Distinct cytokine production by lung and blood neutrophils from children with cystic fibrosis

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Submitted 20 May 2002; accepted in final form 13 January 2003

Cystic fibrosis (CF) is one of the most frequent lethal autosomal hereditary disorders in Caucasian populations (10). It is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene, resulting in defective cAMP-dependent chloride ion conductance. In pa-tients with CF, lung disease is the major cause of mor-bidity and mortality (9, 16, 24). The progressive decline of pulmonary function is due to a vicious cycle of airway infection and inflammation. Indeed, there is now evidence that inflammation plays a pivotal role and may be present very early in life, even before the onset of respira-tory manifestations (2, 8, 11).

The inflammatory process in the CF lung is dominated by a polymorphonuclear neutrophil influx (7). Accumulation of neutrophils in the airways is associ-ated with high concentrations of neutrophil-derived mediators, in particular proinflammatory cytokines such as IL-8 and TNF-α (5). Neutrophils also release numerous toxic agents, e.g., proteases and reactive oxygen species, which contribute to the damage of lung tissue (4, 20, 32). If the consequences of the neutrophil-domi-nated inflammation in CF with an altered repair of the respiratory structures can be explained by an overwhelming neutrophil toxicity, the mechanisms leading to neutrophil accumulation and activation in the CF airways are poorly understood (34). Several possibilities can be discussed. The proinflammatory and anti-inflammatory imbalance with excessive concentra-tions of the neutrophil chemotactic cytokine IL-8 certainly plays an important role in the influx of neu-trrophils in the inflamed airways (14, 19). Locally, bac-terial toxins and inflammatory mediators can directly activate the neutrophils to carry out their cytotoxic activities. In addition, in CF patients, impaired neu-trrophil functions may contribute to an abnormal re-release of inflammatory mediators. Recently, Witko-Sarsat et al. (33) provided data suggesting that myeloperoxidase-dependent oxygenation activities are altered in blood neutrophils from CF heterozygotes and homozygotes.

Therefore, in the airways of children with CF, it is unclear whether the excessive presence of neutrophils is solely a consequence of an increased influx of these cells or whether it is associated with a cellular dysfunc-tion. To address this question we examined in the present study the capacity of neutrophils to release the major neutrophil pro- and anti-inflammatory cyto-

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kines, respectively, IL-8 and IL-1-receptor antagonist (ra) (23). We compared the production of these molecules by neutrophils isolated from the sputum and from the blood of children with CF. The capacity of neutrophils from CF children to release IL-8 and IL-1ra was also compared with cytokine production by blood neutrophils obtained from control subjects and by airway neutrophils obtained from children with chronic pulmonary disease related to dyskinetic cilia syndrome. In addition, we analyzed the response of airways and blood neutrophils to the anti-inflammatory action of dexamethasone.

MATERIALS AND METHODS

Study populations and samples. The CF population included 15 children, seven boys and eight girls (mean age: 12.6 ± 0.4 yr). In all patients, the diagnosis of CF was confirmed by sweat chloride concentration >60 meq/l (21, 25). Over a 3-mo period, all CF children who visited the outpatient department were invited to participate in the study. The criteria for eligibility were the ability to produce an adequate volume of sputum and the absence of pulmonary exacerbation at the time of the study. Results of physical examination, chest radiographs, and pulmonary function tests with determination of forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), oxygen saturation, and sputum quantitative bacterial cultures were recorded at the time of the study. The main characteristics of the CF patients are summarized in Table 1 with the results of genetic analysis, the clinical score determined by the Schwachman-Kulczycki score (60 ± 9), the results of the FVC and the FEV1 (57 ± 16% and 43 ± 18%, respectively), as well as the presence of Pseudomonas aeruginosa.

Cytokine production by blood neutrophils from CF patients was compared with control subjects, five healthy young adults (mean age: 30.8 ± 3.2 yr) from the medical staff without history of lung disease and with normal lung function. Indeed, for ethical reasons, it was not possible to obtain blood samples from healthy young children or children with dyskinetic cilia syndrome.

