Investigating the Pathogenicity of *Acanthamoeba* through their immunotranscriptome

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Acanthamoeba castellanii is a facultative free-living protozoan amoeba distributed worldwide and ubiquitous in the environment, with strains isolated from water, dust, shower heads, swimming pools, and human tissue. Despite the ubiquitous nature of this organism and the presumed frequent exposure to it, all forms of diseases are rare in humans. One reason for this observation is that only some strains of *Acanthamoeba* are known to be pathogenic (notably in the T4, T3 and T2 lineages). The precise mechanisms accounting for pathogenicity are not known, although it can be postulated that genetic variability, environmental adaptation and host factors likely contribute. The host immune system is clearly an important extrinsic attribute in this respect as the organism causes granulomatous amoebic encephalitis (GAE) in immune-compromised humans. However, *Acanthamoeba* is also a facultative parasite causing *Acanthamoeba* keratitis (AK) even in immunocompetent humans supporting amoeba strain differences are important too. We hypothesise that differences in the manner that each *Acanthamoeba* strain interact with the immune response determines their pathogenicity. In this study, we compare the immune response of bone marrow derived macrophages to two strains of *Acanthamoeba,* the non-pathogenic *A. castellanii* (NEFF strain) and the pathogenic *A. castellanii* (50370 strain). RNA sequencing (RNA-seq) was used to identify differences in gene transcript expression in bone marrow-derived macrophages (BMDMs) six hours post-infection with each strain, enabling a pathway-level analysis of infected cells. Immunological assays were subsequently employed to validate a number of bioinformatic findings. Analysis of global gene expression, ‘immuno-transcriptome’, revealed marked heterogeneity in macrophage responses between the 2 groups. Notably, infection with *A. castellanii* (50370 strain) was associated with an M2-like transcriptional profile, whereas infection with the *A. castellanii* (NEFF strain) elicited a predominantly M1-like gene expression. These findings suggest that the pathogenicity of *A. castellanii* (50370) could be due to its ability to polarise macrophages from an M1 to an M2 phenotype, thereby enabling its survival in the absence of effective macrophage antimicrobial activity. Ongoing studies aim to further characterise the ‘immuno-transcriptome’ induced by each strain and to determine if similar differences apply to other strains of pathogenic and non-pathogenic strains of *Acanthamoeba.*