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Increased Monocyte Chemiluminescence in Cystic Fibrosis Patients and in their Parents

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ABSTRACT. We examined the chemiluminescence response of peripheral blood monocytes from patients with cystic fibrosis (CF) and their asymptomatic parental carriers of the CF gene to three different types of stimulation. We found that monocytes from both patients and carriers have increased luminol-dependent chemiluminescence in the first 25 min after stimulation by adherence to glass. These results are consistent with the hypothesis that monocytes from both CF heterozygotes and homozygotes respond to adhesion with increased oxygen radical formation. The increased adherence-induced monocyte chemiluminescence of the parental carriers did not vary with age or length of exposure of the parents to a child with CF. Also, repeated exposure to medications and respiratory secretions of CF patients was not associated with an increase in adherence–induced monocyte chemiluminescence of their nonbiologically related caretakers. Thus, this observed increase in chemiluminescence is not simply secondary to the medications or respiratory dysfunction seen in the patients with CF. Patients with other types of obstructive lung disease did not show increased adherence-induced monocyte chemiluminescence. We conclude that increased early phase adherence-induced monocyte chemiluminescence occurs in patients with cystic fibrosis and the obligate carriers of the CF gene independent of environmental influences. (*Pediatr Res* 20: 619–622, 1986)

Abbreviations

CF, cystic fibrosis
PMA, phorbol myristate acetate
FVC, forced vital capacity
FEV_1, forced expiratory volume at 1s
CPM, counts per min
CL, chemiluminescence

CF is the most common lethal autosomal recessive disease trait found in the Caucasian population, occurring in approximately one of every 2000 live births. The carrier frequency has been estimated to be as high as 5% (1). In the CF homozygote, the disease is characterized by chronic pulmonary disease accompanied by bacterial infection, pancreatic insufficiency associated with malabsorption and malnutrition, and an elevated sweat chloride level. CF heterozygotes appear to be clinically normal. Although long sought, neither the basic biochemical abnormality nor the gene product of CF are known (2–5).

Increased oxygen uptake and mitochondrial electron transport activity have been reported in the skin fibroblasts of both CF homozygotes and heterozygotes (6). The current study was designed to examine whether peripheral blood monocytes, whose oxygen metabolism during phagocytosis involves both mitochondrial and nonmitochondrial pathways (7), would show differences among CF patients, their parents, and controls in oxygen radical production. As a sensitive indicator of oxygen radical production we studied the amount of luminol-amplified CL (8–11) generated when the purified peripheral blood monocytes of CF patients, carriers, and their age-sex matched controls were stimulated by opsonized zymosan, PMA, or by adherence to the sides of glass liquid scintillation vials (12).

METHODS

Human subjects. After informed consent, four groups of individuals were studied. Each individual was compared with a simultaneously examined age-sex matched control selected from the normal population. This study was approved by the Human Subjects Committee at the University of Minnesota.

Group one consisted of 15 CF patients between the ages of 2 and 40. All had elevated sweat chloride levels by the method of Gibson and Cooke (13). All had had evidence of pancreatic insufficiency. Most were regularly receiving bronchodilators, pancreatic enzyme replacement, and antibiotic therapies. All patients had their pancreatic insufficiency controlled at the time of study. FVC varied from 38 to 124% predicted (median = 67%), FEV_1 from 23 to 114% predicted (median = 39%) FEV_1/FVC from 0.38 to 0.87 (median = 0.61) and Brasfield scores (14) from 1 to 19 (median score = 12) at the time of study.

Group two was comprised of 21 parents of CF patients, *i.e.* obligate CF carriers. Nine of these were parents of CF patients who were diagnosed within 1 wk prior to testing. Thus, these individuals were obligate CF carriers who had less exposure to environmental factors associated with living with and caring for a patient with CF such as medications and bacterial flora common in the sputum produced by older patients with CF. These factors could conceivably alter the parents' monocyte CL.