Again for ethical reasons, it was not possible to obtain sputum samples from healthy young children. As we had found in a preliminary study and in agreement with several reports in the literature that induced sputum from healthy subjects do not contain enough neutrophils for isolation and culture (data not shown), we collected the sputum from four children with chronic obstructive lung disease related to dyskinetic cilia syndrome. These four children, two boys and two girls (mean age: 11 ± 0.5 yr), belong to the population of children with dyskinetic cilia syndrome followed in our pulmonary pediatric center and recently described (28). As indicated previously, the diagnosis of dyskinetic cilia syndrome was confirmed by electron microscopy. All children with dyskinetic cilia syndrome who visited the outpatient department over a 3-mo period were invited to participate in the study. The criterion for eligibility was the absence of pulmonary exacerbation at the time of the study. Results of physical examination, chest radiographs, pulmonary function tests, and sputum quantitative bacterial cultures were recorded at the time of the study. In two children, bacterial analysis indicated the presence of Haemophilus influenzae. It is important to point out that the volume of sputum that could be collected from those children was very limited, and the number of neutrophils was reduced compared with the number obtained in the sputum of CF children.

Informed consent was obtained from each group, given either by the parents and the children or the subject in the control group. The ethics committees of St. Antoine University Hospital (Paris, France) approved this study.

Isolation of neutrophils in blood samples. Blood was drawn onto heparin (20 IU/ml) in both groups (12). Ten volumes of blood was mixed with two volumes of glucose dextran (3% glucose; 3% dextran T250; Sigma, Saint Quentin Fallavier, France). After sedimentation, the leukocytes were recovered and diluted 1:2 in RPMI 1640 medium and then layered on Ficoll-Paque (Amersham Pharmacia Biotech, Orsay, France). The ratio was two volumes of leukocytes to one volume of Ficoll-Paque. After centrifugation for 25 min at 15°C and 500 g, the cell pellet was washed and centrifuged once for 5 min at 300 g. Contaminating erythrocytes were lysed after a 5-min incubation of the resuspended cells at 4°C in 5 ml of lysis buffer (8.32 g/l NH4Cl, 0.84 g/l NaHCO3, 43.2 mg/l Na4EDTA). Lysis was stopped by the addition of a large excess of RPMI 1640 medium (Life Technology, Saint Cergy Pontoise, France), and the cells were washed and centrifuged for 10 min at 200 g. The neutrophils were then purified after incubation with pan anti-human human leukocyte antigen (HLA) class II-coated magnetic beads (Dynabeads M450; Dynal, Oslo, Norway) for 20 min at 4°C with gentle rotation.

Table 1. Main characteristics of children with CF

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<th>Patient</th>
<th>Sex</th>
<th>Age, yr</th>
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<th>FEV1, %</th>
<th>P. aeruginosa Colonization</th>
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CF, cystic fibrosis; clinical score, Schwachman-Kulczycki score; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; P. aeruginosa, Pseudomonas aeruginosa; NI, not identified; F, female; M, male.
to deplete monocytes, B cells, and activated T cells (17). Cells were counted, and viability was assessed by the trypan blue dye exclusion test. The purity of the neutrophil suspension was >99%, as assessed by staining with May-Grünwald-Giemsa.

Isolation of neutrophils in sputum samples. Each child with CF or with dysskinetic cilia syndrome was asked to rinse his or her mouth, swallow the water, and blow his or her nose to minimize contamination with saliva and postnasal drip. The sputum was then collected in sterile cups and processed immediately.

The sputum was transferred in a petri dish and weighed. As described by Pang et al. (15), an aliquot of 10 ml of trypsin-EDTA containing 0.05% trypsin and 0.50 mM EDTA (Sigma) was added to each gram of sputum. The mixture was shaken vigorously and then incubated at 37°C on a rotator for 30 min. The procedure was repeated after the addition of an equal volume of fresh trypsin-EDTA. The cell suspension was filtered through a 40-µm nylon gauze (Falcon) to remove debris and then centrifuged for 5 min at 300 g and 4°C. The cells were incubated 5 min with trypsin inhibitor (volume equal to the volume of trypsin-EDTA) and then washed three times with cold phosphate-buffered saline (PBS).

As described for blood samples, the neutrophils were then purified after incubation with pan anti-human HLA class II-coated magnetic beads. Cells were counted, and viability was assessed by the trypan blue dye exclusion test. The purity of the neutrophil suspension was >99%, as assessed by staining with May-Grünwald-Giemsa.

