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Molecular analyses of genes involved in the pathogenesis of *Entamoeba histolytica*

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Objectives of Research

Our main objective is to characterize the molecules and regulatory mechanisms which the amoeba parasite uses to kill mammalian cells and to cause invasive disease in humans. Analysis of genes differentially expressed in virulent *E. histolytica* with those of an isogenic amoeba, which is incapable of killing host cells, is yielding interesting new insights.

Recent findings

Amoebapores (AP) and the killing of target cells: We have earlier shown that the phenotype of amoeba trophozoites transfected with a plasmid construct coding for antisense mRNA of AP-A was significantly less virulent both in vitro as well as in vivo, demonstrating for the first time that AP-A is a crucial virulence factor. Surprisingly, transfectants in which AP-A was overexpressed four fold were also found to be incapable of killing mammalian or bacterial cells and could not induce liver lesions in hamsters. Fluorescent microscopy revealed that the AP-A was dispersed in the cytoplasm and not only in the typical granules, suggesting that the correct localization of the AP-A protein in the trophozoite is important for its toxic function on target cells (Fig. 1).

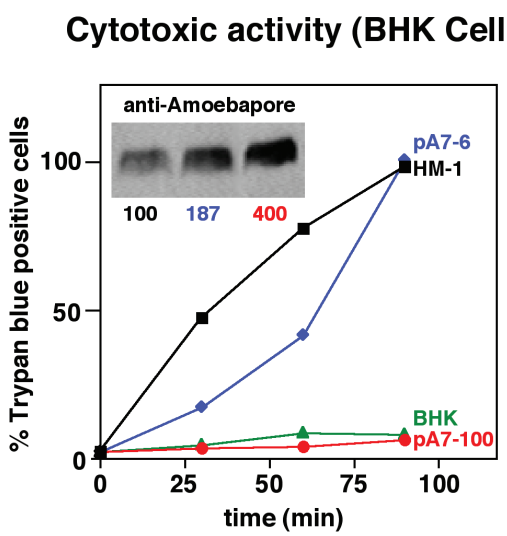

Fig. 1 *Virulence of transfectans overexpressing amoebapore A.*

Silencing of the expression of Amoebapore A gene: In an attempt to overexpress with a plasmid construct, in which the AP-A gene was placed under the regulation of the promotor of the endogenous EhAP-A gene instead of the usual RP-L21 gene, the transfectants obtained were, surprisingly, devoid of AP-A protein and avirulent. Our hypothesis is that the plasmid's upstream AP-A segment is sequestering an essential factor that prevents the transcription of the endogenous AP-A gene and a search for putative DNA binding proteins is currently under way.

Preparation of Recombinant Amoebapore A: Recombinant AP-A was successfully prepared for the first time as a GST-fusion protein in bacteria. The fused protein retained the ability of AP-A to disrupt artificial membranes. This will enable preparation of AP-A crystals and the study of its interaction with membranes.

The 35 kDa light subunit (LGL) of the Gal-specific surface lectin (GL) is a putative virulence factor: We have previously shown that inhibition of expression of the LGL gene (~60%) by antisense RNA caused a very significant decrease in virulence of a pathogenic strain, but did not affect the Gal-sensitive adherence of the amoeba to the target cells. A series of plasmids overexpressing mutated forms of the LGL were prepared: a C-truncated LGL lacking the putative GPI substitution site, and an N-truncated LGL lacking the first 55 amino acids of the mature protein. Transfectants lacking the N-terminus were shown to displace the native LGL from the heterodimeric lectin and displayed a reduced virulence as well as reduced adherence capability to mammalian cells probably due to a dominant-negative effect (Fig. 2). The C-truncated LGL subunit was unable to bind to the heavy (170 kDa) subunit of the Gal-lectin. No effect was observed on virulence or adherence of the c-truncated transfectant. Additional mutated forms of the LGL are currently being studied .

Genes involved in the modulation of amoebic virulence following cultivation with E. coli: Cultivation of a pathogenic strain of amoeba with *E. coli* serotype 055, which avidly binds to the GL, was previously shown to cause a drastic decrease in the virulence of the parasite. A transcription differential analysis

between the virulent and avirulent amoeba revealed several transcripts that were underexpressed in the bacteria-associated amoeba. One of these, coding for an NSF-like protein, has been recently cloned and characterized. The function of the *E. histolytica* NSF as well as its relation to parasite virulence are currently under investigation.

* In memoriam of Tamara Stolarsky. Passed away, Sept 27, 2001.

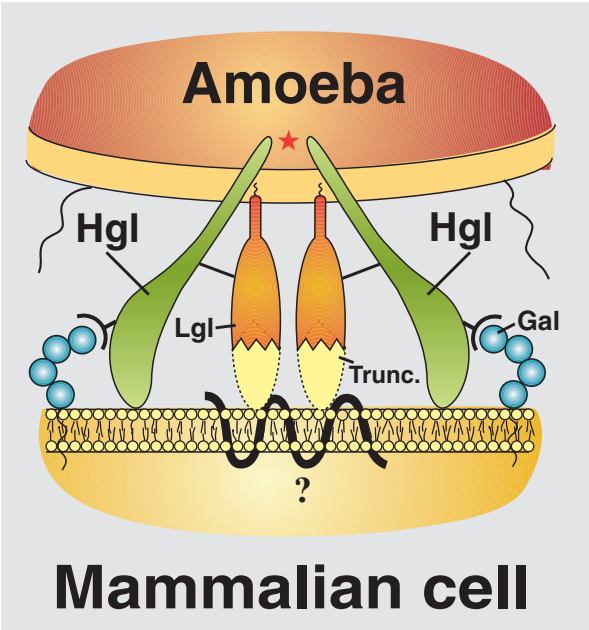

Fig. 2 *Proposed model of function of the Gal-lectin subunit with target cells*

Selected Publications

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Ankri, S., Stolarsky, T., and Mirelman, D. (1998) Antisense inhibition of expression of cysteine proteinases does not affect *Entamoeba histolytica* cytopathic or hemolytic activity but inhibits phagocytosis. Mol. Microbiol. 28, 777-785.

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