(Form4-2)

JOINT RESEARCH PROJECT

FINAL REPORT
For Japan-Korea Joint Research Project

AREA	1. Mathematics & Physics 2. Chemistry & Material Science 3. Biology 4. Informatics & Mechatronics 5. Geo-Science & Space Science [6.] Medical Science 7. Humanities & Social Sciences

1. Research Title:

Pathogenesis of the enteric protozoan parasite *Entamoeba histolytica*

2. Term of Research: From July1, 2009 To June 30, 2011

3. Total Budget

a. Financial Support by JSPS: Total amount: 2,400 thousand yen

1st Year 900 thousand yen 2nd Year 1,200 thousand yen

3rd Year 300 thousand yen

b. Other Financial Support : Total amount: 0 thousand yen

4. Project Organization

a. Japanese Principal Researcher	
Name	Tomoyoshi Nozaki
Institution / Department Position	National Institute of Infectious Diseases, Department of Prasitology, Director
b. Korean Principal Researcher	
Name	Myeong Heon Shin
Institution / Department Position	Yonsei University College of Medicine, Department of Environmental Medical Biology & Institute of Tropical Meicine, Associate Professor

c. List of Japanese-side Participants (Except for Principal Researcher)

Name	Institution/Department	Position
Yumiko Saito-Nakano	National Institute of Infectious Diseases, Department of Prasitology	Senior Researcher
Kumiko Nakada-Tsukui	National Institute of Infectious Diseases, Department of Prasitology	Senior Researcher
Atsushi Furukawa	Gunma University Graduate School of Medicine, Department of Prasitology	PhD student

d. List of Korean-side Participants (Except for Principal Researcher)

Name	Institution/Department	Position
Young Ah Lee	Yonsei University College of Medicine, Department of Environmental Medical Biology & Institute of Tropical Meicine	PhD student
Kyeong Ah Kim	Yonsei University College of Medicine, Department of Environmental Medical Biology & Institute of Tropical Meicine	PhD student
Tai Soon Yong	Yonsei University College of Medicine, Department of Environmental Medical Biology & Institute of Tropical Meicine	Professor

5. Number of Exchanges during the Final Fiscal Year*

a. from Japan to Korea *Japanese fiscal year begins April 1.

Name	Home Institution	Duration	Host Institution
Tomoyoshi Nozaki	National Institute of Infectious Diseases	5.23.2011-5.26.2011	Yonsei University College of Medicine
Yumiko Saito-Nakano	National Institute of Infectious Diseases	5.23.2011-5.25.2011	Yonsei University College of Medicine

For Final Fiscal Year(FY2011) Total: 2 persons	For Final Fiscal Year(FY2011) Total: 7 man-days
Numbers of Exchanges during the past fiscal years	
FY2009: Total 1 persons FY2010: Total 0 persons	

b. from Korea to Japan

Name	Home Institution	Duration	Host Institution
For Final Fiscal Year(FY2011) Total: 0 persons		For Final Fiscal Year(FY2011) Total: 0 man-days	
Numbers of Exchanges during the past fiscal years			
FY2009: Total 5 persons FY2010: Total 6 persons			

6. Objective of Research

<General background> Amebiasis, caused by *Entamoeba histolytica,* is the major cause of death only after malaria among infections caused by protozoa, and affects 1% of the world population and kills 40,000-110,000 annually. The disease is regarded as one of "neglected tropical diseases (NTD)" and G8 countries committed their efforts to reduce the burden caused by the disease.
<Introduction> When *E. histolytica* colonizes the large intestine and invades colonic epithelial tissues, *E. histolytica* induces apoptosis in host immune cells such as T lymphocytes to evade immunity and sustain parasitism.
<Objective of the proposed study>
We will establish the molecular basis of this mechanism to elucidate immune evasion and virulence mechanisms of *E. histolytica*.
<Uniqueness of the proposed study>
Our proposal is based on a tight collaboration between two groups with different expertise. The Japanese Principal Researcher, Nozaki, is an expert on the virulence mechanisms of *E. histolytica* and established that vesicular trafficking of the major virulence factor cysteine proteases plays a pivotal role in the pathogenesis. In contrast, the Korean Principal Researcher, Shin, is an expert on host responses during amebic infection. Shin discovered that *E. histolytica* induces apoptosis in T cells during adherence.
<Contribution to Korea-Japan cooperation>
We will continue to mutually exchange PhD students (Gunma U. and Yonsei U.) to promote international collaboration. This proposal will strengthen the international partnership in parasitology

7. Methodology

1. Production of *E. histolytica* lines where intracellular trafficking of cysteine proteases (CPs) is impaired
   a. Construction of plasmids for transfection of *E. histolytica*
      The *E. histolytica* genome encodes two isotypes of the intrinsic inhibitors of CPs (ICP1 and ICPs), which is localized to the cytosol and lysosomes, respectively. We constructed plasmids to overexpress ICP1 and ICP2 with the epitope tag, e.g., hemaglutinin (HA) or repress their expression.
   b. Transfection and establishment of *E. histolytica* lines to overexpress ICPs
      We produced *E. histolytica* transformant lines, where ICP expression is either augmented or repressed. The level of ICP and CP expression and intracellular distribution was examined by immunoblots, fractionation, and immunofluorescence analyses.

2. Analysis of signaling events in apoptosis caused by *E. histolytica*
   a. Identification of signaling pathways involved in apoptosis caused by *E. histolytica*
      We investigated intracellular signaling molecules such as caspases, calpain, cathepsin, NADPH oxidase-derived ROS, involved in host cell death of colon epithelial cells by adherence of *Entamoeba histolytica*.
   b. Examination of ability of secretory components secreted by *E. histolytica* to induce activation of pro-apoptotic signaling machineries in colon epithelial cells.

3. Functional analysis of overexpression and repression of ICPs on apoptosis
   a. Examination of overexpression and repression of ICPs on the *E. histolytica*'s ability to induce apoptosis in Jurkat T cells and colon epithelial cells
      We examined the effects using real-time imaging of the interaction of the amoeba transformants and Jurkat cells..

4. Analysis of signaling events in apoptosis caused by *E. histolytica*
   a. Identification of signaling pathways involved in apoptosis caused by *E. histolytica*
      We examined whether siRNA-mediated repression of calpain or NADPH oxidase in host cells can inhibit *E. histolytica*-induced cell death. In addition, they will examine whether there is a cross-talk between calcium and ROS in colon cells stimulated with *E. histolytica*.
   b. Identification of the pro-apoptotic role of amoebic-secreted cysteine proteases