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ScienceDirect
Molecular & Biochemical Parasitology 151 (2007) 118–128
MOLECULAR & BIOCHEMICAL PARASITOLOGY

Transcriptomic comparison of two *Entamoeba histolytica* strains with defined virulence phenotypes identifies new virulence factor candidates and key differences in the expression patterns of cysteine proteases, lectin light chains, and calmodulin

Paul H. Davis [a,b,1], Jochen Schulze [c], Samuel L. Stanley Jr. [a,b,*]

[a] *Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA*
[b] *Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, USA*
[c] *University of Hamburg, Biozentrum Klein Flottbek, Molekulare Phytopathologie und Genetik, Hamburg, Germany*

Received 19 June 2006; received in revised form 23 October 2006; accepted 25 October 2006
Available online 20 November 2006

Abstract

The availability of Rahman, and the virulent HM-1:IMSS strain of *E. histolytica*, provides a powerful tool for identifying virulence factors of *E. histolytica*. Here we report an attempt to identify potential virulence factors of *E. histolytica* by comparing the transcriptome of *E. histolytica* HM-1:IMSS and *E. histolytica* Rahman. With phenotypically defined strains, we compared the transcriptome of Rahman and HM-1:IMSS using a custom 70mer oligonucleotide based microarray that has essentially full representation of the *E. histolytica* HM-1:IMSS genome. We find extensive differences between the two strains, including distinct patterns of gene expression of cysteine proteinases, AIG family members, and lectin light chains.

Keywords: *E. histolytica*; HM-1:IMSS; Rahman; microarray; Strain comparison; Proteomics

Entamoeba histolytica infection is a constant threat to health in much of the world. *E. histolytica* trophozoites infect the colon, causing amebic dysentery, and can spread through the portal circulation to the liver, where they cause amebic liver abscess [1]. Interestingly, infection with *Entamoeba histolytica* does not always result in disease. One possible explanation for the varied outcome of infection may be differences in the underlying virulence of *E. histolytica* isolates. One strain known to exhibit reduced virulence is *E. histolytica* Rahman, which was isolated from an asymptomatic patient in England in 1972 [2]. The conserved nucleotide sequence of its of 5.8S rRNA indicates that Rahman is *E. histolytica*, yet in various in vitro assays it appears to be less virulent than the prototype *E. histolytica* HM-1:IMSS strain [3]. Recently, we have shown that Rahman exhibits defects in phagocytosis and shows significantly reduced virulence in a human intestinal xenograft model of amebic colitis [4].

Here we report an attempt to identify potential virulence factors of *E. histolytica* by comparing the transcriptome of *E. histolytica* HM-1:IMSS and *E. histolytica* Rahman. We used phenotypically defined laboratory strains that are known to differ in virulence, and compared the transcriptome of Rahman and HM-1:IMSS using a custom 70mer oligonucleotide based microarray that has essentially full representation of the *E. histolytica* HM-1:IMSS genome. Our data indicates that there are a number of potentially significant transcriptional differences between Rahman and HM-1:IMSS, including differences in the expression of genes linked to virulence, and new candidate virulence genes.

1. Materials and methods

1.1. Entamoeba strains

Entamoeba histolytica strain HM-1:IMSS was originally obtained as an uncloned line from Victor Tsutsumi at Cinvestav,

* Corresponding author at: Department of Medicine, Washington University School of Medicine, Campus Box 8051, 660 S. Euclid Avenue, St. Louis, MO 63110, USA. Tel. +1 314 286 0432; fax: +1 314 362 3525.
E-mail address: sstanley@id.wustl.edu (S.L. Stanley Jr.).
[1] Present address: Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA.

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doi:10.1016/j.molbiopara.2006.10.014

and has been passaged through animal livers to maintain virulence [5]. Trophozoites used for mRNA had been passaged through mice approximately 30 days prior to use. Strain Rahman was obtained from ATCC, #50738. Both were maintained in culture medium BI-S-33 [6].

1.2. Microarray comparison

For microarray comparison, we designed a 70-base oligonucleotide array to analyze 6242 genes uniquely. The entire genome dataset available from the TIGR/Sanger *Entamoeba histolytica* sequencing project (http://www.tigr.org/tdb/e2k1/eha1/) in February 2004, with additional immune-related and "housekeeping" genes chosen from model organisms and minus highly repetitive sequences (mainly LINE/SINEs) was compiled and input into ArrayOligoSelector (http://arrayoligosel.sourceforge.net/) to generate 70mer oligonucleotides which have similar binding properties, and hybridize uniquely to one transcript. Oligonucleotides were manufactured by Illumina (San Diego, California) and were printed in triplicate on 100 Cel Associates Epoxy slides (Santa Clara, California) by the Washington University School of Medicine Microarray Core Facility. The average computed T_ms for all oligos was 80.8 °C, with a standard deviation of 2.73 (range 70.5–95.5 °C). Following the publication of the *E. histolytica* genome [7], printed oligo sequences were BLASTed against the database at NCBI (http://www.ncbi.nlm.nih.gov) and ascribed NCBI accession numbers. Additional annotations were computed for remaining genes representing hypothetical proteins using NCBI blastx against the nr database excluding *Entamoeba* genus with a cutoff of expectation value $<1 \times 10^{-10}$, and Interpro domain and feature characterizations. The microarray was tested for hybridization using labelled *E. histolytica* HM-1:IMSS genomic DNA, which detectably hybridized to 99.4% of the *Entamoeba* microarray elements.

RNA was isolated from approximately 5×10^6 pre-stationary phase *E. histolytica* HM-1:IMSS and Rahman each simultaneously grown in 15-ml glass flasks using the Qiagen RNeasy kit (Valencia, California) following the manufacturer's protocol, including the DNase treatment. RNA quantity and quality were obtained from an absorbance ratio at 260 and 280 nm. RNA quality was confirmed for each sample using an Agilent 2100 Bioanalyzer (Palo Alto, California) according to the manufacturer's instructions. Cy3 and Cy5 labelled cDNA was created using the Genispehere 3DNA array350 kit (Hatfield, Pennsylvania). Six samples (three Rahman and three HM-1:IMSS) were competitively hybridized on six individual chips. Each biological replicate was hybridized to two chips in which the Cy fluorescent channel was alternated in order to reduce dye-specific effects (dye swap). The primary hybridization was performed by adding 48 μl of sample to the microarray under a supported glass coverslip (Erie Scientific, Portsmouth, NH) at 70 °C for 16–20 h at high humidity in the dark. Prior to the secondary hybridization, slides were gently submerged into 2× SSC, 0.2% SDS (at 70 °C) for 11 min, transferred to 2× SSC (at room temperature) for 11 min, transferred to 0.2× SSC (at room temperature) for 11 min, and then spun dry by centrifugation. Secondary hybridization was carried out using the complimentary capture reagents provided in the 3DNA Array 350 kit. Slides were scanned on a ScanArray Express HT scanner (Perkin-Elmer, Boston, MA) to detect Cy3 and Cy5 fluorescence. Laser power was kept constant for Cy3/Cy5 scans and PMT was varied for each experiment based on optimal signal intensity with lowest possible background fluorescence. Gridding and analysis of images was performed using ScanArray v3.0 (Perkin-Elmer). Log_2 ratios of HM-1:IMSS versus Rahman samples were calculated, local background subtracted, and Loess normalized. Data was imported into a MySQL database (www.mysql.org) and data analysis was accomplished with custom SQL scripts and Spotfire DecisionSite software (Somerville, MA). Averages and standard deviation were calculated for each transcript, and transcripts considered significant showed a Student's *t*-test *p*-value of <0.01, two-fold or more increase or decrease from HM-1:IMSS, and a normalized standard deviation ratio (standard deviation/average) <1 to eliminate overly varying probes between biological and technical (dye-swapped and triplicately plated) replicates.

