Title: INHIBITORY EFFECT OF CYSTIC FIBROSIS SERUM ON PSEUDOMONAS PHAGOCYTOSIS BY RABBIT AND HUMAN ALVEOLAR MACROPHAGES

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SUMMARY

This report presents experimental observations indicating the presence of an inhibitory activity in cystic fibrosis (CF) serum which impairs phagocytosis of Pseudomonas aeruginosa by rabbit as well as human alveolar macrophages. Of the 49 patient serum samples studied, 40 consistently showed ≥ 60% inhibition, 3 showed no inhibition and 6 were in the range of 20-60% inhibition of Pseudomonas phagocytosis. In parallel studies, the phagocytosis of S. aureus and S. marcescens was found not to be inhibited by CF serum. Mixing of CF serum with normal serum could not overcome the inhibitory effect, indicating the presence of an inhibitory factor rather than the lack of a necessary component. The inhibitory activity is not lost upon exposure of serum to glass, upon freezing the serum once, or upon heating at 56°C for 30 minutes.

SPECULATION

The serum of cystic fibrosis patients selectively inhibits alveolar macrophage function in vitro; the expression of this inhibitory activity in vivo may compromise effective host control of infection. Investigation of the origin, nature and pathophysiologic role of the activity may suggest new approaches to the control of Pseudomonas pulmonary infection.

Pulmonary infection is a major factor in the morbidity and mortality associated with cystic fibrosis (CF) (6). Pseudomonas, a ubiquitous organism in the environment, is usually not pathogenic for healthy individuals. However, individuals with the chronic lung disease of CF are particularly susceptible to opportunistic Pseudomonas aeruginosa infections. The frequency of this organism in CF pulmonary infections is inadequately explained. It is well known that most CF patients have elevated levels of Pseudomonas antibodies in their sera and pulmonary secretions (12,14). While recently there has been an indication that a lymphocyte unresponsiveness to Pseudomonas may be acquired as the infection progresses (18,19), no other immunologic abnormality has been consistently observed (5,10). Extrapulmonary infection is extremely rare and sepsis is almost never seen after the first months of life (22). This unusual susceptibility to Pseudomonas despite apparently normal systemic humoral and cellular immunity, suggests that local pulmonary host defense mechanisms are defective in CF. Several recent studies have indicated that lung defenses can, to a certain extent, function independently of systemic humoral and cell mediated immune systems (9,15,20,21).

Lung defenses include mucociliary transport as well as phagocytic cells, lymphocytes, and immunoglobulin secretion. Since mucociliary transport in some CF patients is compromised (5), clearing of the bacteria becomes more dependent on the efficient action of the phagocytic cells. Previous studies in our laboratory (2) and by Biggar, et al. (1) have shown that CF serum impairs phagocytosis of Pseudomonas by rabbit alveolar macrophages. This report presents experimental observations indicating the presence of an inhibitory activity in CF serum which impairs phagocytosis of Pseudomonas by human as well as rabbit alveolar macrophages.

MATERIALS AND METHODS

All studies were carried out under an approved human investigation protocol and after securing informed consent from all participants.

Preparation of Rabbit Alveolar Macrophages

Rabbit alveolar macrophages were obtained by pulmonary lavage according to the method of Myrvik, et al. (13) as modified by Brain and Frank (3). Disease-free New Zealand rabbits weighing 3-4 kg were sacrificed with pentobarbital and the lungs excised and placed in a 37°C water bath. The lungs were lavaged with sterile normal saline and the lavage fluid passed through a blood filter (Travenol Laboratories, Inc., Deerfield, Ill.). Seven to eight lung washings were pooled (ca 400 ml), centrifuged at 400g for 10 minutes; whereupon the cell pellet was washed three times with normal saline. The average yield per rabbit was 3.9×10^7 cells (range $1.4 - 7.3 \times 10^7$). More than 95% of the cells isolated were macrophages, and greater than 95% of these excluded trypan blue.

Preparation of Human Alveolar Macrophages

Alveolar macrophages were obtained from normal, healthy adult volunteers by fiberoptic bronchoscopy. Brief medical histories were obtained on all volunteers, none of whom had recent acute pulmonary infection or a history of chronic pulmonary disease. A flexible bronchoscope was passed transnasally with topical anesthesia (lidocaine or cocaine) into a segment of the lingula. With the tip of the bronchoscope wedged into a segmental or subsegmental airway, aliquots (60-75 ml) of sterile 0.9% saline were alternately instilled by gravity and withdrawn by gentle aspiration with a syringe. A total volume of 400-600 ml was usually employed, of which all but 100-150 ml was recovered. The lavage procedure required approximately 10 minutes. No subject experienced adverse effects. Bronchoscopic findings were within normal limits, except in some of the cigarette smokers who had evidence of increased secretions in the airways. The smokers in the study smoked between 10-20 cigarettes per day. Smokers were used because of the greater cell yield and previous demonstration of no significant difference in phagocytosis of Pseudomonas by alveolar macrophages from smokers and non-smokers (16). The average yield from non-smokers was 7.9×10^6 cells (range $2.6 \times 10^6 - 18 \times 10^6$) and from smokers 5.6×10^7 cells (range $0.8 \times 10^7 - 13 \times 10^7$). More than 89% of the cells isolated were macrophages as determined by a nonspecific esterase stain (11), and greater than 95% of these excluded trypan blue.

Establishment of Macrophage Cultures

Macrophages were cultured in tissue culture chambers (2 or 4 chamber units, Lab-Tek Products, Div. Miles Laboratories, Inc., Naperville, Ill.) containing McCoys 5A medium (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal calf serum, 2.9 mg/ml L-glutamine, and antibiotics (gentamicin sulfate, penicillin, streptomycin and amphotericin B). The two chamber slides were seeded with 1×10^6 cells per chamber and the four chamber slides with 5×10^5 cells per chamber.[1] Cultures were incubated for 24 hours at 37°C in a humidified 5% CO_2 atmosphere. To insure comparability of cell cultures, individual cultures were checked for uniformity of the cell population by examination with an inverted microscope at 100X magnification.

Preparation of Radioactive Bacteria

Four strains of Pseudomonas aeruginosa were obtained from CF sputum or throat cultures and two non-CF strains were obtained from urine cultures. The same non-CF strain of P. aeruginosa was used for all assays except those comparing different strains (see Table 3). In some experiments, organisms were used in addition to the P. aeruginosa; and these include a single strain of Serratia marcescens, from Dr. Paul Quie at the University of Minnesota and a single strain of Staphylococcus aureus, a CF sputum isolate. The isolate of P. aeruginosa and S. marcescens were grown 15-18 hours at 37°C with shaking in minimal salts (Koser Citrate) supplemented with 0.5% Casamino Acids (Difco Laboratories, Detroit, Michigan). Staphylococcus aureus was grown in minimal salts supplemented with 0.5% yeast extract. The cultures were diluted to mid log phase and 150 uCi of ^{3}H-leucine (specific activity 30-50 Ci/mmol, ICN Pharmaceuticals, Inc., Irvine, California) were added to P. aeruginosa and S. marcescens, while 250 uCi of ^{3}H-thymidine (specific activity 40-60 uCi/mmol, Amersham/Searle, Arlington Heights, Illinois) were added to S. aureus. After 1 to 2 hours further incubation, the bacteria were harvested by centrifugation at 3,000g for 10 minutes and washed with normal saline until no radioactivity was detected in the wash. The specific activity of the bacteria was always greater than 10^{-4} cpm/bacterium.

Patient and Normal Control Serum Samples

Controls were age and sex matched to patients using serum samples from children on the orthopedic wards and adult hospital workers. Serum was also collected from eight obligate heterozygotes (Mothers of CF patients used in the study). The age range of the patients was 9 months to 31 years and the range of the controls was 3 years to 40 years. CF was diagnosed by a positive sweat test and either typical pulmonary or gastrointestinal symptoms or a confirmed family history of CF. Respiratory tract cultures are obtained on these patients approximately every six weeks. Clinical evaluation was based on a modification (7) of the Shwachman and Kulczycki (16) system and on pulmonary function tests. Patients were classified into three categories as follows in consultation with each patient's physician:

A - Good clinical condition: x-ray and physical exam (PE) 19 or above; pulmonary function - vital capacity (VC) 80% or above, and residual volume/total lung capacity (RV/TLC) 0.35 or below.

B - Moderate clinical condition: x-ray and PE = 12 to 18; VC = 55-80%, and RV/TLC = 0.35 - 0.5.

C - Poor clinical condition: x-ray and PE < 12; VC < 55% and RV/TLC > 0.5.

All patients were off antibiotics for at least 48 hours prior to obtaining blood samples.

Serum Samples

Depending on the age and size of the subject, 10 to 30 ml of blood were obtained for the study. Initially the blood was collected in plastic tubes. Subsequent samples were collected in glass when this proved more satisfactory. After clotting at room temperature, the blood was centrifuged at 1,500g and the serum decanted. The serum was than aliquoted and samples which were not used immediately were frozen at -70°C. In some experiments pooled normal human sera (Grand Island Biological Company) were used as controls.

Phagocytosis Assay

The 24-hour macrophage monolayer cultures were washed three times with Hank's balanced salt solution (BSS) before adding the reaction mixture which consisted of 0.1 ml of a 1:1 dilution of a CF or a normal serum sample in BSS, 0.1 ml of the bacterial suspension, 0.8 ml of BSS. All components were preincubated individually at 37°C. The ratio of bacteria to cells varied between 10:1 and 100:1. The concentration of the bacterial inoculum was estimated by optical density at 520 nm and was confirmed by subsequent plate counts. The variation in the ratio of bacteria to cells over this range did not affect the comparison of phagocytosis in the presence of CF and normal serum since the ratio was constant within each experiment. The effect of CF serum on phagocytosis was compared with a parallel study of normal serum; each assay included duplicate tests of both CF and normal serum samples. The chambers were then incubated at 37°C with gentle shaking. After 30 minutes the supernatant was decanted and the cell layer washed six times with cold BSS. One ml of cold distilled water was then added to each culture to lyse the cell monolayer. The cells were allowed to swell for at least 30 minutes in the cold and then disrupted by vigorous pipetting. Observations of the culture vessels with an inverted microscope (100X) routinely revealed all the cells to be disrupted by this procedure. One tenth ml of the lysate was then counted in Triton X-100 toluene scintillation fluid. Calculations were as follows:

$$\frac{\text{cpm in lysate from each culture}}{\text{cpm of bacteria added to each culture}} \times 100 = \% \text{ phagocytosis}$$

$$\left[1 - \frac{\% \text{ phagocytosis (CF serum)}}{\% \text{ phagocytosis (control serum)}}\right] \times 100 = \% \text{ inhibition of phagocytosis}$$

If more than one control was used in an experiment, the median control was used in the calculation. For statistical evaluation, the paired t test was used to compare the per cent phagocytosis in the presence of CF serum to the per cent phagocytosis in the presence of normal control serum.

Agglutinating Antibody Titer

The antibody titer of the serum samples against the bacteria used in the phagocytosis assay was determined by slide agglutination. All serum samples were titered against a single preparation of the antigen which was prepared by growing the bacteria overnight at 37°C in 50 ml of Mueller-Hinton broth. The culture was centrifuged, washed extensively with normal saline, and the final pellet resuspended in 10 ml normal saline. Dilutions of the bacterial suspension were mixed with undiluted serum samples and optimal agglutination used to select the standard antigen dilution. Each serum was diluted serially whereupon 50 λ of each dilution was placed on a glass slide and mixed with 50 λ of the standard bacterial suspension. The slide was gently rotated and the endpoint read as the last dilution which produced +2 agglutination.

