INFECTION AND IMMUNITY, Jan. 2009, p. 565–566 Vol. 77, No. 1
0019-9567/09/$08.00+0 doi:10.1128/IAI.01160-08

Letter to the Editor

What Is the Relevance of Lung Epithelial Cells during the Dissemination of Spores in Inhalational Anthrax?

A recent paper published by Russell et al. suggests that *Bacillus anthracis* spores can be taken up by lung epithelial cells that then participate in the transport and diffusion of pathogens (6). In a model of BALB/c mice infected by the Sterne strain, the authors focus on data from 2 and 4 h after infection, which limits their analysis to the very early stages of infection. They analyzed spore-cell contact on a "crude lung cell suspension" by using confocal microscopy. Although a powerful technique, confocal microscopy simply does not enable observation of histological tissue structure or spatial orientation for spore contact. As a result, it cannot be concluded from what is shown that spores traverse (from outside to inside) the lung epithelium in vivo. One of the main questions unanswered is whether spores are only phagocytosed or are phagocytosed and then traverse throughout the epithelial cells.

Beside these technical flaws, the number of epithelial cells with intracellular spores is strikingly low, estimated at 20,163 for 3.3×10^6 epithelial cells ($0.62\% \pm 0.16\%$). This is to be compared to $>60\%$ of spore-phagocytosing alveolar macrophages after 10 min of infection in a recent report (2). The authors acknowledge in their Discussion that in vivo internalization frequency is only about 0.3% of the total inhaled spores. Although the authors' observations are interesting, it seems that the capture of anthrax spores by lung epithelial cells is a rather marginal phenomenon.

It should be stressed that epithelial cells are not motile per se. As *B. anthracis* spores and bacilli are not motile either, the bacterial spores need a host-provided vehicle for traversing the epithelial wall in their journey to the draining lymph nodes. The authors' hypothesis suggesting that "spores may potentially enter the pulmonary capillaries by a paracellular route" is simply not realistic according to what is known about spore and bacillus motility. Most data published so far on inhalational anthrax pathogenesis have shown that either macrophages or dendritic cells play the vehicle role (2–5). Such results mean that even if spores traverse the lung wall through the epithelial cells, the spores still need to be captured by a macrophage or dendritic cell for the rest of their journey to the draining lymph nodes.

Clearly, the role of lung epithelial cells in spore transport during inhalational anthrax is still debatable. It does not mean that the lung epithelial cells do not contribute to pathogenesis, because the lung microenvironment certainly plays a crucial role in controlling the immune system (7). Spore capture by the epithelium can subsequently stimulate chemokine secretion (1), leading to the recruitment of proinflammatory cells (polymorphonuclear cells, monocytes) as well as trigger the antimicrobial protein secretion. The lung epithelial cells could then play a central role during the recruitment of immune effectors in the early steps of anthrax infection that facilitates host defense.

REFERENCES

1. **Chakrabarty, K., W. Wu, J. L. Booth, E. S. Duggan, N. N. Nagle, K. M. Coggeshall, and J. P. Metcalf.** 2007. Human lung innate immune response to *Bacillus anthracis* spore infection. Infect. Immun. **75:**3729–3738.
2. **Cleret, A., A. Quesnel-Hellmann, A. Vallon-Eberhard, B. Verrier, S. Jung, D. Vidal, J. Mathieu, and J. N. Tournier.** 2007. Lung dendritic cells rapidly mediate anthrax spore entry through the pulmonary route. J. Immunol. **178:**7994–8001.
3. **Cote, C. K., K. M. Rea, S. L. Norris, N. van Rooijen, and S. L. Welkos.** 2004. The use of a model of in vivo macrophage depletion to study the role of macrophages during infection with *Bacillus anthracis* spores. Microb. Pathog. **37:**169–175.
4. **Cote, C. K., N. Van Rooijen, and S. L. Welkos.** 2006. Roles of macrophages and neutrophils in the early host response to *Bacillus anthracis* spores in a mouse model of infection. Infect. Immun. **74:**469–480.
5. **Guidi-Rontani, C., M. Weber-Levy, E. Labruyere, and M. Mock.** 1999. Germination of *Bacillus anthracis* spores within alveolar macrophages. Mol. Microbiol. **31:**9–17.
6. **Russell, B. H., Q. Liu, S. A. Jenkins, M. J. Tuvim, B. F. Dickey, and Y. Xu.** 2008. In vivo demonstration and quantification of intracellular *Bacillus anthracis* in lung epithelial cells. Infect. Immun. **76:**3975–3983.
7. **Tournier, J. N., and M. Mohamadzadeh.** 2008. Microenvironmental impact on lung cell homeostasis and immunity during infection. Expert Rev. Vaccines **7:**457–466.

