Tr. J. of Medical Sciences
29 (1999) 461-465

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Neutrophil chemotaxis in acutely infected and clinically stable cystic fibrosis patients

Received: May 11,1998

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Abstract: Brochopulmonary infection in cystic fibrosis (CF) patients is associated with chronic progressive lung disease. The role of host factors, such as neutrophil functions, in the progressive pathologic processes in the respiratory tract of these patients, is poorly defined. Neutrophil chemotaxis is one of the first mechanisms of host defense to become activated after bacterial invasion has occurred. This study was aimed to evaluate the role of neutrophil chemotaxis in CF and also to determine whether an acute bacterial infection and the nutritional status can affect neutrophil chemotaxis or not. Twelve acutely infected and 12 clinically stable CF patients and 10 healthy age-matched controls were studied. Neutrophil chemotaxis and random migration were investigated in-vitro in the peripheral blood of the subjects by the Boyden-chamber method and the results were expressed as chemotactic index (CI). The nutritional status of the cases was evaluated as basal mass index (BMI). The CI values in the acutely infected group were found to be significantly lower than the clinically stable and the healthy control groups ($p<0.05$ and $p<0.05$, respectively). There was no significant difference between the clinically stable CF group and the healthy control group ($p>0.1$). No significant correlation was detected between the CI and BMI of the two groups of CF patients ($p>0.05$). The present study confirms that neutrophil chemotaxis and random migration are normal in clinically stable CF patients. The decreased CI in acutely infected patients indicates the possible role of infection itself on neutrophil chemotaxis.

Key Words: *Cystic fibrosis, neutrophils, chemotaxis*

Introduction

Cystic fibrosis (CF) is a genetically linked multisystem disease, accompanied by chronic bronchopulmonary infection and progressive lung injury. A variable period of chronic pulmonary infection and progressive lung injury is associated with considerable morbidity (1). Lung injury is mainly caused by a continuous and exuberant host inflammatory response to chronic endobronchial bacterial infection. Once bronchopulmonary infection is established, the single most consistently observed abnormality in CF lung defense is the inability to eradicate these organisms (2). In CF there is no convincing evidence to suggest a defect in host defenses although there are reports of individual cases of abnormality (3,4).

The combination of all the immunological abnormalities described in CF appear to have a final common pathway through effects on neutrophils (4). Neutrophils play an important role in host defense through their ability to remove pathogens and antigenic material from affected tissues. Chemokinetic properties of circulating phagocytic cells have been shown to be important in the host response to bacterial infection (5). The role of neutrophil functions in the progressive pathologic processes in the respiratory tract of CF patients is poorly defined and variable (6-10). Greater knowledge of the host response in CF patients would be of considerable advantage in the control of the inflammatory processes in the lung of these cases. This study was aimed primarily to evaluate the role of neutrophil chemotaxis in acutely infected and in clinically stable CF patients, and also to evaluate any possible role of the nutritional status of CF patients on neutrophil chemotaxis.

Material and Methods

Patients: Twelve CF patients with acute bacterial infection, 12 CF patients without evidence of infection (cilinically stable) and 10 healthy age-matched controls were included into the study. The acute bacterial infection

was diagnosed according to the presence of increased sputum production and purulence, increased cough or respiratory distress, fever, increased erythrocyte sedimentation rate, increased white blood cell count and changes in the chest X-ray. The clinically stable CF patients were the cases who applied to the hospital for routine monthly check-ups, without clinical and laboratory evidence of infection. Subjects under antibiotic or antiinflammatory therapy within the last 10 days were excluded from the study.

Basal mass index (BMI), which is an indicator of the nutritional status, was calculated and percentile values were determined for each patient, according to the standard BMI values (11) and these were correlated with the CI values in the study groups.

Microbiology: The bacteriological evaluation of the semiquantitative sputum cultures and smears from the acutely infected CF patients and throat cultures from the clinically stable CF patients was performed according to the standard microbiological techniques (12).