Group three consisted of 11 people who routinely cared for CF patients but were biologically unrelated. Included were two respiratory therapists, four spouses of CF patients, one private day care attendant, and six foster or adoptive parents. These individuals were routinely exposed to CF environmental factors but were not known CF carriers.

To determine whether obstructive lung disease and the use of bronchodialators and antibiotics by these patients could alter CL, a fourth group of 13 individuals without evidence of CF but with obstructive pulmonary disease were studied. Eight had chronic obstructive pulmonary disease with $FEV_1/FVC < 0.80$

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and chronic cough. Five had asthma with obstructive pulmonary disease significantly reversible by β-agonists.

Individuals with evidence of acute or chronic bacterial infection were excluded from groups two, three, and the control group. They were not excluded from group four. Individuals who had symptoms of acute viral infection or who were immunodeficient were excluded from the study.

Monocyte preparation. Monocytes were purified using preconditioned plastic flasks as previously described (15, 16). The resulting cells were at least 95% monocytes by nonspecific esterase staining and greater than 90% viable by the trypan blue exclusion method.

Fifty-one of the 67 adherence assays were performed without the technician knowing whether samples were from experimental or control individuals. Before identifying the donors, data pairs were rejected if the raw CPM for any given vial was less than two times the background levels, or if their coefficient of variation from duplicates exceeded 30%.

Chemiluminescence assays. To measure zymosan-induced CL, 2×10^4 monocytes suspended in 1 ml HBSS with 0.1% gelatin (w/v) were added to duplicate scintillation vials containing zymosan, preopsonized in pooled human serum for 10′ at 37° C and 1.3 μM luminol in 4.5 ml HBSS. Vials were counted in a Beckman LS100 scintillation counter using an open window and one photomultiplier tube set out of coincidence. They were counted approximately every 4.1 min until after the peak in light flux.

PMA-induced CL was measured in the same manner as zymosan–induced CL, but 2.2 μg PMA in place of zymosan was added to vials containing 4.5 ml HBSS with 1.3 mM luminol.

To measure adherence-induced CL, 2.5×10^5 monocytes in 1 ml HBSS were added to vials containing 1.3 μM luminol in 4.5 ml HBSS. Because the presence of protein can inhibit leukocyte adherence to glass (12), no gelatin was present in the reaction mixture. All vials had been dark-adapted, background CL levels had been recorded, and all monocytes were added in the dark. Time 0 was the time of addition of monocytes. Vials were counted approximately every 4.1 minutes in a Beckman LS100 liquid scintillation counter using an open window and one photomultiplier tube set out of coincidence. The vials were removed from the scintillation counter after 25 min, rinsed once gently with HBSS, and the adherent cells counted by releasing the nuclei with Zap-o-globin II (Coulter Diagnostics). Results are expressed as peak scintillation CPM per adherent cell; the average of the duplicates is reported.

RESULTS

When opsonized zymosan was used as a stimulant, monocytes from 11 CF patients exhibited similar CL to their matched controls [mean difference in CPM per cell ± 1 SEM = 3.3 ± 1.6, $p_\alpha(t) = 0.065$]. Zymosan-induced CL from nine CF carriers also was not significantly different from controls [mean difference in CPM per cell ± 1 SEM = −0.19 ± 0.87, $p_\alpha(t) \geq 0.30$].

PMA-induced CL was slightly lower in monocytes from 11 CF patients than controls [mean difference in CPM per cell ± 1 SEM = −2.03 ± 0.65, $p_\alpha(t) = 0.01$]. PMA-induced CL from nine CF carriers showed no significant difference when compared with controls [mean difference in CPM per adherent cell ± 1 SEM = 0.87 ± 1.10, $p_\alpha(t) > 0.30$].