1.3. Real-time PCR

Real-time PCR assays were conducted by reverse transcribing one randomly chosen biological replicate and its microarray pair using Invitrogen (Carlsbad, CA, USA) Superscript III without RNase and Invitrogen oligo dT primers per manufacturer's instructions. cDNA was treated with Invitrogen RNase H to degrade remaining RNA. Treated cDNA was then diluted and amplified using SYBR Green Master Mix 2× reagent (Applied Biosystems) in an Applied Biosystems 7500 Real-time analyzer in a total of 25 μl per reaction, per manufacturer's instructions. All primer sets were run in triplicate, and primer dissociation curves were analyzed to ensure that the primers used were not amplifying multiple products. Up to four reference gene transcripts were measured from each sample and calculated using the Excel (Microsoft, Redmon, WA, USA) add-in geNorm (http://medgen.ugent.be/~jvdesomp/genorm/) to normalize overall transcript abundance between strains [8]. Reference genes were chosen from a list of transcripts which showed the lowest normalized variability from over 15 microarray experiments. Graphs were constructed using Microsoft Excel. Primers were designed with and obtained through PrimerQuest (www.idtdna.com) using sequence information from NCBI, and NCBI BLAST was used to confirm primer specificity against the current *Entamoeba* dataset. Primer sequences can be found in supplementary data, at http://stanleylab.wustl.edu/data/rahmanarray/.

2. Results and discussion

2.1. Comparing the transcriptome of E. histolytica Rahman and E. histolytica HM-1:IMSS

The goal of our work was to compare two *E. histolytica* strains with defined virulence phenotypes to identify potential virulence factors. *E. histolytica* strain HM-1:IMSS is the prototype viru-

lent strain, and our strain is routinely passaged through animal models to maintain virulence. While clonal variation is a potential concern in this kind of experiment, there do not appear to be significant differences between our isolate of HM-1:IMSS and other HM-1:IMSS clones, based on a recent proteomic comparison which showed only one protein concentration difference out of more than 2000 recognized protein spots (Davis PH, et al., manuscript in preparation). *E. histolytica* Rahman shows greatly reduced virulence compared to HM-1:IMSS (results by us and others are listed in [4]), and our recent phenotypic analysis showed that Rahman has significant deficits in erythrophagocytosis, and a marked decrease in the ability to cause amebic colitis in the SCID-hu-intestinal model of disease when compared to *E. histolytica* HM-1:IMSS [4].

To identify the differences between these defined strains of *E. histolytica* Rahman and HM-1:IMSS at the molecular level, we compared the baseline transcriptomes of the two organisms. We used a microarray with 6209 70mer oligonucleotides encompassing approximately 90% of the unique genes found in the *E. histolytica* genome dataset as of February 2004. We did not include oligonucleotides representing many of the highly repetitive sequences such as tRNAs and LINE/SINE sequences within the genome [7]. Importantly, given the large number of multicopy genes within the *E. histolytica* genome, we were able to design oligonucleotides that should discriminate between many members of physiologically important gene families, such as the heavy and light chain of the Gal/GalNAc lectin, amoebapores, and cysteine proteinases.

Using this array, and analyzing three biological replicates for Rahman and for HM-1:IMSS, we detected 353 gene transcripts that showed a two-fold or greater difference between Rahman and HM-1:IMSS with a Students' *t*-test statistical significance *p*-value score <0.01. As shown in Table 1, we found 152 transcripts that were higher in HM-1:IMSS, and 201 transcripts that were expressed at higher levels in Rahman (Table 2). These tables do not show data for those highly repetitive or hypothetical genes that we could not group via BLAST or InterPro functional protein domain discovery. The results for those genes, as well as the entire data set, are found in supplementary data http://stanleylab.wustl.edu/data/rahmanarray. We used Real-time PCR to confirm the differential expression of 10 of the genes (marked in Tables 1 and 2), and found that all 10 correlated to microarray results with an R^2 of 0.92.

Recently, McFarlane and Singh used a DNA-based microarray containing contigs from the *E. histolytica* sequencing project that contains an estimated 2110 unique genes to compare *E. histolytica* HM-1:IMSS with *E. histolytica* Rahman and *E. dispar* [9]. In contrast to our results, only 32 genes with lower expression in Rahman than in HM-1:IMSS were detected in their analysis. Among the 19 non-highly repetitious genes identified in their study as showing differential expression, 8 also showed differential expression in our study. The increased sensitivity of our assay probably reflects the differences in the two arrays, with our oligonucleotide-based array providing coverage of more genes (greater than 6000 genes representing 90% of the genome), and the ability to distinguish between members of the same gene family, leading to the detection of allelic differences between Rahman and HM-1:IMSS. In addition, we compared strains with defined virulence phenotypes, which may have increased the number of detectable differences between our *E. histolytica* HM-1:IMSS and Rahman. It is worth noting that in addition to microarray analyses, representational differential analysis (RDA) has also been used to study transcriptional differences between HM-1:IMSS and Rahman, and identified three genes, *S*-adenosylhomocysteine hydrolase, aldose 1-epimerase, and one member of the light chain subunit of the Gal/GalNAc lectin family, that were expressed at lower levels in Rahman compared to HM-1:IMSS [10].

