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Successful Treatment with Aerosolized Pentamidine of *Pneumocystis carinii* Pneumonia in Rats

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We examined both the therapeutic efficacy and tissue distribution of aerosolized pentamidine in immunosuppressed rats with *Pneumocystis carinii* pneumonia. In rats immunosuppressed by 5 weeks of pretreatment with dexamethasone, a 2-week course of 5 mg of aerosolized pentamidine per kg per day, administered free or encapsulated in the drug carrier system (liposomes), eradicated *P. carinii* pneumonia in 75% of treated animals. At this dose, extrapulmonary drug uptake as measured by a sensitive high-pressure liquid chromatography assay was negligible. No significant differences in tissue distribution were noted between aerosolized free and liposome-encapsulated pentamidine. In rats receiving dexamethasone for 6 weeks prior to treatment with pentamidine, both lung uptake and therapeutic efficacy of aerosolized pentamidine (5 mg/kg per day) were substantially reduced. Aerosolized pentamidine appears to be an effective therapy for *P. carinii* pneumonia in rats and produces significantly lower extrapulmonary drug deposition than parenteral administration. The severity of *P. carinii* involvement at the time of treatment influences both the level of drug delivery to the lung and the response to aerosolized pentamidine therapy.

Recently, *Pneumocystis carinii* pneumonia has emerged as the most common life-threatening infection associated with the acquired immunodeficiency syndrome (AIDS). *P. carinii* pneumonia is refractory to current therapy in up to 40% of all cases (11). Treatment of *P. carinii* pneumonia in AIDS patients produces a high frequency of adverse drug reactions and often requires prolonged courses of therapy (4, 11, 17). Pentamidine is an effective but highly toxic agent used in the treatment of *P. carinii* pneumonia (17). Because *P. carinii* organisms are largely confined to the alveolar space (6), site-specific drug delivery to the lungs may both improve therapeutic efficacy and reduce the high incidence of extrapulmonary side effects associated with parenteral therapy. Aerosolized pentamidine produces high, sustained drug levels in the lungs of normal animals and reduces systemic drug uptake compared with intramuscular drug administration (15). Furthermore, aerosolized pentamidine, both free and encapsulated in phospholipid bilayer vesicles (liposomes), produces significantly higher drug levels in the terminal bronchioles and alveoli of healthy animals than high-dose intravenous (i.v.) drug administration (R. J. Debs, R. J. Straubinger, E. N. Brunette, E. J. Lin, J. M. Lin, and D. Papahadjopoulos, Am. Rev. Respir. Dis., in press).

We therefore examined the therapeutic efficacy and biodistribution of pentamidine administered i.v. and by aerosolization to rats with *P. carinii* pneumonia. Aerosol delivery of both free and liposome-encapsulated pentamidine was assessed.

MATERIALS AND METHODS

Liposome preparation. Pentamidine isethionate was a gift from Lyphomed, Melrose Park, Ill. Phospholipids were obtained from Avanti Polar Lipids, Birmingham, Ala. Multilamellar vesicles were prepared from lipid mixtures consisting of either (i) dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylglycerol (DPPG), and alpha-tocopherol (alpha-T) at molar ratios of 8:2:0.1 or (ii) egg yolk phosphatidylcholine (PC), bovine brain phosphatidylserine (PS), cholesterol (chol), and alpha-T at molar ratios of 8:2:5:0.1. Lipids suspended in chloroform were placed in a 100-ml round-bottomed flask and evaporated to dryness on a rotary evaporator. Liposomes composed of DPPC-DPPG-alpha-T were prepared at 50°C and of PC-PS-chol-alpha-T at room temperature (20°C). A 100-mg/ml solution of pentamidine in sterile water was added to the dried lipid film at a ratio of 2.5 mg of pentamidine per μmol of phospholipid. The mixture was then vortexed for 20 min and subsequently extruded through polycarbonate membranes (0.2-μm-diameter pore size) (14). Pentamidine-containing liposomes were separated from unencapsulated drug by passage over a Sephadex G-75 column. The amount of pentamidine present in liposomes was determined by optical density measurement at 262 nm. Liposomes were kept at room temperature and used within 2 h of column separation.