RESULTS

Serum Effects on Phagocytosis

The phagocytosis of Pseudomonas by rabbit alveolar macrophages was investigated in the presence of serum from 49 CF patients and matched controls (assayed in parallel). The comparison of phagocytosis in the presence of the two types of serum revealed significant inhibition by the CF serum ($P < .001$). Table 1 shows results from all of these patients. Of the 49 patients' serum samples studied, 40 consistently showed ≥60% inhibition, 3 showed no inhibition and 6 were in the range of 20-60% inhibition of Pseudomonas phagocytosis. Figures 1A and 1B show microscopically the effect of CF and normal serum on phagocytosis. Bacteria are associated with alveolar macrophages incubated in normal serum, in contrast, few bacteria are associated with cells in the presence of CF serum. The phagocytosis of S. aureus and S. marcescens was also evaluated, using 13 and 6 serum samples respectively (Table 2). Statistical analysis using the paired t test showed no significant CF serum inhibition of phagocytosis of either organism.

Table 1 also shows the percent inhibition of Pseudomonas phagocytosis by human alveolar macrophages in the presence of serum from 13 CF patients. The cross-species correlation is excellent. The 12 patients who showed inhibition in the rabbit system also inhibited in the human system, while one patient (patient 7) failed to inhibit in either system. The inhibition of CF serum of Pseudomonas phagocytosis was observed with both smoker and non-smoker cells. The paired t test was not performed in these studies because all of the serum samples tested with human cells were selected on the basis of previous experience with rabbit cells.

The assay (rabbit cells) was repeated using 41 of the 49 patients and again significant inhibition of phagocytosis was demonstrated ($P < .001$). Only 4 patient serum samples (patients 7, 42, 43, A.S.) have been found to consistently show no inhibition. All of these individuals were in good clinical condition. None of the four donors had mucoid Pseudomonas. One was an infant without Pseudomonas and two had had non-mucoid Pseudomonas only rarely. A second infant (A.S.), who was not in the original statistical evaluation, did not show inhibition and was also without Pseudomonas. In the group which consistently demonstrated inhibition of Pseudomonas phagocytosis, there were 7 individuals without evidence of chronic Pseudomonas infection of the lung. One individual (patient 22) in particular has never had a positive culture in over 19 years since diagnosis of CF, and yet his serum consistently inhibited Pseudomonas phagocytosis.

A plot of per cent inhibition of Pseudomonas phagocytosis versus agglutinating antibody titer for 42 patients is shown in Figure 2. There does not appear to be a correlation between the degree of inhibition of phagocytosis and agglutinating antibody titer. In fact, there were individuals whose serum showed marked inhibition in both the low and high titer groups. However, the individuals who did not show inhibition had low titers (points a, b, and c in Figure 2). The serum titers of 25 control subjects were determined, demonstrating that 16 had no titers, 6 had titers of 1:2, and 3 had titers of 1:4.

Effect of CF Patient History

The culture history of each of the 50 CF patients used in the study was reviewed (Table 1). The 4 patients who showed no inhibition were discussed previously. Of the remaining 46, 29 patients had mucoid Pseudomonas, 10 had only non-mucoid Pseudomonas, 5 had non-mucoid Pseudomonas intermittently, and 2 never had Pseudomonas cultured. However, of these latter two, one was newly diagnosed (patient 44, age 8 years).

As determined by x-ray score, physical exam, and pulmonary function data, 35 of the 49 patients were in good clinical condition, 13 patients were in moderate condition and one patient was in poor condition. The patients were preselected on the basis of being able to come off antibiotics for 48 hours before the blood was drawn. Thus, few patients in poor condition were used. All of the patients whose serum consistently did not show inhibition were in good condition (category A as defined above).

While there is no correlation between age and degree of inhibition, individuals who failed to show inhibition were generally younger. Sex also did not correlate with inhibition. Serum samples from 8 obligate heterozygotes also did not demonstrate the inhibitory activity.

