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# Potential Effects of Erythromycin on Host Defense Systems and Virulence of *Pseudomonas aeruginosa*

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**We evaluated several potential effects of erythromycin (EM) on host defense systems and the virulence of *Pseudomonas aeruginosa*. Peritoneal macrophages obtained from mice given 250 mg of EM per kg of body weight for 7 days by the intraperitoneal, intravenous, subcutaneous, or oral route produced significantly greater amounts of thymocyte-activating factors. These data suggest that EM enhances the in vivo production of cytokines, such as interleukins 1 and 6. Treatment of *P. aeruginosa* D4 with subinhibitory concentrations of EM enhanced the association of bacteria with murine Kupffer cells in vitro and increased bacterial clearance from the blood in mice. EM suppressed the in vitro production of exotoxin A, total protease, elastase, and phospholipase C by *P. aeruginosa* D4; exotoxin A production by *P. aeruginosa* PA-103; and total protease production by *P. aeruginosa* B16 and PAO1 in a generally dose-dependent manner. These data demonstrate that EM produces various effects in addition to its direct antimicrobial activity, suggesting that it has potential as an immunomodulator or bacterial virulence-suppressing agent against *P. aeruginosa* and other infections.**

Erythromycin (EM), a macrolide antibiotic, is used to treat a variety of common infections and for some specific indications, e.g., *Mycoplasma* and *Legionella* infections (12). It has a broad spectrum of antimicrobial activity against most gram-positive bacteria. In contrast, members of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa* are generally resistant to EM (12, 31).

However, it has been reported that EM improves the clinical symptoms and prognosis of patients with diffuse pulmonary panbronchiolitis associated with *P. aeruginosa* infection (21). Moreover, we have shown recently that erythromycin lactobionate (EML) enhances the survival rates of mice with *P. aeruginosa* bacteremia (16). Although the other effects of EM, in addition to its direct antimicrobial activity, seem to be involved in providing protection against such infections, the protective mechanism is not well understood.

Recently, several antimicrobial agents have been shown to affect host immunological responses, such as neutrophil chemotaxis and cytokine production (3, 4, 14, 21, 29, 30). On the other hand, treatment of bacteria with subinhibitory concentrations of antimicrobial agents frequently increases bacterial phagocytosis, intracellular killing, and susceptibility to serum (1, 2) and suppresses the production of bacterial virulence factors, including extracellular enzymes (13, 34).

Therefore, in the present study, we evaluated the potential effects of EM on both host defense systems and the virulence of *P. aeruginosa*.

## MATERIALS AND METHODS

**Antimicrobial agents.** EML (Dainabot Co., Osaka, Japan) for injection and EM base were used for animal and in vitro studies, respectively. Both forms of EM were provided by Dainippon Pharmaceutical Co., Ltd., Osaka, Japan. EML was dissolved in pyrogen-free water and was then suspended in saline for injection. The endotoxin content of the EML preparation was less than 0.02 endotoxin units (EU) per ml (6.9 pg/ml) by the chromogenic *Limulus* test (Toxicolor system; Seikagaku Co., Tokyo, Japan). EM was dissolved in a small amount of ethanol and was then suspended in sterile water.

**Bacterial strains.** *P. aeruginosa* D4 was used in this study; it was isolated from the blood of a neutropenic mouse with bacteremia (17). *P. aeruginosa* B16 was a clinical isolate from a blood sample from Nagasaki University Hospital, Nagasaki, Japan. *P. aeruginosa* PA-103 (23) and PAO1 (18) were kindly provided by B. H. Iglewski, University of Rochester School of Medicine and Dentistry, Rochester, N.Y.

**In vitro susceptibility.** MICs were determined by a broth microdilution method (32) by using Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with calcium and magnesium at concentrations of 50 and 25 μg/ml, respectively. Bacteria were inoculated at a final concentration of $5.0 \times 10^5$ CFU/ml.

**Treatment of mice with EML and preparation of peritoneal macrophages.** Specific-pathogen-free male ddY mice (weight, 20 to 24 g) were administered 250 mg of EML per kg of body weight or saline (control) by intraperitoneal injection daily for 7 days. Mice were sacrificed by ether inhalation and were given 5 ml of Hanks balanced salt solution (HBSS) by injection into the peritoneal cavity. Peritoneal lavage fluids were obtained after gentle massage. There was no difference in the number of peritoneal exudative cells between EML-treated and control mice (16). The cells in the fluids were washed with HBSS and were then suspended in HBSS containing 10% fetal calf serum at a concentration of $1.0 \times 10^6$ cells per ml. One milliliter of cell suspension was transferred to 24-well tissue culture plates (Falcon 3047; Becton Dickinson Labware, Oxnard, Calif.), and the plates were incubated at 37°C in 5% $CO_2$ for 1 h. Nonadherent cells

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were removed by gentle washing. More than 95% of the adherent cell population were monocytes, as judged by nonspecific esterase staining.

