In Vitro Evaluation of the Interaction of Salmonella with Human Macrophages

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ABSTRACT

Salmonella, a gram-negative rod, is one of the most common bacterial pathogens to humans. There are more than 2,500 serotypes among Salmonella. S. Typhi and S. Paratyphi cause typhoid and paratyphoid fevers in humans, respectively, while other non-typhoid Salmonella, such as S. Typhimurium, S. Enteritidis, and S. Choleraesuis, usually cause gastrointestinal infections and bacteremia. In this study, we used human macrophage cell line, THP-1, and different serotypes of Salmonella to investigate the Salmonella- host cell interactions. All serotypes of Salmonella appeared to survive within THP-1 cells following the ingestion. Nevertheless, S. Choleraesuis, compared to S. Typhimurium, S. Typhi, and S. Enteritidis, expressed higher ability to survive and replicate within macrophages. Flow cytometry with Annexin V-FITC/PI double staining was used to evaluate the cell death of macrophages caused by Salmonella. At 12- and 14- hr post-infection, S. Choleraesuis provoked 16% and 18% of THP-1 macrophages to cell death. Only the PMA-activated THP-1 cells exhibited significant level of apoptosis, compared to non-activated THP-1 cells following Salmonella infection. Transmission electron microscope demonstrated typical morphological changes of apoptosis in THP-1 cells, including nuclear condensation and cytoplasmic vacuolization. We also used Oligo GEArray (Superarray) to assess the gene expression profiles of macrophages that underwent apoptosis following ingestion of Salmonella. S. Choleraesuis SC-B67 apparently induced the expression of genes associated with apoptosis in THP-1, including caspase-3, caspase-8, caspase-9, Bax and Bad. Our data suggested that Salmonella induced the late-phase apoptosis of macrophages mainly through intrinsic pathway. The ability to modulate the activity of effector caspases may represent an unexploited avenue for therapeutic intervention in Salmonella infections.

Keywords : Salmonella, Salmonella enterica serotype Choleraesuis, Macrophage, Flow Cytometry, Transmission electron microscope, Microarray, Apoptosis