Chemotaxis assay: A modified Boyden-chamber method was used for the assessment of neutrophil (PMN) chemotaxis and random migration (13). Peripheral blood was collected in heparinized (10 U/ml) syringes from the subjects. Erythrocytes were allowed to settle for 1 hour at 37°C and the top leucocyte rich plasma was layered over 5 ml Ficoll-Hypaque (density=1077 g/ml, Sigma Chemical Co., St. Louis, MO, USA) and centrifuged at 400 g for 30 min. PMN cell pellet was then washed twice, the residual erytrocytes were lysed and the remaining PMNs were resuspended in RPMI-1640 (Sigma Chemical Co.). Cell count was adjusted to $5x10^6$ cells/ml. Viability of the PMNs was assessed by Trypan blue test. For the asessment of random migration and chemotaxis of PMNs the lower compartment of the Boyden-chambers was filled with RPMI-1640 and zymosan-activated serum (ZAS), respectively. The upper compartments were filled with PMN suspension. The upper and lower chambers were seperated by a nitrocellulose membrane with a mean pore diameter of 3 μm (Millipore Corp., USA). The chambers were incubated at 37°C in 5-10% CO_2 for 60 min. The membrane was then removed and stained with Harris haematoxylin stain. Estimation of migration was undertaken by the leading front technique (14). The results were expressed as chemotactic index (CI): motility with chemoattractant (ZAS)/random migration. Each assay was run in duplicate.

Statistical analysis: Mann-Whitney test analysis between groups was applied for the evaluation of statistical significance in terms of relation between chemotaxis and presence of infection. Sperman rank correlation test was used to determine the correlation between CI and BMI percentile of the cases.

Results

The mean age±s.e.m. of the patients in the clinicaly stable group was 5.36±1.20 years (range=1.14 years), in the acutely infected group was 4.48±1.12 years (2-17 years) and in the healthy control group was 6.40±1.54 years (3-10 years). The male to female ratio was 3/9 in the infected group, 8/4 in the stable group and 6/4 in the control group.

The distribution of the isolated bacteria in the acutely infected and the clinically stable CF patients is presented on Table 1. The isolation of bacteria in the clinically stable group was considered as "colonization" according to the clinical evaluation of the case and microscopic evaluation of the specimen. *Pseudomonas aeruginosa* was the most frequent bacterial pathogen isolated from the acutely infected cases (66.66%).

The chemotactic index of the circulating PMNs, expressed as mean CI±s.e.m., from the acutely infected CF patients (1.44±0.05) was found to be markedly decreased when compared to that of the clinically stable CF patients (1.83±0.12), or the age-matched controls (1.93±0.08), ($p<0.05$) (Fig. 1). The CI values of the clinically stable CF patients were not significantly different than the values of the healthy controls ($p>0.05$).

Random migration of the PMNs from the acutely infected group (38.33±2.09) was not different from that of the clinically stable CF patients (33.74±1.42) and the

Bacteria isolated	Acutely infected group (n)	Clinically stable group (n)
Pseudomonas aeruginosa	8	2
Staphylococcus aureus	3	3
Haemophilus influenzae	2	0
Klebsiella pneumoniae	1	0
Escherichia coli	0	1

Table 1. Distribution of bacteria isolated from the two groups of CF patients.

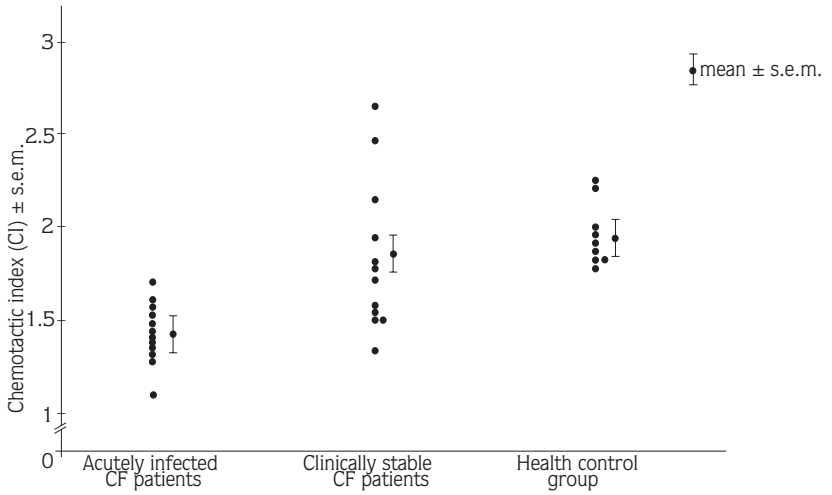

Figure 1. Mean chemotactic index±s.e.m. in acutely infected and clinically stable CF patients and in the healthy control group.

	BMI Percentiles (%)					
Study group	5	10	25	50	75	90
Acutely infected group (n=12)	2	2	1	4	-	3
Clinically stable group (n=12)	-	2	4	4	1	1

Table 2. Distribution of the BMI (Basal Mass Index) Percentiles of the Cases in Two Groups of the CF Patients.

healthy controls (34.72±2.88). No significant statistical difference was observed between the random migration values of the three groups (p>0.05).

