Protein Expression Profiling of Infected Murine Macrophage Cells (RAW 264.7) by *Bacillus anthracis* Spores

Gwi-Moon Seo, Jeong-Ah Nam, Kwang-Gun Oh[1], Young-Gyu Chai[*]

Dept. of Biochem. & Mol. Biol., Hanyang Univ., Ansan, Kyunggi-do, 425-791, Korea.

[1]Cell & Gene Co., Ansan Technopark, Ansan, Kyunggi-do, 425-971, Korea

Summary

Current therapeutic strategies for anthrax have had no significant impact on anthrax mortality over the last several decades. This study used a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) discovery platform to generate protein expression profiles in search of overexpressed proteins in murine macrophage cells (RAW264.7) which infected with *Bacillus anthracis* spores as potentially novel molecular targets. Two differentially expressed proteins were identified in infected murine macrophage cells as Syndapin and CDC46, respectively. Syndapins are potential links between the cortical actin cytoskeleton and endocytosis. Other two proteins were identified from murine macrophage cells infected with avirulent spores as ITBG-2 (CD18) and HSPA5, respectively. These data demonstrate the feasibility of using a MALDI-TOF platform to generate protein expression profiles and identify potential molecular targets for anthrax therapeutics.

Introduction

Bacillus anthracis, the causal agent of anthrax, is a Gram positive, endospore-forming, aerobic bacterium that colonizes its host using a virulence determinant and causes bacteraemia and toxemia. Virulence genes are located on two large plasmids, pXO1 (185kb) and pXO2 (95kb) (11). The pXO1 contains the three structural genes for the anthrax toxin proteins, *pagA* (protective antigen; PA), *lef* (lethal factor; LF), and *cya* (edema factor; EF) (14). The toxin proteins combine to form two binary exotoxins. Lethal toxin (LeTx) consists of PA, the moiety that is responsible for cellular binding and entry, and LF, which is a zinc metalloprotease. LeTx consists of PA and LF, the entry of macrophages has an effect of host death (3,7). Edema toxin (EdTx) consists of PA and EF, a calmodulin-dependent adenylate cyclase (9). The regulatory gene *atxA* is present on pXO1 and encodes a *trans*-acting protein that is required for the bicarbonate-mediated regulation of toxin and capsule expression (10). Bacterial survival in macrophages occurs by several routes. These include: [1] prevention of lysosome-phagosome fusion, [2] escape into the cytoplasm before phagosome-lysosome fusion occurs, and [3] resistance to antimicrobial response (4,13,15). In the case of *B.*

anthracis, it uses macrophage as a Trojan horse to enter the host (5). The immediate early stage of anthrax occurs when pathogenic endospores interact with macrophage cells at the initial site of entry into the host (5). Once phagocytosed, endospores are transported to the regional lymph nodes, germinating inside macrophage cells *en route*. During later stages of the disease, growth of vegetative bacilli occurs extra-cellularly with titers approaching 10^8 organisms per ml of blood. *B. anthracis* vegetative cells must survive the hostile intracellular environment of the macrophage phagosome and ultimately be released from the phagocyte for infection to spread (2,5,6).

For this study, we developed a mass spectrometry-based platform to generate protein expression profiles from macrophage cells infected with virulent *B. anthracis* spores in an effort to discover and identify novel molecular targets for anthrax.

Results and Discussion

B. anthracis Sterne or delta-Sterne spores was infected to murine macrophage RAW 264.7 cells according to the method of Dixon *et al.* (2). Infected spores were observed macrophage cells within 15 min. In this study, spores uninfected states have been removed by gentamycin washing step. In this result, at 90 min, infected spores in RAW 264.7 cells were germinated. After 2 hr, the morphology of macrophage cells was dramatically changed to cell death (7).

To elucidate that the early events of macrophage cells infected with *B. anthracis* spores, we performed 2DE (Fig. 1). In the 2-DE map, we got the 200 spots from delta Sterne, and 183 spots from Sterne. Molestina group published the 2-DE map of proteins from HEp-2 cells infected with *Chlamydia pneumoniae* using silver staining and approximately 600 protein spots could be detected by utilizing nonlinear pH 3 to 10 IPG strips (12).
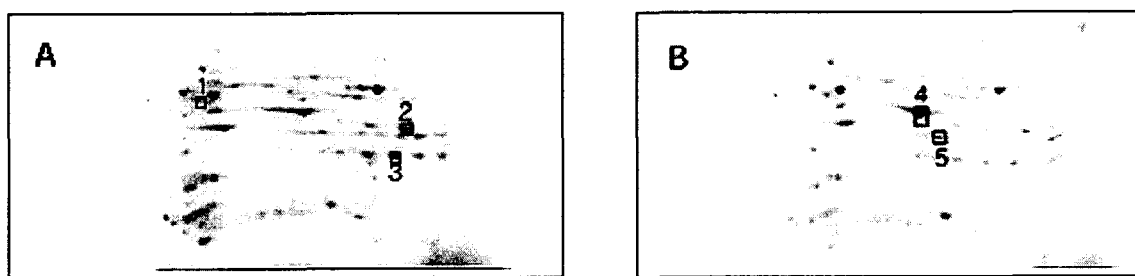

