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Strategies used by Entamoeba histolytica, the agent of amoebiasis, to invade human tissues

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Amoebiasis is characterized by intestinal diarrhea, dysentery and liver abscess with specific clinical manifestations for each of these pathologies. The agent of amoebiasis is the amoeba parasite *Entamoeba histolytica*. Amoeba-induced inflammation triggers recruitment of neutrophils and macrophages to the invasion site. These cells could inhibit pathogen multiplication by releasing a variety of effector molecules; i.e. nitric oxide (NO) that exert nitrosative effects leading to inactivation of glycolytic enzymes and reduction of ATP levels. To understand the cellular and molecular mechanisms of infection, we are involved in the functional analysis of genes that are up-regulated or exclusively expressed in highly virulent strains, induced following contact of parasites with compounds of the inflammatory response (TNF, NO, genotoxic agents) or suppressed in non-virulent strains. General transcriptome analysis of mRNA from virulent amoebae and parasites treated with TNF, UV light or NO allowed us to discover an unprecedented set of six proteins whose lysine contents are higher than 25% (KRiP= **K** (lysine) **Ri**ch **P**roteins) and that are up-regulated during virulence and NO treatment. We have also observed a gene expression profile marked by (i) an extreme stress response (involving the chaperones Hsp-70, Hsp-90 and Hsp101) which is known to reactivate aggregated proteins; (ii) the activation of DNA repair mechanisms; and (iii) an increase of glycolytic enzyme gene transcription. To study the link of KRiP factors with these phenotypes we focused on KERP1 and show that it interacts with human enterocytes. Using molecular beacons and qRT-PCR, we revealed a strong decrease of *kerp1* mRNA in the first days of liver infection and an up-regulation after three days of abscess formation. Diminution of the KERP1 amounts in engineered trophozoites led to a reduced pathogenicity, loss of adherence to and cytotoxicity for human cells. KERP1 is a lysine- and glutamic acid-rich protein of 21 kDa in which cationic residues are particularly concentrated in the central and C-terminal part of its primary sequence. Based on the hypothesis that KERP1 plays a role in the disruption of host cell membranes during the interaction with *E. histolytica* we are examining the structural features of this factor by dynamic light scattering, circular dichroism and analytical ultracentrifugation.

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