Individuals with cystic fibrosis do not display impaired endothelial function or evidence of oxidative damage in endothelial cells exposed to serum

Lawrence T. McGrath*, Damien McCall*, Colm G. Hanratty*, Siobhan Brennan†, Adrian Devine*, Daniel F. McCauley*, Bernard Silke* and Stuart Elborn‡

*Department of Therapeutics and Pharmacology, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, U.K., †Department of Geriatric Medicine, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, U.K., and ‡Adult Cystic Fibrosis Unit, Belfast City Hospital, Lisburn Road, Belfast BT9 7AB, Northern Ireland, U.K.

ABSTRACT

Heightened systemic oxidative stress is increasingly recognized as a feature of cystic fibrosis (CF). The consequences of long-term exposure to free radical attack include a predisposition to diseases such as cancer and atherosclerosis. An increased incidence of malignancy among adult patients with CF has been reported, but the absence of atherosclerotic disease is well described. The aim of the present study was to assess endothelial function in vivo and relate this to the potential of serum from patients with CF to induce oxidative-mediated damage in cultured human endothelial cells. A group of 11 CF patients was matched with a group of healthy volunteers with regard to age and sex. Endothelial function was assessed as endothelium-dependent and -independent vasodilation by measuring forearm blood flow in response to infused acetylcholine and sodium nitroprusside respectively. Confluent monolayers of cultured human endothelial cells were exposed to serum from CF patients and control subjects. Following exposure, cell death was assessed by lactate dehydrogenase release, and the degree of lipid peroxidation in the membrane was assessed by measuring the content of lipid hydroperoxides, malondialdehyde and 4-hydroxynonenal. Endothelial monolayers exposed to serum from CF patients released significantly less lactate dehydrogenase following exposure than those exposed to serum from healthy controls (1.8% and 3.0% respectively; mean difference −1.2%; 95% confidence intervals −1.9% to −0.1%; P < 0.05) and contained significantly less 4-hydroxynonenal (0.75 and 3.41 μmol/g of protein respectively; mean difference −2.66 μmol/g; 95% confidence intervals −5.10 to −0.22 μmol/g; P < 0.05). There was no significant difference between patients and controls in the extent of serum-induced membrane peroxidation, as assessed by malondialdehyde or lipid hydroperoxides, or in endothelial function, as assessed by forearm blood flow. In conclusion, despite evidence for heightened systemic oxidative stress in CF, patients displayed no impairment of endothelial function, and their serum caused significantly less damage to human endothelial cells than that from matched controls.

Key words: atherosclerosis, cystic fibrosis, oxidative stress.

Abbreviations: CF, cystic fibrosis; CI, confidence intervals; EBM, endothelial cell basal medium; EGM, endothelial cell growth medium; FBF, forearm blood flow; 4-HNE, 4-hydroxynonenal; LD, lactate dehydrogenase; MDA, malondialdehyde; NOS, nitric oxide synthase; VEGF, vascular endothelial growth factor.

Correspondence: Dr L. T. McGrath (e-mail l.mcgrath@qub.ac.uk).
INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disorder affecting multiple organ systems and resulting in premature death. The condition is characterized by abnormalities in cAMP-dependent chloride ion movement across apical epithelial membranes [1]. A consistent feature of CF is the chronic inflammatory/immune response to airway sepsis, which results in elevated levels of pro-inflammatory cytokines, with subsequent ‘spillover’ into the circulation [2]. Elevated breath levels of pentane and ethane, breakdown products of lipid hydroperoxides, support the presence of an increased oxidative burden within the airways [3]. Heightened levels of oxidative stress in CF are not limited to the respiratory system alone. As with the pulmonary inflammatory response, there is likely to be a spillover of reactive oxygen species into the circulation. In addition, potential intracellular sources of free radicals have also been identified generally. These are mainly characterized by leakage of mitochondrial electrons into the cytosol, with consequent formation of superoxide anion [4,5].