When allowed to adhere to glass liquid scintillation vials (Fig. 1), monocytes from the 11 CF patients and 21 CF carriers, generated significantly higher early CL than their respective matched controls (Fig. 2) [mean difference from control in peak CPM per adherent cell ± 1 SEM: CF patients *versus* controls 2.70 ± 0.51, $p_\alpha(t) < 0.001$; CF carriers *versus* controls 1.69 ± 0.40, $p_\alpha(t) < 0.001$; Fig. 3]. The kinetics of the CL response during the first 25 min was similar in all groups. There was no significant difference in number of monocytes adhering at 25′ between CF and control pairs [mean difference = -1.22×10^4 cells, SEM = 1.2×10^4, $n = 15$, $P_\alpha(t) = 0.33$]. Nor was there a significant difference in adherent CF carrier monocytes at 25′ compared to control [mean difference = -0.73×10^4 cells, SEM = 0.37×10^4, $n = 21$, $P_\alpha(t) = 0.06$]. To control for environmental influences, nine of these carriers who were parents of newly diagnosed patients were separately analyzed and they too generated significantly higher CL [mean difference from control in peak CPM/cell ± 1 SEM = 0.78 ± 0.33, $p_\alpha(t) = 0.045$]. Moreover, when all carriers were analyzed there was no association between the difference in CPM from control and the length of time they were exposed to a CF patient.

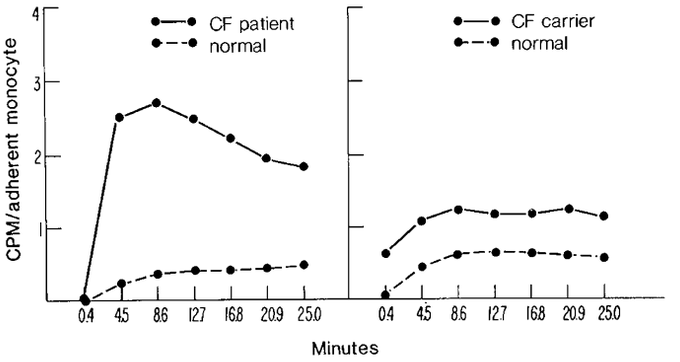

Fig. 1. Adherence-induced CL: a single experiment. Normal controls were age- and sex-matched to the CF patient and carrier. Glass adherence produced an early plateau or initial small peak occurring before 25 min, followed by a gradual increase in light production per adherent monocyte. Discrimination between CF patients, carriers, and controls was obtained by comparing the initial peaks or the highest rate of light production within the first 25 min using a two-tailed paired *t* test.

Finally, monocytes from caretakers of CF patients who were not biologically related to them (group 3) showed no difference from matched controls in their adherence-induced CL [mean difference in peak CPM per adherent cell ± 1 SEM = 0.02 ± 0.39, $p_\alpha(t) > 0.30$, Fig. 3]. Similarly the presence of obstructive pulmonary disease in patients who did not have cystic fibrosis (group 4) did not increase the adherence-induced CL over matched control values [mean difference in peak CPM per adherent cell ± 1 SEM = 0.26 ± 0.28, $p_\alpha(t) > 0.30$, Fig. 3]. Interestingly, the five patients with asthma had a small but significantly decreased adherence-induced CL compared to their matched controls [mean difference in peak CPM per adherent cell ± 1 SEM = −0.40 ± 0.12 $p_\alpha(t) = 0.03$] and none had CL above that of his matched control. In both of these groups the probability (β) of not detecting a difference as large as that seen between CF carriers and controls was ≤10%.

