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Effect of Phagocyte Membrane Stimulation on Antibiotic Uptake and Intracellular Bactericidal Activity

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Phagocytosis stimulated a substantial increase in clindamycin uptake and a smaller increase in rifampin accumulation by polymorphonuclear leukocytes. The effect of this increased antibiotic uptake on intraphagocytic bactericidal activity was evaluated. Although zymosan stimulated antibiotic uptake by polymorphonuclear leukocytes, neither zymosan nor formyl-methionyl-leucyl-phenylalanine enhanced the ability of clindamycin or rifampin to kill ingested staphylococci. Properties other than antibiotic uptake are important in determining intraphagocytic bactericidal activity.

Infections caused by pathogenic bacteria that survive within phagocytes pose special problems in antimicrobial therapy (10). In this setting, an antibiotic must not only be active against the organism but must also reach the intraphagocytic bacteria and exert its effect under the conditions that are present in this environment (11, 14, 15). Although many antibiotics do not penetrate phagocytes well, lipid-soluble drugs such as rifampin achieve intracellular levels several times greater than extracellular concentrations (6, 12, 16). A few antibiotics, especially clindamycin and erythromycin, are markedly concentrated within phagocytes by active energy-requiring processes. Clindamycin transport is mediated by the plasma membrane nucleoside transport system (7, 18).

Phagocytosis and nonparticulate membrane stimulation of phagocytes are accompanied by dramatic changes in the cell membrane and metabolic processes of leukocytes (1, 2, 4, 13, 17). We found that cell membrane stimulation may also affect antibiotic uptake by phagocytes (18). In the present study, we asked whether phagocyte stimulation, with concomitant increased uptake of clindamycin and rifampin, alters intracellular bactericidal activity in the presence of either antibiotic. Polymorphonuclear leukocytes (PMN) ingested quantities of *Staphylococcus aureus* that modestly augmented phagocyte antibiotic uptake and superoxide generation and were then challenged with opsonized zymosan or formyl-methionyl-leucyl-phenylalanine (FMLP) in doses that further enhanced antibiotic uptake or superoxide generation or both.

For studies of phagocyte function, peripheral blood PMN were isolated by Ficoll-Hypaque density gradient centrifugation as previously described (3, 16, 18). *S. aureus* was grown overnight in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with or without [^{3}H]thymidine (10 μCi/ml). Standard-sensitivity antibiotic powders (clindamycin and rifampin) were obtained from their manufacturers.

Tube-dilution antibiotic susceptibility assays with *S. aureus* (~10^6 organisms per ml) were performed in tissue culture medium 199–0.1% gelatin–5% fresh normal human serum, the same medium used in the PMN phagocytic-bactericidal assay described below. The MICs and MBCs of clindamycin and rifampin against the test strain of *S. aureus* were as follows. The MIC and MBC of clindamycin were 0.1 and 50 to 100 μg/ml, respectively, and the MIC and MBC of rifampin were <0.1 and ~1 μg/ml, respectively.

For antibiotic uptake studies, PMN (5×10^6 cells per ml) were incubated with ingestible particles (*S. aureus* and opsonized zymosan) or FMLP for 30 min at 37°C, washed, and suspended in tissue culture medium 199-normal human serum (18). In some instances, the cells were challenged with a second stimulus (zymosan or FMLP) for 30 min before determination of antibiotic uptake. Cellular concentrations of radiolabeled drugs, [^{3}H]clindamycin hydrochloride (The Upjohn Co., Kalamazoo, Mich.) and [^{14}C]rifampin (CIBA-GEIGY Corp., Summit, N.J.), were assessed by a velocity gradient centrifugation technique (6, 12, 16). Antibiotic uptake by PMN was expressed as the ratio of cellular to extracellular antibiotic concentration (C/E).

Since uptake of antibiotics by PMN was the same at 1 and 3 h, combined results are shown in Table 1. Ingestion of *S. aureus* or opsonized zymosan increased clindamycin uptake by PMN. Exposure of PMN to zymosan after *S. aureus* ingestion caused a substantial increase in clindamycin uptake compared with that of PMN incubated with *S. aureus* alone. Cellular concentrations of rifampin were slightly increased after incubation with either *S. aureus* or zymosan particles. FMLP had no effect on uptake of either antibiotic (data not shown).