Jean-Nicolas Tournier*
Aurélie Cleret
Anne Quesnel-Hellmann
CRSSA
Département de Biologie des Agents Transmissibles
Groupe Interactions Hôte-Agent Pathogène
La Tronche, France

*Phone: 33 476636848
Fax: 33-476636917
E-mail: jntournier@gmail.com

Authors' Reply

Based on confocal image analysis of immunofluorescently labeled lung sections and quantitative examination of lung epithelial cells (LEC) isolated from spore-challenged mice, we concluded in our paper that spores were inside lung epithelial cells at early stages of infection. In Discussion, we contemplated what this intracellular presence of spores might mean to *B. anthracis*; (i) it may provide an alternative portal for breaching the epithelium, based on the observation that *B. anthracis* could translocate across LECs from the apical side to the basolateral side (7), (ii) it may provide a niche for spore persistence in the lung, based on the finding that internalized *B. anthracis* could survive in LECs (7), and (iii) it may activate intracellular signaling pathways and influence the innate immune response, based on a report by Chakrabarty et al. (1).

Regarding dissemination, it should be noted that there are significant gaps in the pathways via alveolar macrophages and lung dendritic cells. Phagocytosis, trafficking to lymph nodes, and microbial killing are parts of a default pathway of the immune system to defend against microbes. Evidence so far suggests that *B. anthracis* has not deviated from this default path (2, 4–6, 8). If phagocytosis and migration to lymph nodes result in the killing of *B. anthracis*, then this route is disadvantageous to the bacteria. In fact, one may argue that the more efficient phagocytosis is, the poorer the outcome for the bacteria. The increased susceptibility to *B. anthracis* in macrophage-depleted mice seems to support this notion (3). Thus, the role of alveolar macrophages and lung dendritic cells in the dissemination process remains unclear. One possible scenario is that although most phagocytosed *B. anthracis* bacteria are killed, the few survivors are sufficient to cause infec-

tion when released into the circulation. Studies using methods that allow quantification of live and dead bacteria along the trafficking route will help to resolve the issue. Also, a mechanism of *B. anthracis* escaping from phagocytes has yet to be clearly established. We note that the number of spores inside LECs was obtained from wild-type mice in which phagocytes had the opportunity to take up spores for several hours before we stopped the experiments. Therefore, we think that the number is meaningful and can have a real impact on the outcome of infections.

Regarding "motility," there has been no evidence indicating that motility is a requirement for pathogens to migrate through/between epithelial/endothelial cells. However, what happens to *B. anthracis* when it emerges from the basolateral side of the epithelium requires consideration. Being taken up by macrophages or dendritic cells is one possibility. The possibility of penetrating the capillary endothelium by paracellular migration cannot be excluded either. Another question is whether the journey through LECs has any impact on how the bacteria interact with other types of cells, such as phagocytes. Answers to these questions and to the question of biological significance will likely come from a better understanding of the underlying mechanisms. With this in mind, we believe that our findings are both significant and relevant to the pathogenesis of *B. anthracis*.

REFERENCES

1. **Chakrabarty, K., W. Wu, J. L. Booth, E. S. Duggan, N. N. Nagle, K. M. Coggeshall, and J. P. Metcalf.** 2007. Human lung innate immune response to *Bacillus anthracis* spore infection. Infect. Immun. **75:**3729–3738.
2. **Cleret, A., A. Quesnel-Hellmann, A. Vallon-Eberhard, B. Verrier, S. Jung, D. Vidal, J. Mathieu, and J. N. Tournier.** 2007. Lung dendritic cells rapidly mediate anthrax spore entry through the pulmonary route. J. Immunol. **178:**7994–8001.
3. **Cote, C. K., N. Van Rooijen, and S. L. Welkos.** 2006. Roles of macrophages and neutrophils in the early host response to *Bacillus anthracis* spores in a mouse model of infection. Infect. Immun. **74:**469–480.
4. **Guidi-Rontani, C., M. Weber-Levy, E. Labruyere, and M. Mock.** 1999. Germination of *Bacillus anthracis* spores within alveolar macrophages. Mol. Microbiol. **31:**9–17.
5. **Hu, H., Q. Sa, T. M. Koehler, A. I. Aronson, and D. Zhou.** 2006. Inactivation of *Bacillus anthracis* spores in murine primary macrophages. Cell. Microbiol. **8:**1634–1642.
6. **Kang, T. J., M. J. Fenton, M. A. Weiner, S. Hibbs, S. Basu, L. Baillie, and A. S. Cross.** 2005. Murine macrophages kill the vegetative form of *Bacillus anthracis*. Infect. Immun. **73:**7495–7501.
7. **Russell, B. H., R. Vasan, D. R. Keene, T. M. Koehler, and Y. Xu.** 2008. Potential dissemination of *Bacillus anthracis* utilizing human lung epithelial cells. Cell. Microbiol. **10:**945–967.
8. **Welkos, S., A. Friedlander, S. Weeks, S. Little, and I. Mendelson.** 2002. In-vitro characterization of the phagocytosis and fate of anthrax spores in macrophage and the effects of anti-PA antibody. J. Med. Microbiol. **51:**821–831.

Yi Xu*
Brooke H. Russell
Center for Extracellular Matrix Biology
Institute of Biosciences and Technology
Texas A&M University Health Science Center
2121 West Holcombe Blvd.
Houston, Texas 77030

*E-mail: yxu@ibt.tamhsc.edu