation. Data were absorbances measured under 490 nm. The experiments were performed in triplicate three times

Exp.	Medium	CHO	Lysed CHO	Lysed <i>A. culbertsoni</i>	CHO + <i>A. culbertsoni</i>	CHO + <i>A. culbertsoni</i> + polyclonal serum	CHO + <i>A. culbertsoni</i> + DG11
1 <sup>st</sup>	0.738	1.541	2.911	0.820	3.200	1.770	1.867
2 <sup>nd</sup>	0.742	1.563	2.955	0.827	3.255	1.899	1.809
3 <sup>rd</sup>	0.759	1.712	2.886	0.770	3.304	1.919	1.898
Mean	0.746	1.605	2.917	0.806	3.253	1.863	1.858
SD	0.011	0.093	0.035	0.031	0.052	0.081	0.045

*culbertsoni* MBP in 2018 (Kang et al., 2018). Antibodies against *Naegleria fowleri* antigens, another pathogenic free-living amoeba have proven useful to diagnose infections in experimental animals (Ryu and Im, 1992; Lee, 2007). In this study, protective effects were analyzed using the monoclonal antibody to *A. culbertsoni* MBP by quantification and were also compared with other free-living amoebae.

*A. culbertsoni* trophozoites (ATCC NO. 30171; Kim et al., 1988; Kong et al., 1993) were grown without shaking in 12 mL of PYG medium (proteose peptone 0.75% (w/v) (Kisan Bio, Seoul, Korea), yeast extract 0.75% (w/v) (Kisan Bio, Seoul, Korea) and glucose 1.5% (w/v) (Sigma-Aldrich Co., St. Louis, MO, USA)) in a 75T culture flask at 25 °C. *A. castellanii* (ATCC NO. 50492), In addition, briefly, pathogenic *N. fowleri* (Carter Nf69 strain, ATCC 30215) was axenically cultured at 37 °C in Nelson's medium (Seong et al., 2017). CHO cells were cultured with Earle's minimum essential medium (EMEM) containing 10% fetal bovine serum at 37 °C in a 5% CO<sub>2</sub> incubator (Kang et al., 2005).

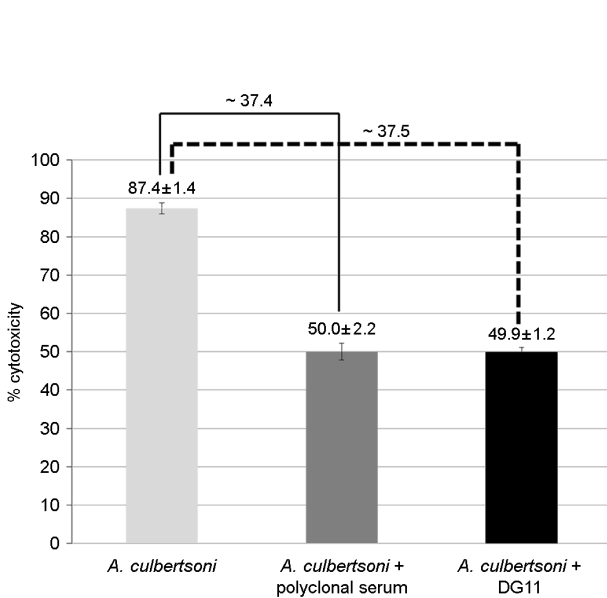
To determine the protective effects of the monoclonal antibody which was produced and cloned, that is, DG11, cytotoxicity assay was performed as previously described (Sissons et al., 2005). The DG11 monoclonal antibody was classified with IgM isotype (Kang et al., 2018). In brief, for the amoebial cytotoxicity to the target cell, amoeba trophozoites ( $2 \times 10^5$  amoebae/0.5 mL/well) were incubated with CHO cells in EMEM. The plates were observed periodically for monolayer disruptions under a phase-contrast microscope for up to 24 h. For the protective effects of antibodies,

amoebae were pre-incubated with them for 4 h and then added to the target cells. After 24 h, the supernatants were collected and examined for host cell cytotoxicity by measuring lactate dehydrogenase (LDH) release (cytotoxicity detection kit; Roche Applied Science, Lewes, East Sussex, UK). In brief, the supernatants of the co-cultures were assessed for the presence of lactate dehydrogenase (LDH), the release of which is considered an estimate of cell death. The percentage LDH release which was measured by absorbance under 490 nm was calculated as follows: experimental LDH  $\times$  100% / maximum LDH = % cytotoxicity.

In results, with regarding the cytotoxicity of *A. culbertsoni*, CHO cells were shown morphologically severe destruction as observed at 24 h incubation (data not shown). The destruction of the CHO cells was described with LDH release shown at Table 1. The CHO cells by co-incubation of *A. culbertsoni* trophozoites were proven mostly death (2.917 absorbance) as similar with whole death of LDH complete lysis (3.253 absorbance). The LDH release was calculated into % cytotoxicity (Fig. 1). That is, the cytotoxicity of *A. culbertsoni* to the CHO cells showed about 87.4%. When the monoclonal antibody of DG11 was pre-incubated with *A. culbertsoni*, the amoebial cytotoxicity was remarkably decreased as shown at LDH release (1.858 absorbance) in Table 1, which was represented with about 49.9% at Fig. 1. On the other hand, polyclonal serum obtained from BALB/c mice 4 weeks post-injection with MBP antigen also showed protective effects (about 50%) as similar with the monoclonal antibody of DG11 at Fig. 1. Based on the % cytotoxicity at

Fig. 1, the protective effects of the monoclonal antibody of DG11 were shown about 37.5%. Taken together, it suggested that the monoclonal antibody of DG11 be important to inhibit the cytotoxicity of *A. culbertsoni* trophozoites to the target cell.