Animal model of *P. carinii* pneumonia. Sprague-Dawley rats weighing 150 to 200 g (Harlan Laboratories, Indianapolis, Ind.) were placed on a low-protein diet (8% protein) and received dexamethasone (2 mg/liter) and tetracycline (500 mg/liter) in their drinking water throughout the experiments. This induction regimen has been reported to produce *P. carinii* pneumonia in 90 to 100% of rats treated (8). Pentamidine was administered daily for 2 weeks following either 6 weeks (experiment 1) or 5 weeks (experiment 2) of immunosuppression by dexamethasone administration.

Administration of pentamidine. For i.v. therapy, 5 mg of pentamidine (in histidine buffer [0.5 ml of NaCl with 10 mM histidine]) per kg was injected into the tail vein of unanesthetized rats. For aerosol therapy, groups of eight rats were placed in a 13-liter enclosed plastic chamber connected by plastic tubing to an Acorn nebulizer (Syncor International, Sylmar, Calif.) driven by compressed air at 15 to 20 lb/in^2.

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TABLE 1. Assessment of *P. carinii* pneumonia in rats receiving various treatments for 2 weeks after 6 weeks of immunosuppression

Group[a]	Treatment	No. of animals with *P. carinii* lung involvement[b]:			
		None	Minimal	Focal	Extensive
i	Control	0	0	3	5
ii	Aerosol DPPC-DPPG-alpha-T liposomes (buffer)	0	0	3	5
iii	Aerosol DPPC-DPPG-alpha-T pentamidine in liposomes	0	1	1	5
iv	Aerosol PS-PC-chol-alpha-T pentamidine in liposomes	0	0	1	7
v	Aerosol free pentamidine	1	0	0	6
vi	i.v. pentamidine	1	2	5	0

[a] One rat each from groups iii and v was cannibalized.
[b] Response rate (the number of animals with no or minimal disease) did not differ significantly between the control group and any of the treated groups ($P > 0.1$ by the Wilcoxon test).

Liposome-encapsulated or free pentamidine suspended in 10 ml of histidine buffer was aerosolized over 30 min. The chamber was vented by an outlet port connected to a 0.2-μm filter. Aerosols of both free and liposome-encapsulated pentamidine were sized with a seven-stage cascade impactor (Intox Products, Albuquerque, N.M.). Mass median aerodynamic diameter and geometric standard deviation were 2.38 ± 2.31 μm and 2.00 ± 2.63 μm for free and liposomal pentamidine, respectively. Mean diameter did not differ significantly between the two.

Histologic analysis of *P. carinii* involvement. Animals were sacrificed in a carbon dioxide chamber at 1 and 24 h following the last dose of therapy, and the lungs were removed immediately. Histologic sections were made after Formalin fixation and stained with silver methamine and hematoxylin-eosin. The slides were coded and reviewed by one of us (W.B.), who did not know from which groups they came. The presence of *P. carinii* on the histologic sections was categorized as extensive, focal, minimal residual, or none. Extensive disease was defined as the presence of alveolitis with intra-alveolar *P. carinii* in more than 50% of the alveoli. Focal disease was characterized by alveolitis in less than 50% of the alveoli. The demarcation between extensive and focal disease was usually clearcut. In some rats, there was no alveolitis, but a few *P. carinii* cysts were found after extensive study of the silver methamine-stained material. These cases are referred to as minimal residual disease and are believed to represent instances of nearly complete recovery. Except for the latter category, the classification scheme is essentially that of Hughes and Smith (7). A similar scheme was used by Kluge et al. (10).

Measurement of pentamidine in *P. carinii*-infected rats. After completing a 14-day course of therapy, animals were scarificed 1 or 24 h after receiving their last dose of pentamidine. Immediately after sacrifice, samples of lung, liver, kidney, and blood were frozen at −70°C. Phosphate-buffered saline (0.5 ml) was added to each organ after thawing. Organs were then homogenized and analyzed for pentamidine content as follows. Proteins were precipitated with acetonitrile containing the internal standard hexamidine. After purification with a C-8 Bond Elut cartridge column (Analytichem, Harbor City, Calif.), pentamidine was separated by high-pressure liquid chromatography (HPLC). Pentamidine concentrations in tissue were determined by comparison with a standard curve for the drug and with hexamidine as an internal standard (13).