The production of superoxide anion by PMN was measured as the superoxide dismutase-inhibitable reduction of ferricytochrome *c* (5). PMN (5×10^6 cells per ml) suspended in tissue culture medium 199 without phenol red were incubated with or without *S. aureus*, washed, suspended in medium with 100 μM ferricytochrome *c* (Sigma Chemical Co., St. Louis, Mo.), and challenged with either zymosan or FMLP. Duplicate samples contained 50 μg of superoxide dismutase (Sigma) per ml. Specimens were incubated for 30 min, and after centrifugation optical transmission of the supernatants was measured at 550 nm in a spectrophotometer (Carey 219).

Generation of superoxide in culture medium alone was 6.5 nmol/5×10^6 PMN in 1 h. Incubation of PMN with *S. aureus* increased superoxide generation to 15.1 nmol/5×10^6 PMN. Incubation with either FMLP or zymosan markedly en-

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TABLE 1. Effect of *S. aureus* and zymosan exposure on clindamycin and rifampin uptake by human PMN[a]

Exptl group	Clindamycin			Rifampin		
	Uptake (C/E)	*P*	No. of expts	Uptake (C/E)	*P*	No. of expts
PMN (control)	9.53 ± 0.42		22	4.10 ± 0.50		5
PMN + *S. aureus*	12.73 ± 0.71	0.0005	22	5.57 ± 0.62	0.05	10
PMN + zymosan	15.69 ± 1.38	0.00001	10	5.65 ± 0.69	0.05	8
PMN + *S. aureus* + zymosan	21.12 ± 2.07	0.00001	12	7.28 ± 0.95	0.006	10

[a] Antibiotic uptake is the mean ± standard error of the mean of observations. *P* values show differences between control and stimulated PMN.

hanced superoxide production to 72.5 and 59 nmol/5 × 10^6 PMN, respectively.

Experiments were performed to evaluate the effects of the membrane stimulation-induced increase in antibiotic uptake on intraphagocytic bactericidal activity. PMN (5 × 10^6/ml) were incubated for 30 min at 37°C with 2 × 10^7 radiolabeled *S. aureus* cells. After centrifugation at 300 × *g* some PMN pellets were washed twice, lysed in distilled water, and cultured to measure viable intracellular bacteria or processed for quantitation of radioactivity. Ingestion of organisms by phagocytes was assessed as previously described (8, 9). After the ingestion period there were 1.19 × 10^6 ± 0.22 × 10^6 viable intraphagocytic organisms per ml. Zymosan, FMLP, or no stimulus was added for 15 min to the remaining PMN suspensions. Phagocytes were then incubated with clindamycin (10 μg/ml), rifampin (20 μg/ml), or no antibiotic, and at 1 and 3 h, samples of PMN were washed, lysed in distilled water, and cultured to measure viable intracellular bacteria.

At each time point after clindamycin was added, the antibiotic slightly enhanced the ability of PMN to eradicate intracellular staphylococci (Fig. 1; compare PMN with PMN plus clindamycin). However, exposure of PMN to zymosan or FMLP had no additional effect on intracellular bactericidal activity.

Rifampin enhanced intraphagocytic bactericidal activity to a somewhat greater extent than did clindamycin (Fig. 2). The addition of zymosan or FMLP had no further effect on intracellular killing by PMN incubated with rifampin.

In this study, we have evaluated the effects of cell membrane-stimulating agents on antibiotic uptake, superoxide generation, and intracellular bactericidal activity by human PMN. Although phagocytosis of zymosan greatly augmented PMN uptake of clindamycin, slightly increased rifampin uptake, and markedly enhanced superoxide generation, killing of intracellular staphylococci in the presence of antibiotic was not increased by phagocytosis. FMLP increased superoxide generation by PMN but also had no effect on intraphagocytic survival of bacteria.