In the present work, blood and airway neutrophils were isolated by a procedure based on the method described by Regli et al. (17). This procedure, which includes an immunomagnetic depletion of cells other than neutrophils in sputum and blood samples, allows the collection of highly purified populations of neutrophils, with <1% of contaminating cells. The influence of this technique of isolation on the capacity of neutrophils to produce IL-8 was evaluated. Neutrophils were isolated from samples obtained from CF patients and control subjects by the dextran-Ficoll technique alone or dextran-Ficoll technique followed by immunomagnetic depletion. They were then cultured in medium without or with LPS, and the production of IL-8 was measured. In all samples tested, the use of immunomagnetic depletion was associated with a lower concentration of IL-8, confirming the elimination of contaminating cells. In the presence of LPS, the magnitude of increase of IL-8 secretion was similar in the two protocols, indicating that the functional response of neutrophils was not modified by the use of immunomagnetic beads (data not shown).

Also, trypsin-EDTA had no effect on IL-8 production by blood neutrophils (data not shown).

Neutrophil cultures. Neutrophils were cultured in RPMI 1640 medium supplemented with 1-glutamine and antibiotics (100 IU/ml penicillin, 100 µg/ml streptomycin; Life Technology) and 5% heat-inactivated normal human serum (a previously described using a monoclonal anti-human IL-8 antibody graciously provided by N. Vita (Sanofi Recherche, Labege, France) (13). The sensitivity of the ELISA was 3 pg/ml.

We set up an ELISA specific for IL-1ra using a monoclonal antibody (no. 84.1, prepared by Dr. J. C. Mazie) against a recombinant human IL-1ra (Synergon, Boulder, CO) and a rabbit polyclonal anti-human IL-1ra antiserum. Immunization of rabbits with recombinant human IL-1ra was performed with IL-1ra in complete Freund’s adjuvant, and two boosters at 3-wk intervals were performed in incomplete Freund’s adjuvant. Ammonium sulfate precipitation was performed on sera, and immunoglobulins were stored in 50% glycerol (20 mg/ml). Microtitration plates (96 wells, Maxisorp; Nuncion, Rockville, Denmark) were coated with 100 µl/well of mouse monoclonal anti-human IL-1ra (5 µg/ml in carbonate buffer, 0.05 M, pH 9.6) overnight at 4°C. After five cycles of washing with PBS-Tween 0.1% buffer, we carried out a protein blocking step using 100 µl/well of bovine serum albumin (BSA, 2% in carbonate buffer) for 60 min at 37°C. After additional washings, standards of recombinant human IL-1ra, diluted in PBS-0.1% Tween-1% BSA containing 10% RPMI 1640 medium were added in triplicate (100 µl/well). Cell supernatants of activated cell cultures in RPMI 1640 medium, tested 1:10 in PBS-Tween-BSA, were added in duplicate (100 µl/well). Incubation was performed for 2 h at 37°C. After being washed, 100 µl/well of rabbit polyclonal anti-human IL-1ra (diluted 1/375 in PBS-0.1% Tween-1% BSA) were added for 90 min at 37°C. After five cycles of washing, 100 µl/well of a peroxidase-labeled goat anti-rabbit immunoglobulin (Silenus-AMRAD Biotech, Victoria, Australia; 1/2,500 in PBS-0.1% Tween-1% BSA) were added. After 1 h of incubation at 37°C, plates were washed five times, and enzymatic activity was revealed by the addition of 100 µl/well ortho-phenylene-diamine substrate (1 mg/ml; Sigma) extemporaneously prepared in 0.05 M citrate buffer, pH 5, containing 0.06% hydrogen peroxide. Plates were kept in the dark, and the reaction was stopped by the addition of 50 µl/well 3 N HCl. Optical density at 490/630 nm was measured on microplate reader spectrophotometer (Dynex Technologies, Compiegne, France). The lower limit of sensitivity of the assay (blank ± 2 SD) was 40 pg. The coefficients of variation of intra-assay and interassay were 5.6 ± 3% and 13 ± 8.4%, respectively, for samples ranging from 40 pg/ml to 3 ng/ml. The ELISA was specific for IL-1ra and was negative for other cytokines such as IL-1α and IL-1β (used up to 50 ng/ml). IL-1ra measurements in samples were similar (r = 0.94) to those obtained with a commercially available kit (R&D Systems, Abingdon, UK).

Statistical analysis. Results are expressed as means ± SE. Statistical comparison was evaluated by analysis of variance followed by the Wilcoxon test. A P value <0.05 was considered significant.