RESULTS

Experiment 1. After 6 weeks of immunosuppression, groups of eight rats received the following treatments daily for two weeks: (i) no therapy; (ii) aerosolized DPPC-DPPG-alpha-T liposomes containing histidine buffer only; (iii) pentamidine (5 mg/kg) in aerosolized DPPC-DPPG-alpha-T liposomes; (iv) aerosolized pentamidine (5 mg/kg) in PS-PC-chol-alpha-T liposomes; (v) aerosolized pentamidine as free drug (5 mg/kg); or (vi) 5 mg of pentamidine per kg by i.v. injection. We administered parenteral drug by the i.v. route because subcutaneous or intramuscular pentamidine administration produces local tissue necrosis and abscess formation (10), which may result in erratic drug absorption from sites of injection.

The majority of animals in groups i through v had extensive *P. carinii* involvement at sacrifice. Although i.v. pentamidine appeared to be more effective than aerosolized drug in treating *P. carinii* pneumonia, no treatment group demonstrated a statistically significant improvement in survival compared with untreated controls (Table 1). Drug levels in the lung following i.v. administration of pentamidine were 7.2- to 11.5-fold higher than those achieved in the aerosol groups (Table 2). Kidney and liver levels produced by i.v. pentamidine ranged from 41 to 175 times and 34 to 210 times higher, respectively, than those of the aerosol groups. Blood levels were undetectable (below 2 ng of pentamidine per ml) for animals receiving aerosolized drug. Pentamidine levels in the lung remained relatively constant from 1 to 24 h following drug administration. Thus, drug deposited in the lung after i.v. or aerosol administration was retained there. Neither lung nor extrapulmonary pentamidine levels differed significantly between the free and liposome-encapsulated aerosol groups.

Experiment 2. We next initiated treatment after 5 rather than 6 weeks of immunosuppression, with escalating doses of aerosolized pentamidine. Seven of eight untreated animals had *P. carinii* pneumonia (Table 3). Aerosol pentamidine at 5 mg/kg per day, a relatively ineffective dose in experiment 1, eliminated *P. carinii* pneumonia in 75% of treated animals. No significant differences in response rate ($P > 0.1$ by the Wilcoxon test) were noted between any of the aerosol pentamidine treatment groups.

Increasing the dose of aerosolized drug significantly increased pentamidine uptake both in the lung and in extrapulmonary sites (Table 4). Raising the aerosolized pentamidine dose 10 times (either free or in liposomes) increased peak lung uptake approximately 10-fold and renal uptake up to 15-fold. A 30-fold increase in the free aerosol pentamidine dose increased peak lung uptake 20-fold and peak renal uptake 48-fold. Increases in liver uptake and higher blood levels also were associated with the higher aerosol pentamidine doses. Liver levels were low compared with lung and kidney levels.

Lung levels produced by comparable 5-mg/kg per day

TABLE 2. Organ levels of pentamidine in rats receiving pentamidine i.v. or by aerosol (free or liposome encapsulated) for 2 weeks after 6 weeks of immunosuppression

Group	Treatment	Mean pentamidine concn ± SD[a]							
		Lung (μg/g)		Liver (μg/g)		Kidney (μg/g)		Blood (μg/ml)	
		1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h
iii	Aerosol DPPC-DPPG-alpha-T pentamidine in liposomes	2.60 ± 0.97	2.17 ± 0.69	0.23 ± 0.22	0.01 ± 0	0.96 ± 0.45	1.32 ± 0.31	—[b]	—
iv	Aerosol PS-PC-cholalpha-T pentamidine in liposomes	1.46 ± 0.35	1.67 ± 0.18	0.26 ± 0.16	0.02 ± 0.01	0.88 ± 0.16	2.05 ± 0.87	—	—
v	Aerosol free pentamidine	2.15 ± 0.68	2.71 ± 0.69	0.05 ± 0.04	0.05 ± 0.01	0.76 ± 0.22	2.71 ± 0.91	—	—
vi	I.v. pentamidine	15.70 ± 3.43	19.50 ± 4.40	10.5 ± 0.70	1.69 ± 0.22	133 ± 18.0	110 ± 14.0	0.024 ± 0.003	0.013 ± 0.003