E. histolytica cysteine proteinases (EhCP) have been unequivocally linked to virulence in in vitro and in vivo models of amebiasis [1,11]. Using oligonucleotides designed to identify specific members of the *E. histolytica* cysteine protease family, we found distinctly different patterns in protease gene expression between *E. histolytica* HM-1:IMSS and *E. histolytica* Rahman (Fig. 1). It is first worth noting that many of the cysteine proteinases we examined are among the most abundant transcripts detected in our microarray analysis, present at a higher level (e.g. EhCP1) than 99.2% of all *E. histolytica* HM-1:IMSS transcripts measured (Fig. 1A). Others, such as EhCP15 and EhCP18, are expressed at much lower levels in both *E. histolytica* strains. With this perspective, we found expression of the genes encoding EhCP4, EhCP6, and EhCP7 was statistically significantly higher (approximately three-fold) in HM-1:IMSS than in Rahman (Fig. 1B). Increased expression of EhCP17 and EhCP12 were seen in *E. histolytica* HM-1:IMSS compared to Rahman, but these differences did not attain statistical significance. The most striking difference was seen in the expression of EhCP3, which was approximately 100-fold higher in Rahman than HM-1:IMSS. (Fig. 1B). In this case EhCP3, which is a relatively low level transcript in HM-1:IMSS, is an abundant transcript in Rahman (Fig. 1A). Interestingly, EhCP3 has also been reported to be expressed at higher levels in *Entamoeba dispar* compared to *Entamoeba histolytica* [12]. The expression of the genes encoding EhCP8 (2.2-fold, p-value = 0.02), and EhCP112 (3.5-fold, p-value = 0.002) was also higher in Rahman than in HM-1:IMSS (Fig. 1B). Unexpectedly a higher expression of EhCP5 (which has been linked to virulence) [13–15] was seen in Rahman than HM-1:IMSS (Fig. 1B), but this difference was not statistically significant, and it is clear that EhCP5 is a relatively abundant transcript in both strains (Fig. 1A). In a recent transcriptional analysis comparing *E. histolytica* HM-1:IMSS trophozoites isolated from mouse intestine compared to cultured *E. histolytica* HM-1:IMSS trophozoites, both EhCP4 and EhCP6 were higher in trophozoites in mouse gut [16]. This is consistent with the concept that increased expression of EhCP4 and EhCP6 could be associated with virulence. The physiologic significance of the different pattern of protease expression between HM-1:IMSS and Rahman remains unknown, especially since the major proteases (EhCP1, EhCP2, and EhCP5) did not show statistically significant differences in transcript levels [12]. It would be of great interest to determine whether EhCP4, EhCP6, EhCP7, EhCP3, EhCP8, or EhCP112 have distinct functions, whether these transcriptional differences lead to significant differences in protease activity, and whether the distinct patterns of protease

Table 1
Genes expressed at higher levels in HM-1:IMSS compared to Rahman

Gene accession	Gene name	Fold change	*p*-value	PCR
Protein degradation				
XM_652272.1	EhCP6; cysteine proteinase 6	3.4	1.73E−04	4.2
XM_651510.1	EhCP4; cysteine protease 4	3.0	8.86E−04	3.0
XM_644785.1	Oligopeptidase A	2.7	3.01E−03	
XM_646849.1	Serine peptidase, clan SP, family S59 [*L. major* e-33]	2.5	2.74E−03	
XM_646489.1	EhCP7; cysteine proteinase 7	2.5	1.90E−05	11.0
Associated with bacterial interaction/killing				
XM_648115.1	AIG1 family protein	20.0	6.61E−05	
XM_643009.1	AIG1 family protein	4.6	3.09E−03	
XM_643614.1	Lysozyme, putative	3.2	1.88E−05	4.5
XM_646533.1	Cecropin domain	3.1	1.23E−03	
Plasmodium-related				
XM_652032.1	Erythrocyte binding protein 2 [*P. falciparum*] e-23	52.0	4.33E−05	
XM_648718.1	Mature parasite-infected erythrocyte surface antigen [*P. falciparum*] e-15	38.0	1.54E−06	
Signaling and stress response				
XM_648655.1	Similar to (e-11) dictyostelium protein kinase	22.5	2.33E−05	
XM649600.1	Protein kinase, putative	16.9	7.69E−06	
XM_644550.1	Protein kinase, putative	11.8	3.31E−08	
XM_649553.1	Calmodulin, putative	6.1	2.11E−04	6.9
XM_650791.1	Rho GTPase activating protein, putative	4.5	5.03E−04	
XM_649555.1	Protein kinase, putative	4.4	1.07E−07	
XM_646898.1	Putative protein kinase	4.1	1.21E−04	
XM_649369.1	Putative ADP-ribosylation	4.1	2.11E−03	
XM_646573.1	Protein kinase, putative	3.0	1.01E−04	
XM_651503.1	Protein kinase, putative	2.5	4.22E−03	
XM_650778.1	Protein tyrosine phosphatase, putative	2.4	2.37E−04	
XM_647630.1	Protein phosphatase, putative	2.3	2.12E−03	
XM_649405.1	*N*-Acetylglucosaminyl-phosphatidylinositol de-*N*-acetylase, putative	2.2	3.44E−06	
XM_648787.1	70 kDa heat shock protein, putative	2.2	2.72E−03	
XM_645348.1	Ras family GTPase	2.1	1.44E−03	
XM_648642.1	WD repeat protein	2.1	6.65E−03	
XM_646354.1	Rho family GTPase	2.1	6.25E−04	
Nucleic acid interaction				
XM_642791.1	RNA modification enzymes, MiaB-family	5.8	1.96E−04	
XM_646583.1	Sulfotransferase, putative	4.1	1.94E−04	
XM_648447.1	P-loop containing nucleoside triphosphate hydrolases	3.8	2.02E−04	
XM_646353.1	Splicing factor 3B subunit 10, putative	2.8	8.34E−06	
XM_646796.1	Pumilio family RNA-binding protein	2.6	5.77E−04	
XM_648788.1	U2 snRNP auxiliary factor, putative	2.5	4.24E−06	
XM_643575.1	Cyclin, putative	2.2	1.23E−03	
XM_646274.1	HMG-box	2.1	9.92E−03	
XM_651915.1	SMC5 protein, putative	2.1	1.86E−03	
XM_651980.1	5′–3′ exonuclease, putative	2.1	7.71E−04	
XM_651992.1	RNA-binding protein, putative	2.1	1.65E−03	
XM_650092.1	Similar to DEK oncogene (DNA binding) [*Tribolium castaneum*]2.27e-07	2.1	2.19E−03	
XM_650103.1	Zinc finger protein, putative	2.0	2.20E−03	
XM_651368.1	Cell cycle control protein cwf12, putative	2.0	5.81E−06	
Carbohydrate, lipid, and other metabolism				
XM_649080.1	Metallo-beta-lactamase, most similar to *Pseudomonas*	4.7	2.36E−04	
XM_646387.1	Alpha-amylase, putative	2.7	5.31E−05	
XM_650718.1	Epimerase	2.6	1.05E−04	
XM_644988.1	Mannosyltransferase, putative	2.4	2.57E−03	
XM_643579.1	Phosphoglucomutase/phosphomannomutase family protein	2.0	3.03E−03	

Table 1 (*Continued*)