Decreased antioxidant defences brought about by pancreatic malfunction and deficiencies in selenium-dependent antioxidant enzymes exacerbate this pro-oxidant balance in CF [6,7]. The effects (if any) of systemic oxidative stress in CF have received relatively little attention. Neglia et al. [8] reported the likelihood of an elevated risk of malignancy in adult CF patients, a prediction limited by the small proportion of sufferers aged over 25 years. However, as intensive therapeutic regimens continue to improve survival in the condition, it is estimated that the life expectancy of a newborn with CF is currently of the order of 40 years. Any elevated risk of cancer may, therefore, soon become more apparent, affecting multiple organ systems and resulting in premature death. The condition is characterized by abnormalities in cAMP-dependent chloride ion movement across apical epithelial membranes [1].

Atherosclerosis is increasingly recognized as an inflammatory response to endothelial injury [10]. The association of established cardiovascular risk factors with increased circulating free radical activity suggests that oxidative stress is an important mediator of endothelial injury in vivo. In this context, the absence of early vessel disease (in the form of fatty streaks) from the aortas of CF patients examined at post mortem is unexpected [11,12]. This reduced incidence in CF cannot be explained by a limited life expectancy in this condition, since an extensive post-mortem study in 15–19-year-old accident victims reported intimal lesions in all (608) aortas examined [13]. It would appear that, paradoxically, the endothelial lining of individuals with CF is protected from damage and dysfunction. The purpose of the present study was to assess endothelial function in patients with CF and to relate this to the ability of their serum to induce oxidative damage in cultured human endothelial cells.

METHODS

Subjects
A total of 11 patients with CF (eight men) and 11 healthy age-matched controls (eight men) were recruited from the Adult Cystic Fibrosis Unit, Belfast City Hospital, and from among staff and students of the Department of Therapeutics and Pharmacology, QUB. Patients with CF had a prior diagnosis by genotype and/or sweat testing (sweat chloride concentration > 70 mmol/l). All patients were receiving vitamin E supplementation (200 mg per day). None of the patients or controls was receiving any other antioxidants, or smoked. All patients were well and were not subject to acute respiratory exacerbation. Control subjects had no personal or immediate family history of CF and no evidence of illness at the start of the study. The study was approved by the local ethics committee, and conformed with the principles outlined in the Declaration of Helsinki (1989) of the World Medical Association. The aims and details of the study were provided to all subjects, and written informed consent was obtained.

Sample collection and treatment
Samples of 20 ml of blood were collected from subjects, and divided between a glass tube without any anticoagulant, a heparinized tube and a tube containing EDTA. Samples were gently mixed, packed in ice and prepared immediately. All samples were maintained at 4°C throughout preparation. Samples were spun at 3000 g for 5 min at 4°C. Heparinized plasma was set aside for analysis of reduced protein thiols and protein carbonyls. Plasma containing EDTA was set aside for analysis of malondialdehyde (MDA) and vitamin E. Aliquots of 50 µl of the heparinized plasma for analysis of carbonyls and 500 µl of EDTA-containing plasma for analysis of MDA were mixed with 50 µl of 0.2% (w/v) ethanolic butylated hydroxytoluene as antioxidant. Serum was removed from the clot and filtered through an Acrodisc® 0.2 µm filter into a sterile 2 ml vial. All of these samples were stored at −80°C and analysed within 4 weeks.

Chemicals
All chemicals, unless otherwise stated, were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Merck diagnostic cholesterol colour reagent was purchased from Merck Ltd. (Lutterworth, Leics., U.K.). Hepes-buffered saline solution, 0.025% trypsin/0.01% EDTA, trypsin-neutralizing solution and material for
preparation of growth medium were purchased from BioWhittaker (Walkersville, MD, U.S.A.)

Materials for cell culture
Human aortic endothelial cells (BioWhittaker) were received cryopreserved. Growth medium was purchased as endothelial cell growth medium-2 (EGM*-2) Bulletkits®. Each kit contained a 500 ml bottle of endothelial cell basal medium-2 (EBM*-2) and EGM*-2 SingleQuots® containing hydrocortisone, human re-combinant fibroblast growth factor, vascular endothelial growth factor (VEGF), insulin-like growth factor-1, ascorbic acid, heparin, fetal bovine serum and human epidermal growth factor. EGM was prepared by adding the Singlequots to EBM.