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REFERENCES

1. Antonsson, B. and J. C. Martinou. 2000. The Bcl-2 protein family. Exp. Cell Res. 256:50-57. 2. Arends, M. J., R. G. Morris, and A. H. Wyllie. 1990. Apoptosis. The role of the endonuclease. Am. J. Pathol. 136:593-608. 3. Borner, C., R. Olivier, I. Martinou, C. Mattmann, J. Tschopp, and J. C. Martinou. 1994. Dissection of functional domains in Bcl-2 alpha by site-directed mutagenesis. Biochem. Cell Biol. 72:463-469. 4. Buchmeier, N. A. and F. Heffron. 1991. Inhibition of macrophage phagosome-lysosome fusion by Salmonella typhimurium. Infect. Immun. 59:2232-2238. 5. Buchmeier, N. A., S. J. Libby, Y. Xu, P. C. Loewen, J. Switala, D. G. Guiney, and F. C. Fang. 1995. DNA repair is more important than catalase for Salmonella virulence in mice. J. Clin. Invest 95:1047-1053. 6. Chaubal, L. H. and P. S. Holt. 1999. Characterization of swimming motility and identification of flagellar proteins in Salmonella pullorum isolates. Am. J. Vet. Res. 60:1322-1327. 7. Chittenden, T., C. Flemington, A. B. Houghton, R. G. Ebb, G. J. Gallo, B. Elangovan, G. Chinnadurai, and R. J. Lutz. 1995. A conserved domain in Bak, distinct from BH1 and BH2, mediates cell death and protein binding functions. EMBO J. 14:5589-5596. 8. Chiu, C. H., T. Y. Lin, and J. T. Ou. 1999. Predictors for extraintestinal infection of non-typhoidal Salmonella in patients without AIDS. Int. J. Clin. Pract. 53:161-164. 9. Chiu, C.H., T.L. Wu, L.H. Su, C. Chu, J.H. Chia, A.J. Kuo, M.S. Chien, T.Y. Lin. 2002. The emergence in Taiwan of fluoroquinolone resistance in Salmonella enterica serotype Choleraesuis. New Engl .J. Med. 346: 413-9. 10. Cohen, J. I., J. A. Bartlett, and G. R. Corey. 1987. Extra-intestinal manifestations of Salmonella infections. Medicine (Baltimore) 66:349-388. 11. Ekins, S., E. Kirillov, E. A. Rakhmatulin, and T. Nikolskaya. 2005. A novel method for visualizing nuclear hormone receptor networks relevant to drug metabolism. Drug Metab Dispos. 33:474-481. 12. Finlay, B. B., B. Gumbiner, and S. Falkow. 1988. Penetration of Salmonella through a polarized Madin-Darby canine kidney epithelial cell monolayer. J. Cell Biol. 107:221-230. 13. Forsberg, M., R. Blomgran, M. Lerm, E. Sarndahl, S. M. Sebti, A. Hamilton, O. Stendahl, and L. Zheng. 2003. Differential effects of invasion by and phagocytosis of Salmonella typhimurium on apoptosis in human macrophages: potential role of Rho-GTPases and Akt. J. Leukoc. Biol. 74:620-629. 14. Green, D. R. and J. C. Reed. 1998. Mitochondria and apoptosis. Science 281:1309-1312. 15. Gray, J. T., P. J. Fedorka-Cray, T. J. Stabel, and T. T. Kramer. 1996. Natural transmission of Salmonella choleraesuis in swine. Appl. Environ. Microbiol. 62:141-146. 16. Gray, J. T. and P. J. Fedorka-Cray. 2001. Long-term survival and infectivity of Salmonella choleraesuis. Berl Munch. Tierarztl. Wochenschr. 114:370-374. 17. Guiney, D. G. 2005. The role of host cell death in Salmonella infections. Curr. Top. Microbiol. Immunol. 289:131-150. 18. Haimovich, B. and M. M. Venkatesan. 2006. Shigella and Salmonella: death as a means of survival. Microbes. Infect. 8:568-577. 19. Hengartner, M. O. 2000. The biochemistry of apoptosis. Nature 407:770-776. 20. Herr, I. and K. M. Debatin. 2001. Cellular stress response and apoptosis in cancer therapy. Blood 98:2603-2614. 21. Holden, D. W. 2002. Trafficking of the Salmonella vacuole in macrophages. Traffic. 3:161-169. 22. Kerr, J. F., A. H. Wyllie, and A. R. Currie. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer 26:239-257. 23. Knodler, L. A. and B. B. Finlay. 2001. Salmonella and apoptosis: to live or let die? Microbes Infect. 3:1321-1326. 24. Larrick, J. W., D. G. Fischer, S. J. Anderson, and H. S. Koren. 1980. Characterization of a human macrophage-like cell line stimulated in vitro: a model of macrophage functions. J. Immunol. 125:6-12. 25. Lundberg, A. S. and R. A. Weinberg. 1999. Control of the cell cycle and apoptosis. Eur. J. Cancer 35:1886-1894. 26. Luo, X., I. Budihardjo, H. Zou, C. Slaughter, and X. Wang. 1998. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell 94:481-490. 27. Matsuda, H., F. R. Strebel, T. Kaneko, L. C. Stephens, L. L. Danhauser, G. N. Jenkins, N. Toyota, and J. M. Bull. 1996. Apoptosis and necrosis occurring during different stages of primary and metastatic tumor growth of a rat mammary adenocarcinoma. Anticancer Res. 16:1117-1121. 28. Mattson, M. P., Q. Guo, K. Furukawa, and W. A. Pedersen. 1998. Presenilins, the endoplasmic reticulum, and neuronal apoptosis in Alzheimer's disease. J. Neurochem. 70:1-14. 29. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607-625. 30. Monack, D. M., D. Hersh, N. Ghori, D. Bouley, A. Zychlinsky, and S. Falkow. 2000. Salmonella exploits caspase-1 to colonize Peyer's patches in a murine typhoid model. J. Exp. Med. 192:249-258. 31. Nakagawa, T., H. Zhu,

