

Epidemiological and pathological investigations on free-living amoebae (FLA)

1. INTRODUCTION

Some of the free-living amoebae (FLA) such as *Acanthamoeba* spp. and *Naegleria fowleri*, harbour the potential to induce opportunistic and non-opportunistic infections in humans and animals. In fact, *Acanthamoeba* spp. are opportunistic protozoan pathogens causing infections of the central nervous system, lungs, sinuses and skin, mainly in immunocompromised individuals. Moreover, *Acanthamoeba* spp. also causes *Acanthamoeba* keratitis in immunocompetent humans. *N. fowleri* on the other hand causes an acute and fulminating, mostly fatal primary amoebic meningoencephalitis in immunocompetent children and young adults. Being free-living and widely distributed in nature, these protozoan parasites represent a large and important part of the fauna of practically any environmental biotope.

2. OUR RESEARCH

2.1 Screening of Swiss water bodies for free-living amoebae and analysis of the pathogenic potential of respective isolates (research until 2009)

Human infections with pathogenic FLA have been increasingly documented in various countries. In Switzerland, so far, the occurrence and distribution of potentially pathogenic FLA has not been investigated yet. For this reason, a selection of Swiss water biotopes, including swimming pools, lakes, rivers and ponds, has recently been screened by us for the presence of FLA, and subsequent assessments of their potential of pathogenicity for a mammalian host has been undertaken. Thus, in a first examination a variety of isolates were recovered by in vitro cultivation from these different aquatic biotopes (Gianinazzi et al., 2009a). Characterization by sequence analysis of *Acanthamoeba* spp.-specific and `FLA`-specific PCR products amplified from 18s rDNA, by morphological traits, thermotolerance, and cytotoxicity towards murine fibroblasts cells yielded the following findings: *Echinamoeba exundans*, *Hartmannella* spp., *Vannella* spp., *Protacanthamoebica bohemica*, *Acanthamoeba castellanii*, and *Naegleria* spp.. *N. fowleri* and other pathogenic FLA i.e. *Balamuthia mandrillaris* and *Sappinia pedata* did not range amongst these isolates. None of the isolates exhibited pronounced cytotoxicity upon 5-day exposure to murine L929 fibroblasts, all failed to grow at 42°C, and therefore do not present any potential of CNS-pathogenicity for humans.

Conversely, another investigation focused on public heated swimming pools in Switzerland in fact led to the isolation of a potentially human-pathogenic strain identified as *Acanthamoeba lenticulata* (Gianinazzi et al., 2009b). This strain turned out to be thermotolerant, cytotoxic and caused a lethal brain pathology in nasally-infected Rag2-immunodeficient mice. Similar results were achieved when a screening for potentially pathogenic FLA in four hot spring resorts in Switzerland was performed (Gianinazzi et al., 2009c). Water samples were taken from water filtration units and from the pools, respectively. Amoebae isolated from samples taken during, or before, the filtration process

were demonstrated to be morphologically and phylogenetically related to *Stenoamoeba* sp., *Hartmannella vermiformis*, *Echinamoeba exundans*, and *Acanthamoeba healyi*. Further investigations showed that the isolates morphologically and phylogenetically related to *A. healyi* displayed a pronounced thermotolerance, exhibited a marked *in vitro* cytotoxicity, and was able to kill Rag2-immunodeficient mice.

In summary, isolates related to *A. healyi* and *A. lenticulata* were suggestive of being potentially pathogenic to immunocompromised persons. As far as *A. healyi* is concerned, the presence of respective isolates was limited to the filtration units in the hot spring resorts, and an effective threat for health can therefore be excluded.

2.2. Molecular and biochemical investigation of pathogenesis in the free-living amoeba *Naegleria fowleri* (current and future research)

Recently, we have initiated a study that is focused on the cellular functions involved in pathogenicity of *N. fowleri*. Current knowledge on this subject is restricted to preliminary findings indicating that pore-forming proteins and proteases might play an important role in the pathogenesis of the parasite. However, the particular functions of these proteins, and other as yet unidentified pathogenic factors within this process, still remain to be elucidated.

In our investigations on pathogenesis of *N. fowleri*, we use a well established apathogenic versus pathogenic *in vitro* model of the parasite that includes two important experimental characteristics: (i) *N. fowleri* trophozoites continuously grown under axenic conditions are completely apathogenic and (ii) xenic cultivation (co-cultivation of trophozoites in presence of murine L929 fibroblasts) leads to a conversion of such trophozoites to a highly pathogenic form. In order to identify potential pathogenicity factors of *N. fowleri*, we now have started to compare the gene/protein expression patterns among these two isogenic, but phenotypically distinct, cell lines. Detection of differentially expressed genes/proteins occurs by means of both transcriptomics (microarray and quantitative real-time reverse transcription PCR) and proteomics (2D gel electrophoreses in combination with MALDI-TOF mass spectrometry) technologies.

In an initial approach, we have already analysed by quantitative RT-PCR the differential expression of known genes putatively involved in pathogenesis such as genes encoding naegleriapores A and B, or serine and cysteine proteases. Interestingly, among these genes only the naegleriapore B gene exhibited a massively increased expression in pathogenic trophozoites. This finding suggested naegleriapore B to be a pathogenicity factor that certainly needs further investigation e. g. regarding its possible tissue-destructive function during cerebral naegleriasis in the experimental murine and natural human host.

3. PROJECT-RELATED PUBLICATIONS IN 2009:

Gianinazzi C, Schild M, Wüthrich F, Ben Nour N, Füchslin HP, Schürch N, Gottstein B, Müller N: Screening Swiss water bodies for potentially pathogenic free-living amoebae. *Res Microbiol.* 160: 367-374 (2009a).

Gianinazzi C, Schild M, Wüthrich F, Müller N, Schürch N, Gottstein B: Potentially human pathogenic

Acanthamoeba isolated from a heated indoor swimming pool in Switzerland. *Exp Parasitol.* 121:180-186 (2009b).

Gianinazzi C, Schild M, Zumkehr B, Wüthrich F, Nüesch I, Ryter R, Schürch N, Gottstein B, Müller N: Screening of Swiss hot spring resorts for potentially pathogenic free-living amoebae. *Exp Parasitol* Dec 29 (2009c). [Epub ahead of print]