Gene accession	Gene name	Fold change	*p*-value	PCR
Oxidation and reduction chemistry				
XM_649604.1	Nitroreductase family protein *Desulfovibrio* e-7	16.5	4.94E−05	
XM_650038.1	Iron-sulfur flavoprotein, putative	13.5	2.29E−05	
XM_643978.1	EhADH3	3.9	8.79E−05	
XM_643978.1	EhADH3	3.0	9.77E−06	
XM_647032.1	Iron-sulfur flavoprotein, putative	2.9	2.71E−03	
XM_650105.1	Flavoprotein	2.4	5.93E−03	
XM_650943.1	Short chain dehydrogenase family protein	2.4	6.08E−05	
XM_643476.1	Malate dehydrogenase, putative	2.4	6.57E−03	
XM_647661.1	Alcohol dehydrogenase, iron-containing, putative	2.3	4.41E−03	
Transport				
XM_651269.1	Importin alpha, putative	34.3	2.74E−09	
XM_646243.1	Nucleoporin nup189, putative [*Cryptococcus neoformans* e-38]	2.9	1.95E−03	
XM_651559.1	Zinc transporter, putative	2.7	8.45E−04	
XM_650578.1	Copine	2.2	6.46E−05	
Other				
XM_650615.1	Similar to *Y. pestis* putative membrane protein	20.1	1.85E−06	
XM_647692.1	Similar to BRCA2 and CDKN1A-interacting protein isoform	10.3	1.60E−05	
XM_650580.1	Acetyltransferase, putative	5.5	4.40E−03	
XM_652394.1	BspA-like leucine rich repeat protein, putative	4.0	1.13E−03	
XM_644228.1	Lipopolysaccharide-induced TNF factor (*Danio rerio*) e-13	3.6	1.55E−04	
XM_645714.1	Nedd4 binding protein 2 [*Homo sapiens*] e-09	3.0	6.91E−05	
XM_652357.1	Calponin homology domain protein e-16	3.0	2.35E−04	
XM_647739.1	Acetyltransferase, putative	3.0	2.40E−03	
XM_648211.1	Alkyl sulfatase, putative	2.8	4.31E−04	
XM_645042.1	Predicted protein	2.8	1.76E−03	
XM_643369.1	Septum formation protein maf, putative	2.6	1.00E−03	
XM_649260.1	Latent nuclear antigen, putative	2.5	9.60E−07	
XM_651591.1	Dopey domain protein, putative	2.4	4.77E−05	
XM_644224.1	Calcium-binding protein, putative	2.3	4.39E−03	
XM_646797.1	Similar to C29H12.2 [*Tribolium castaneum*]	2.3	3.13E−04	
XM_645230.1	Accumulation-associated protein [*Staphylococcus epidermidis*] e-09	2.3	3.10E−04	
XM_645318.1	EF_HAND	2.2	6.56E−04	
XM_643478.1	Alpha/beta hydrolase, putative	2.2	4.15E−03	
XM_647832.1	ARM repeat	2.2	1.14E−03	
XM_649642.1	Villin-related	2.2	4.10E−03	
XM_648632.1	Calcium-binding protein	2.1	2.77E−04	
XM_644804.1	Metal dependent phosphohydrolase, putative	2.1	1.33E−03	
XM_649881.1	CXXC-rich protein	2.1	2.57E−03	
XM_646423.1	Vacuolar ATP synthase subunit d, putative	2.1	1.81E−04	
XM_644199.1	Beta adaptin, putative	2.1	5.62E−03	
XM_644806.1	Brix domain protein, putative	2.0	8.88E−05	
XM_644357.1	Protein FAM38A [*Strongylocentrotus* e-58]	2.0	7.93E−04	
XM_643718.1	CXXC-rich protein	2.0	1.05E−04	

Microarray results representing *Entamoeba histolytica*-specific transcripts which were at least two-fold more abundant in strain HM-1:IMSS compared to Rahman. Bad elements (microarray elements failing triplicate or dye-swap replication) were removed. Unique probes covering a single transcript were averaged. All transcripts are statistically significant, having a p-value < 0.01. The gene accession number, gene name, fold change, and p-value expressed exponentially are shown. Where gene expression levels were studied by Real-time PCR the fold change obtained by Real-time PCR is shown (PCR column). Genes are classified by putative functions of the gene product; or, in the case of "Plasmodium-related", represent aligned protein homologies most similar to those found in *Plasmodium* spp.

Table 2
Genes expressed at higher levels in Rahman compared to HM-1:IMSS

Gene accession	Gene name	Fold change	*p*-value	PCR
Protein degradation				
XM_648162.1	CP3; cysteine protease 3	101.2	5.37E−07	155
XM_647901.1	EhCP112	3.5	1.82E−03	1.5
XM_649631.1	Predicted metal-dependent membrane protease	2.2	4.67E−04	
Surface-associated				
XM_649244.1	Lgl3	21.9	3.22E−04	26.0
XM_645597.1	PFAM: LRR	2.3	8.45E−03	
Plasmodium-related				
XM_645291.1	Erythrocyte binding protein 3 [*Plasmodium falciparum*]	155.5	7.14E−06	217
XM_646183.1	Asparagine-rich antigen [*Plasmodium falciparum*] e-15	3.6	1.07E−04	
XM_646183.1	235 kDa rhoptry protein [*Plasmodium yoelii* yoelii]	2.8	8.98E−04	
XM_648401.1	rpoD [*Plasmodium falciparum*] e-08	2.6	2.47E−03	
XM_650530.1	*P. falciparum* RESA-like protein with DnaJ domain e-23	2.1	3.95E−04	
Signaling				
XM_646619.1	Rho guanine nucleotide exchange factor, putative	9.3	3.45E−05	
XM_650709.1	Ras guanine nucleotide exchange factor, putative	6.8	2.44E−04	
XM_648368.1	heat shock protein DnaJ homologue Pfj2 [*Plasmodium falciparum*] e-09	4.6	5.12E−04	
XM_644491.1	Protein kinase, putative	4.5	1.04E−03	
XM_643898.1	Ras family GTPase	3.9	7.53E−03	
XM_649777.1	Rap/Ran GTPase activating protein, putative	3.6	3.66E−06	
XM_649440.1	Protein kinase, putative	3.5	6.23E−04	
XM_648550.1	Protein kinase, putative	3.4	5.26E−05	
XM_646706.1	Phosphatidylinositol 3- and 4-kinase family	3.4	8.29E−03	
XM_646706.1	Phosphatidylinositol 3- and 4-kinase family	3.4	1.30E−04	
XM_652186.1	C2 domain protein, putative	3.2	1.00E−03	
XM_647152.1	Protein phosphatase, putative	2.9	1.27E−04	
XM_651834.1	Rho_GAP	2.8	2.40E−04	
XM_650127.1	Rho GTPase activating protein, putative	2.8	1.86E−04	
XM_649262.1	hsp70 family protein	2.7	4.10E−03	
XM_651861.1	Ras guanine nucleotide exchange factor, putative	2.6	4.49E−03	
XM_649440.1	Protein kinase, putative	2.6	2.70E−03	
XM_648409.1	Protein kinase, putative	2.6	1.25E−03	
XM_652334.1	IkappaB kinase complex-associated protein, putative	2.4	1.76E−03	
XM_646245.1	70 kDa heat shock protein	2.4	9.78E−03	
XM_644487.1	Protein kinase, putative	2.4	1.32E−03	
XM_643700.1	Protein kinase, putative	2.4	4.58E−03	
XM_644493.1	Rho_GAP	2.2	4.46E−03	
XM_644898.1	GTPase activating protein, putative	2.2	4.65E−03	
XM_648895.1	Protein kinase, putative	2.2	7.02E−03	
XM_651538.1	Protein kinase, putative	2.2	7.17E−03	
XM_648888.1	TNF receptor-like	2.2	4.10E−03	
XM_644968.1	Rho guanine nucleotide exchange factor, putative	2.2	1.30E−06	
XM_642769.1	Ras family GTPase	2.1	1.55E−04	
XM_646117.1	Ras_GEF	2.1	2.30E−06	
XM_647365.1	Rho family GTPase	2.0	1.06E−03	
XM_651446.1	Protein phosphatase, putative	2.0	5.57E−05	
XM_645598.1	Protein kinase, putative	2.0	6.19E−05	
XM_651809.1	Rho_GAP	2.0	1.43E−04	
Nucleic acid interaction				
XM_642877.1	Phosphatidate cytidylyltransferase, putative [*Oryza sativa* e-20]	9.7	6.34E−03	
XM_646562.1	DNA mismatch repair protein mutS, putative	6.4	2.21E−04	

