Step I: White Paper Concept Approval Request

Project Title: Genome sequencing of clinical strains of Entamoeba histolytica

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It has been shown recently by our group using the paired-samples from 3 countries (Bangladesh, Italy and USA) that parasite genotypes in the intestine and aspirated pus samples of amebic liver abscess (ALA) patients are different in the same patient (Ali et al, 2008). This suggests that either the initial intestinal infection was with more than one strain (or genotype) of E. histolytica, but only one of these strains (which has to be a minor population) had the ability to migrate and cause liver abscess in the infected patient or a DNA recombination event is taking place during the migration of ameba from intestine to the liver. A comparison of genomic sequences between intestinal and liver abscess strains from the same ALA patient may provide vital information as to what is actually happening. And if it is indeed a DNA recombination event, then this comparison might provide clues on how it is helping render the parasite capable of migrating to the liver site.

E. histolytica strains can be maintained in laboratory either xenically (i.e., in presence of bacteria) or axenically (in the absence of bacteria). Many investigations such as parasite virulence or gene expression analysis are being carried out using the axenic strains of E. histolytica, and as a result, we do not know how accurately this mimics the parasite in their actual host environment (intestine) where they encounter host microbiome. Also, it has been demonstrated for certain E. histolytica strains that they display increased virulence in in vitro or mouse model experiments if they are cultured in xenic condition. A genome sequencing of representative strains before and after axenization may provide information on whether axenization may result in a change in the genome

13. Tibayrenc M, Kjellberg F, Ayala FJ (1990) A clonal theory of parasitic protozoa: the population structures of Entamoeba, Giardia, Leishmania, Naegleria, Plasmodium, Trichomonas, and Trypanosoma and their medical and taxonomical consequences. Proceedings of National Academy of Science, USA 87:2414-2418.
14. Ali IK, Solaymani-Mohammadi S, Akhter J, Clark CG, Roy S, et al. (2008) Tissue Invasion by Entamoeba histolytica: Evidence of Genetic Selection and/or DNA Reorganization Events in Organ Tropism. PLoS Neglected Tropical Diseases 2:e219.

Demonstration of the relevant scientific community's size and depth of interest in the proposed sequencing or genotyping data for this organism or group of organisms.

There are several laboratories in the world working on amebiasis, and quite a few of them are interested in sequencing or genotyping data for Entamoeba histolytica strains. The laboratory of Dr. William A. Petri, Jr. at the University of Virginia supports the genotyping of E. histolytica strains from clinical specimens. Dr. Petri's lab has a collaboration with a number of other renowned labs working on amebiasis world-wide including Bangladesh, Japan, India, Nepal, Turkey etc. In Bangladesh, there is an excellent field study going on of amebiasis at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) by Dr. Rashidul Haque, in collaboration with the University of Virginia, and clinical specimens for genome sequencing will be available from there. In addition, 4 axenic clinical strains for sequencing are available from Dr. Petri's lab.

Utility of the new sequencing or genotyping information

Successful sequencing of the proposed strains will help the scientific community to better understand the genomic basis and difference between asymptomatic and disease-causing E. histolytica strains.

Certain SNPs may be identified that will show association with a particular clinical sample group, which will ultimately help discover a new genotyping system with a very high predictive value for clinical outcome.

Genomic sequences of xenic versus corresponding axenic strains will help us understand if axenization causes change in genome sequences. If this is so, it would be interesting to look into sequences of known virulence factors, such as cysteine proteases, amebapores, lectins etc to learn why axenic strains are generally less virulent.

Genomic sequences of cyst-stage versus corresponding excysted trophozoite will help us understand if excystation causes change in genome sequences, and it may explain how a new genotype of E. histolytica evolves in actual world.

Status of other projects on the same organism.

I do not know the details of sequencing project at the Liverpool University in the UK. I recently learnt from Graham Clark that they have sequenced *E. histolytica* Rahman strain and in the process of sequencing another strain “2592100”.

Arrangements for deposit of resulting reagents, resources, and datasets in NIAID approved repositories.

The resulting genome sequences will be deposited to the NIAID approved repositories.

List availability of other funding sources for the project.

Not known.

Nature, Availability & Source of Reagents/Samples:

Indicate availability of laboratory strains and clinical isolates. State sample types to be

Stanford University for the strain SAW891 (which I know that it encysts, although inefficiently, at Gretchen's hand in there).

6. There is a possibility that we would like paired-strains' DNA (i.e. DNA purified from the intestinal strain as well as corresponding LA pus strain from the same ALA patient) from ICDDR,B. However, the isolation of strain from the patient's LA pus material has been unsuccessful so far.

Collaborator Role:

List all potential project collaborators and their role in the project

Dr. Rashidul Haque, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), will provide xenic and any liver abscess strains of *E. histolytica* and will also provide DNA samples isolated from the purified *E. histolytica* cysts.

Dr. Upinder Singh, Stanford University, will provide xenic *E. histolytica* strain SAW891 or its DNA, as well as cyst DNA from this strain, if necessary.

NIAID's Genomic Sequencing Center Reagent, Data & Software Release Policy:

Accept

http://www3.niaid.nih.gov/research/resources/mscs/data.htm

Accept Decline

Describe arrangements for deposit of resulting reagents, resources, and datasets in NIAID approved repositories.

Investigator Signature: