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Phagocytosis of *Pseudomonas aeruginosa* by Polymorphonuclear Leukocytes and Monocytes: Effect of Cystic Fibrosis Serum

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It has been shown previously that serum from chronically infected patients with cystic fibrosis inhibits the phagocytosis of *Pseudomonas aeruginosa* by both normal and cystic fibrosis alveolar macrophages. In the present study, the ability of peripheral monocytes and polymorphonuclear leukocytes from normal volunteers and cystic fibrosis patients to phagocytize *P. aeruginosa* was shown not to be inhibited in the presence of serum from cystic fibrosis patients.

The serum of cystic fibrosis (CF) patients chronically colonized with *Pseudomonas aeruginosa* inhibits the phagocytosis of *P. aeruginosa* by both normal and CF alveolar macrophages in vitro (7, 9). Phagocytosis by the pulmonary polymorphonuclear leukocyte (PMN) population has not been adequately studied. In CF patients, most of the cells obtainable from the respiratory tract are in a degenerating state, and in normal individuals, PMNs are not usually recoverable from the lung. Ultrastructural studies have suggested, however, that CF pulmonary PMNs, in contrast to the macrophage (9), is actively engaged in phagocytosis. Circulating PMNs from normal individuals have been tested for phagocytic ability in the presence of CF and normal sera and found to be functional (1). The ability of CF peripheral monocytes and PMNs to phagocytize *Pseudomonas* has not been investigated. Because the pulmonary phagocyte is recruited, in part, from the peripheral blood, we studied the ability of peripheral monocytes and PMNs from normal volunteers and CF patients to phagocytize *P. aeruginosa* in the presence of CF serum, under the same experimental conditions as those used previously to test the alveolar macrophage. Phagocytosis of *Staphylococcus aureus* and *Serratia marcescens* by CF and normal peripheral cells was also compared. We conclude that phagocytosis of *Pseudomonas* by PMNs and monocytes is not inhibited by CF serum.

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For isolation of monocytes and PMNs, 30 to 90 ml of heparinized venous blood was obtained from healthy normal volunteers or CF patients. CF patients participating in the study were in good clinical condition (8) and not acutely ill at the time of the study. After dextran sedimentation to remove erythrocytes, the leukocytes were separated on Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N.J.) gradients (2).

Monocytes were cultured on 12-mm cover slips in 35-mm culture dishes containing McCoy 5A medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 20% fresh human serum from normal volunteers and antibiotics as previously described (7). Cover slips were seeded with 7.5×10^5 cells in 0.2 ml of medium, incubated for 45 to 60 min and washed gently with Hanks balanced salt solution to remove non-adherent cells, and then an additional 1.5 ml of medium was added for overnight culture in a humidified 5% CO_2 atmosphere at 37°C. Stained cultures demonstrated that greater than 90% of the cells were monocytes at the time of the assay. PMNs were seeded at 1.25×10^6 cells on 18-mm cover slips in 0.4 ml of medium and incubated for 60 min as above and then used in the phagocytosis assay.

Alveolar macrophages were obtained from normal, healthy adult volunteers by fiberoptic bronchoscopy as previously described (7). Macrophage cultures were seeded at 5.0×10^5 cells on 12-mm cover slips in 0.2 ml of McCoy 5A medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics. The cover slips were incubated for 60 min, and an additional 1.5 ml of medium was added for overnight culture. The 24-h macrophage monolayers were then used in the phagocytosis assay.

TABLE 1. Comparison of percent inhibition determined by phagocytic index and by uptake of radioactively labeled bacteria in normal volunteer serum and CF serum.