In subsequent studies, EML was given by different routes, including intravenous or intramuscular injection and oral administration. Peritoneal macrophages were obtained from the EML-treated mice as described above.

**Stimulation of macrophages with heat-killed *P. aeruginosa* and thymocyte costimulation assay.** Macrophages in culture plates were incubated with or without heat-killed *P. aeruginosa* D4 at a concentration of $10^5$, $10^6$, or $10^7$ cells per ml of medium for 24 h as described above. The supernatants were passed through a 0.22-μm-pore-size filter and were then used as samples for the thymocyte costimulation assay (11, 26). Thymuses were obtained aseptically from 6-week-old male C3H/HeJ mice, and single-cell suspensions were prepared in RPMI 1640 medium by gently passaging them through a stainless wire mesh. After being washed, the cells were suspended at a density of $1.0 \times 10^7$ cells per ml in RPMI 1640 medium containing 10% fetal calf serum, 100 U of penicillin, 100 μg of streptomycin per ml, and $1.0 \times 10^{-4}$ M 2-mercaptoethanol. One hundred microliters of cell suspension was transferred ($1.0 \times 10^6$ cells per well) to flat-bottom 96-well tissue culture plates (Falcon 3072). Thymocytes were incubated for 72 h with 2 μg of phytohemagglutinin per ml and serial dilutions of test samples in a final volume of 200 μl. Cultures were pulsed with 0.5 μCi of [*methyl*-$^3$H]thymidine per well for the final 18 h of incubation. The cells were then collected with an automatic cell microharvester, and the [*methyl*-$^3$H]thymidine incorporation was measured in a scintillation counter. The data presented are the mean ± standard deviation counts per minute determined in triplicate.

**Influence of EM on clearance of *P. aeruginosa* D4 from blood in mice.** *P. aeruginosa* D4, which was relatively resistant to clearance from blood of normal mice (17), was incubated in Mueller-Hinton broth at 37°C for 24 h. Cells were then diluted 1:10 in Mueller-Hinton broth without EM or with EM at concentrations of 0.2, 2.0, and 20 μg/ml; and the solutions were incubated for 5 h. Cells were washed three times and suspended in pyrogen-free saline at a concentration of $1.0 \times 10^8$ CFU/ml. Clearance from blood was evaluated as described previously (17). Briefly, specific-pathogen-free male ddY mice (weight, 20 to 24 g) were injected in the tail vein with 0.2 ml of bacterial suspension ($2.0 \times 10^7$ CFU per mouse, which was three times the 50% lethal dose, by intravenous injection). At 5, 10, 15, and 30 min after injection, 20-μl samples of blood were obtained from the retro-orbital plexus, and the blood was diluted in saline and plated onto Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.). The values at time zero were calculated by estimating the blood volume as 8% (vol/wt) of the body weight of each mouse used (17). The data presented are the mean ± standard deviation percent recoveries of five experiments.

**Preparation of Kupffer cells and visual assessment of association with bacteria.** Kupffer cells were harvested from ddY mice by perfusion and digestion of liver with collagenase and DNase as described previously (9, 17). Tissue culture chamber slides (LabTek; Miles Laboratories, Inc., Naperville, Ill.) containing Kupffer cells at a density of $1.0 \times 10^6$ cells per well were incubated with untreated *P. aeruginosa* D4 or *P. aeruginosa* D4 treated with 20 μg of EM per ml, as described above, at a concentration of $10^8$ CFU/ml in HBSS at 37°C for 60 min with gentle rocking. After being washed five times with HBSS, the chambers were removed from the slides and the slides were air dried, fixed with methanol, and stained with acridine orange (Vacutainer; Becton Dickinson). The slides were examined at ×400 magnification with oil immersion under a fluorescence microscope. A total of 200 Kupffer cells were examined, and the percentage of bacterium-associated Kupffer cells and the number of bacteria per Kupffer cell were recorded. Examinations were run in duplicate, and data are expressed as the means of two examinations.

