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.


**Selected Publications**


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