Pulmonary Nonspecific Defense Mechanisms in Cystic Fibrosis

I. Phagocytic Capacity of Alveolar Macrophages and Neutrophils

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Summary

Cystic fibrosis (CF) is the commonest cause of chronic obstructive lung disease within the first three decades of life. Because patients suffer from repeated pulmonary infections, we were interested in studying the nonspecific antibacterial mechanisms of the lungs of CF children. Fifteen CF children, 13 nontuberculous pulmonary-infected (I) children and 4 noninfected (N-I) children, who were diagnosed as having a foreign body in the air passages, were submitted to a bronchopulmonary washing. The absolute number, viability, and differential count of the cells were determined, and the phagocytic assay using the Lehrer's technique was carried out on the harvested cells.

The materials obtained from CF and I patients were mucopurulent, whereas the ones from the N-I patients were not mucopurulent. The number of cells retrieved from CF and I patients was higher than that from the N-I patients. This was attributed to an increase in the absolute number of polymorphonuclear leukocytes. The phagocytic capacity of polymorphonuclear leukocytes was not different for the three groups studied, whereas that of alveolar macrophages was higher for the CF and the I patients than for the N-I ones. Inasmuch as there was no significant difference between the CF and the I patients, we conclude that the nonspecific phagocytic behavior of alveolar macrophages and pulmonary polymorphonuclear leukocytes of CF patients might be similar to the one coming from other bacterial infectious states of the lung.

Speculation

Although cystic fibrosis children suffer from chronic progressive pulmonary infections, they show the normal population risk for other infections outside the lungs. Inasmuch as the immune system appears to be intact in these patients, a failure in one of the nonspecific antibacterial defense mechanisms of the lung may be considered.

The major cause of morbidity and mortality in cystic fibrosis (CF) is unquestionably the severe, chronic progressive pulmonary infections due to bacterial pathogens, particularly *Pseudomonas aeruginosa* and *Staphylococcus aureus* (18). An important feature of these infections is their striking localization in pulmonary tissue, with only very rare spread via the bloodstream to other sites of the body. Although this fact would tend to suggest that there is not a primary genetic defect in immunologic defense mechanisms in CF (4, 11), the overwhelming role of infection in the disease has led to an exhaustive search for acquired or local pulmonary-immunologic defect in this inherited disease.

The nonrespiratory activity of purifying inhaled air and keeping lung tissues free of infection has been collectively termed "lung

host-defense mechanisms" (5); that includes mechanical barriers, mucociliary clearance, immunoglobulins, and inflammatory response involving polymorphonuclear leukocytes (PMN). In addition, the alveolar macrophages (AM) play a principal role during phagocytosis in the airways and during their scavenging activity on the alveolar surfaces (6).

Several studies (7, 17) have suggested the compartmentalization of cell-mediated immunity, i.e., both respiratory tract and systemic cell-mediated immunity exist and are relatively independent of each other. This concept would suggest an hereditary or acquired immunologic defect localized solely in CF lung.

Because of our interest in the lung defense mechanisms, the present study was undertaken to investigate the specific question of whether there are any defects in pulmonary AM and PMN nonspecific phagocytic function in CF.

SUBJECTS AND METHODS

SUBJECTS

Fifteen CF patients, aged 1 to 17 years (9 females and 6 males) were studied with a diagnostic or therapeutic proposal; CF was diagnosed by clinical signs and by elevated sweat electrolytes; clinical grading according to Shwachman and Kulczycki score ranged from 65 to 90. All the children were on normal diets and receiving appropriate therapy. Thirteen control subjects, aged 1 to 13 years (6 females and 7 males), were investigated for variable reasons and were subsequently found to have nontuberculous infectious pulmonary involvement and no positive sweat test. Four children who had no lung infection were also investigated; these subjects were diagnosed as patients with foreign body in airways; none received antibiotics for at least 48 hr before bronchopulmonary lavage, and none was found to have a positive sweat test or CF familiar history.

These studies were carried out with the proper consent from parents of the children involved, according to the principles of the Declaration of Helsinki.