**Influence of EM on exoenzyme production. (i) Culture conditions.** *P. aeruginosa* cells were incubated in each type of broth medium overnight at each optimal temperature and were then diluted 1:20 in fresh medium with or without various concentrations of EM (13). After 24 h of growth on a reciprocal shaker (180 cycles per min), a portion of each culture was taken for determination of bacterial viability by plate counts on Trypticase soy agar. The supernatants were collected by centrifugation, passed through a 0.22-μm-pore-size filter, and used as samples. The assay was run in duplicate for each flask, and data are expressed as the amount of exoenzyme per CFU (means of five replicate flasks).

**(ii) Assay of total protein and exoenzymes.** The protein concentration was determined by a dye-binding assay (protein assay; Bio-Rad Laboratories, Richmond, Calif.) with bovine serum albumin used as a standard (6).

Exotoxin A was measured by a sandwich-type enzyme-linked immunosorbent assay by using goat and rabbit anti-exotoxin A antibodies. Purified *P. aeruginosa* exotoxin A (List Biological Laboratories, Inc., Campbell, Calif.) was used to make a standard curve. Bacteria were incubated in Trypticase soy broth dialysate (TSBD-C) at 32°C (19) for the measurement of exotoxin A. The iron content in TSBD-C after treatment with Chelex 100 (Bio-Rad) was 0.09 μg/ml, as determined with bathophenanthroline.

Proteolytic activities were measured by the method of Woods et al. (36). Bacteria were incubated in brain heart infusion broth (Difco) at 37°C. For the total protease assay, the culture supernatant was incubated with the substrate hide powder azure (Sigma Chemical Co., St. Louis, Mo.) at 37°C for 60 min with vigorous shaking. After centrifugation, the $A_{595}$ values of the supernatants were compared with those of a standard curve prepared with purified *P. aeruginosa* protease (Nagase Biochemicals Ltd., Kyoto, Japan). For assay of elastase activity, elastin Congo red (Sigma) was used as a substrate, and $A_{495}$ values were compared with those of a standard curve derived by using purified *P. aeruginosa* elastase (Nagase).

Phospholipase C activity was measured by the method of Berka et al. (5), with modifications. Bacteria were grown in tryptose minimal medium (33) at 32°C, and the supernatant was incubated with *p*-nitrophenylphosphorylcholine (Sigma) as a substrate. Purified *Clostridium perfringens* phospholipase C (Sigma) was used as a standard.

**Statistics.** Student's *t* test was used to compare the means, and a level of 5% was considered significant.

## RESULTS

**In vitro susceptibility.** The MICs of EM for *P. aeruginosa* D4, B16, PAO1, and PA-103 were 512, 256, 256, and 256 μg/ml, respectively.

**Influence of in vivo treatment with EML on production of thymocyte-activating factors by murine peritoneal macrophages.** When macrophages obtained from mice which had been given EML by intraperitoneal injection were cultured with increasing concentrations of heat-killed *P. aeruginosa*

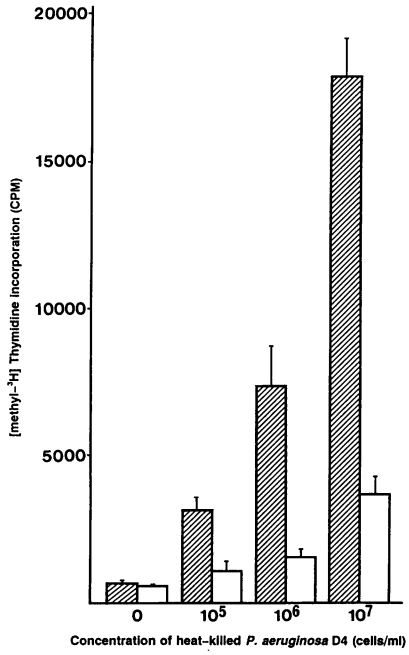


FIG. 1. Influence of EML on production of thymocyte-activating factor by murine peritoneal macrophages. Mice received 250 mg of EML per kg (hatched bars) or saline (open bars) injected intraperitoneally daily for 7 days. Peritoneal macrophages were triggered by the indicated concentrations of heat-killed *P. aeruginosa* D4. The results are expressed as the means of triplicate counts per minute. The bars indicate 1 standard deviation.