Effect of Bacterial Characteristics

To determine whether this inhibitory phenomenon was specific for the source of Pseudomonas, 4 CF isolates and 2 non-CF isolates were investigated (Table 3). Serum samples from six patients demonstrated similar results using different organisms regardless of the source of the organism. The only exception was patient 33, whose serum produced less inhibition of phagocytosis of a non-CF Pseudomonas than with his own organism. Serum samples from 3 other patients showed relatively equal inhibition with this non-CF organism as compared with organisms from CF patients.

Characteristics of Inhibiting Activity

Serum inhibition of phagocytosis can arise from either the presence of an inhibitory factor or from a serum deficiency. To distinguish these possibilities, 4 paired serum samples from CF patients and controls were mixed in equal parts. The results shown in Table 4 demonstrate that normal sera could not overcome the inhibitory effect. This indicates the presence of an inhibitory factor rather than the lack of a necessary component. The inhibitory activity could, however, be eradicated by serial dilution of CF serum into normal serum. A serum from the high inhibitory group demonstrating 94% inhibition must be diluted with normal serum more than 40 fold for complete loss of activity while a serum demonstrating 37% inhibition shows loss of activity at a 4 fold dilution in the same normal serum.

The inhibitory activity is not lost upon exposure of serum to glass or upon freezing the serum once. Table 5 shows the results with 7 serum samples before and after heating at 56°C for 30 minutes; none had a significant change in the per cent inhibition of phagocytosis after heating.

DISCUSSION

The alveolar macrophage is an important component in the clearance of bacteria from the lung (8); efficient functioning of this cell is essential in control of infection. We have shown that phagocytosis of Pseudomonas by both rabbit and human alveolar macrophages is significantly inhibited by CF serum. This serum effect is apparently specific for Pseudomonas since phagocytosis experiments with S. aureus and S. marcescens demonstrated no significant inhibition. The impairment appears to be the result of the presence of a factor(s) rather than a lack of an opsonin as Biggar, et al. (1) postulated because the inhibitory effect could not be overcome in mixing experiments with CF and control sera. Unlike some other "CF factors" (4), the inhibitory factor is stable to freezing, heating and is unaffected by exposure to glass.

Of the CF patients studied to date, 6 patients have intermediate inhibitory activity of 20-60% in their serum and 4 patients do not exhibit the inhibitory effect. It may be that patients with 20-60% inhibition have lower levels of the factor. The experiments using diluted serum samples support this hypothesis. Of the 4 patients who do not exhibit the inhibitory factor, 2 are infants from whom Pseudomonas has never been cultured and the other two do not have mucoid Pseudomonas. Every patient who had mucoid Pseudomonas has demonstrated inhibition, although a few inhibitory serum samples were from patients who had non-mucoid Pseudomonas. Chronic Pseudomonas infection does not appear to be a necessary prerequisite, but the presence of the inhibitory activity correlates well with a positive Pseudomonas culture history. To evaluate the role of infection, the patients without the activity are being followed longitudinally to determine if they acquire the activity after chronic infection with mucoid Pseudomonas. Obligate heterozygotes do not have the activity in their serum. To further characterize the source of the factor, we are investigating non-CF patients with chronic Pseudomonas infections and non-CF patients with chronic lung disease without Pseudomonas.

Since all CF serum samples do not exhibit inhibition, this phenomenon may not be a direct result of a primary or intrinsic CF defect but rather may be an acquired characteristic. The phenomenon could result from a Pseudomonas induced alteration of the host defense system, such as non-opsonizing antibodies or other less well defined serum components which alter either opsonization or the regulation of macrophage function (e.g., lymphokines). CF serum does not have a direct toxic effect on the macrophages since the cells remain functional as evidenced by their ability to phagocytize S. aureus and S. marcescens. Complement is probably not involved since heat does not affect the inhibition. The presence of the inhibitory activity also does not appear to correlate with antibody titer as determined by bacterial agglutination. The serum titers of the CF patients seem low but they were determined only against the Pseudomonas used in the phagocytosis assay. A patient may have a higher titer to his own organism.