CELL RETRIEVAL

Under light general anesthesia achieved by inhalation of halothane (19), a rigid bronchoscope was introduced into the trachea and the main stem bronchi. A radiopaque catheter was passed through the bronchoscope as deeply as to the tip wedged into a segmental or subsegmental airway within a zone which previously had not shown any pathologic involvement to X-rays.

Aliquots of sterile 0.15 M NaCl solution were alternatively instilled and withdrawn by gentle aspiration with a syringe. The total volume of washing solution to be instilled was determined according to the age and the lung size of the patient, calculated

from radiologic parameters (range, 50 to 200 ml). The complete lavage procedure required approximately 5 min, and there were no complications during or after performing it.

PROCEDURE

All samples of lung washing suspension were shaken vigorously during 2 min; a 5-ml aliquot was treated with 1 ml of a 20% solution of *N*-acetylcystein (20) and incubated 10 min at 37°C with constant shaking. The total number of cells, the viability as assessed by exclusion of trypan blue dye, and the differential cell count on smears obtained by sedimentation and Giemsa staining were determined.

The remainder of the material was sedimented 2 min at $1 \times g$ and decanted; the decanted solution was centrifuged 5 min at $200 \times g$. The viability of these cells was not different from those of the *N*-acetylcystein-treated cells. From the pellet obtained, 4×10^5 cells were scattered in Leighton tubes in 1 ml of tissue culture medium (21) added to 10% of fresh human serum obtained from a pool of normal subjects.

After 3 hr incubation, the adhered cells were rinsed twice with warm tissue culture medium, and varying concentrations of *Candida albicans* were added to give a cell:yeast ratio of 1:4, 1:6, 1:8, 1:12, and 1:16. In this way, a modified phagocytic assay of Lehrer (9) was carried out by incubating the cell-yeast mixture for 30 min. The results of phagocytic capacity were expressed for the two principal phagocytic cellular types (AM and PMN) as the number of *Candida* yeasts clearly engulfed per 100 cells.

When a pair of means for samples distributed normally had to be compared, a Student's *t* test for independent samples was applied with significant levels of $P < 0.01$ and $P < 0.05$. If the samples were not distributed normally and there was no homoscedasticity, the Mann-Whitney *U* test was applied (14).

RESULTS

The recovery of washing solution was more effective in CF patients than in the noninfected (N-I) ones; the scattering of the data from nontuberculous pulmonary-infected (I) individuals made impossible the detection of any difference between this

group and the N-I patients (Table 1). The total number of free cells harvested by bronchial lavage (Table 1) was higher in the I patients and was attributed principally to a near 200-fold increase in the absolute number of PMN, as is shown from the proportion

Table 1. Percentage of wash fluid recovered and total cell content and cell viability in wash fluid obtained by bronchopulmonary lavage in CF children and control subjects

	Patients					
	N-I (n = 4)		CF (n = 15)		I (n = 13)	
	$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$	Range
Recovery of wash fluid (%)	29.80	20.0–38.0	45.25 ¹	27.5–58.8	40.20	22.5–75.0
Cell no. ($\times 10^6$)	3.32	1.0–9.3	49.63 ²	14.4–138.0	63.98 ²	1.5–144.0
Viability (%)	96.2	88–95	90.4	80–100	89.1	70–97

¹ Significantly different from the N-I patient group ($P < 0.01$).

² Significantly different from the N-I patient group ($P < 0.05$).

Table 2. Differential cell count made on Giemsa-stained smears obtained by sedimentation of samples of lung wash suspensions

	Patients					
	N-I (n = 4)		CF (n = 15)		I (n = 13)	
	$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$	Range
AM	84	76–89	16 ¹	3–47	20 ¹	1–56
Foamy cells	4	1–10	2	1–6	1	0–4
Giant cells	4	2–7	1	0–4	2	0–7
PMN	7	4–11	79 ¹	43–94	75 ¹	37–98
Lymphocytes	1	0–2	1	0–4	1	0–2
Eosinophils	0		1	0–9	1	0–4

¹ Significantly different from N-I patient group ($P < 0.01$).