Preparation and exposure of human aortic endothelial cell monolayers
Human aortic endothelial cells, on receipt, were taken out of cryopreservation, grown up in bulk, divided into portions in EBM containing DMSO (10%, v/v) and fetal bovine serum (20%, v/v), and stored at the third subcultivation in liquid nitrogen (500000 cells/ml). For the preparation of monolayers for addition of serum, each CF patient and a matched control, third-subcultivation human aortic endothelial cells were seeded into 10 ml of EGM in four 25 cm² flasks at a density of 4000 cells/cm², and incubated at 37 °C in 95% air/5% CO₂. Culture medium was changed at 24 h. The cells were examined microscopically, and when greater than 90% confluent were further subcultivated between twelve 25 cm² flasks and used for the study.

For each experiment, each set of cells (exposed to serum from one patient or one control, or unexposed) was studied in triplicate, with one flask used to calculate total release of lactate dehydrogenase (LD) (see below). EGM was removed from all flasks and replaced with 5.0 ml of 10% (v/v) serum from a CF patient or a matched control in EBM, or EBM alone for unexposed cells. Flasks were incubated at 37 °C in 95% air/5% CO₂. After 1 h the diluted serum/EBM was removed and retained. Flasks were washed twice with pre-warmed EBM, 5 ml of fresh EBM was added and the incubation was continued for a further 6 h, after which EBM was removed and retained. Each flask was rinsed twice with ice-cold PBS and a further 2 ml of PBS was added. Cells were removed by scraping, centrifuged at 1000 g for 10 min at 4 °C, and the pellets were stored at −80 °C until analysis.

Analysis of LD release
LD activity was measured using a commercial kit based upon the oxidation of lactate, with measurement of the rate of increase in absorbance at 340 nm. One flask each for unexposed cells or cells exposed to patient or control serum was washed with PBS and the cells were lysed with 1.0 ml of 0.5% (v/v) Triton X-100 in PBS. This was used to calculate the total LD content of cells. LD activity was measured in EBM and in diluted patient/control serum before and after exposure of cells for 1 h. LD activity was also measured in EBM from flasks after the 6 h exposure. This was then expressed as percentage of total LD released.

Analysis of markers of lipid peroxidation and vitamin E
Pellets were allowed to thaw at room temperature for 5 min, then 300 µl of 0.1% Triton X-100 (peroxide- and carbonyl-free) in PBS was added to each pellet. The pellet, in Triton X-100, was sonicated at 30% of full power for 2 × 10 s bursts, with a period of 15 s of cooling on ice between bursts. MDA and 4-hydroxynonenal (4-HNE) were measured in dissociated pellet and plasma using a specific spectrophotometric assay (Calbiochem-Novabiochem, Nottingham, U.K.). Membrane lipid hydroperoxides were measured using a colorimetric assay [14]. Total plasma thiols and protein carbonyls were assayed spectrophotometrically [3,15]. Vitamin E was measured using an HPLC method [16]. Cholesterol was measured enzymically [17].

For each experiment, each set of cells (exposed to serum from one patient or one control, or unexposed) was studied in triplicate, with one flask used to calculate total release of lactate dehydrogenase (LD) (see below). EGM was removed from all flasks and replaced with 5.0 ml of 10% (v/v) serum from a CF patient or a matched control in EBM, or EBM alone for unexposed cells. Flasks were incubated at 37 °C in 95% air/5% CO₂. After 1 h the diluted serum/EBM was removed and retained. Flasks were washed twice with pre-warmed EBM, 5 ml of fresh EBM was added and the incubation was continued for a further 6 h, after which EBM was removed and retained. Each flask was rinsed twice with ice-cold PBS and a further 2 ml of PBS was added. Cells were removed by scraping, centrifuged at 1000 g for 10 min at 4 °C, and the pellets were stored at −80 °C until analysis.