The distribution of the BMI percentile values of the cases in the acutely infected and clinically stable groups was shown on Table 2. No significant correlation was detected between the CI and BMI percentiles of the cases in both groups (p>0.05).

There was no significant correlation between the CI and mean age (p>0.05) of the three groups (p>0.05).

Discussion

Most of the morbidity and mortality associated with CF is due to chronic progressive pulmonary disease. Several studies have suggested an association between the severity of the pulmonary disease of CF and measures of chronic inflammation, morbidity and mortality (2,4). The role of the immune fucntions that contribute to the increased incidence of infections in patients with CF has not been clearly defined yet. The relationship of the immune response to age, chronic malnutrition and the basic defect in CF is not established. The immunological abnormalities that have been described in patients with CF include decreased lymphocyte responsiveness, defects in opsonic activity and increased circulating immune complexes (6).

Chemotaxis of PMNs is an important event in the host defense mechanism against bacterial invasion (5). It has been previously shown that PMN chemotaxis is variably affected in CF. Church et al showed that PMN chemotaxis was normal both in clinically stable and in acutely infected CF patients (8,9). On the other hand Hill et al detected markedly increased PMN chemotaxis in CF patients with symptomatic pulmonary infection and also detected the return of leukotactic activity to normal levels as active pulmonary infection disappeared (10). Leucocyte locomotion was found to be significantly depressed in CF patients studied by Zielinski et al., but the relation of acute pulmonary infection with leukocyte locomotion was not pointed out clearly (7). Kurland et al., documented defective chemotaxis in an infant with CF and polymicrobial bacterial sepsis (15).

The present study confirms that neutrophil chemotaxis and random migration are normal in clinically stable CF patients. Thus a primary role of a congenital neutrophil chemotaxis defect does not appear to be likely as also indicated in some of the previous studies (8,10).

On the other hand in the acutely infected CF patients, though random migration values are not significantly different from the other two groups, the chemotaxis index values are different significantly, indicating the possible role of infection itself on neutrophil chemotaxis. It has been previously shown that neutrophil chemotaxis is decreased during the course of recurrent infections, such as lower respiratory tract infections (16,17). It is known that pulmonary infections in CF are associated with chronic colonisation by bacterial pathogens and episodes of acute debilitating exacerbations usually due to bacteria (1,4). Thus it can be speculated that chemotactic inhibitors may originate from the chronically colonised foci in the airways. This also raises the possibility that repeated infections since early infancy may have maintained a sufficiently large antigenic mass to result in immunologic hyporesponsiveness, such as depressed PMN chemotaxis. The clinical and microbiological follow-up of these acutely infected and chemotactically depressed patients showed that they have been chronicity of the infection may be a reason for depressed PMN chemotaxis seen in these patients. *P. aeruginosa* was the most frequently isolated bacteria in the acutely infected group. It is known that rhamnolipid, elastase and alkaline protease which are the products of *P. aeruginosa,* decrease neutrophil chemotaxis (13), and this fact may also explain decreased neutrophil chemotaxis in the acutely infected CF patients in our study, as 66.66% of the infections were due to *P. aeruginosa*.

It has been previously shown that malnutrition may result in alterations in immune functions, including polymorphonuclear leucocyte chemotaxis and killing (19). Most of the CF patients are known to be malnourished (2). Kurland et al reported a CF case with malnutrition and sepsis, with a transient defect in neutrophil chemotaxis, but were not able to clarify the role of malnutrition on neutrophil chemotaxis (15). When BMI values were evaluated in our patients, it was determined that no correlation existed between the BMI and CI values, indicating no significant effect of the nutritional status of the caes on neutrophil chemotaxis. In fact our CF patients did not have severe malnutrition, therefore it is not likely to propose that nutritional status has no effect on neutrophil chemotaxis in CF patients.

The previous studies on PMN chemotaxis in CF patients revealed variable results. Conflicting data on the degree of impairment of neutrophil locomotion in CF may be explained by differences between techniques used for assessing locomotion, or by the diverging states of disease activity, age of patients studied and presence and chronicity of infection.

It can be concluded that decreased neutrophil chemotaxis may be a consequence of recurrent respiratory infections and ineffective clearing of the offending endobronchial microorganisms, due to depressed neutrophil chemotaxis, may have a role in the pathogenesis and progression of pulmonary dysfunction in CF. Unregulated inflammation may interfere with normal airway anatomy and function, ultimately leading to tissue injury. These findings provide the basis for further studies on the role of the neutrophils in the inflammatory processes seen in CF.

Acknowledgement

We are grateful to Ergun Karaoğlu, Professor, PhD for statisticall analysis.

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