An alternate hypothesis is that a bacterial property of the Pseudomonas could directly affect the macrophage or simply protect the bacteria from phagocytosis. However, if the inhibitory effect is the result of a bacterial product, it does not appear to be strain specific since both CF and non-CF Pseudomonas isolates give the same results in the phagocytosis assay. Non-mucoid isolates were used in the experiments presented in this paper because of the inability to maintain the mucoid property. We have since isolated a stable strain which will be used in subsequent studies.

Further studies are in progress which will hopefully provide answers to some of these questions concerning the origin and the identity of the activity. Since complete eradication of Pseudomonas in the CF patient is virtually impossible with current modes of therapy, an understanding of this inhibitory phenomenon may provide insight into ways for improving host defense mechanisms in the CF patient and thereby achieving effective control of infection.

Footnote

[1]Different culture vessels were used because of limited availability of chamber slides from the supplier.

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Table 1. Characteristics of CF Patients and Inhibition of Pseudomonas Phagocytosis by Rabbit and Human Alveolar Macrophages (AM)[a]

Patient	Sex	Age	Culture History[b]	Clinical Condition[c]	Rabbit AM % Inhibition	Human AM[d] %Inhibition
1	F	13	P	A	76	82(N)
2	F	28	M	B	87*	77(N)
3	M	12	M	B	81	76(N)
4	F	12	P	A	66	66(N)
5	F	14	M	A	87*	81(N)
6	M	12	M	B	87*	70(S)
7	F	13	I	A	0	0(N)
8	M	23	M	B	74	80(S)
9	F	31	M	A	97	88(N)
10	M	22	M	B	96	85(S)
11	M	25	M	A	93	85(S)
12	F	12	M	A	96	88(S)
13	F	20	I	A	80	65(N)
14	F	3	P	A	82	
15	M	11	M	A	70	
16	M	25	M	A	50	
17	F	14	M	B	83	
18	F	23	M	A	67	
19	F	15	M	A	83	
20	M	16	P	A	41	
21	F	24	M	B	87	
22	M	22	N	A	83	
23	F	20	M	C	65	
24	M	19	M	A	87	
25	M	18	M	A	75	
26	F	18	P	A	83	
27	F	15	I	A	41	
28	F	18	M	B	95 (87*)	
29	F	14	P	A	83	
30	F	18	M	A	78*	
31	M	26	M	B	85*	
32	M	16	M	A	76	
33	F	13	P	A	74	
34	M	16	M	B	82	
35	F	11	P	B	53*	
36	F	17	I	A	86	
37	M	10	P	A	92	
38	M	25	M	A	86	
39	M	18	M	A	83	
40	F	15	M	B	84*	
41	F	11	M	A	79*	
42	M	9	I	A	O	
43	M	< 1	N	A	O	
44	M	8	D	B	47*	
45	F	15	M	A	87	
46	M	10	P	A	58	
47	M	15	I	A	88	
48	F	15	M	A	83	
49	F	16	I	A	78	
A.S.	F	< 1	N	A	0	

[a] All assays were carried out with 1 X 10^6 macrophages except (*); these were carried out with 5 X 10^5 cells

[b] P = non-mucoid Pseudomonas

M = mucoid Pseudomonas

I = intermittent positive cultures for Pseudomonas

N = no known Pseudomonas

D = newly diagnosed; therefore no culture history is available

[c] see text for exact classification;

A = good, B = moderate, C = poor

[d] (N) = non smoker AM

(S) = smoker AM

Table 2. Comparison of Inhibition of Pseudomonas Phagocytosis by Rabbit Alveolar Macrophages with Phagocytosis of Staphylococcus and Serratia in the Presence of CF Serum[a]

Patient Number	Pseudomonas Inhibition	Staphylococcus Inhibition	Serratia Inhibition
1	76	0	0
2	87*	14*	NT[b]
6	87*	0	NT
7	0	0	0
14	82	NT	0
15	70	40	0
16	50	0	0
17	83	0	13
23	65	0	NT
24	87	0	NT
25	75	5	NT
26	83	8	NT
27	41	31	NT
32	76	0	NT

[a] All assays were carried out with 1 X 10^6 macrophages except (*); these were carried out with 5 X 10^5 cells

[b] NT = not tested

Table 3. Phagocytosis of Various Strains of Pseudomonas by Rabbit Alveolar Macrophages in the Presence of CF Serum.

