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Protein profiles and immunoreactivities of *Acanthamoeba* morphological groups and genotypes

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HIGHLIGHTS

- *Acanthamoeba* protein profiles correlate to genotype system.
- *Acanthamoeba* protein profiles correlate to gross morphological groups.
- Group I acanthamoebae show only very weak immunoreactivities.

GRAPHICAL ABSTRACT

Group	Genotype	Strain	Origin	Major protein bands (kDa)	Major immunoreactive bands (kDa)	Comments
I	T7	Pb30/40	Physiotherapy pool	38, 42	42	Weak immunoreactivities, particularly for IgM and IgA
I	T9	Pj	Soil	(35), 38, 42	(35), 42	Weak immunoreactivities, particularly for IgM and IgA
II	T4	2HH	Keratitis patient, cornea	42, 44, 62	29, 33, 38, 39, 42, 46, 130, 154	High immunoreactivities, particularly for IgM and IgA
II	T4	1BU	Keratitis patient, cornea	33, 42, 44, 50	29, 33, 36, 38, 42, 47, 63	High IgA immunoreactivities
II	T4	3ST	Keratitis patient, cornea	33, 42, 44, 50	29, 33, 36, 38, 47	High IgA immunoreactivities
II	T4	PAT06	Keratitis patient, cornea	33, 42, 44, 50	29, 33, 38, 42, 71	IgM immunoreactivities in the high range
II	T4	9GU	Contact lens case	33, 42, 44, 50	29, 33, 36, 38,47	High IgA immunoreactivities
II	T4	4CL	Contact lens case	33, 42, 44, 50	29, 33, 36, 38, 47	High IgA immunoreactivities
II	T4	NEFF	Soil	33, 42, 44, 50	29, 33, 38	Weak immunoreactivities
II	T11	ZOO9	Anaconda tissue	42	42, 46, 130	Weak immunoreactivities, particularly for IgM and IgA
II	T12	BUD 9	Hot tub	(35), 42, 47, 62	(35), 42, 47	Weak IgM immunoreactivities
III	T5	72/2	Mouse brain	42, 47, 62	39, 42, 47, 130, 154	High IgA immunoreactivities
III	T6	11DS	Keratitis patient, cornea	42, 47, 62	29, 39, 42, 47, 130, 154	High IgA immunoreactivities

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ABSTRACT

Acanthamoeba is a free-living protozoan found in a wide variety of habitats. A classification of *Acanthamoeba* into currently eighteen genotypes (T1–T18) has been established, however, data on differences between genotypes on the protein level are scarce. The aim of this study was to compare protein and immunoreactivity profiles of *Acanthamoeba* genotypes. Thirteen strains, both clinical and non-clinical, from genotypes T4, T5, T6, T7, T9, T11 and T12, representing three morphological groups, were investigated for their protein profiles and IgG, IgM and IgA immunoreactivities. It was shown that protein and immunoreactivity profiles of *Acanthamoeba* genotypes T4, T5, T6, T7, T9, T11 and T12 are clearly distinct from each other, but the banding patterns correlate to the morphological groups. Normal human sera revealed anti-*Acanthamoeba* antibodies against isolates of all investigated genotypes, interestingly, however only very weak IgM and virtually no IgA immunoreactivity with T7 and T9, both representing morphological group I. The strongest IgG, IgM and IgA immunoreactivities were observed for genotypes T4, T5 and T6. Differences of both, protein and immunological patterns, between cytopathic and non-cytopathic strains, particularly within genotype T4, were not at the level of banding patterns, but rather in expression levels.

1. Introduction

Acanthamoebae are found in various habitats and are known as potential pathogens (Boost et al., 2008; Booton et al., 2009). In healthy individuals, *Acanthamoeba* can cause *Acanthamoeba* keratitis (AK), a seriously progressing inflammation of the cornea occurring predominantly in contact lens wearers (Illingworth and Cook, 1998). In immunocompromised individuals, acanthamoebae can cause several highly destructive disseminating infections, including skin lesions, pneumonitis and granulomatous amoebic encephalitis (GAE) (Martinez and Visvesvara, 1997).

Acanthamoebae are widely spread throughout the environment, thus exposure of humans to *Acanthamoeba* antigens and

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the presence of antibodies against them in serum samples of healthy individuals are common (Cursons et al., 1980; Walochnik et al., 2001a,b). The genus *Acanthamoeba* has been classified into three groups, based on size and shape of the cysts (Pussard and Pons, 1977), and into 18 genotypes, based on ⩾5% of 18S rDNA sequence dissimilarity between genotypes (Corsaro and Venditti, 2010; Gast et al., 1996; Hewett et al., 2003; Horn et al., 1999; Qvarnstrom et al., 2013; Stothard et al., 1998; Nuprasert et al., 2010). The genotype classification, however, in most cases does not correlate with the morphology-based species designations, nor with pathogenicity or virulence traits. The aim of this study was to evaluate possible differences in protein and immunoreactivity profiles of different *Acanthamoeba* genotypes, including both clinical and non-clinical isolates. A previous study, including three different genotypes, indicated considerable differences in immunoreactivities between the genotypes (Walochnik et al., 2001a). We now examined the profiles of 13 strains from seven different genotypes, including T4, T5, T6, T7, T9, T11 and T12.