There was an obvious discrepancy between the marked increase in uptake of clindamycin after phagocytosis and failure of the drug to kill intraphagocytic bacteria. Theoretically, inability of this bacteriostatic drug to kill *S. aureus* might account for this phenomenon. However, the calculated cellular concentration of clindamycin after ingestion of *S. aureus* (130 μg/ml; C/E = 13) and of both *S. aureus* and zymosan (218 μg/ml; C/E = 21.8) exceeded the MBC (50 to 100 μg/ml) of the drug for *S. aureus*.

Other factors may alter the activity of clindamycin against intraphagocytic bacteria. We have examined the possibility

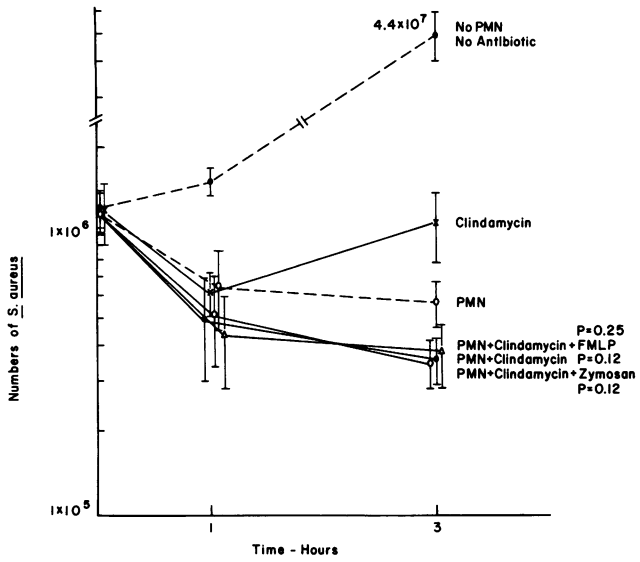

FIG. 1. Influence of exposure to clindamycin alone or with zymosan or FMLP on the viability of *S. aureus* ingested by human PMN or in the absence of phagocytes. Results are the mean ± standard error of the mean of observations at each time point. Differences between control (PMN alone) and experimental (PMN + clindamycin) groups are expressed by *P* values.

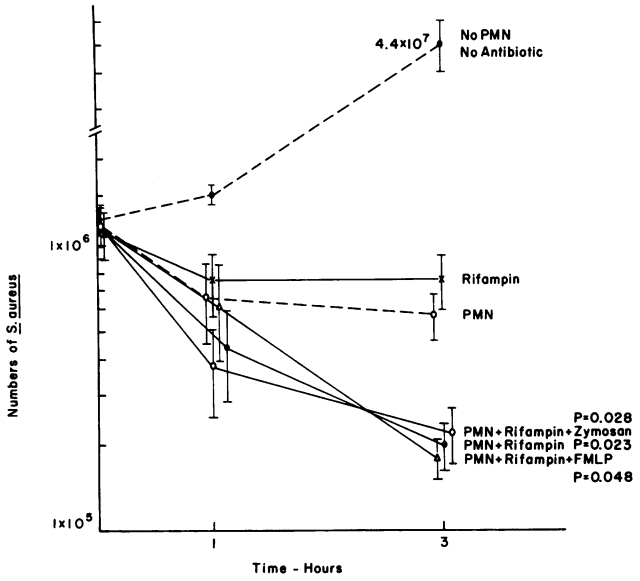

FIG. 2. Effect of rifampin alone or with zymosan or FMLP on the viability of *S. aureus* ingested by human PMN or in the absence of phagocytes. Results are the mean ± standard error of the mean of observations at each time point. Differences between control (PMN alone) and experimental (PMN + rifampin) groups are shown by *P* values.

that clindamycin might adversely affect phagocyte function and found that this antibiotic inhibits oxidative respiratory burst activity (superoxide and hydrogen peroxide production) in stimulated human PMN (W. L. Hand, D. L. Harper, and N. L. King-Thompson, unpublished observations). These findings suggest that intracellular antibiotic activity depends not only on the drug concentration but also on the biological properties of the agent in the cellular environment.

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