	Per Cent Inhibition of Phagocytosis					
Patient	PS 4	PS 5	PS 7	PS 33	Non-CF 1	Non-CF 2
4	98	97	-	-	95	85
7	-	-	-	0	0	0
8	94	96	-	-	94	82
31	61	96	-	-	75	90
33	-	-	-	86	74	47
36	-	-	86*	-	86*	-

[a] All assays were carried out with 1 X 10^6 macrophages except (*); these were carried out with 5 X 10^5 cells.

[b] Number designations refer to patients from whom the particular Pseudomonas strain was isolated.

Table 4. Effect of Mixing CF and Normal Control (C) Serum on Per Cent Inhibition of Phagocytosis by Rabbit Alveolar Macrophages[a]

Sera	% Inhibition
0.1 ml C-1	0
0.1 ml C-2	0
0.1 ml C-3	0
0.05 ml C-1 + 0.05 ml C-2	0
0.1 ml CF-1	96
0.05 ml CF-1 + 0.05 ml C-1	93
0.05 ml CF-1 + 0.05 ml C-2	91
0.1 ml CF-2	95
0.05 ml CF-2 + 0.05 ml C-1	93
0.05 ml CF-2 + 0.05 ml C-2	93
0.1 ml CF-3	74
0.05 CF-3 + 0.05 C-3	83
0.1 ml CF-4	86
0.05 CF-4 + 0.05 C-3	80

