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Role of NADPH Oxidase and ERK1/2 MAPK in Neutrophil Apoptosis Induced by Protozoan Parasite *Entamoeba histolytica*

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Entamoeba histolytica is a tissue-invasive protozoan parasite that causes amoebic dysentery and liver abscess in human beings. To establish successful attachment and invasion of the amoeba in vivo, *E. histolytica* must to bind to the large intestinal epithelium and destroys the tissues. In vitro live trophozoites of *E. histolytica* have been well known to induce apoptosis of host cells including neutrophils, T lymphocytes and macrophages. Host cell apoptosis by the parasite pathogens might be of particularly important for both the parasite as a survival mechanism and the host as a defense mechanism for the subsequent clearance of apoptotic neutrophils by the macrophages recruited at the inflammation sites.

Mitogen-activated protein kinase (MAPK) cascades are protein kinase transduction pathways that are deeply involved in the signaling for various immune responses including apoptosis. In mammalian cells, there are at least three MAPK subtypes, such as extracellular signal-regulated kinase (ERK1/2), p38 MAPK and c-Jun N-terminal kinase (JNK). The ERK1/2 cascade is activated through receptor-mediated signaling stimuli including growth factors, and is associated with cell proliferation, differentiation and survival. Reactive oxygen species (ROS), such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-), have recently been regarded as important intracellular signaling messengers inducing apoptosis. Intracellular ROS have been reported to directly activate MAPK in cell death systems.

Neutrophils are recruited to the inflammatory sites as a first line of strong defense against microbes including *E. histolytica*. Circulating neutrophils have a short life span in vivo, and aged cells in vitro undergo a spontaneous death within 1-2 days of culture in the absence of growth factors. In spite of fact that MAPK and ROS have been found to be powerful signaling molecules responsible for mediating neutrophil apoptosis, the possible role of ROS and MAPK in host cell apoptosis induced by *E. histolytica* is not totally understood.

In this study, we investigated the role of ROS and their interaction with MAPK in the neutrophil apoptosis induced by *E. histolytica*. The neutrophils incubated with live trophozoites of *E. histolytica*

revealed a marked increase of receptor shedding of CD16 as well as phsophatidylserine (PS) externalization on the cell surface. The *Entamoeba*-induced apoptosis was effectively blocked by pretreatment of cells with DPI, a flavoprotein inhibitor of NADPH oxidase. A large amount of intracellular ROS was detected after exposure to viable trophozoites, and the treatment with DPI strongly inhibited the *Entamoeba*-induced ROS generation. However, a mitochondrial inhibitor rotenone did not attenuate the *Entamoeba*-induced ROS generation and apoptosis. Although *E. histolytica* strongly induced activation of ERK1/2 and p38 MAPK in neutrophils, the activation of ERK1/2 was closely associated with ROS-mediated apoptosis. Pretreatment of neutrophils with MEK1 inhibitor PD98059, but not p38 MAPK inhibitor SB202190, prevented *Entamoeba*-induced apoptosis. Moreover, DPI almost completely inhibited *Entamoeba*-induced phosphorylation of ERK1/2, but not phosphorylation of p38 MAPK. These results suggest strongly that NADPH oxidase derived ROS-mediated activation of ERK1/2 is required for the *Entamoeba*-induced neutrophil apoptosis.

In summary, we have presented evidence that NADPH oxidase generated ROS (a non-mitochondrial source of ROS) induces activation of ERK1/2 MAPK, which is essential for neutophil apoptosis induced by live trophozoites of *E. histolytica*. The comprehension of the molecular signaling mechanisms in the neutrophil apoptosis caused by *E. histolytica* can provide a better understanding of the fine tuning systems in the host-parasite specific interaction, which can also be of large benefit for treatment of host organisms involved in parasitic infections.

References

1. Stanley Jr., S. L. 2003. Amoebiasis. *Lancet 361:1025.*
2. Stanley Jr., S. L., and S. L. Reed. 2001. Microbes and microbial toxins: Paradigms for microbial-mucosal interactions VI. *Entamoeba histolytica:* parasite-host interactions. *Am. J. Physiol. Gastrointest. Liver Physiol. 280:G1049.*
3. Petri Jr., W. A., R. Haque, and B. J. Mann. 2002. The bittersweet interface of parasite and host: lectin-carbohydrate interactions during human invasion by the parasite *Entamoeba histolytica. Annu. Rev. Microbiol. 56:39.*
4. Velazquez, C., M. Shibayama-Sales, J. Aguirre-Garcia, V. Tsutsumi, and J. Calderon. 1998. Role of neutrophils in innate resistance to *Entamoeba histolytica* liver infection in mice. *Parasite Immunol. 20:255.*
5. Sim, S., K. A. Kim, T. S. Yong, S. J. Park, K. I. Im, M. H. Shin. 2004. Ultrastructural observation of human neutrophils during apoptotic cell death triggered by *Entamoeba histolytica. Korean J. Parasitol.* 42:205.
6. Huston, C. D., E. R. Houpt, B. J. Mann, C. S. Hahn, and W. A. Petri Jr. 2000. Caspase 3-dependent killing of host cells by the parasite *Entamoeba histolytica. Cell. Microbiol. 2:617.*