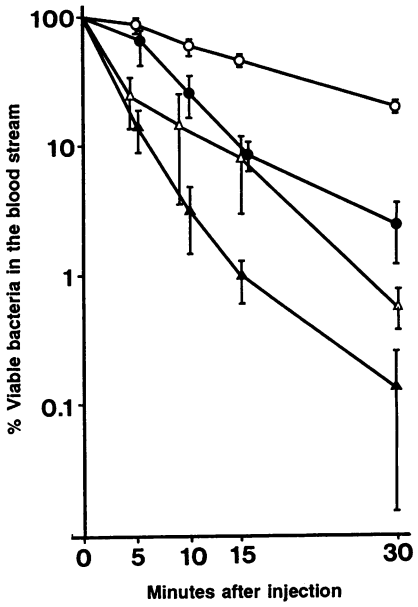


FIG. 2. Influence of EM on the clearance of *P. aeruginosa* D4 from the blood of mice. Bacteria were incubated with different concentrations of EM for 5 h before inoculation into the mice. The results are expressed as the mean ± standard deviation percent recovery from five mice. EM-treated bacteria were rapidly eliminated from the blood at all time points ($P < 0.01$); clearance was significant, except at 5 min after injection, when 0.2 μg of EM per ml was used. Symbols: ○, control; ▲, treated with 20 μg of EM per ml; △, treated with 2.0 μg of EM per ml; ●, treated with 0.2 μg of EM per ml.

D4, a significant dose-dependent increase in the amount of thymocyte-activating factor was observed (Fig. 1). The maximal [*methyl*-$^3$H]thymidine incorporation level achieved by culture with $10^7$ CFU of heat-killed bacteria per ml was 17,902.2 ± 1,259.6 and 3,694.5 ± 561.2 cpm for macrophages obtained from EML-treated and control mice, respectively. Table 1 shows that enhancement of thymocyte proliferation was also observed when mice were given EML by different routes. The maximal level was achieved by peritoneal injection.

**Influence of EM on blood clearance and association with Kupffer cells of *P. aeruginosa* D4.** As shown in Fig. 2, pretreatment of *P. aeruginosa* D4 with EM significantly enhanced the clearance of this strain from blood at all time points in a dose-dependent manner ($P < 0.01$). For example, 30 min after bacterial challenge, only 0.23% ± 0.23% of viable bacteria were recovered after treatment with 20 μg of EM per ml, whereas 20.01% ± 1.52% of viable bacteria were recovered from control.

In the absence of EM, 68.0% of Kupffer cells were associated with bacteria (1.81 bacteria per Kupffer cell). Pretreatment of bacteria with 20 μg of EM per ml enhanced the percentage of bacterium-associated Kupffer cells to 94.5% and the number of bacteria per Kupffer cell to 7.13.

**Influence of EM on exoenzyme production.** In the absence of EM, *P. aeruginosa* D4 produced (0.56 ± 0.15) × $10^{-9}$ μg of exotoxin A per CFU, (9.37 ± 2.27) × $10^{-9}$ μg of total protease per CFU, (2.96 ± 0.52) × $10^{-9}$ μg of elastase per CFU, and (24.38 ± 3.88) × $10^{-9}$ U of phospholipase C per CFU (means ± standard deviations). EM significantly decreased the viable bacterial cell numbers in brain heart infusion broth and tryptose minimal medium when EM was present at concentrations of more than 8.0 μg/ml (at 8.0 μg/ml, 62.36 and 33.89% of controls in brain heart infusion broth and tryptose minimal medium, respectively). Concentrations of EM greater than 32 μg/ml also decreased cell numbers in TSBD-C (35.56% of control at 32 μg/ml). EM decreased total protein synthesis in TSBD-C at a concentration of 128 μg/ml (the mean protein level was 64.04% of that of control). No significant decrease in total protein was found in brain heart infusion broth (for proteases) or tryptose minimal medium (for phospholipase C) up to an EM concentration of 128 μg/ml.

As shown in Fig. 3, EM suppressed exotoxin A and total protease production in a dose-dependent manner. The minimum concentrations of EM which significantly decreased exoenzyme production were 1.0 and 0.25 μg/ml for exotoxin A and total protease, respectively. EM also suppressed

TABLE 1. Influence of EML administered by different routes on thymocyte-activating factor production by peritoneal macrophages

| Route of administration | [*methyl*-$^3$H]thymidine incorporation (cpm)[a] | |
|---|---|---|
| | EML | Saline (control) |
| Intraperitoneal injection | 33,579.3 ± 3,403.0 | 4,473.3 ± 382.7 |
| Intravenous injection | 27,241.0 ± 1,519.2 | 2,184.3 ± 682.6 |
| Intramuscular injection | 15,884.7 ± 1,419.6 | 1,905.7 ± 7.5 |
| Oral administration | 14,588.0 ± 2,395.0 | 2,477.0 ± 243.6 |

[a] Macrophages were stimulated with $10^7$ cells of heat-killed *P. aeruginosa* D4 per ml for 24 h. Results are expressed as the means ± standard deviations of three assays.