Table 2 (*Continued*)

Gene accession	Gene name	Fold change	*p*-value	PCR
XM_648023.1	DNA mismatch repair protein mutS, putative	5.7	1.84E−03	
XM_647744.1	Adenosine deaminase, putative	4.6	2.22E−05	
XM_648147.1	Small nuclear ribonucleoprotein F, putative	4.6	1.59E−06	
XM_646207.1	Uridine-cytidine kinase, putative	4.1	1.80E−04	
XM_652405.1	Polynucleotide kinase-3'-phosphatase, putative	3.9	9.43E−04	
XM_645326.1	Cysteine-rich DNA binding domain, DM domain	3.1	1.90E−07	
XM_650320.1	C2H2 and C2HC zinc fingers	2.9	2.20E−03	
XM_644570.1	SMC domain protein	2.6	1.14E−05	
XM_650100.1	Dihydrouridine synthase 1-like	2.5	2.55E−04	
XM_650455.1	Non-structural maintenance of chromosome element 4 e-14	2.4	5.54E−04	
XM_648085.1	DNA clamp	2.2	7.08E−03	
XM_647442.1	DNA mismatch repair protein mutS, putative	2.2	1.17E−03	
XM_645838.1	Myb family DNA-binding protein	2.2	2.00E−03	
XM_646466.1	Ribonucleases P/MRP protein subunit, putative [*C. neoformans* e-15]	2.1	1.02E−03	
XM_643619.1	Helicase, putative	2.1	7.11E−03	
XM_652084.1	Zinc finger protein, putative	2.1	6.05E−06	
XM_646970.1	Apyrase, putative	2.0	9.19E−04	
XM_648658.1	Topoisomerase, putative	2.0	1.20E−03	
Carbohydrate, lipid, and other metabolism				
XM_650451.1	Ceramide synthase, putative	2.3	1.77E−03	
XM_651264.1	Carbohydrate degrading enzyme, putative	2.2	5.73E−03	
XM_647204.1	Chitinase Jessie, putative	2.2	7.17E−04	
XM_645653.1	Patatin-like phospholipase, putative	2.1	5.65E−03	
XM_651057.1	Lecithin:cholesterol acyltransferase, putative	2.1	1.88E−03	
XM_644489.1	Protein farnesyltransferase alpha subunit, putative	2.1	6.72E−05	
XM_651407.1	Sphingomyelinase C, putative	2.1	6.41E−05	
XM_652341.1	Ceramide synthase, putative	2.0	1.28E−03	
Oxidation and reduction chemistry				
XM_642833.1	D-3-Phosphoglycerate dehydrogenase, putative	5.9	3.82E−05	
XM_651283.1	Nitroreductase family protein	4.8	1.51E−05	
XM_651443.1	Zinc-containing alcohol dehydrogenase, putative	4.2	1.55E−03	
XM_651412.1	Oxidoreductase, putative	2.9	1.17E−03	
XM_651412.1	Oxidoreductase, putative	2.7	2.61E−03	
XM_647747.1	Fe-hydrogenase, putative	2.5	6.98E−04	
XM_643772.1	Glutamate dehydrogenase, putative	2.2	6.61E−03	
XM_650899.1	Ferric-chelate reductase (Fre2), putative [*Aspergillus fumigatus* Af293]	2.2	1.35E−06	
Transport				
XM_644292.1	ABC transporter, putative	102.9	4.56E−07	
XM_646404.1	ABC transporter, putative	86.7	2.35E−06	
XM_650291.1	Phosphoserine aminotransferase, putative	14.0	5.70E−08	
XM_645566.1	Amino acid transporter, putative	3.3	5.95E−03	
XM_646862.1	M271permease of the major facilitator superfamily [*Leptospira interrogans*]	2.8	6.75E−04	
XM_652378.1	Permease-like protein [*Leishmania major*] e-95	2.6	5.25E−03	
XM_645599.1	Prefoldin	2.5	4.07E−05	
XM_649615.1	Amino acid transporter, putative	2.3	3.37E−03	
XM_647098.1	Membrane transporter, putative	2.3	4.30E−04	
Other				
XM_646601.1	HMW glutenin subunit [*Thinopyrum bessarabicum*] e-19	51.4	9.46E−06	149
XM_645479.1	Similar to tripartite motif-containing 35 isoform 1 [*Danio rerio*] e-35	39.2	4.75E−05	
XM_649962.1	Viral A-type inclusion protein repeat, putative	11.6	1.76E−05	

Table 2 (*Continued*)

Gene accession	Gene name	Fold change	*p*-value	PCR
XM_646695.1	Beige/BEACH domain protein, putative	7.3	1.07E−04	
XM_651413.1	Alpha/beta-Hydrolases	4.8	2.94E−04	
XM_647605.1	Similar to tripartite motif-containing 35 isoform 1 [*Danio rerio*] e-52	4.5	3.60E−06	
XM_650859.1	158792 GrfA protein, putative	4.3	1.31E−08	
XM_649327.1	Coronin, putative	3.6	5.89E−05	
XM_651370.1	AAA family ATPase, putative	3.3	1.70E−03	
XM_651211.1	t-snare	3.1	8.33E−06	
XM_646197.1	WD40_like	2.9	9.38E−04	
XM_651297.1	Hrf1 domain	2.8	4.42E−04	
XM_646563.1	Protein with DENN and LIM domains	2.6	3.53E−03	
XM_647502.1	Peroxisome assembly protein [*Trypanosoma cruzi*] e-08	2.4	4.28E−05	
XM_643542.1	Starch-binding domain	2.4	4.55E−04	
XM_650783.1	Bromodomain protein, putative	2.4	2.93E−04	
XM_647859.1	PCTP-like protein, putative	2.4	7.76E−05	
XM_649049.1	ARM repeat	2.4	3.79E−04	
XM_644562.1	GS1 protein, putative	2.3	2.43E−04	
XM_651435.1	Vacuolar protein sorting 35, putative	2.3	8.06E−04	
XM_643303.1	ARM repeat	2.3	1.23E−04	
XM_650020.1	Suppression of tumorigenicity 5 [*Danio rerio*] e-27	2.3	6.82E−05	
XM_644469.1	NHL repeat domain protein [*Geobacter sulfurreducens* e-11]	2.2	4.54E−03	
XM_650974.1	Nedd4 binding protein 2 [*Mus musculus*]	2.2	1.85E−03	
XM_647831.1	PREDICTED: similar to nuclear receptor coactivator 7 [*Rattus norvegicus*]1.29741e-18	2.2	3.20E−03	
XM_652470.1	ARM repeat	2.2	1.94E−04	
XM_648598.1	WW_DOMAIN_1	2.2	2.12E−04	
XM_647022.1	Acetyltransferase, putative	2.1	3.64E−05	
XM_645850.1	Predicted hydrolase [*Clostridium tetani* e-29]	2.1	7.23E−03	
XM_650963.1	Tetratricopeptide repeat domain containing protein [*Rickettsia felis* e-7]	2.1	2.62E−04	
XM_645314.1	Actin binding protein, putative	2.1	2.31E−06	
XM_646075.1	Protein UNC-89–*Caenorhabditis elegans* e-28	2.1	7.29E−06	
XM_647427.1	SMAD_FHA	2.1	7.76E−06	