Cell source[a]	Phagocytic index		% Inhibition	Radioactivity (dpm ± SD)[b]		% Inhibition
	Normal serum	CF serum		Normal serum	CF serum	
NV-PMN	95	86	9	6,474 ± 37	6,438 ± 6	1
NV-PMN	68	90	−32	10,020 ± 2,007	13,848 ± 2,746	−38
NV-PMN	72	78	−8	6,660 ± 336	6,528 ± 1,645	2
NV-PMN	52	43	17	10,395 ± 1,188	6,876 ± 1,485	34
NV-PMN	52	60	−15	8,910 ± 1,753	8,367 ± 2,182	6
NV-PMN	59	45	24	52,431 ± 4,827	36,355 ± 3,131	31
CF-PMN	81	97	−20	14,975 ± 1,451	13,311 ± 587	11
CF-PMN	93	84	10	25,384 ± 4,881	24,736 ± 3,918	2
CF-PMN	58	64	−10	13,874 ± 3,717	13,536 ± 2,956	2
NV-AM	57	23	60	8,451 ± 2,071	3,191 ± 242	62
NV-AM	86	59	32	6,361 ± 939	3,382 ± 1,496	47
NV-AM	60	37	39	18,038 ± 938	8,767 ± 892	51
NV-AM	46	19	59	21,758 ± 490	7,908 ± 1,252	64

[a] NV-PMN, Normal volunteer polymorphonuclear leukocytes; CF-PMN, cystic fibrosis polymorphonuclear leukocytes; NV-AM, normal volunteer alveolar macrophage.

[b] Results are expressed as mean dpm ± standard deviation.

The same clinical isolate of *P. aeruginosa* used previously with alveolar macrophages (7) was used in all assays. A clinical isolate of *S. marcescens* and a strain of *S. aureus* (Wood 46 [ATCC 10832]) were also included. In some experiments, *P. aeruginosa* was radioactively labeled with [^{3}H]leucine (ICN Pharmaceuticals, Inc., Irvine, Calif.) as previously described (7). The ratio of bacteria to cells in the assay varied between 25:1 and 50:1. The concentration of the bacterial inoculum was estimated by the optical density at 520 nm and was confirmed by plate counts.

In preparation for the phagocytosis assay, the 60-min PMN cultures and 24-h monocyte or macrophage cultures were washed twice with Hanks balanced salt solution before adding the reaction mixture which consisted of 0.2 ml of a 1:1 dilution of a CF or normal serum sample (collected as previously described [7]) in unsupplemented McCoy medium, 0.2 ml of the bacterial suspension, and 1.6 ml of unsupplemented McCoy medium. All components were preincubated individually at 37°C. Cells with the reaction mixture were incubated for 30 min at 37°C with gentle rotation. In some experiments, parallel cultures were incubated at 4°C to differentiate attachment from ingestion. (The cells are relatively inactive at 4°C and events requiring substantial energy production, such as ingestion, will not occur.) Phagocytosis was stopped by washing with cold Hanks balanced salt solution. Cover slips were stained with tetrachrome and 100 to 200 cells were counted for phagocytic index. Phagocytic index is defined as the ratio of cells with associated bacteria to total cells counted. If radioactivity was included, triplicate cover slips were each placed in 1.0 ml of 0.2 N NaOH, blended in a Vortex mixer, and 9 ml of Formula 963 scintillation fluid (New England Nuclear Corp., Boston, Mass.) and 0.2 ml of 3 N HCl were added. Percent inhibition (or enhancement) of phagocytosis was calculated as:

$$\left[1 - \frac{^3\text{H bacterial uptake (CF serum)}}{^3\text{H bacterial uptake (control serum)}}\right] \times 100$$

or

$$\left[1 - \frac{\text{phagocytic index (CF serum)}}{\text{phagocytic index (control serum)}}\right] \times 100$$

Phagocytosis in CF and normal serum was assessed by the phagocytic index and the uptake of radioactively labeled bacteria (Table 1). The number of associated bacteria per cell was not scored. In contrast, phagocytosis determined by uptake of radioactive bacteria includes the total number of cell-associated bacteria for the entire cell population. The two methods of determining percent inhibition correlated well ($r = 0.923$) for the experiments shown in Table 1. From these experiments, it appeared that the determination of phagocytic index was sufficient to assess the inhibition of phagocytosis in CF serum. Because determining radioactive incorporation required more cells, we used only phagocytic index in experiments with limited cell numbers (monocytes) and to screen large numbers of serum

TABLE 2. Comparison of *P. aeruginosa* phagocytosis by normal and CF PMN and monocytes and normal alveolar macrophages[a]