N. Morishima, E. Li, J. Xu, B. A. Yankner, and J. Yuan. 2000. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. Nature 403:98-103. 32. Nicholson, D. W. and N. A. Thornberry. 1997. Caspases: killer proteases. Trends Biochem. Sci. 22:299-306. 33. Niedergang, F., J. C. Sirard, C. T. Blanc, and J. P. Kraehenbuhl. 2000. Entry and survival of Salmonella typhimurium in dendritic cells and presentation of recombinant antigens do not require macrophage-specific virulence factors. Proc. Natl. Acad. Sci. U. S. A 97:14650-14655. 34. Popoff, M. Y., J. Bockemuhl, and L. L. Gheesling. 2003. Supplement 2001 (no. 45) to the Kauffmann-White scheme. Res. Microbiol. 154:173-174. 35. Reed, J. C. 1998. Bcl-2 family proteins. Oncogene 17:3225-3236. 36. Ryan, K. M. and G. D. Birnie. 1996. Myc oncogenes: the enigmatic family. Biochem. J. 314 (Pt 3):713-721. 37. Saraste, A. and K. Pulkki. 2000. Morphologic and biochemical hallmarks of apoptosis. Cardiovasc. Res. 45:528-537. 38. Schwan, W. R., X. Z. Huang, L. Hu, and D. J. Kopecko. 2000. Differential bacterial survival, replication, and apoptosis -inducing ability of Salmonella serovars within human and murine macrophages. Infect. Immun. 68:1005-1013. 39. Shiratsuchi, A., S. Osada, S. Kanazawa, and Y. Nakanishi. 1998. Essential role of phosphatidylserine externalization in apoptosing cell phagocytosis by macrophages. Biochem. Biophys. Res. Commun. 246:549-555. 40. Sizemore, D. R., E. A. Elsinghorst, L. C. Eck, A. A. Branstrom, D. L. Hoover, R. L. Warren, and F. A. Rubin. 1997. Interaction of Salmonella typhi strains with cultured human monocyte-derived macrophages. Infect. Immun. 65:309-312. 41. Stennicke, H. R. and G. S. Salvesen. 1998. Properties of the caspases. Biochim. Biophys. Acta 1387:17-31. 42. Su, L.H., C.H. Chiu, C. Chu, J.T. Ou. 2004. Antimicrobial resistance in nontyphoid Salmonella serotypes: a global challenge. Clin. Infect. Dis. 39:546-51. 43. Tauxe, R. V. 1997. Emerging foodborne diseases: an evolving public health challenge. Emerg. Infect. Dis. 3:425-434. 44. Tibbetts, M. D., L. Zheng, and M. J. Lenardo. 2003. The death effector domain protein family: regulators of cellular homeostasis. Nat. Immunol. 4:404-409. 45. Tollefson, L., S. F. Altekruse, and M. E. Potter. 1997. Therapeutic antibiotics in animal feeds and antibiotic resistance. Rev. Sci. Tech. 16:709-715. 46. Vermes, I., C. Haanen, H. Steffens-Nakken, and C. Reutelingsperger. 1995. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. J. Immunol. Methods 184:39-51. 47. Villa, P., S. H. Kaufmann, and W. C. Earnshaw. 1997. Caspases and caspase inhibitors. Trends Biochem. Sci. 22:388-393. 48. Yershov, G., V. Barsky, A. Belgovskiy, E. Kirillov, E. Kreindlin, I. Ivanov, S. Parinov, D. Guschin, A. Drobishev, S. Dubiley, and A. Mirzabekov. 1996. DNA analysis and diagnostics on oligonucleotide microchips. Proc. Natl. Acad. Sci. U. S. A 93:4913-4918. 49. Yoshikawa, T. T., P. Herbert, and P. A. Oill. 1980. Salmonellosis. West J. Med. 133:408-417. 50. Zimmermann, K. C., C. Bonzon, and D. R. Green. 2001. The machinery of programmed cell death. Pharmacol. Ther. 92:57-70. 51. Zou, H., Y. Li, X. Liu, and X. Wang. 1999. An APAF-1 cytochrome c multimeric complex is a functional apoptosome that activates procaspase-9. J. Biol. Chem. 274:11549-11556.