Microarray results representing disproportional *Entamoeba histolytica*-specific transcripts which were at least two-fold more abundant in strain Rahman compared to HM-1:IMSS. Bad elements (microarray elements failing triplicate or dye-swap replication) were removed. Unique probes covering a single transcript were averaged. All transcripts are statistically significant, having a p-value <0.01. The gene accession number, gene name, fold change, and p-value expressed exponentially are shown; where gene expression levels were studied by Real-time PCR the fold change obtained by Real-time PCR is shown (PCR column). Genes are classified by putative functions of the gene product; or, in the case of "Plasmodium-related", represent aligned protein homologies most similar to those found in *Plasmodium* spp.

expression play a role in the ability of *E. histolytica* trophozoites to cause disease.

Another marked difference between *E. histolytica* HM-1:IMSS and *E. histolytica* Rahman lies in the expression of one of the genes encoding the light chain (Lgl) of the galactose-binding lectin. The light chain of the lectin is linked to the carbohydrate-binding heavy chain by disulfide bonds, and may play a role in signal transduction [17]. As noted above, studies using differential display reported decreased expression of Lgl1 in Rahman compared to HM-1:IMSS, and showed that inhibition of Lgl1 gene expression by antisense, or expression of a dominant negative Lgl1 construct in HM-1:IMSS, resulted in a reduced virulence phenotype, including a decreased ability to phagocytose red blood cells [18,19]. We found no difference in the levels of expression of Lgl1 between HM-1:IMSS and Rahman in our study, but found that the expression of Lgl3 was significantly higher (22-fold) in Rahman than in HM-1:IMSS (Table 2). It is possible that this simply represents an allelic difference between the strains, and Lgl3 functions similarly to Lgl1, but it also raises the interesting question of whether the Lgl3 isotype might function less well in signaling than Lgl1 (acting like a dominant negative) and the higher levels of Lgl3 in Rahman could explain both our observation of reduced phagocytosis and decreased virulence in Rahman [4].

E. histolytica ingests and kills bacteria. We found higher expression of lysozyme, an enzyme that can kill Gram positive bacteria, in HM-1:IMSS than in Rahman (Table 1). We also found higher expression of a gene encoding a protein with a cecropin (antibacterial peptide) domain, in HM-1:IMSS than Rahman. We found that several genes with homology to members of the AIG1 gene family were expressed at much higher levels in HM-1:IMSS than in Rahman. The AIG1 (aviru-