Figure 1. Two-dimensional gels of murine macrophage RAW264.7 cells for 1 hour after infection by *Bacillus anthracis* delta-Sterne spores (A) and by *Bacillus anthracis* Sterne spores (B). The indicated spots were chosen for MALDI-TOF analysis (1: ITBG-2, 2: CDC46, 3: HSPA5, 4: Syndapin 1, 5: GNA 13).

After the image analyses, some spot up-/down regulated by spores were identified by MALDI-TOF mass spectrometry. Patterns of measured masses were matched against theoretical masses of proteins found in the annotated database matrix science (http://www.matrixscience.com/).

Early intracellular events of *B. anthracis* spores and macrophage cells have determined the expression of ITBG-2, HSPA5, GNA-13, Syndapin 1, and CDC46. ITBG-2 and HSPA5 proteins

were highly expressed delta-Sterne than Sterne in 2-DE analysis. From this result, ITBG-2 mediates to resist phagocytosis from macrophage cells due to the infection of *B. anthracis* delta Sterne. In the infection of *M. tuberculosis* to macrophages, ITBG-2 level was decreased with live vegetative cells, however, it was not changed with heat-killed *M. tuberculosis* (1). Syndapin is associated with enhanced phagocytosis. In conclusion, the observations suggest that spores of Sterne are associated with enhanced phagocytosis and increased subsequent rate of germination, and that spores of delta Sterne strain are somewhat resisted phagocytosis.

References

1. DesJardin, L.E., T.M. Kaufman, B. Potts, B. Kutzbach, H. Yi, and L.S. Schlesinger. 2002. *Microbiology,* 148, 3161-3171.

2. Dixon, T.C., A.A. Fadl, T.M. Koehler, J.A. Swanson, and P.C. Hanna. 1999. *Cell Microbiol.* 6, 453-463.

3. Duesbery, N.S., C.P. Webb, S.H. Leppla, V.M. Gordon, K.R. Klimpel, T.D. Copeland, N.G. Ahn, M.K. Oskarsson, K. Fukasawa, K.D. Paull, and G.F. Vande Woude. Copeland. 1998. *Science* 280, 734-737.

4. Finlay, B.B., and P. Cossary. 1997. *Science* 276, 718-725.

5. Guidi-Rontani, C. 2002. *Trends Microbiol.* 10, 405-409.

6. Guidi-Rontani, C., M. Weber-Levy, E. Labruyere, and M. Mock. 1999. *Mol. Microbiol.* 31, 9-17.

7. Hanna, P.C., S. Kochi, and R.J. Collier. 1992. *Mol. Biol. Cell* 3, 1269-1277.

8. Ireland, J.A. and P.C. Hanna. 2002. *Infect. Immun.,* 10, 5870-5872.

9. Leppla, S.H. 1982. *Proc. Natl. Acad. Sci. USA,* 79, 3162-3166.

10. Makino, S., C. Sasakawa, I. Uchida, N. Terakado, and M. Yoshikawa. 1998. *Mol. Microbiol.* 2, 371-376.

11. Mock, M. and A. Fouet. 2001. *Annu. Rev. Microbiol.* 55, 647-671.

12. Molestina, R.E., J.B. Klein, R.D. Miller, W.H. Pierce, J.A. Ramirez, and J.T. Summersgill. 2002. *Infect. Immun.* 6, 2976-2981.

13. Nakachi, N., K. Mazsunaga, T.W. Klein, H. Friedman, and Y. Yamamoto. 2000. *Infect. Immun.* 68, 6069-6072.

14. Okinaka, R.T, K. Cloud, O. Hampton, A.R. Hoffmaster, K.K. Hill, P. Keim, T.M. Koehler, G. Lamke, S. Kumano, J. Mahillon, D. Manter, Y. Martinez, D. Ricke, R. Svensson, and P.J. Jackson. 1999. *J. Bacteriol.* 181, 6509-6515.

15. Slauch, J., R. Taylor, and S. Maloy. 1997. *Genes Dev.* 11, 1761-1774.

16. Zeger, D.L., N. Osman, M. Hennings, I.F. McKenzie, D.W. Sears, and P.M. Hogarth. 1990. *Immunogenetics* 31, 191-197.