[a] Each value represents the mean ± standard deviation for at least three animals. Animals were sacrificed 1 or 24 h after receiving the last dose of a 2-week course of pentamidine at 5 mg/kg per day.
[b] —, Less than 2 ng of pentamidine per ml.

doses of aerosolized pentamidine differed significantly between experiments 1 and 2. At 24 h after aerosol administration, most of the drug recovered from the lung represents material deposited in the terminal bronchioles and alveoli (1). At this time, lung levels achieved by 5-mg/kg free and liposome-encapsulated aerosolized pentamidine doses in experiment 2 were 4.3-fold ($P < 0.05$ as determined by Student's t test) and 7.6-fold ($P < 0.05$) higher, respectively, than those produced in experiment 1. Kidney and liver levels did not differ significantly between the two experiments. These observations suggest that access of aerosolized drug to the respiratory unit is reduced in the setting of more extensive *P. carinii* pneumonia, but that systemic drug uptake is not significantly affected by the severity of infection.

Lung levels 24 h after aerosol administration of 50 mg of free pentamidine per kg were 3.3 times higher than those observed after i.v. injection of the drug in experiment 1. Peak kidney and liver levels produced by this aerosol dose were 20 and 8%, respectively, of the levels achieved by a 5-mg/kg dose injected i.v. Even at 150 mg/kg per day, an aerosol dose which produced 6.7-fold higher levels of pentamidine in the lung than i.v. drug administration, peak kidney and liver levels were reduced 50 and 75%, respectively, compared with the i.v. group. It is unlikely that an additional week of immunosuppression would significantly alter kidney, liver, or blood levels produced by i.v. pentamidine administration. Although pentamidine may cause tissue trauma after local injection in both animals (10) and humans (11), we did not observe airway inflammation in lung sections from treated animals even at the highest aerosol doses. The animals appeared to be comfortable throughout aerosol administration.

DISCUSSION

Most agents administered via the airway are cleared rapidly from the lungs (2), and certain drugs are absorbed much more quickly from the lung than from the gut (3). In these studies, aerosolized pentamidine, both free and encapsulated in liposomes, was retained in the lungs from 1 to 24 h after administration. Prolonged lung deposition of aerosolized pentamidine should enhance its activity against *P. carinii* pneumonia and may account for the low systemic drug levels observed after aerosol administration. Increasing the aerosol drug doses enhanced lung uptake of pentamidine and also resulted in significantly higher systemic levels. However, 150 mg of aerosolized pentamidine per kg, which achieved a 6.7-fold increase in lung uptake compared with i.v. administration, still produced significantly lower systemic drug levels than i.v. drug injection.

The levels of aerosolized pentamidine in the lung in the present study differ from those reported by other investigators (15; E. M. Bernard, H. J. Donnelly, H. P. Koo, and D. Armstrong, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 552, 1985). These differences may occur for several reasons. First, different methods (fluorometric, bioassay, and HPLC) were used to assay pentamidine. Second, we assessed immunosuppressed ani-

TABLE 3. Assessment of *P. carinii* pneumonia in rats receiving various forms of therapy for 2 weeks after 5 weeks of immunosuppression

Group	Treatment and dose (mg/kg per day)	No. of animals with *P. carinii* lung involvement:			
		None	Minimal	Focal	Extensive
i	Control (no therapy)	1	0	4	3
ii[a]	DPPC-DPPG-alpha-T pentamidine in liposomes (5)	5	1	1	1
iii	DPPC-DPPG-alpha-T pentamidine in liposomes (50)	4	1	2	1
iv[a]	Free pentamidine (5)	5	1	1	1
v[a]	Free pentamidine (50)	7	1	0	0
vi[a]	Free pentamidine (150)	5	1	2	0

[a] Response rate (the number of animals with no or minimal disease) was significantly greater than that of control group ($P < 0.05$ by the Wilcoxon test). However, there were no significant differences in the response rate when the different treatment groups were compared with each other ($P > 0.1$ by the Wilcoxon test).