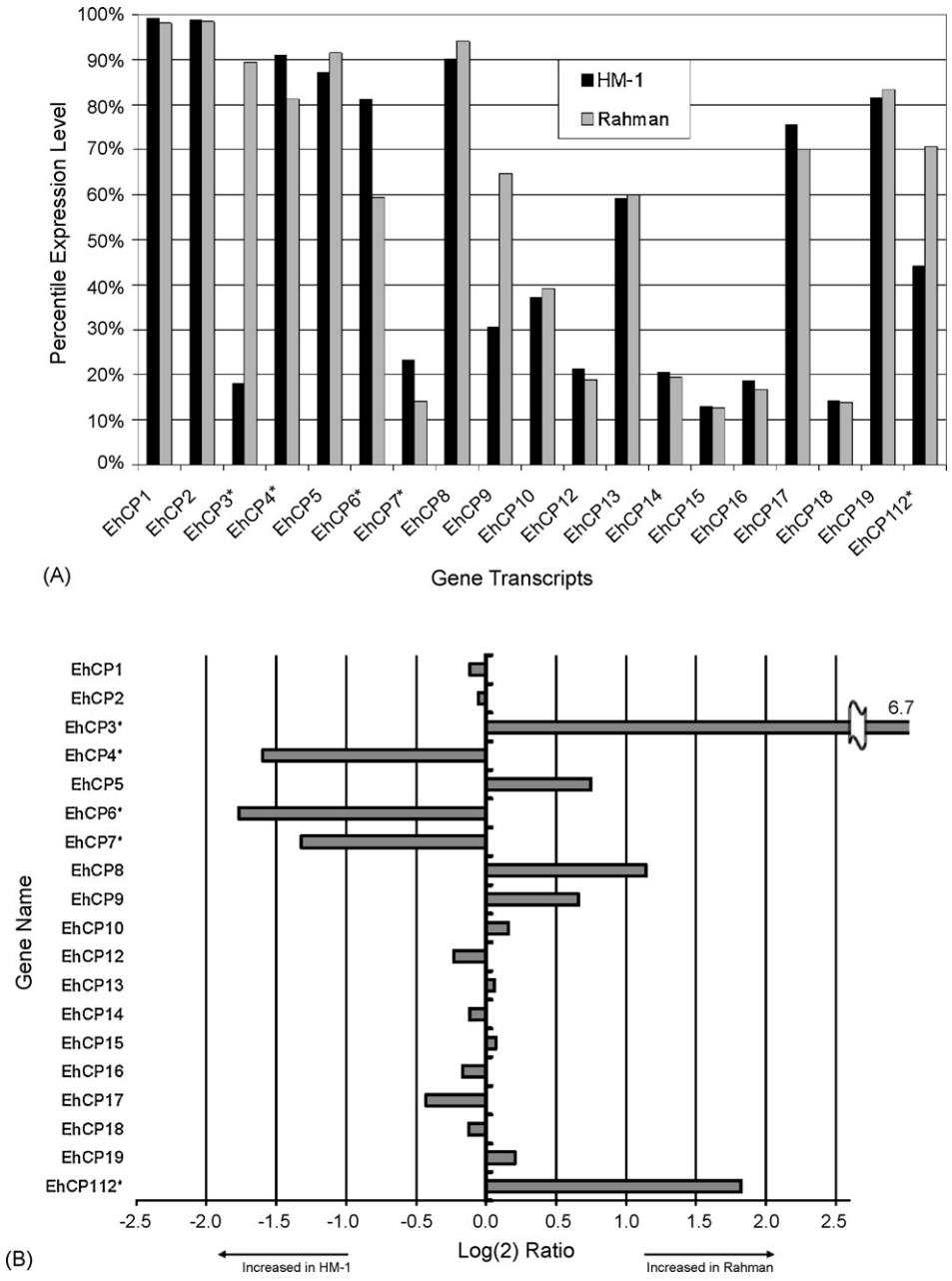

Fig. 1. Cysteine proteinase genes show a distinct pattern of expression between HM-1:IMSS and Rahman. (Panel A) Relative ranked expression levels of cysteine proteinase genes. Relative values were calculated by rank scaling averaged intensities between 0 and 1 for all genes per channel following Loess normalization. These values are expressed as a percentile (e.g. for HM-1:IMSS, the transcript for EhCP1 is more abundant than 99.2% of HM-1:IMSS transcripts). Genes with transcript levels that differed significantly ($p < 0.01$) between strains HM-1:IMSS (HM-1) and Rahman are marked with an asterisk. (Panel B) Fold difference in expression between cysteine proteinase genes in *E. histolytica* Rahman and HM-1:IMSS. The relative difference in expression levels expressed in the $\log_2$ ratio is shown. Those genes with a statistically significant ($p < 0.01$) difference in expression levels are marked with an asterisk.

lence induced genes) family originally described in *Arabidopsis thaliana* consists of proteins containing GTPase domains that are involved in plant resistance to bacteria [20]. Orthologous genes, such as immunity-associated nucleotide 7 protein are found in higher mammals [21,22]. It is not yet known whether each of these proteins (lysozyme, the cercropin-domain protein and the AIG1-like proteins) are actually linked to antibacterial activity in *E. histolytica*, or whether, like the amoebapores, they could also play a role in damaging host cells. Their higher expression in HM-1:IMSS would appear to be more consistent with the latter interpretation, since there is no a priori reason to suspect that Rahman would have a deficit in bacterial killing. Transcripts from *E. histolytica* HM-1:IMSS trophozoites obtained in a murine model of amebic colitis showed increased expression of AIG1 genes, consistent with a potential role for this family in virulence [16].

The *E. histolytica* genome project delineated the large repertoire of potential signal transduction pathways in *Entamoeba* [7,23]. All of the major families contained with the eukaryotic protein kinase superfamily are represented within the

E. histolytica genome, including tyrosine kinases with SH2 domains, tyrosine kinase-like protein kinases, and putative receptor Ser/Thr kinases. We detected differences in the expression of multiple genes linked to signaling or stress response between Rahman and HM-1:IMSS (Tables 1 and 2), including protein kinases, RhoGTPase activating proteins, and protein phosphatases. Many of the signaling molecules are members of multigene families, and various representatives of each family were found expressed at higher levels in HM-1:IMSS or Rahman, suggesting that most are probably allelic differences. One notable exception was the finding of higher levels of calmodulin in *E. histolytica* HM-1:IMSS compared to Rahman (Table 1). A prior study demonstrated that inhibition of calmodulin activity in *E. histolytica* HM-1:IMSS reduced trophozoite phagocytosis and cytotoxic activity in vitro [24]. Thus, decreased calmodulin activity in Rahman would be consistent with the in vitro phenotype of decreased phagocytic activity we observed in Rahman [4].

E. histolytica trophozoites express high levels of GPI-anchored proteophosphoglycans (PPG) on their surface that appear to play an important role in amebic pathogenesis [25–28]. Interestingly, differences in the type and quantity of GPI-anchored propeophosphoglycans on the surface of HM-1:IMSS compared to Rahman have been reported [27,29]. The light chain of the Gal/GalNAc lectin also has a GPI anchor, which is required for assembly of the heavy and light chains into the lectin heterodimer [30]. With one exception, all of the genes encoding proteins linked to GPI synthesis showed increased expression in *E. histolytica* HM-1:IMSS compared to Rahman, and for two genes, GPI-deacetylase and mannosyltransferase-I, this difference was statistically significant. A recent study showed that decreasing expression of the GPI-deacetylase gene in *E. histolytica* HM-1:IMSS caused a decrease in endocytosis and trophozoite adhesion to host cells, indicating that lower levels of GPI-deacetylase expression could be associated with a reduced-virulence phenotype [31].

While we were able to identify a comparatively large number of genes that show differential expression between Rahman and HM-1:IMSS, many of these differences may not be directly related to virulence. Additional strain comparisons may help determine transcriptional differences which might be due to normal strain variation and those more directly involved in virulence. Because the sequence of the oligonucleotides on our microarray is derived from the *E. histolytica* HM-1:IMSS genome, we do not know how much of the transcriptional differences we detected represent differences at the genomic level between HM-1:IMSS and Rahman. There could be HM-1:IMSS genes that are simply not present in the Rahman genome, or sequence differences in homologous genes in Rahman and HM-1:IMSS that give rise to different hybridization efficiencies leading to "false positive" differences in transcript levels. However, it should be noted that in the two genes sequenced in both Rahman and HM-1:IMSS to date [4,32], genomic sequences were identical, and a comparison of genomic sequences between *E. histolytica* HM-1:IMSS and Rahman using a HM-1:IMSS-based genomic microarray showed only 5 of 1817 genes as being highly or significantly divergent [33].

In summary, our analysis identified a large number of genes that are differentially expressed between *E. histolytica* Rahman and *E. histolytica* HM-1:IMSS. The finding of multiple transcriptional differences between the two strains is consistent with the relatively broad physiologic differences that we, and others, have detected between Rahman and HM-1:IMSS [9,27,34,35]. Taken as a whole, these data indicate that there is not be a single gene product responsible for the differences in virulence between Rahman and HM-1:IMSS, but rather that multiple pathways, possibly involving signal transduction, antibacterial activity, cytoskeletal rearrangements, and protease production or secretion, differ between the two strains.

Acknowledgements

This work was supported by NIH grants AI37977 and AI30084 to SLS, AI0717225 to PHD, and U54AI057160, the Midwest Regional Center of Excellent for Biodefense and Emerging Infectious Diseases Research. SLS is a Burroughs Wellcome Scholar in Molecular Parasitology.