DISCUSSION

During phagocytosis, peripheral blood monocyte oxygen consumption increases dramatically (7, 17). This oxygen is rapidly converted into superoxide anion, hydrogen peroxide, and hydroxyl radical via one and two electron transfers to oxygen, with concurrent oxidation of NADH or NADPH (16). These oxygen products can be measured with high efficiency by luminol–dependent chemiluminescence assays (8–10, 18). Luminol, however, will emit detectable photons whenever it is oxidatively dioxygenated (19). Thus, oxidants other than oxygen radicals, per se, might be responsible for the effects we observed. For example, peroxidases present in freshly isolated monocytes will likely produce HOCl from H_2O_2 and Cl^-. The combination of HOCl and H_2O_2 significantly increases the Cl from luminol over that with H_2O_2 generating systems alone. Moreover, OH^-, O_2^-, and $^1O_2$ do not seem critical to the production of luminol CL by HOCl with H_2O_2 (20). The major alternative source to oxidase generated oxygen radicals and peroxidase products would be

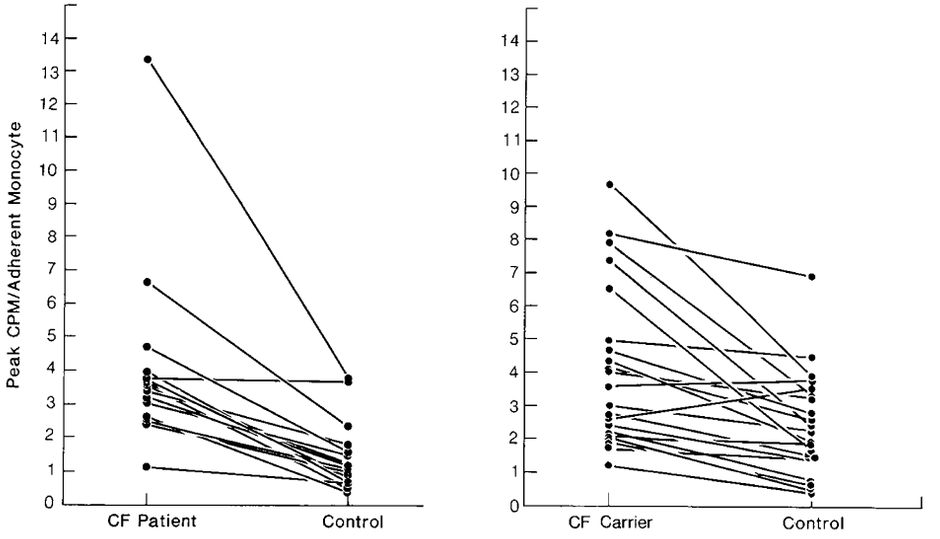

Fig. 2. Adherence-induced CL: each *point* represents the peak CPM/adherent monocyte for each CF patient connected to his/her age- and sex-matched control's value. Values are based on duplicate determinations after background CL was subtracted. Similarly, each parental carrier's peak CPM/adherent monocyte is connected to his/her age- and sex-matched control's value. For statistical analysis of the differences see Figure 3.