Cell type	Serum	Phagocytic index[b] (no. of serum samples)	
		Normal	CF
PMN	CF	57.1 ± 17.0 (40)	58.4 ± 15.5 (34)
	Normal	65.1 ± 18.0 (39)	65.0 ± 19.9 (25)
Monocyte	CF	53.6 ± 21.0 (16)	48.0 ± 10.7 (22)
	Normal	51.6 ± 23.4 (11)	48.2 ± 14.1 (20)
Alveolar macrophage	CF	20.8 ± 7.2[c] (41)	ND
	Normal	45.2 ± 9.9 (41)	ND

[a] These experiments were carried out on PMNs and monocytes from 15 normal individuals and 11 CF patients and on alveolar macrophages from 17 normal volunteers.
[b] Results are expressed as mean phagocytic index ± standard deviation. ND, Not done.
[c] Phagocytosis by normal alveolar macrophages in the presence of CF serum is significantly different from phagocytosis in normal serum ($P < 0.001$). No other comparisons were statistically significant.

samples. The mean of parallel experiments at 4°C showed less than 7% of the cells with associated bacteria. This low level of attachment suggests that this method may not be optimal for distinguishing the processes of attachment and ingestion of bacteria. This observation is consistent with the findings of Rabinovitch (6). He found that both attachment and ingestion are temperature dependent.

The phagocytosis of *P. aeruginosa* by CF and normal PMNs and monocytes in the presence of CF and normal serum were compared with results obtained with normal alveolar macrophages (Table 2). The same CF serum samples which demonstrated inhibition of the alveolar macrophages were tested with PMNs and monocytes in this study. No phagocytic inhibitory effect of CF serum was demonstrated for normal and CF PMNs and monocytes. In contrast, CF serum inhibited phagocytosis by alveolar macrophages ($P < 0.001$). Comparison by Student's *t* test of CF and normal peripheral cells showed no significant difference in their ability to phagocytize *Pseudomonas* under these experimental conditions. The phagocytic indices of monocytes and macrophages were slightly lower than those for the PMNs in both normal and CF serum. For PMNs, the increased phagocytic index was coupled with an increased number of bacteria per cell for most experiments.

To assess the functional ability of CF cells to phagocytize a gram-positive strain and an unrelated gram-negative strain, we compared the uptake of *S. aureus* and *S. marcescens* by CF and normal PMNs and monocytes. CF sera did not inhibit the uptake of either bacterium by CF or normal cells. The phagocytic indices of PMNs with *S. aureus* (range 87 to 90) were higher than for *P. aeruginosa* (57 to 65) or *S. marcescens* (43 to 59). Uptake by monocytes was also highest

TABLE 3. Comparison of *S. aureus* and *S. marcescens* phagocytosis by normal and CF PMNs and monocytes[a]

Bacterium phagocytized	Cell type	Serum	Phagocytic index[b] (no. of serum samples)	
			Normal	CF
S. aureus	PMN	CF	87.1 ± 10.5 (14)	88.3 ± 7.5 (12)
		Normal	90.0 ± 11.0 (10)	87.0 ± 10.6 (11)
	Monocyte	CF	72.2 ± 12.0 (14)	70.8 ± 6.0 (9)
		Normal	75.9 ± 14.0 (12)	73.4 ± 10.7 (7)
S. marcescens	PMN	CF	55.6 ± 15.0 (16)	44.5 ± 19.6 (11)
		Normal	58.8 ± 19.9 (12)	42.6 ± 20.5 (10)
	Monocyte	CF	40.2 ± 10.9 (13)	45.1 ± 11.9 (15)
		Normal	47.2 ± 12.2 (10)	44.8 ± 10.4 (8)

[a] These experiments were carried out on PMNs and monocytes from 9 normal individuals and 9 CF patients.
[b] Results are expressed as mean phagocytic index ± standard deviation.

for *S. aureus* (71 to 76), whereas *P. aeruginosa* (48 to 54) and *S. marcescens* (40 to 47) were similar.