TABLE 4. Organ levels of pentamidine in rats receiving various forms of pentamidine for 2 weeks after 5 weeks of immunosuppression[a]

Group	Treatment and dose (mg/kg per day)	Mean pentamidine concn ± SD							
		Lung (μg/g)		Liver (μg/g)		Kidney (μg/g)		Blood (μg/ml)	
		1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h
ii	DPPC-DPPG-alpha-T pentamidine in liposomes (5)	6.43 ± 4.81	17.7 ± 2.2	0.16 ± 0.07	—	2.15 ± 0.40	1.4 ± 0.5	—	0.009 ± 0.008
iii	DPPC-DPPG-alpha-T pentamidine in liposomes (50)	63.1 ± 9.90	64.57 ± 21.54	0.79 ± 0.38	0.50 ± 0.22	25.2 ± 3.90	26.23 ± 3.17	—	0.009 ± 0.0008
iv	Free pentamidine (5)	9.58 ± 4.11	8.1 ± 4.9	0.14 ± 0.06	—	1.45 ± 0.27	1.95 ± 0.67	—	—
v	Free pentamidine (50)	56.1 ± 17.5	64.4 ± 0.20	0.87 ± 0.03	0.47 ± 0.15	22.3 ± 3.3	22.3 ± 2.4	0.018 ± 0.004	0.005 ± 0.003
vi	Free pentamidine (150)	128.65 ± 58.9	130.0 ± 12.5	2.72 ± 1.41	1.35 ± 0.25	69.5 ± 17.0	60.5 ± 8.7	0.042 ± 0.001	0.018 ± 0.007

[a] See Table 2, footnotes *a* and *b*.

mals infected with *P. carinii* rather than healthy animals. Third, differences in the aerosol delivery systems, including the nebulizer used, the ratio of air flow in the chamber to the minute ventilation of the animals, and the duration of exposure to the aerosol, can produce significant alterations in lung uptake of the drug.

Both pulmonary uptake and the therapeutic efficacy of aerosolized pentamidine were reduced by initiating treatment after 6 rather than 5 weeks of dexamethasone induction. Hughes and colleagues have previously shown that 5 versus 6 weeks of dexamethasone administration produces very different response rates to identical trimethoprim-sulfamethoxazole (TMP-SMX) and dapsone treatment regimens in *P. carinii*-infected rats (7, 8). After 6 weeks of dexamethasone pretreatment, they found that 50 to 250 mg of TMP-SMX per kg did not cure any of the treated animals (7), whereas a similiar regimen cured seven of nine animals who had received dexamethasone for 5 weeks (8). Similarly, dapsone at 25 and 125 mg/kg produced 0 to 20% cure rates in animals treated for 6 weeks with dexamethasone. Dapsone at 25 and 100 mg/kg as well as TMP at 60 mg/kg plus dapsone at 25 mg/kg each cured 100% of treated animals that received 5 weeks of dexamethasone preinduction (7). Response rates to TMP-SMX or TMP plus dapsone treatment in humans with *P. carinii* pneumonia (11, 12, 17) are similiar to those seen in rats receiving dexamethasone for 5 weeks (7, 8).

Our pharmacokinetic data provide at least a partial explanation for the dramatic differences in response rates observed at 5 versus 6 weeks. Access of aerosolized pentamidine to the lung is significantly reduced in the 6-week group, presumably due to more severe underlying pulmonary disease. Lung uptake of agents administered via the airway has previously been shown to be reduced in areas of pulmonary consolidation (9). The severity of host immunosuppression, which increases progressively with continued dexamethasone administration (16), may also influence response rates.

In summary, aerosolized pentamidine eliminated *P. carinii* pneumonia in the majority of treated animals, at a time when 88% of untreated control animals demonstrated significant infection. Furthermore, aerosolized administration of pentamidine resulted in substantially higher lung levels than those produced by i.v. injection, with lower kidney, blood, and liver uptake. No obvious ill effects were observed in animals receiving high-dose aerosolized pentamidine, and no histopathologic evidence of airway inflammation or damage was present in treated animals. Therefore, this study suggests that aerosol administration of pentamidine may prove effective in the treatment of human *P. carinii* pneumonia and should reduce the high incidence of extrapulmonary toxicity associated with parenteral administration of this drug. The severity of *P. carinii* pneumonitis at the time of treatment may have a significant effect on the outcome of aerosol therapy. However, several factors, including minute ventilation and breathing patterns, which differ between rats (5) and humans (1), may alter lung distribution of aerosolized pentamidine in the setting of *P. carinii* pneumonia. Therefore, pharmacokinetic studies of aerosolized pentamidine in human patients appear warranted prior to human clinical trials.