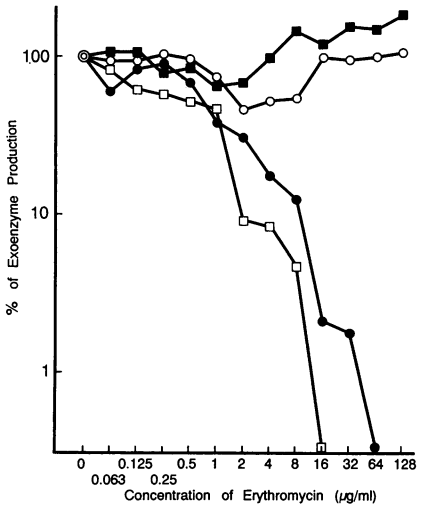


FIG. 3. Influence of EM on exoenzyme production by *P. aeruginosa* D4. Bacteria were cultured with the indicated dose of EM for 24 h. The results are expressed as the mean percentages of exoenzyme production in five replicate flasks compared with that in controls. Symbols: ●, exotoxin A; □, total protease; ○, elastase; ■, phospholipase C.

elastase at concentrations of between 1 and 8 μg/ml and phospholipase C at concentrations of between 1 and 4 μg/ml. However, high concentrations of EM did not suppress the amount of elastase and phospholipase C per CFU because suppression of viable cell numbers was greater than that of the enzymes per milliliter of supernatant.

As shown in Table 2, EM suppressed exotoxin A production of hyper-toxin-producing strain PA-103 in a dose-dependent manner. No significant changes were observed in viable bacterial cell numbers or amounts of total protein in supernatants at EM concentrations of between 0 and 32 μg/ml.

EM suppressed total protease production by strains B16 and PAO1 (Tables 3 and 4, respectively) in a generally dose-dependent manner. EM did not affect the viable bacterial cell numbers or the amounts of total protein of these strains when it was present at concentrations of up to 32 μg/ml.

## DISCUSSION

In our previous study (16), EML reduced the mortality rate of mice systemically infected with *P. aeruginosa*, although EML levels in serum, liver, and stool were apparently lower than the MIC for the infecting organism. Moreover, early treatment was effective in both the endogenous and intravenous infection models, especially before infection in the latter model, excluding a direct antimicrobial effect of EM against the pathogen (16).

Macrolide antibiotics penetrate and concentrate in polymorphonuclear neutrophils and macrophages (20, 28). Some investigators have reported that EM enhances such polymorphonuclear neutrophil functions as chemotaxis, ingestion, and bactericidal activity (8, 10), although opposite results have also been reported (7, 25). Since EML was effective in both normal and granulocytopenic mice with infections in our previous study (16), EM may possess immunostimulating activity in addition to its effect on polymorphonuclear neutrophils.

Various antimicrobial agents administered in vivo or in vitro have been reported to modify host immune responses (14, 30), including cytokine production (3, 4, 22, 29). Roche et al. (29) have shown that high concentrations (100 μg/ml) of EM enhance extracellular interleukin-1 (IL-1) activity from human monocytes in vitro. Kita et al. (21) have shown that the administration of EM to mice enhanced the production of IL-1 by macrophages and production of IL-2 and IL-4 by splenocytes (21). Bailly et al. (4) have reported that EM increases IL-6 production by human monocytes in vitro. IL-1 is known to increase the survival rate of mice systemically infected with *P. aeruginosa* (27). Elevated levels of IL-6 are found in the body fluids of patients with acute local bacterial infections and in the sera of patients with gram-negative bacteremia (15). In this study, peritoneal macrophages obtained from EM-treated mice produced greater amounts of thymocyte-activating factors in the presence of phytohemagglutinin following culture with heat-killed *P.*

TABLE 2. Influence of EM on exotoxin A production by *P. aeruginosa* PA-103

| EM concn (μg/ml) | Exotoxin A in supernatant ($10^{-9}$ μg/CFU)[a] | % Activity remaining |
|---|---|---|
| 0 | 0.536 ± 0.193 | 100.00 |
| 0.5 | 0.394 ± 0.046 | 73.59 |
| 2 | 0.231 ± 0.141 | 43.04[b] |
| 8 | 0.166 ± 0.063 | 30.98[c] |
| 32 | 0.016 ± 0.001 | 3.01[c] |

[a] Results are expressed as means ± standard deviations for five replicate flasks.
[b] Significantly less than the value in the absence of EM ($P < 0.02$).
[c] Significantly less than the value in the absence of EM ($P < 0.01$).