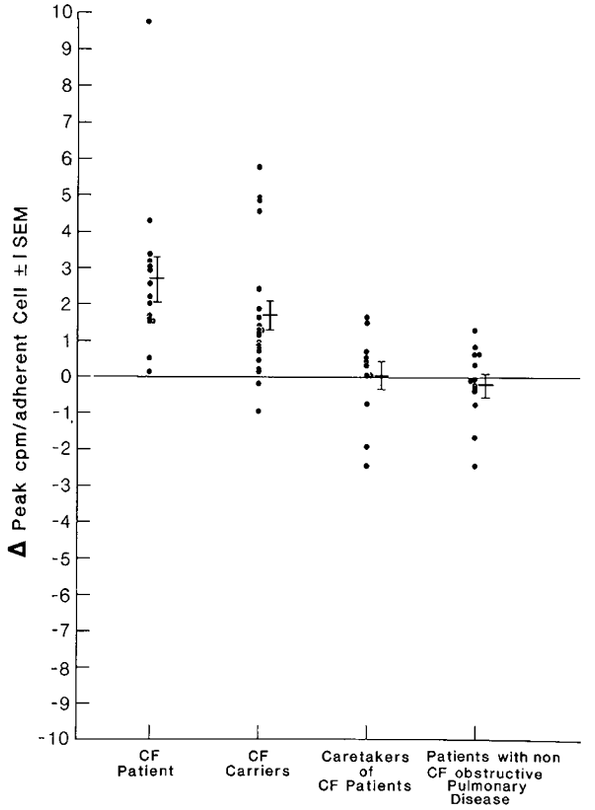

Fig. 3. Adherence-induced CL: each *point* is the difference between an individual's initial peak CPM/adherent monocyte in an experimental group and that of his age- and sex-matched control. Values are based on duplicate determinations and after background CL was subtracted. CF patients had a mean difference from control in peak CL/cell ± 1 SEM of 2.70 ± 0.57 [$p_\alpha(t) < 0.001$]; CF carriers of 1.69 ± 0.40 [$p_\alpha(t) < 0.002$]. Caretakers of CF patients who were not biologically related and non-CF patients with obstructive pulmonary disease showed mean differences from matched controls 0.02 ± 0.39 and −0.20 ± 0.26, neither of which differ significantly from 0. The mean of the differences in the carriers differed significantly from that of the caretakers [$p_\alpha(t) < 0.001$] and from that of patients with non-CF obstructive pulmonary disease [$p_\alpha(t) < 0.001$].

products of arachidonic acid cyclooxygenase and endoperoxidation. This pathway has been shown to be active in CL generated by platelets on exposure to exogenous fatty acids (21). However, the efficiency of the interaction of these products with luminol to produce CL has not been defined and no exogenous fatty acids were added in our system. Our results therefore suggest that during the first 25 min after adherence to glass liquid scintillation vials, monocytes from CF patients and CF carriers either generated increased amounts of oxygen radicals when compared with normals or secreted more enzyme(s) such as peroxidase that would catalyze the generation of oxygen radicals. However, monocytes from CF carriers and normals generated essentially equivalent amounts of these putative oxygen radicals when stimulated with opsonized zymosan or PMA. This difference among differing stimuli could relate to differences in membrane-surface interaction, membrane receptor-surface interaction, ionic flux, second messenger stimulation, compartmentalization of the oxidases involved in the oxygen radical formation (22), or differences in the oxidase pathways. Further investigation by use of other stimuli, alternative luminogenic probes, and metabolic inhibitors is currently underway.

It should be emphasized that the CL difference between CF patients and controls and between CF carriers and controls were produced by monocytes that had been purified by adhering to preconditioned plastic flasks. It is possible that this step selected a particular monocyte subset and/or activated the surface membrane of these monocytes permitting the detection of these differences from control. However, since there was no difference in the application of this purification technique to any of the experimental or control groups, the technique itself cannot explain the differences in CL we have observed.

Our finding of increased CL of CF and CF carrier phagocytic cells is consistent with the finding that CF and CF carrier fibroblasts show increased O_2 uptake and a variant NADH dehydrogenase (6), but we have no direct evidence at this time that this particular enzyme system is involved in the phenomenon we have observed. The significance of this early increase in presumed oxygen radical formation and/or release for host defense against microbes or for oxidative damage to host tissues remains to be defined.

The fact that the phenotypically normal parents of patients with CF showed an increase in adherence-induced CL and that the differences from control did not vary with age or length of exposure of a carrier to a child with CF argue strongly that this

phenomenon is not simply secondary to either the medications or the respiratory dysfunction seen in the patients with CF; the fact that patients with other types of obstructive lung disease did not show an increase also indicates that it may be unique to CF. Moreover, repeated exposure to medications and respiratory secretions of CF patients was not associated with an increase in adherenced-induced CL in monocyte samples from their non-biologically related caretakers nor did the biological parents of patients with CF show increasing adherence CL with increasing exposure time to the drugs and therapies prescribed for their children. We conclude that increased early phase adherence-induced monocyte CL occurs in patients with CF and the obligate parental carriers of the CF gene independent of environmental influences.

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