2. Materials and methods

2.1. Amoeba strains

A total of 13 *Acanthamoeba* isolates, both clinical and non-clinical, of seven different genotypes representing all three morphological groups were examined. Summary information of all investigated strains is given in Table 1. Briefly, strain NEFF (T4) was isolated from soil more than 50 years ago (Neff, 1957) and was purchased from the ATCC. Strains 72/2 (T5) and Pb30/40 (T7) were kindly provided by Dr. Michel (Koblenz, Germany). Strain 72/2 (T5) was originally isolated from the nasal mucosa of a healthy individual but had proven to be highly virulent in mice (Michel et al., 1982; De Jonckheere and Michel, 1988). The strain used in this study is the reisolate from the mouse brain. Strain Pb30/40 (T7) was originally isolated from a greenhouse (Michel et al., 2004). Strains 4CL (T4), 9GU (T4), 2HH (T4), 1BU (T4), 3ST (T4), 11DS (T6) were isolated in our laboratory between 1997 and 1999 (Walochnik et al., 2000a,b) and strains PAT06 (T4), Pj (T9) (Ertabaklar et al., 2007), ZOO9 (T11) and Bud9 (T12) were isolated in our laboratory in the years 2006 and 2009, respectively.

2.2. Cultivation of amoebae

All amoeba strains except Pj (T9) and Bud9 (T12) were cultured in sterile filtrated proteose peptone yeast extract-glucose medium (Visvesvara and Balamuth, 1975) in 150 cm^2 tissue culture flasks (ASAI Glass, Osaka, Japan) at room temperature (RT). Trophozoites were harvested from extensively growing cultures by centrifugation at 2,500 rpm for 10 min. The cell pellets were washed 3 times with PBS and then used for protein extraction.

Strains Bud9 (T12) and Pj (T9) do not grow axenically and were thus cultured on plates (Walochnik et al., 2000b) and harvested at extensive growth using several parallel plate cultures for one preparation to assure sufficient cell numbers. Trophozoites were washed 3 times with PBS using centrifugation at 1,000 rpm for 10 min and then the pellets were used for protein extraction.

2.3. Isolation of proteins

Proteins of 10^7 trophozoite cells were extracted using trichloroacetic acid (TCA)/Acetone (Leitsch et al., 2005). Cell pellets were resuspended in 10% (w/v) TCA in acetone followed by vortexing. Proteins were precipitated at −20 °C for 90 min and pelleted by centrifugation at 12,000 rpm for 30 min at 4 °C. The pellets were washed twice with 90% (v/v) acetone in ddH_2O. Each washing step

Table 1
Detailed information on the studied *Acanthamoeba* strains, their major protein as well as immunoreactive bands (given in approximate kDa values).

Group	Geno-type	Strain	Origin	Major protein bands (kDa)	Major immunoreactive bands (kDa)	Comments	ATCC number	GenBank accession number	Source of references
I	T7	Pb30/40	Greenhouse	38, 42	42	Weak immunoreactivities, particularly for IgM and IgA	–	–	Michel et al. (2004)
I	T9	Pj	Soil	(35), 38, 42	(35), 42	Weak immunoreactivities, particularly for IgM and IgA	–	DQ185605	Ertabaklar et al. (2007)
II	T4	2HH	Keratitis patient, cornea	42, 44, 62	29, 33, 38, 39, 42, 46, 130, 154	High immunoreactivities, particularly for IgM and IgA	PRA-113	AF260722	Walochnik et al. (2000b)
II	T4	1BU	Keratitis patient, cornea	33, 42, 44, 50	29, 33, 36, 38, 42, 47, 63	High IgA immunoreactivities	PRA-105	AF260721	Walochnik et al. (2000b)
II	T4	3ST	Keratitis patient, cornea	33, 42, 44, 50	29, 33, 36, 38, 47	High IgA immunoreactivities	PRA-114	AF260723	Walochnik et al. (2000b)
II	T4	PAT06	Keratitis patient, cornea	33, 42, 44, 50	29, 33, 38, 42, 71	IgM immunoreactivities in the high range	–	EF429131	Koehsler et al. (2008)
II	T4	9GU	Contact lens case	33, 42, 44, 50	29, 33, 36, 38, 47	High IgA immunoreactivities	PRA-108	AF251938	Walochnik et al. (2000a)
II	T4	4CL	Contact lens case	33, 42, 44, 50	29, 33, 36, 38, 47	High IgA immunoreactivities	PRA-107	AF260724	Walochnik et al. (2000b)
II	T4	NEFF	Soil	33, 42, 44, 50	29, 33, 38	Weak immunoreactivities	50373	U07416	Neff (1957)
II	T11	ZOO9	Anaconda tissue	42	42, 46, 130	Weak immunoreactivities, particularly for IgM and IgA	–	–	Pumidonming et al. (2010)
II	T12	BUD 9	Hot tub	(35), 42, 47, 62	(35), 42, 47	Weak IgM immunoreactivities	–	–	New isolate
III	T5	72/2	Mouse brain	42, 47, 62	39, 42, 47, 130, 154	High IgA immunoreactivities	50704	U94732	Michel et al. (1982)
III	T6	11DS	Keratitis patient, cornea	42, 47, 62	29, 39, 42, 47, 130, 154	High IgA immunoreactivities	PRA-112	AF251939	Walochnik et al. (2000a)