The results of this study demonstrate that phagocytosis of *P. aeruginosa* by peripheral PMNs and monocytes from both normal volunteers and CF patients is not inhibited by CF serum as compared to normal serum. Comparison of CF to normal cells, regardless of serum source, resulted in the following observations: (i) phagocytosis of *P. aeruginosa, S. aureus*, and *S. marcescens* by CF cells was comparable to phagocytosis of the same organisms by normal cells; and (ii) phagocytosis of *S. aureus* is greater for PMNs and monocytes than phagocytosis of *P. aeruginosa* or *S. marcescens*. These data suggest that no defect exists in the phagocytic ability of CF peripheral cells under the same conditions that the alveolar macrophages were tested under. The ability of the peripheral cells to efficiently phagocytize *P. aeruginosa* corroborates the clinical observation that sepsis in CF is rare after the first few months of life (10). The nature of the cellular specificity of this phenomenon is not clear. Limited studies comparing cellular receptors of alveolar macrophages and monocytes (5) have been carried out and have not revealed significant differences between the two. However, the specificity of the inhibitory activity of CF serum for alveolar macrophages may be related to some undetermined receptor differences between monocytes and macrophages. Because the inhibitory activity is specific for *Pseudomonas* and is associated with *Pseudomonas* infection (CF patients without *Pseudomonas* don't have the activity and non-CF patients with pulmonary *Pseudomonas* infection have the activity), we believe the activity is antibody in nature. Immunoglobulin G antibodies from CF serum have been reported to be ineffective opsonins for alveolar macrophages (3); the peripheral cells have not been evaluated in this system.

Hoidal (4) recently compared the phagocytic ability of alveolar macrophages with that of peripheral monocytes and PMNs from the same normal individual in normal serum. PMNs were shown to have higher phagocytic rates, capacity, and bactericidal activity than monocytes or alveolar macrophages. Our results also indicate that PMNs have a higher phagocytic capacity than alveolar macrophages in this assay. Further studies are necessary to evaluate the effect of the CF-altered lung environment on the phagocytic ability of the peripheral cells.

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LITERATURE CITED

1. **Biggar, W. D., B. Holmes, and R. A. Good.** 1971. Opsonic defect in patients with cystic fibrosis of the pancreas. Proc. Natl. Acad. Sci. U.S.A. **68**:1716–1719.
2. **Boijum, A.** 1967. Isolation of mononuclear cells and granulocytes from human blood: isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation of 1g. Scand. J. Clin. Lab. Invest. **21**(Suppl. 97):77–89.
3. **Fick, R. B., G. P. Naegel, R. A. Matthay, and H. Y. Reynolds.** 1981. Cystic fibrosis Pseudomonas opsonins. J. Clin. Invest. **68**:899–914.
4. **Hoidal, J. F., D. Schmeling, and P. K. Peterson.** 1981. Phagocytosis, bacterial killing, and metabolism by purified human lung phagocytes. J. Infect. Dis. **144**:61–71.
5. **Morahan, P. S.** 1980. Macrophage nomenclature: where are we going? RES J. Reticuloendothel. Soc. **27**:223–245.
6. **Rabinovitch, M.** 1967. The dissociation of the attachment and ingestion phases of phagocytosis by macrophages. Exp. Cell Res. **46**:19–28.
7. **Thomassen, M. J., B. Boxerbaum, C. A. Demko, P. J. Kuchenbrod, D. G. Dearborn, and R. E. Wood.** 1979. Inhibitory effect of cystic fibrosis serum on Pseudomonas phagocytosis by rabbit and human alveolar macrophages. Pediatr. Res. **13**:1085–1088.
8. **Thomassen, M. J., C. A. Demko, B. Boxerbaum, R. C. Stern, and P. J. Kuchenbrod.** 1979. Multiple isolates of *Pseudomonas aeruginosa* with differing antimicrobial susceptibility patterns from patients with cystic fibrosis. J. Infect. Dis. **140**:873–880.
9. **Thomassen, M. J., C. A. Demko, R. E. Wood, B. Tandler, D. G. Dearborn, B. Boxerbaum, and P. J. Kuchenbrod.** 1980. Ultrastructure and function of alveolar macrophages from cystic fibrosis patients. Pediatr. Res. **14**:715–721.
10. **Wood, R. E.** 1976. Pseudomonas: the compromised host. Hosp. Pract. **11**:91–100.