

Experimental Models to Study the Pathogenesis of Amoebiasis: Contribution of Morphological Methodologies.

Victor Tsutsumi and Mineko Shibayama

Department of Experimental Pathology, Center for Research and Advanced Studies, National Polytechnic Institute, Mexico City, Mexico. E-mail: vtsutsu@cinvestav.mx

For several decades, one of the research interests in amoebiasis has been the understanding of the mechanisms of tissue damage, either intestinal or hepatic. In vitro studies performed in the protozoan parasite *Entamoeba histolytica* have contributed importantly on the knowledge of the parasite itself, and diverse molecules have been reported in the process of damage; however, studies directly related to the tissue invasion and the establishment of the amoebic lesions have required the use of in vivo experimental models. Therefore, it is increasingly clear that neither the host nor the parasite are passive participants but that the infection process alters them both. The main interest of our group has been the study of the pathogenesis of intestinal and hepatic amoebiasis using both susceptible and resistant laboratory animals. In the case of hamster as susceptible rodent for hepatic infection, we reported about 20 years ago that the host inflammatory response to amoebic infection plays an important role in tissue destruction. By electron microscopy analysis, we demonstrated that lysis of inflammatory cells are clearly related with the liver destruction. Similar hepatic studies in gerbils, and in experimental intestinal lesions produced also in gerbils, guinea pigs and hamsters have shown the important role of inflammatory cells in host tissue destruction. Using immunocytochemical methods, we demonstrated that amoebic antigen can be diffused at some distance from the trophozoites and acute and chronic inflammatory cells are capable to capture this antigen. Moreover, in the very early hepatic infection we analyzed some molecules related with the activation of sinusoidal endothelial cells. These cells, beside capturing amoebic antigen, produced the activation of some molecules known as pro-inflammatories, such as adhesion (ICAM-1 and ICAM-2), and blood clotting Von Willebrand factor. Regarding to the role of nitric oxide on the pathogenesis of hepatic amoebiasis, and contrarily to the reported in vitro studies, our in vivo experiments performed in hamsters showed that this molecule does not have any amoebicidal effect. Currently, we are studying other amoebic molecules that may or may not regulating the production of some cytokines (TNF, IFN, and others) produced by host inflammatory cells. More recently, we studied a lectin that has been closely related to the adhesion of the parasite to target cells and participates importantly during the early stages of pathogenesis of intestinal as well as hepatic amoebiasis. This 170 kDa lectin has been immunolocalized by cytochemistry and electron microscopy at the plasma membranes and at membranes of cytoplasmic vacuoles and appeared to be secreted by the parasite. Its role in the process of tissue damage is still unclear, although some data suggest that it may be participating during the apoptotic phenomenon observed in the target cell death.

The use of experimental animals resistant to develop amoebic lesions has also been very useful for understanding some processes related to protection against amoebic infection. For this purposes we have used guinea pigs, rats and more recently different species of mice, including Balb/c, C3H/HeJ and C57BL/6J. Intestinal and hepatic inoculations of *E. histolytica* trophozoites in these rodents have suggested the important role of the non-specific or natural immune response by rejecting the amoebic infection, mainly mediated by polymorphonuclear neutrophils.

In conclusion, morphological studies that include light microscopy, histochemistry, immunocytochemistry, transmission and scanning electron microscopy and immunoelectron microscopy, have all contributed importantly for a better understanding the mechanisms of pathogenesis of *E. histolytica*. Although various in vitro studies for the analysis of host-parasite interaction have been performed, our objectives have been mainly focused on the physiopathological processes or phenomena occurring in vivo models and based on their susceptibility or resistance to amoebic infection.

1. Tsutsumi V, Martínez-Palomo A. Inflammatory reaction in experimental hepatic amebiasis: An ultrastructural study. *Amer. J. Pathol.* 130:112-119. 1988.
2. Shibayama-Salas M, Navarro-García F, López-Revilla R, Martínez-Palomo A, Tsutsumi V. *In vivo* and *in vitro* intestinal amebiasis in gerbils. *Parasitol. Res.* 83:170-176, 1997.
3. Pacheco J, Shibayama M, Campos R., Beck DL, Houpt E, Petri WA Jr, Tsutsumi V. In vitro and in vivo interaction of *Entamoeba histolytica* Gal/GalNAc lectin with various target cells: An immunocytochemical analysis. *Parasitol. International.* 53:35-47, 2004.