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LITERATURE CITED

1. **Brain, J. D., and P. A. Valberg.** Deposition of aerosols in the respiratory tract. Am. Rev. Respir. Dis. **120**:1325–1373.
2. **Enna, S. J., and L. S. Schankar.** 1972. Absorption of saccharides and urea from the rat lung. Am. J. Physiol. **222**:409–414.
3. **Enna, S. J., and L. S. Schankar.** 1972. Absorption of drugs from the rat lung. Am. J. Physiol. **223**:1227–1231.
4. **Gordin, F. M., G. L. Simon, C. B. Wofsy, and J. Mills.** 1984. Adverse reactions to trimethoprim-sulfamethoxazole in patients with the acquired immunodeficiency syndrome. Ann. Intern. Med. **100**:495–499.
5. **Guyton, A. C.** 1947. Measurement of the respiratory volumes of laboratory animals. Am. J. Physiol. **150**:70–77.
6. **Henshaw, N. G., J. L. Carson, and A. M. Collier.** 1985. Ultrastructural observations of *Pneumocystis carinii* attachment to rat lung. J. Infect. Dis. **151**:181–186.
7. **Hughes, W. T., and B. L. Smith.** 1984. Efficacy of diaminodiphenylsulfone and other drugs in murine *Pneumocystis carinii* pneumonitis. Antimicrob. Agents Chemother. **26**:436–440.
8. **Hughes, W. T., B. L. Smith, and D. P. Jacobus.** 1986. Successful treatment and prevention of murine *Pneumocystis carinii* pneumonitis with 4,4′-sulfonylbisformanilide. Antimicrob. Agents

Chemother. **29**:509–510.
9. **Jakob, G. H., and G. M. Green.** 1973. Effects of pneumonia on intrapulmonary distribution of inhaled particles. Am. Rev. Respir. Dis. **107**:675–678.
10. **Kluge, R. A., D. M. Spaulding, and A. J. Spain.** 1978. Combination of pentamidine and trimethoprim-sulfamethoxazole in the therapy of *Pneumocystis carinii* pneumonia in rats. Antimicrob. Agents Chemother. **13**:975–978.
11. **Kovacs, J. A., J. W. Hiemenz, A. M. Macher, D. Storer, H. Murray, J. Shelhamer, C. Lane, C. Urmacher, C. Honig, D. Longo, M. Parker, C. Natanson, J. Parrillo, A. Fauci, S. P. Pizzo, and H. Masur.** 1984. *Pneumocystis carinii* pneumonia: a comparison between patients with the acquired immunodeficiency syndrome and patients with other immunodeficiencies. Ann. Intern. Med. **100**:663–671.
12. **Leoung, G. S., J. Mills, P. C. Hopewell, W. Hughes, and C. Wofsy.** 1986. Dapsone-trimethoprim for *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. Ann. Intern. Med. **105**:45–48.
13. **Lin, J. M., R. J. Shi, and E. J. Lin.** 1986. High performance liquid chromatography determination of pentamidine in plasma. J. Liq. Chromatogr. **9**:2035–2046.
14. **Olson, F., C. A. Hunt, F. C. Szoka, W. T. Vail, and D. Papahadjopoulos.** 1979. Preparation of liposomes of defined size distribution by extrusion through polycarbonate membranes. Biochim. Biophys. Acta **557**:9–23.
15. **Waldman, R. H., D. E. Pearce, and R. A. Martin.** 1973. Pentamidine isothionate in lungs, livers, and kidneys of rats after aerosol or intramuscular administration. Am. Rev. Respir. Dis. **108**:1004–1006.
16. **Walzer, P. D., M. LaBine, T. Redington, and M. T. Cushion.** 1984. Predisposing factors in *Pneumocystis carinii* pneumonia: effects of tetracycline, protein malnutrition, and corticosteroids on hosts. Infect. Immun. **46**:747–753.
17. **Wharton, J. M., D. C. Coleman, C. B. Wofsy, J. M. Luce, W. Blumenfeld, K. Hadley, L. Ingram-Drake, P. A. Volberding, and P. C. Hopewell.** 1986. Trimethoprim-sulfamethoxazole or pentamidine for *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. Ann. Intern. Med. **105**:37–44.