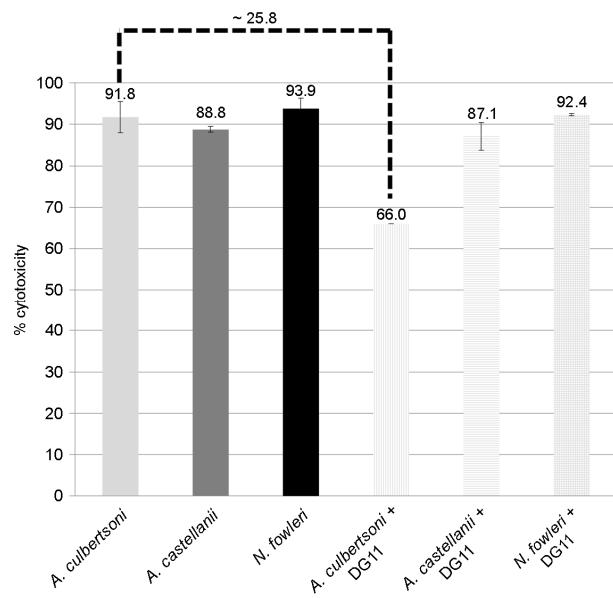
The monoclonal antibody of DG11 is reported *A. culbertsoni* MBP-specific. To understand the cross-reactivity by the cytotoxicity assay, LDH assay was performed using other pathogenic free-living amoebae, e.g., *A. castellanii*, *N. fowleri*. The LDH release could be useful to analyze the cytotoxicity and protectivity with numerical value for the quantification. As shown at Table 1 and Fig. 1, the cytotoxicity of *A. culbertsoni* and protective effects of the monoclonal antibody of DG11 were similar in Table 2 and Fig. 2.



**Fig. 1. Cytotoxicity of *A. culbertsoni* and protective effects of DG11 to the amoeba.** Based on the LDH assay shown at Table 1, the cytotoxicity was calculated with percent value.

With *A. culbertsoni*, *A. castellanii* and *N. fowleri* induced huge cytotoxicity over about 87%. However, the protective effects of the monoclonal antibody of DG11 were not observed in the cytotoxicity of *A. castellanii* and *N. fowleri* at Table 2 and Fig. 2. It implied that the monoclonal antibody of DG11 be *A. culbertsoni* MBP-specific.

Contact-dependent pathway of *A. culbertsoni* via saccharides or proteins has been of interest. It was reported that other pathogenic *A. castellanii* have strong associated with MBP in the contact-dependent pathway (Kim et al., 2012). Recently, MBP of *A. culbertsoni* was purified and the monoclonal antibody of DG11 was produced and characterized



**Fig. 2. Comparison of cytotoxicity by other free-living amoebae and the protective effects of DG11.** Based on the LDH assay shown at Table 2, the cytotoxicity was calculated with percent value.

**Table 2. Comparison of protective effects of DG11 using other free living amoebae.** Other three pathogenic strains were applied. Data were absorbances measured under 490 nm. The experiments were performed in triplicate three times as shown at Table 1

Exp.	Medium	CHO	Lysed CHO	Lysed <i>A. culbertsoni</i>	Lysed <i>A. castellanii</i>	Lysed <i>N. fowleri</i>	CHO + <i>A. culbertsoni</i>	CHO + <i>A. castellanii</i>	CHO + <i>N. fowleri</i>	CHO + <i>A. culbertsoni</i> + DG11	CHO + <i>A. castellanii</i> + DG11	CHO + <i>N. fowleri</i> + DG11
1 <sup>st</sup>	0.555	1.231	2.999	0.598	0.515	0.502	3.000	2.875	3.131	2.100	2.920	2.989
2 <sup>nd</sup>	0.458	1.234	2.567	0.675	0.599	0.563	3.215	2.901	3.011	2.313	2.888	2.991
3 <sup>rd</sup>	0.525	1.451	2.498	0.700	0.612	0.591	2.997	2919	2.984	2.208	2.719	3.003
Mean	0.513	1.305	2.688	0.658	0.575	0.552	3.071	2.898	3.042	2.207	2.842	2.994
SD	0.050	0.126	0.272	0.053	0.053	0.046	0.125	0.022	0.078	0.107	0.108	0.008

with IgM (Kang et al., 2018). The monoclonal antibody of DG11 possessed kappa chain and about 83 kDa (Kang et al., 2018).

In *A. culbertsoni* trophozoite, MBP concentrated in the movement would be associated with the adhesion of the amoeba to a host cell, leading to amoebial cytotoxicity. It was guided that if treated with antibodies, the cytotoxicity of *A. culbertsoni* would be inhibited. The protective effects of the monoclonal antibody of DG11 were shown about 37.5%, which implied that MBP should be absolutely associated with adhesion and further amoebial cytotoxicity.

Rather than optical finding such as immunofluorescence for the cross-reactivity and specificity, quantification is needed for better understanding. The LDH release showed numerical results of the amoebial cytotoxicity and protective effects of the monoclonal antibody of DG11. As a result, other amoebae of *A. castellanii* and *N. fowleri* did not show its protective effect against *A. culbertsoni*-specific MBP.

The antibody will be applied into an *in vivo* functional analysis, which would help to develop therapeutics.

#### ACKNOWLEDGMENT

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017-R1D1A1A02018651).

#### CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

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<https://doi.org/10.15616/BSL.2018.24.4.435>

**Cite this article as:** Park AY, Kang AY, Jung SY. Protective Effects of a Monoclonal Antibody to a Mannose-Binding Protein of *Acanthamoeba culbertsoni*. *Biomedical Science Letters*. 2018. 24: 435-438.