[a] All assays were carried out with 1 X 10^6 macrophages

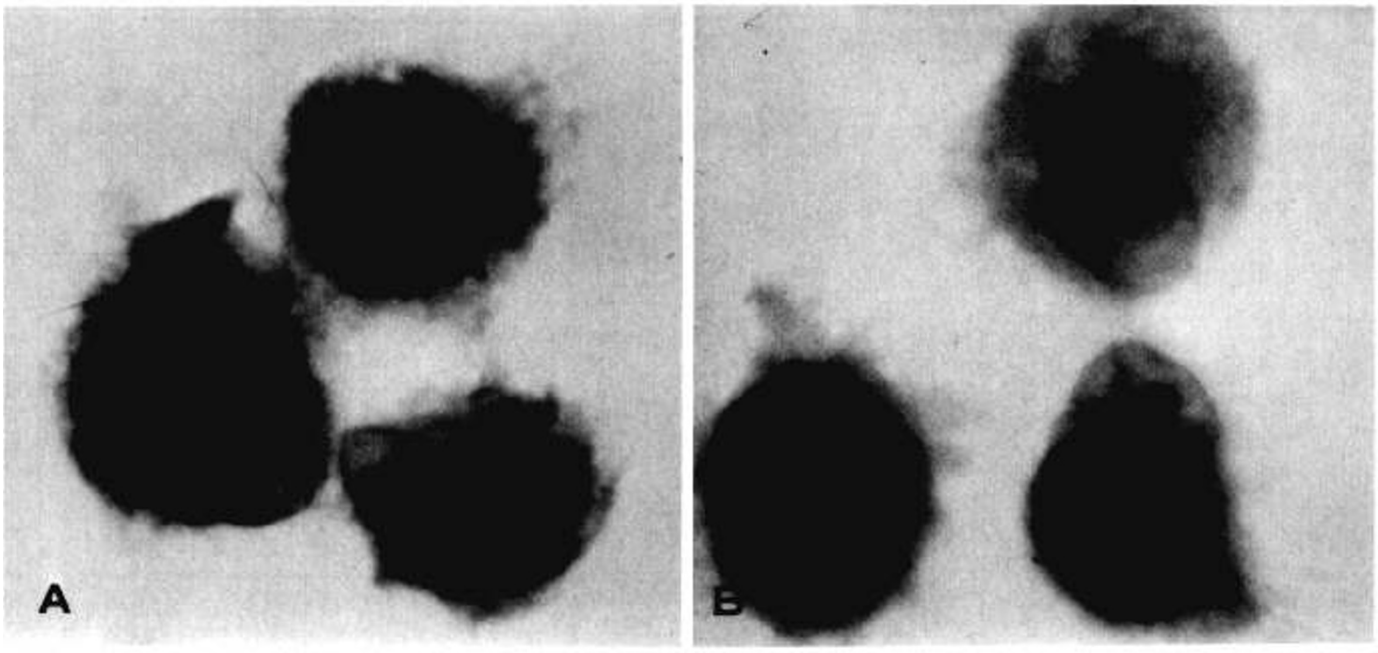

Figure 1. Photomicrographs of normal human alveolar macrophages from a non-smoker after incubation with Pseudomonas in the presence of normal serum (A) and in the presence of CF serum (B) x 2000.

Table 5. Effect of Heat Inactivation on Per Cent Inhibition of Phagocytosis by Rabbit Alveolar Macrophages[a].

Patient	Before Heating	After Heating
3	90	91
6	75	89
7	0	14
26	88	71
29	83	72
36	61	57
37	78	74

[a] All assays were carried out with 1×10^6 macrophages

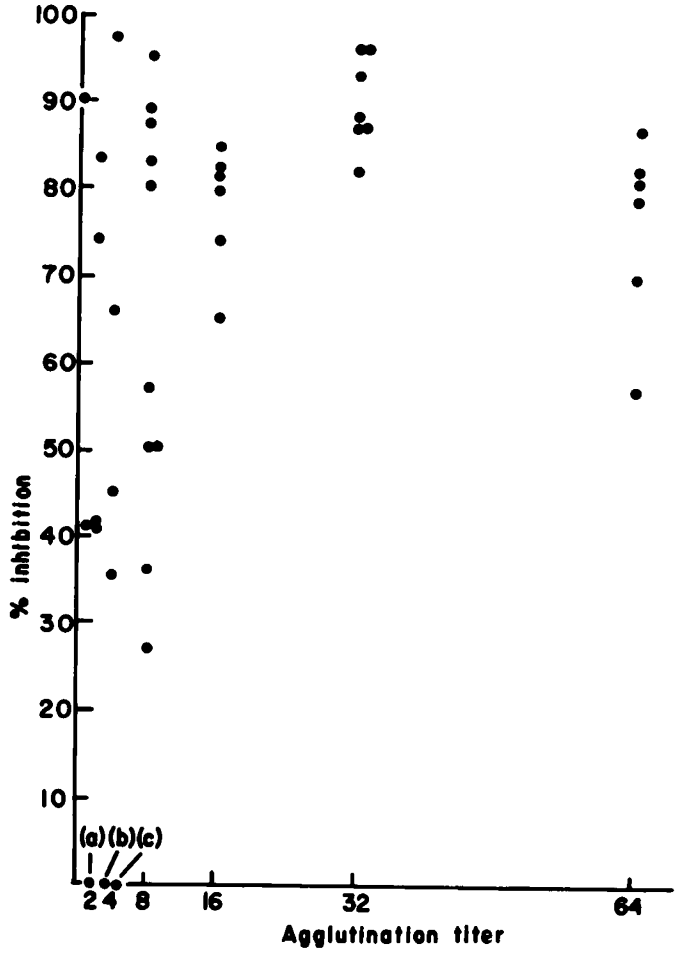

Figure 2. Comparison of inhibition of phagocytosis with agglutinating titer against the test organism. See Table 1 for details concerning points (a) - patient 7, (b) - patient 42, and (c) - patient A.S.

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