7. Huston, C. D., D. R. Boettner, V. Miller-Sims, and W. A. Petri Jr. 2003. Apoptotic killing and phagocytosis of host cells by the parasite *Entamoeba histolytica. Infect. Immun. 71:964.*
8. Tong, C., R. J. Davis, and R. A. Flavell. 2002. MAP kinases in the immune responses. *Annu. Rev. Immunol. 20:55.*
9. Stanciu, M., Y. Wang, R. Kentor, N. Burket, S. Watkins, G. kress, I. Reynold, E. Klann, M. R. Angiolieri, J. W. Johnson, and D. B. DeFranco. 2000. Persistent activation of ERK contributes to glutamate-induced oxidative toxicity in a neuronal cell line and primary cortical neuron cultures. *J. Biol. Chem. 275:12200.*
10. Wang, X., J. L. Martindale, Y. Liu, and N. J. Holbrook. 1998. The cellular responses to oxidative stress: influences of mitogen-activated protein kinase signaling pathways on cell survival. *Biochem. J. 333:291.*
11. Forman, H. J., and M. Torres. 2002. Reactive oxygen species and cell signaling. *Am. J. Respir. Crit. Care Med. 166:S4.*
12. Zhang, X., P. Shan, M. Sasidhar, G. L. Chupp, R. A. Flavell, A. M. Choi, and P. J. Lee. 2003. Reactive oxygen species and extracellular signal-regulated kinases 1/2 mitogen-activated protein kinase mediate hyperoxia-induced cell death in lung epithelium. *Am. J. Respir. Cell. Mol. Biol. 28:305.*
13. Seydel, K. B. T. Zhang, and S. L. Stanley Jr. 1997. Neutrophils play a critical role in early resistance to amoebic liver abscess in severe combined immunodeficient mice. *Infect. Immun. 65:3951.*
14. Dransfield, I., A. Buckle, J. S. Savil, A. McDowel, C. Haslett, and N. Hogg. 1994. Neutrophil apoptosis is associated with a reduction in CD16 (FcγRIII) expression. *J. Immunol. 153:1254.*
15. Takeda, Y., H. Watanabe, S. Yonehara, T. Yamashita, S. Saito, and F. Sendo. 1993. Rapid acceleration of neutrophil apoptosis by tumor necrosis factor-α. *Int. Immunol. 5:691.*
16. Kasahara, Y., K. Iwai, A. Yachie, K. Ohta, A. Konno, H. Seki, T. Miyawaki, and N. Taniguchi. 1997. Involvement of reactive oxygen intermediates in spontaneous and CD95 (Fas/APO-1)-mediated apoptosis of neutrophils. *Blood 89:1748.*
17. Frasch, S. C., J. A. Nick, V. A. Fadok, D. L. Bratton, G. S. Worthen, and P. M. Henson. 1999. p38 Mitogen-activated protein kinase-dependent and –independent intracellular signal transduction pathways leading to apoptosis in human neutrophils. *J. Biol. Chem. 273:8389.*
18. Lundqvist-Gustafsson, H., S. Norman, J. Nilsson, and A. Wilsson. 2001. Involvement of p38-mitogen-activated protein kinase in *Staphylococcus aureus*-induced neutrophil apoptosis. *J. Leukoc. Biol. 70:642.*
19. Savill, J., and C. Haslett. 1995. Granulocyte clearance by apoptosis in the resolution of inflammation. *Semin. Cell. Biol. 6:385.*

20. Lundqvist-Gustafsson, H., and T. Bengtsson. 1999. Activation of the granule pool of the NADPH oxidase accelerates apoptosis in human neutrophils. *J. Leukoc. Biol. 65:196.*
21. Karlsson, A., and C. Dahlgren. 2002. Assembly and activation of the neutrophil NADPH oxidase in granule membranes. *Antioxid. Redox Signal. 4:49.*
22. Zhang, B., J. Hirahashi, X. Cullere, and T. N. Mayadas. 2003. Elucidation of molecular events leading to neutrophil apoptosis following phagocytosis. *J. Biol. Chem. 278:28443.*