TABLE 3. Influence of EM on total protease production by *P. aeruginosa* B16

| EM concn (μg/ml) | Total protease in supernatant ($10^{-9}$ μg/CFU)[a] | % Activity remaining |
|---|---|---|
| 0 | 3.464 ± 0.987 | 100.00 |
| 0.5 | 3.217 ± 0.855 | 60.07 |
| 2 | 2.149 ± 0.553 | 40.13[b] |
| 8 | 2.013 ± 0.609 | 37.58[b] |
| 32 | 1.033 ± 0.217 | 19.30[c] |

[a] Results are expressed as means ± standard deviations for five replicate flasks.
[b] Significantly less than the value in the absence of EM ($P < 0.02$).
[c] Significantly less than the value in the absence of EM ($P < 0.01$).

TABLE 4. Influence of EM on total protease production by *P. aeruginosa* PAO1

| EM concn (μg/ml) | Total protease in supernatant ($10^{-9}$ μg/CFU)[a] | % Activity remaining |
|---|---|---|
| 0 | 0.481 ± 0.089 | 100.00 |
| 0.5 | 0.370 ± 0.057 | 76.96[b] |
| 2 | 0.438 ± 0.072 | 91.14 |
| 8 | 0.319 ± 0.058 | 66.43[c] |
| 32 | 0.189 ± 0.017 | 39.28[c] |

[a] Results are expressed as means ± standard deviations for five replicate flasks.
[b] Significantly less than the value in the absence of EM ($P < 0.05$).
[c] Significantly less than the value in the absence of EM ($P < 0.01$).

*aeruginosa*. These factors may include IL-1 and/or IL-6, which are the major cytokines produced by mononuclear phagocytes, and may induce the proliferation of thymocytes (24, 35). These data demonstrate that EM enhances the in vivo potency of macrophages for producing cytokines.

On the other hand, Adinolfi and Bonventre (1) and Andreana et al. (2) have demonstrated that sub-MICs of antibiotics enhance phagocytosis, killing by macrophages, and the susceptibilities of bacteria to serum. In this study, treatment of *P. aeruginosa* with subinhibitory concentrations of EM enhanced the bacterial association with murine Kupffer cells in vitro and resulted in increased clearance of bacteria from the blood of mice.

Grimwood et al. (13) and Warren et al. (34) have reported that sub-MICs of the cephems, aminoglycosides, and quinolones suppress the expression of several exoenzymes. Because EM interferes with bacterial protein synthesis, it is possible that a subinhibitory concentration of this antibiotic may alter superficial components and affect extracellular products, and consequently, it may reduce the virulence of *P. aeruginosa*. Kita et al. (22) have recently shown that erythromycin stearate suppresses the production of proteases and leukocidin by *P. aeruginosa* without affecting cell growth. They showed that the optimal EM concentration for inhibiting *Pseudomonas* protease production was 0.5 μg/ml and that the inhibition efficacy was lower at higher concentrations of EM. In our study, EM suppressed the in vitro production of several exoenzymes in a generally dose-dependent manner. It is possible that the optimal concentration of EM for suppressing exoenzyme production may be strain dependent.

Woods et al. (36) previously reported that bacteremic strains of *P. aeruginosa* produced greater amounts of total protease and exotoxin A than did other clinical strains, such as isolates from sputum and urine, suggesting that these exoenzymes play an important role in the occurrence of *P. aeruginosa* bacteremia. Interestingly, suppression of exoenzymes by EM was strong with regard to total protease and exotoxin A. Therefore, suppression of these exoenzymes by EM may contribute to the protection of mice against *P. aeruginosa* bacteremia.