References

[1] Stanley Jr SL. Amoebiasis. Lancet 2003;361:1025–34.
[2] Mattern CF, Keister DB, Caspar PA. Experimental amebiasis. III. A rapid in vitro assay for virulence of *Entamoeba histolytica*. Am J Trop Med Hyg 1978;27:882–7.
[3] Som I, Azam A, Bhattacharya A, et al. Inter- and intra-strain variation in the 5.8S ribosomal RNA and internal transcribed spacer sequences of *Entamoeba histolytica* and comparison with Entamoeba dispar. *Entamoeba moshkovskii* and *Entamoeba invadens*. Int J Parasitol 2000;30:723–8.
[4] Davis PH, Zhang X, Guo J, et al. Comparative proteomic analysis of two *Entamoeba histolytica* strains with different virulence phenotypes identifies peroxiredoxin as an important component of amoebic virulence. Mol Microbiol 2006;61:1523–32.
[5] Zhang T, Cieslak PR, Stanley Jr SL. Protection of gerbils from amebic liver abscess by immunization with a recombinant *Entamoeba histolytica* antigen. Infect Immun 1994;62:1166–70.
[6] Diamond LS, Harlow DR, Cunnick CC. A new medium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*. Trans R Soc Trop Med Hyg 1978;72:431–2.
[7] Loftus B, Anderson I, Davies R, et al. The genome of the protist parasite *Entamoeba histolytica*. Nature 2005;433:865–8.
[8] Vandesompele J, De PK, Pattyn F, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 2002;3. RESEARCH0034.
[9] MacFarlane RC, Singh U. Identification of differentially expressed genes in virulent and nonvirulent *Entamoeba* species: potential implications for amebic pathogenesis. Infect Immun 2006;74:340–51.
[10] Mirelman D, Ankri S, Katz U, et al. Pathogenesis of *Entamoeba histolytica* depends on the concerted action of numerous virulence factors. Arch Med Res 2000;31:S214–5.
[11] Stanley Jr SL, Reed SL. Microbes and microbial toxins: paradigms for microbial–mucosal interactions. VI. *Entamoeba histolytica*: parasite–host interactions. Am J Physiol Gastrointest Liver Physiol 2001;280:G1049–54.
[12] Bruchhaus I, Loftus BJ, Hall N, et al. The intestinal protozoan parasite *Entamoeba histolytica* contains 20 cysteine protease genes, of which only a small subset is expressed during in vitro cultivation. Eukaryot Cell 2003;2:501–9.
[13] Tillack M, Nowak N, Lotter H, et al. Increased expression of the major cysteine proteinases by stable episomal transfection underlines the impor-

tant role of EhCP5 for the pathogenicity of *Entamoeba histolytica*. Mol Biochem Parasitol 2006;149:58–64.

[14] Zhang Z, Wang L, Seydel KB, et al. *Entamoeba histolytica* cysteine proteinases with interleukin-1 beta converting enzyme (ICE) activity cause intestinal inflammation and tissue damage in amoebiasis. Mol Microbiol 2000;37:542–8.

[15] Jacobs T, Bruchhaus I, Dandekar T, et al. Isolation and molecular characterization of a surface-bound proteinase of *Entamoeba histolytica*. Mol Microbiol 1998;27:269–76.

[16] Gilchrist CA, Houpt E, Trapaidze N, et al. Impact of intestinal colonization and invasion on the *Entamoeba histolytica* transcriptome. Mol Biochem Parasitol 2006;147:163–76.

[17] McCoy JJ, Mann BJ, Vedvick TS, et al. Structural analysis of the light subunit of the *Entamoeba histolytica* galactose-specific adherence lectin. J Biol Chem 1993;268:24223–31.

[18] Katz U, Ankri S, Stolarsky T, et al. *Entamoeba histolytica* expressing a dominant negative N-truncated light subunit of its gal-lectin are less virulent. Mol Biol Cell 2002;13:4256–65.

[19] Ankri S, Padilla-Vaca F, Stolarsky T, et al. Antisense inhibition of expression of the light subunit (35 kDa) of the Gal/GalNac lectin complex inhibits *Entamoeba histolytica* virulence. Mol Microbiol 1999;33:327–37.

[20] Reuber TL, Ausubel FM. Isolation of Arabidopsis genes that differentiate between resistance responses mediated by the RPS2 and RPM1 disease resistance genes. Plant Cell 1996;8:241–9.

[21] Poirier GM, Anderson G, Huvar A, et al. Immune-associated nucleotide-1 (IAN-1) is a thymic selection marker and defines a novel gene family conserved in plants. J Immunol 1999;163:4960–9.

[22] Krucken J, Schroetel RM, Muller IU, et al. Comparative analysis of the human gimap gene cluster encoding a novel GTPase family. Gene 2004;341:291–304.

[23] Beck DL, Boettner DR, Dragulev B, et al. Identification and gene expression analysis of a large family of transmembrane kinases related to the Gal/GalNAc lectin in *Entamoeba histolytica*. Eukaryot Cell 2005;4:722–32.

[24] rias-Negrete S, Munoz ML, Murillo-Jasso F. Expression of in vitro virulence by *Entamoeba histolytica*: effect of calmodulin inhibitors. APMIS 1999;107:875–81.

[25] Stanley Jr SL, Huizenga H, Li E. Isolation and partial characterization of a surface glycoconjugate of *Entamoeba histolytica*. Mol Biochem Parasitol 1992;50:127–38.

[26] Marinets A, Zhang T, Guillen N, et al. Protection against invasive amebiasis by a single monoclonal antibody directed against a lipophosphoglycan antigen localized on the surface of *Entamoeba histolytica*. J Exp Med 1997;186:1557–65.

[27] Moody-Haupt S, Patterson JH, Mirelman D, et al. The major surface antigens of *Entamoeba histolytica* trophozoites are GPI-anchored proteophosphoglycans. J Mol Biol 2000;297:409–20.

[28] Zhang Z, Duchene M, Stanley Jr SL. A monoclonal antibody to the amebic lipophosphoglycan-proteophosphoglycan antigens can prevent disease in human intestinal xenografts infected with *Entamoeba histolytica*. Infect Immun 2002;70:5873–6.

[29] Moody S, Becker S, Nuchamowitz Y, et al. Identification of significant variation in the composition of lipophosphoglycan-like molecules of *E. histolytica* and *E. dispar*. J Eukaryot Microbiol 1998;45:9S–12S.

[30] Ramakrishnan G, Lee S, Mann BJ, et al. *Entamoeba histolytica*: deletion of the GPI anchor signal sequence on the Gal/GalNAc lectin light subunit prevents its assembly into the lectin heterodimer. Exp Parasitol 2000;96:57–60.

[31] Vats D, Vishwakarma RA, Bhattacharya S, et al. Reduction of cell surface glycosylphosphatidylinositol conjugates in *Entamoeba histolytica* by antisense blocking of *E. histolytica* GlcNAc-phosphatidylinositol deacetylase expression: effect on cell proliferation, endocytosis, and adhesion to target cells. Infect Immun 2005;73:8381–92.

[32] Villalobo E, Wender N, Mirelman D. *Entamoeba histolytica*: molecular characterization of an aldose 1-epimerase (mutarotase). Exp Parasitol 2005;110:298–302.

[33] Shah PH, MacFarlane RC, Bhattacharya D, et al. Comparative genomic hybridizations of *Entamoeba* strains reveal unique genetic fingerprints that correlate with virulence. Eukaryot Cell 2005;4:504–15.

[34] Bujanover S, Katz U, Bracha R, et al. A virulence attenuated amoebaporeless mutant of *Entamoeba histolytica* and its interaction with host cells. Int J Parasitol 2003;33:1655–63.

[35] Dvorak JA, Kobayashi S, Nozaki T, et al. Induction of permeability changes and death of vertebrate cells is modulated by the virulence of *Entamoeba* spp. isolates. Parasitol Int 2003;52:169–73.