RESULTS

IL-8 secretion by blood neutrophils. We first compared IL-8 production of blood neutrophils from control subjects and from CF patients. Results are shown in Fig. 1. In control subjects (Fig. 1A), the release of IL-8 by blood neutrophils was significantly increased in the presence of 1 µg/ml of LPS (P < 0.001). We then tested the inhibitory effect of dexamethasone. Spontaneous and LPS-induced IL-8 production levels were signifi-
cystic fibrosis (CF) patients. Blood neutrophils from control subjects (A) or children with CF (B) were cultured for 18 h (5 × 10⁶ cells/well) at 37°C in basal medium (control) in the presence of 1 μg/ml LPS (LPS), 10⁻⁶ M dexamethasone (Dex), or 1 μg/ml LPS and 10⁻⁶ M dexamethasone (LPS + Dex). IL-8 was measured in the supernatants by ELISA. Data are means ± SE. *P < 0.001 vs. control conditions; #P < 0.001 for results from CF patients vs. results from control subjects.

fig. 1. IL-8 secretion by blood neutrophils from control subjects and cystic fibrosis (CF) patients. Blood neutrophils from control subjects (A) or children with CF (B) were cultured for 18 h (5 × 10⁶ cells/well) at 37°C in basal medium (control) in the presence of 1 μg/ml LPS (LPS), 10⁻⁶ M dexamethasone (Dex), or 1 μg/ml LPS and 10⁻⁶ M dexamethasone (LPS + Dex). IL-8 was measured in the supernatants by ELISA. Data are means ± SE. *P < 0.001 vs. control conditions; #P < 0.001 for results from CF patients vs. results from control subjects.

significantly decreased in the presence of 10⁻⁶ M dexamethasone (P < 0.001).

In CF patients (Fig. 1B), the spontaneous release of IL-8 by circulating neutrophils was dramatically enhanced (P < 0.001) compared with blood neutrophils from control subjects. Nevertheless, the IL-8 secretion could still be significantly increased by the addition of LPS (P < 0.001). As observed for neutrophils from control subjects, dexamethasone (10⁻⁶ M) significantly decreased both the spontaneous and the LPS-induced IL-8 production (P < 0.001).

**IL-8 secretion by airway neutrophils.** Analysis of IL-8 production by neutrophils present in the airways of children with CF revealed a profile different from that documented in blood neutrophils (Fig. 2A). First, the amount of spontaneously released IL-8 was significantly higher than that from blood neutrophils from the same patients (+70%, P < 0.01). Second, addition of LPS failed to further enhance the secretion of IL-8. Third, dexamethasone, when used at 10⁻⁶ M, was unable to repress the production of IL-8 in the absence or presence of LPS. Notably, the levels of IL-8 production were similar in CF patients without or with *P. aeruginosa* colonization.

Interestingly, the profile of IL-8 production by neutrophils present in the airways of children with dyskinetic cilia appeared to be different from that of CF airway neutrophils and to mimic the pattern documented in experiments using blood neutrophils (Fig. 2B). The spontaneous release of IL-8 was significantly lower (−55%, P < 0.05). In addition, it was significantly increased in the presence of 1 μg/ml LPS (+30%, P < 0.05) and decreased in the presence of 10⁻⁶ M dexamethasone (−20%, P < 0.05).

Because dexamethasone at 10⁻⁶ M was unable to repress the production of IL-8 by sputum CF neutrophils, we tested whether it could be modulated by higher concentrations of dexamethasone. Neutrophils were cultured with increasing concentrations of dexamethasone ranging from 10⁻⁶ M to 10⁻³ M (Fig. 3). A significant inhibitory effect of dexamethasone on IL-8 secretion by sputum neutrophils could be observed only at the highest concentration employed, i.e., 10⁻³ M (P < 0.05).

To document whether the failure of dexamethasone to reduce IL-8 secretion by CF airway neutrophils might be due to its inability to act on cells that have been already activated, we performed the following experiments. Blood neutrophils from control subjects were activated by LPS, and dexamethasone (10⁻⁶ M) was added simultaneously or 3–6 h after LPS addition. As shown in Fig. 4, the delayed addition of dexamethasone to the LPS-activated neutrophils was associated with a reduced capacity of dexamethasone to decrease IL-8 secretion.