In conclusion, we demonstrated that EM has various effects on host immune responses and on the virulence of *P. aeruginosa*. The synergistic interactions which occur between the host immune system and antimicrobial agents may contribute to the successful outcome of antimicrobial chemotherapy (8). In this respect, EM may be an excellent agent for many infections caused by organisms that are susceptible to this antibiotic. Moreover, it is possible that EM may also be useful as an immunomodulator or bacterial virulence-suppressing agent in cases of infection caused by other organisms that are resistant to EM, including *P. aeruginosa*.

## REFERENCES


1. **Adinolfi, L. E., and P. F. Bonventre.** 1988. Enhanced phagocytosis, killing, and serum sensitivity of *Escherichia coli* and *Staphylococcus aureus* treated with sub-MICs of imipenem. Antimicrob. Agents Chemother. **32:**1012–1018.
2. **Andreana, A., P. Perna, R. Utili, M. Dilillo, and G. Ruggiero.** 1984. Increased phagocytosis and killing of *Escherichia coli* treated with subinhibitory concentrations of cefamandole and gentamicin in isolated rat livers. Antimicrob. Agents Chemother. **25:**182–186.
3. **Bailly, S., M. Fay, and M. A. Gougerot-Pocidalo.** 1990. Effects of quinolones on tumor necrosis factor production by human monocytes. Int. J. Immunopharmacol. **12:**31–36.
4. **Bailly, S., J. Pocidalo, M. Fay, and M. Gougerot-Pocidalo.** 1991. Differential modulation of cytokine production by macrolides: interleukin-6 production is increased by spiramycin and erythromycin. Antimicrob. Agents Chemother. **35:**2016–2019.
5. **Berka, R. M., G. L. Gray, and M. L. Vasil.** 1981. Studies of phospholipase C (heat-labile hemolysin) in *Pseudomonas aeruginosa*. Infect. Immun. **34:**1071–1074.
6. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
7. **Eyraud, A., J. Descotes, J. Y. Lombard, A. Laschi-Loquerie, P. Tachon, C. Veysseyre, and J. C. Evreux.** 1986. Effects of erythromycin, josamycin, and spiramycin on rat polymorphonuclear leukocyte chemotaxis. Chemotherapy **32:**379–382.
8. **Fernandes, A. C., R. Anderson, A. J. Theron, C. Joone, and C. E. J. van Rensburg.** 1984. Enhancement of human polymorphonuclear leukocyte motility by erythromycin in vitro and in vivo. S. Afr. Med. J. **66:**173–177.
9. **Filice, G. A.** 1988. Antimicrobial properties of Kupffer cells. Infect. Immun. **56:**1430–1435.
10. **Fraschini, F., F. Scaglione, F. Ferrara, O. Marelli, P. C. Braga, and F. Teodori.** 1986. Evaluation of the immunostimulating activity of erythromycin in man. Chemotherapy **32:**286–290.
11. **Gerry, I., R. K. Gershon, and B. H. Waksman.** 1972. Potentiation of the T-lymphocyte response to mitogens. 1. The responding cell. J. Exp. Med. **136:**128–155.
12. **Gribble, M. J., and A. W. Chow.** 1982. Erythromycin. Med. Clin. N. Am. **66:**79–89.
13. **Grimwood, K., M. To, H. R. Rabin, and D. E. Woods.** 1989. Inhibition of *Pseudomonas aeruginosa* exoenzyme expression by subinhibitory antibiotic concentrations. Antimicrob. Agents Chemother. **33:**41–47.
14. **Hauser, W. E., and J. S. Remington.** 1982. Effects of antibiotics on the immune response. Am. J. Med. **72:**711–716.
15. **Helfgott, D. C., S. B. Tatter, U. Santhanam, R. H. Clarick, N. Bhardwaj, N. May, and P. B. Sehgal.** 1989. Multiple forms of IFN-$B_2$/IL-6 in serum and body fluids during acute bacterial infection. J. Immunol. **142:**948–953.
16. **Hirakata, Y., M. Kaku, K. Tomono, K. Tateda, N. Furuya, T. Matsumoto, R. Araki, and K. Yamaguchi.** 1992. Efficacy of erythromycin lactobionate for treating *Pseudomonas aeruginosa* bacteremia in mice. Antimicrob. Agents Chemother. **36:**1198–1203.
17. **Hirakata, Y., K. Tomono, K. Tateda, T. Matsumoto, N. Furuya, K. Shimoguchi, M. Kaku, and K. Yamaguchi.** 1991. Role of bacterial association with Kupffer cells in occurrence of endogenous systemic bacteremia. Infect. Immun. **59:**289–294.
18. **Holloway, B. W., V. Krishnapillai, and A. F. Morgan.** 1979. Chromosomal genetics of *Pseudomonas*. Microbiol. Rev. **43:**73–102.
19. **Iglewski, B. H., and J. C. Sadoff.** 1979. Toxin inhibitors of protein synthesis: production, purification, and assay of *Pseudomonas aeruginosa* toxin A. Methods Enzymol. **60:**780–793.
20. **Ishiguro, M., H. Koga, S. Kohno, T. Hayashi, K. Yamaguchi, and M. Hirota.** 1989. Penetration of macrolides into human polymorphonuclear leucocytes. J. Antimicrob. Chemother. **24:**719–729.
21. **Kita, E., M. Sawaki, F. Nishikawa, K. Mikasa, Y. Yagyu, S. Takeuchi, K. Yasui, N. Narita, and S. Kashiba.** 1990. Enhanced interleukin production after long-term administration of erythromycin stearate. Pharmacology **41:**177–183.
22. **Kita, E., M. Sawaki, D. Oku, A. Hamuro, K. Mikasa, M. Konishi, M. Emoto, S. Takeuchi, N. Narita, and S. Kashiba.** 1991. Suppression of virulence factors of *Pseudomonas aeruginosa* by erythromycin. J. Antimicrob. Chemother. **27:**273–284.
23. **Liu, P. V.** 1974. Extracellular toxins of *Pseudomonas aeruginosa*. J. Infect. Dis. **130:**S94–S99.
24. **Mizell, S. B.** 1982. Interleukin 1 and T cell activation. Immunol. Rev. **63:**51–72.
25. **Nelson, S., W. R. Summer, P. B. Terry, G. A. Warr, and G. J. Jakab.** 1987. Erythromycin-induced suppression of pulmonary antibacterial defenses: a potential mechanism of superinfection in the lung. Am. Rev. Respir. Dis. **136:**1207–1212.
26. **Otterness, I. G., M. L. Bliven, J. D. Eskra, M. Reinke, and D. C.**