Fig. 1. Relationship between the number of intracellular *Candida* per 100 lung cells (ICC) and the number of yeast particles per cell, cultured for 30 min (*C per cell*). A, heavy mean values of the ICC engulfed by both AM and PMN together; B and C, mean values of the ICC engulfed by PMN and AM, respectively. In all cases, $\bar{x} \pm S.D.$

of these cells and AM (Table 2). The viability of the morphologically conserved cells (Table 1) was the same in the three groups; however, it must be noted that all the infected (CF and I) patients showed a high number of partially destroyed cells which were not included in the viability counting.

The phagocytic capacity of the total content of free cells in washing fluid was plotted against the inverse of the cell:yeast ratio, and the results are shown in Figure 1. Each point represents the heavy average of the number of cell-associated yeasts found in 100 phagocytic cells, namely AM and PMN. Recognition of the cell-associated *Candida* was easy, as shown in Figure 2.

Estimated values of phagocytic capacity overlapped in both infected (CF and I) patients, whereas all the N-I patients showed a lesser phagocytic capacity than the infected ones. This difference was not detected when the PMN were considered separately (Fig. 1B); in other words, the phagocytic capacity of PMN was not different for the three groups. However, the difference appeared again when the phagocytic capacity of the AM was studied in isolation (Fig. 1C). It can be noted that the differences were detected between infected (CF and I) and noninfected patients and only attributed to the AM.

Fig. 2. Phagocytic cells of the respiratory tract of CF children. A, two PMN which have engulfed yeasts of *C. albicans* (4 and 3 yeasts in left and right PMN's, respectively); B, alveolar macrophage with one intracellular yeast; C, binucleated giant cell with 3 engulfed yeasts (sedimented cells stained with Giemsa, $\times 1250$).

DISCUSSION

During the last years, the involvement of AM in the antibacterial defense of the lungs (12) has been recognized as one of the most important mechanisms. Because CF patients suffer from persistent lung infection, the study of the lung defense mechanisms in these children has become more important each day, and the investigation in this field related to AM becomes a necessity. In this context, different approaches have been worked out, although the interest of these authors arose primarily in the study of the influence of possible phagocytic inhibitory factors in CF serum (2, 16) or in the decrease of serum factors which normally promote the engulfment of particles by normal nonhuman AM (1). Other studies have been developed to study the mechanisms of intracellular degradation of mucus by AM in CF patients; these results have not been confirmed yet (3, 10).

The present work was directed towards the analysis of the phagocytic capacity of AM and PMN harvested by bronchopulmonary washing of CF children.

Both the CF and the I patients had mucus hypersecretion, which was not only seen macroscopically but detected through the recovery of the wash solutions; this hypersecretion was less in N-I patients than in the other two groups. Because the mucopurulent samples obtained were difficult to process, it was necessary to liquefy the suspensions with a mucolytic agent, whereas non-treated cells partially homogenized by shaking were preferred for functional studies.

As it has been described previously, both CF and I patients showed marked hypercellularity in lung-washing suspensions. This was due to PMN (13, 15). In view of the free cell content of airways, CF does not differ from other nontuberculous infectious states.

Because separation and purification of respiratory cells constitute a considerable technical problem, we decided to apply the method described by Lehrer (9) for evaluating simultaneously the nonspecific phagocytic capacity of AM and PMN.

When the total cell content was analyzed for phagocytic capacity, no significant differences between CF and I patients were found, and in both cases, the cells were more effective than those from N-I patients. This clear difference may be well understood in terms of macrophage activation, which was produced *in vivo* in the infected lung environment. When AM and PMN were investigated separately, however, it was seen that this increase in phagocytic capacity was associated with AM only. The mean values of phagocytic capacity tend to be lower for CF than I patients in almost all the assays performed; however, the differences were not significant, probably due to the dispersion of the data. The results, therefore, indicate that the behavior of CF-AM might not be substantially different from those of the AM found in the airways of patients with nontuberculous infection.

The AM is the only tissue macrophage readily available for study, and therefore, it has been the main source of the relatively limited information on hand on macrophage function in human tissues (8). The present study deals only with the phagocytic cells which can adhere in 3 hr and remain adherent during the assay procedure. Therefore, the results presented here should be carefully interpreted because the population of cells investigated may not be either the predominant one or representative of the *in vivo* whole organ.

Future studies regarding the evaluation of other CF-AM-specific mechanisms would provide further information related to the pathogenesis of the CF lung bacterial involvement.

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