**IL-1ra secretion by blood neutrophils.** Results of IL-1ra secretion by blood neutrophils are shown in Fig. 5. In control subjects (Fig. 5A), the spontaneous IL-1ra release by blood neutrophils was 1,550 ± 250 pg/ml and could be enhanced by the addition of LPS (P < 0.05). Interestingly, dexamethasone failed to reduce both the spontaneous and the LPS-induced production of IL-1ra.

Opposite to the results with IL-8, the spontaneous IL-1ra secretion by blood neutrophils from CF patients
was significantly lower than that by neutrophils from control subjects (P < 0.001), as shown in Fig. 5B. The IL-1ra secretion by blood neutrophils from CF patients could be significantly enhanced by the addition of LPS (P < 0.001), whereas the presence of dexamethasone did not significantly influence either spontaneous or LPS-induced release of IL-1ra.

IL-1ra secretion by airway neutrophils. As observed for IL-8, IL-1ra secretion profile by neutrophils present in the airways of children with CF was different from that from blood neutrophils from the same patients (Fig. 6A). First, the amount of spontaneous IL-1ra was significantly higher than that from blood neutrophils (P < 0.01). Second, addition of LPS failed to further enhance this production. As observed for circulating neutrophils, dexamethasone had no effect on IL-1ra secretion by CF airway neutrophils.

Results of IL-1ra secretion by neutrophils present in the airways of children with dyskinetic cilia syndrome are shown in Fig. 6B. The spontaneous release of IL-1ra was significantly lower than that from CF airway neutrophils (P < 0.05). Treatment with 1 μg/ml LPS resulted in a 35% increase in IL-1ra secretion. As observed for blood neutrophils and CF airway neutrophils, dexamethasone had no effect on IL-1ra secretion by airway neutrophils from children with dyskinetic cilia syndrome.

**DISCUSSION**

An excessive inflammatory response is likely to play an important role in the pathogenesis of lung disease in CF (18, 29, 30). This is supported by several studies that have documented increased levels of proinflammatory mediators in the CF airways and decreased production of anti-inflammatory molecules such as IL-10 (14, 17). Furthermore, the observation of a dramatic accumulation of neutrophils in the CF lung strongly suggests a role of these cells in the perpetuation of an abnormal airway inflammation (34).

The present work is the first report comparing airway and blood neutrophils from children with CF in terms of pro- and anti-inflammatory cytokine production and their respective responsiveness to glucocorticoids. Comparison of airway and blood neutrophils from the same CF patients showed distinct profiles of cytokine production spontaneously and in the presence of LPS, as well as differences in the response to dexamethasone, supporting the view that the local environment may modify the functional properties of the cells. In addition, comparisons of cytokine production by circulating neutrophils from children with CF and controls and by airway neutrophils from children with CF or dyskinetic cilia syndrome revealed significant differences, suggesting that genetic components may also participate in the altered neutrophil function in CF.

Results reported herein indicate that in CF patients the responsiveness of neutrophils was different in the lung and in the systemic circulation. Indeed, we show that the amount of spontaneous release of IL-8 by airway neutrophils was significantly higher than that by blood neutrophils from the same patients and that addition of LPS failed to further enhance the secretion of IL-8. Increased production of IL-8 by airway neutrophils corroborated the elevated levels of IL-8 we have previously reported in the sputum of children with CF (14). For IL-1ra, similar results were observed: the level of IL-1ra produced spontaneously by lung neutrophils was significantly increased compared with the level secreted by circulating cells and was not modified by LPS. There is now cumulative evidence that neutrophils can display distinct functional capacities...
pending on their local environment. Pang et al. (15) analyzed the activity of neutrophils isolated from sputum and blood in bronchial sepsis. They documented higher production of IL-8, IL-1, and TNF-α by sputum neutrophils. They also showed that cytokine production by these cells is constitutive, with little increase in the presence of LPS. In addition, in contrast to blood neutrophils, anti-TNF-α antibodies did not inhibit IL-8 production by sputum cells. Together these data support the view that specific regulatory mechanisms of neutrophil activation may be present in the airways that differ from those involved in the systemic circulation. These mechanisms may vary, depending on the underlying disease (3). In CF, differences in blood and airway neutrophil behavior can be linked to the intense inflammation and infection of the respiratory tract. They can also be related to ionic composition of the airway surface fluid. Tager et al. (26) demonstrated that exposure of neutrophils to elevated chloride concentrations increases IL-8 synthesis and accelerates cell apoptosis and lysis.