**Hanson.** 1988. The pharmacologic regulation of interleukin-1 production: the role of prostaglandins. Cell. Immunol. **114**:385–397.

27. **Ozaki, Y., T. Ohashi, A. Minami, and S.-I. Nakamura.** 1987. Enhanced resistance of mice to bacterial infection induced by recombinant human interleukin-1a. Infect. Immun. **55**:1436–1440.
28. **Pocidalo, J.-J., F. Albert, J. F. Desnottes, and S. Kernbaum.** 1985. Intraphagocytic penetration of macrolides: in-vivo comparison of erythromycin and spiramycin. J. Antimicrob. Chemother. **16**(Suppl. A):167–173.
29. **Roche, Y., M. Fay, and M. A. Gougerot-Pocidalo.** 1988. Interleukin-1 production by antibiotic-treated human monocytes. J. Antimicrob. Chemother. **21**:597–607.
30. **Roche, Y., M. A. Gougerot-Pocidalo, M. Fay, D. Etienne, N. Forest, and J. J. Pocidalo.** 1987. Comparative effects of quinolones on human mononuclear functions. J. Antimicrob. Chemother. **19**:781–790.
31. **Rozgonyi, F., E. Papp-Falusi, J. Varga, and K. Rozgonyi-Szitha.** 1989. In-vitro activity of cefetamet (Ro15-8074) compared with other oral agents. J. Antimicrob. Chemother. **24**:539–546.
32. **Sahm, D. F., and J. A. Washington II.** 1991. Antibacterial susceptibility tests: dilution methods, p. 1105–1116. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
33. **Stinson, M. W., and C. Haydon.** 1979. Secretion of phospholipase C by *Pseudomonas aeruginosa*. Infect. Immun. **25**:558–564.
34. **Warren, R. L., N. R. Baker, J. Johnson, and M. J. Stapleton.** 1985. Selective inhibition of the accumulation of extracellular proteases of *Pseudomonas aeruginosa* by gentamicin and tobramycin. Antimicrob. Agents Chemother. **27**:468–472.
35. **Wong, G. W., and S. C. Clark.** 1988. Multiple actions of interleukin 6 within a cytokine network. Immunol. Today **9**:137.
36. **Woods, D. E., M. S. Schaffer, H. R. Rabin, G. D. Campbell, and P. A. Sokol.** 1986. Phenotypic comparison of *Pseudomonas aeruginosa* strains isolated from a variety of clinical sites. J. Clin. Microbiol. **24**:260–264.