Morphological Change in *Pseudomonas aeruginosa* following Antibiotic Treatment of Experimental Infection in Mice and Its Relation to Susceptibility to Phagocytosis and to Release of Endotoxin

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Recently, in vitro and in vivo studies have shown antibiotic-induced endotoxin release when gram-negative bacteria are exposed to antibiotics, although endotoxin is shed spontaneously during bacterial growth in culture. Antibiotic-induced endotoxin release observed in an in vitro incubation system and experimental infection models has been reviewed previously (2, 3, 6, 7). β-Lactam antibiotics are considered the antibiotics most responsible for the liberation of excessive amounts of endotoxin. Of the many β-lactams studied, imipenem (IPM), an antibiotic of the carbapenem antibiotic subclass, has been shown to induce the release of smaller amounts of endotoxin during bacterial exposure (1, 4, 5, 8, 9). Recently, we have reported that in vitro treatment of *Pseudomonas aeruginosa* with IPM induced much lower levels of endotoxin release than treatment with other β-lactam antibiotics, such as ceftazidime (CAZ) and meropenem, and that the level of antibiotic-induced endotoxin release affected the production of proinflammatory mediators on physiologically relevant cells (9). Moreover, IPM and CAZ treatment of *P. aeruginosa* showed marked differences in the resulting bacterial morphologies. IPM treatment induced rod-shaped bacteria to become rounded, whereas exposure to CAZ induced the formation of long filaments (8, 9). This result is consistent with the findings reported by Jackson and Kropp (4, 5). The morphological changes could be explained by the fact that IPM and CAZ inhibit penicillin binding proteins 2 and 3, respectively (4, 5). On the basis of our preceding work (8, 9), it was of interest to determine whether the low level of endotoxin release in in vitro incubation of *P. aeruginosa* with IPM could be applied to in vivo antibiotic treatment of experimental *P. aeruginosa* infection. In the present study, we first tried to study in vivo antibiotic-induced endotoxin release in a murine experimental model of *P. aeruginosa* infection. Incidentally, we found that round *P. aeruginosa* cells with IPM treatment were phagocytized more by peritoneal cells than were long filamentous ones with CAZ treatment. We describe herein the close relationship between antibiotic-induced morphological changes of *P. aeruginosa* and susceptibility to phagocytosis.

IPM and CAZ were obtained from Banyu Pharmaceutical Co. (Tokyo, Japan) and Tanabe Pharmaceutical Co. (Osaka, Japan), respectively. Stock solutions of IPM and CAZ were prepared by the methods supplied by the manufacturer. The MICs of CAZ and IPM for *P. aeruginosa* PAO-1 were 0.63 and 1.25 μg/ml, respectively. BALB/c mice of approximately 7 weeks of age were purchased from Japan SLC (Hamamatsu, Japan). A single colony of *P. aeruginosa* PAO-1 was selected, suspended in L broth (5 ml), and placed in a test tube with shaking at 37°C overnight. The bacterial suspension (approximately 100 μl) was diluted in Mueller-Hinton broth (10 ml) and cultured for 2 h with shaking. Log-phase bacteria (300 μl) at approximately 1 × 10^6 to 4 × 10^8 were mixed with 10× or 100× the MIC of IPM or CAZ per ml, respectively, and diluted to 1 ml with phosphate-buffered saline. One milliliter of the mixture of *P. aeruginosa* and antibiotic was injected intraperitoneally into mice. Three mice were used for each experimental group. Peritoneal cells were collected by washing the peritoneal cavity with phosphate-buffered saline (2 ml) 2 h after the injection. Peritoneal cells were smeared on slide glasses, fixed with acetone, and stained with Giemsa’s stain. The number of viable bacteria recovered was also determined by colony count on agar plates. Three consecutive experiments were performed, and similar experimental results were obtained. The results from a typical experiment are shown in Table 1. First of all, the number of viable bacteria recovered was determined. There was a marked difference in the recovered colony numbers between treatments with antibiotic and those without. A remarkably low colony number was recovered from mice injected with IPM or CAZ, suggesting an extremely low viability of recovered bacteria. In the resulting bacterial morphologies, there was a significant difference between *P. aeruginosa* cells treated with IMP and those treated with CAZ. Round-shaped *P. aeruginosa* cells became rounded 2 h after IPM treatment, whereas CAZ induced the formation of long filaments (data not shown). The relationship between morphological changes in antibiotic-treated *P. aeruginosa* cells and their susceptibility to phagocytosis was studied.

First, more than 100 peritoneal cells were inspected to de-
Moreover, we found that round morphological changes induced by IPM and CAZ were consistent with the findings reported by Jackson and Kropp (4, 5). Moreover, we found that round P. aeruginosa cells with IPM treatment became susceptible to the phagocytosis and were phagocytized more by peritoneal macrophages. On the other hand, P. aeruginosa cells treated with CAZ became large filamentous rods and were hardly phagocytized by peritoneal macrophages. The susceptibility to phagocytosis might be related to the difference in the size between the bacterial cells treated with IPM and those treated with CAZ. It is possible that filamentous bacteria induced by CAZ might be too large for peritoneal macrophages to phagocytize.

Treatment of experimental P. aeruginosa infection with IPM and CAZ led to differential levels of plasma endotoxin. Furthermore, the high level of endotoxin released from P. aeruginosa cells treated with antibiotics causes high-level production of tumor necrosis factor alpha and nitric oxide (9). The possibility was raised that the higher level of released endotoxin might reduce the phagocytic activity of peritoneal macrophages through higher production of cytokines and other mediators. However, this was unlikely, because there wasn’t sufficient time for released endotoxin to modulate the phagocytic activity of peritoneal cells. The susceptibility of bacteria to phagocytosis was found 2 h after the injection, while the endotoxin release had just started 2 h after the injection and reached its peak 6 to 8 h postinjection (8).

Previously we demonstrated that the in vitro treatment of P. aeruginosa with IPM induced much lower levels of endotoxin release than treatment with CAZ. It was of interest to determine whether or not the in vitro low level of endotoxin release of IPM could be applied to in vivo treatment of experimental P. aeruginosa infection. The present study clearly demonstrated that the therapeutic use of IPM in experimental P. aeruginosa infection resulted in low-level release of endotoxin in vivo. The in vitro and in vivo low-level release of endotoxin by antibiotics seemed to be related to morphological changes, i.e., the round and filamentous shapes, induced by antibiotics (4, 5, 8, 9). The present study raises another possibility: the lower level of in vivo endotoxin release by IPM might be partly due to the high clearance of IPM-treated bacteria by phagocytes.

REFERENCES