Analysis of the effect of dexamethasone on IL-8 production confirmed the difference in the responsiveness of lung and blood neutrophils in CF patients. When used at a concentration effective in reducing IL-8 production by blood neutrophils (10⁻⁶ M), dexamethasone was unable to repress the secretion of IL-8 by airway neutrophils either in the absence or presence of LPS. An inhibitory effect of dexamethasone on these lung cells could be observed only at a very high concentration (10⁻³ M). These results share similarities with the data reported by Pang et al. (15). In their study, culture of sputum neutrophils from patients with chronic bronchial sepsis with various concentrations of the anti-inflammatory cytokine IL-10 showed no significant effect on IL-8 production, whereas a major reduction of IL-8 secretion was observed with blood neutrophils under the same experimental conditions. The resistance of IL-8 secretion by airway neutrophils provides additional evidence that the mechanisms, which regulate neutrophil cytokine production within the inflamed lung, differ from those controlling blood neutrophil response. This conclusion is of importance for the development of anti-inflammatory therapies in CF (29, 35). Clearly, evaluation of the efficacy of drugs aimed at reducing lung inflammation should be performed on cells isolated from the respiratory compartment. Alternatively, the resistance of airway neutrophils to the effects of dexamethasone could be due to their precondition status as a reflection of their activation by either microbial products or inflammatory agents, independent of their compartmentalization. Indeed, the LPS-induced IL-8 production by circulating neutrophils from healthy controls was essentially inhibited by dexamethasone when added simultaneously with LPS. In contrast, the capacity of dexamethasone to decrease IL-8 secretion was dramatically reduced when dexamethasone was added after the activation of the cells with LPS. Accordingly, one can suggest that the altered inhibitory effect of dexamethasone on IL-8 release by airway neutrophils may be due to its inability to act on cells that are already activated.

The present study also showed that neutrophils from CF patients constitutively secrete higher amounts of IL-8 than those from control subjects. Although this difference in neutrophil behavior could be explained by a sustained in vivo exposure of CF cells to various inflammatory mediators, one cannot exclude a genetic component to altered cytokine production by neutrophils in CF (6, 16). In this view, Russell et al. (22) reported that neutrophils from CF patients displayed a decreased responsiveness with regard to L-selectin shedding. They also showed that this reduction in L-selectin responsiveness was not observed in non-CF bronchiectasis patients, supporting the hypothesis that the response of CF neutrophils differs from that of neutrophils from patients without a defective CFTR gene. The results on neutrophil myeloperoxidase-dependent oxygenation activities reported by Witko-Sarsat et al. (31) and on neutrophil elastase release by Taggart et al. (27) also suggest a relationship between altered neutrophil functions and CFTR mutations.

Data reported herein showing significant differences in cytokine production by airway neutrophils from children with two distinct causes of chronic obstructive lung disease, CF and dyskinetic cilia syndrome, provide additional support for a role of genetic component in the altered neutrophil function in CF.

Our results on IL-1ra provide additional evidence for a dysregulation of the inflammatory response in CF. IL-1ra is a major anti-inflammatory cytokine that functions as a specific inhibitor of the two other functional members of the IL-1 family, IL-1α and IL-1β (1). In the present work, the spontaneous IL-1ra secretion by blood neutrophils from CF patients was significantly lower than that by neutrophils from control subjects. The striking point is that the same blood CF neutrophils produced significantly higher concentrations of the proinflammatory IL-8 than control cells, supporting the concept of an altered regulation of neutrophil cytokine production in CF patients. In the airways, CF neutrophils secreted higher amounts of IL-1ra than blood neutrophils. However, from a consideration of the intensity of CF lung inflammation and the altered production of IL-10 reported in several studies, it is likely that the local production of IL-1ra is largely insufficient to control the inflammatory activities. Indeed, the current understanding of the control of the inflammatory response is that the anti-inflammatory agents should be present in far greater concentrations than those of proinflammatory cytokines to inhibit their actions.

To conclude, data reported in the present work provide additional evidence that the persistent and excessive inflammation in the lungs of CF patients involves a failure of the mechanisms that control the inflammatory response. An altered regulation of cytokine production by neutrophils is certainly an important factor that promotes continued inflammation and injury. Development of therapeutic interventions with specific cytokine inhibitors, anti-inflammatory cytokines, as
REFERENCES