Analysis of markers of lipid peroxidation and vitamin E
Pellets were allowed to thaw at room temperature for 5 min, then 300 µl of 0.1% Triton X-100 (peroxide- and carbonyl-free) in PBS was added to each pellet. The pellet, in Triton X-100, was sonicated at 30% of full power for 2 × 10 s bursts, with a period of 15 s of cooling on ice between bursts. MDA and 4-hydroxynonenal (4-HNE) were measured in dissociated pellet and plasma using a specific spectrophotometric assay (Calbiochem-Novabiochem, Nottingham, U.K.). Membrane lipid hydroperoxides were measured using a colorimetric assay [14]. Total plasma thiols and protein carbonyls were assayed spectrophotometrically [3,15]. Vitamin E was measured using an HPLC method [16]. Cholesterol was measured enzymically [17].

For each experiment, each set of cells (exposed to serum from one patient or one control, or unexposed) was studied in triplicate, with one flask used to calculate total release of lactate dehydrogenase (LD) (see below). EGM was removed from all flasks and replaced with 5.0 ml of 10% (v/v) serum from a CF patient or a matched control in EBM, or EBM alone for unexposed cells. Flasks were incubated at 37 °C in 95% air/5% CO₂. After 1 h the diluted serum/EBM was removed and retained. Flasks were washed twice with pre-warmed EBM, 5 ml of fresh EBM was added and the incubation was continued for a further 6 h, after which EBM was removed and retained. Each flask was rinsed twice with ice-cold PBS and a further 2 ml of PBS was added. Cells were removed by scraping, centrifuged at 1000 g for 10 min at 4 °C, and the pellets were stored at −80 °C until analysis.

Analysis of markers of lipid peroxidation and vitamin E
Pellets were allowed to thaw at room temperature for 5 min, then 300 µl of 0.1% Triton X-100 (peroxide- and carbonyl-free) in PBS was added to each pellet. The pellet, in Triton X-100, was sonicated at 30% of full power for 2 × 10 s bursts, with a period of 15 s of cooling on ice between bursts. MDA and 4-hydroxynonenal (4-HNE) were measured in dissociated pellet and plasma using a specific spectrophotometric assay (Calbiochem-Novabiochem, Nottingham, U.K.). Membrane lipid hydroperoxides were measured using a colorimetric assay [14]. Total plasma thiols and protein carbonyls were assayed spectrophotometrically [3,15]. Vitamin E was measured using an HPLC method [16]. Cholesterol was measured enzymically [17].

The protein content of the dissociated pellet was measured using a commercial kit (Sigma Chemical Co.). Endothelial cell MDA, 4-HNE and lipid hydroperoxides were expressed per g of protein. Plasma MDA and vitamin E were lipid-standardized and expressed per mol of total cholesterol.

In vivo assessment of endothelial function
Endothelial function was assessed by measuring forearm blood flow (FBF) by strain-gauge venous-occlusion plethysmography [18]. Briefly, basal FBF was measured 30 min after placing a 27 gauge polyethylene catheter in the non-dominant brachial artery under local anaesthetic. Then sodium nitroprusside was infused intra-arterially in four incremental doses (3, 6, 9 and 12 nmol·min⁻¹) to assess endothelium-independent vasodilation. After a 20 min rest period, basal FBF was measured again, and then acetylcholine was infused in four incremental doses (60, 120, 180 and 240 nmol·min⁻¹) to assess endothelium-dependent vasodilation. FBF was expressed as ml·min⁻¹·100 ml⁻¹ forearm volume. The response to the full dose range of vasoactive substances was calculated as the summary measure of area under the curve change from baseline [19].

Statistical analyses
Data were analysed using the SPSS 8.0 statistical package (SPSS Inc., Chicago, IL, U.S.A.). Comparisons between
RESULTS

Subjects
The average age of the CF patients was 25.0 years (range 18–37 years) and that of the controls was 25.4 years (range 20–35 years). There were no significant differences between patients and controls with regard to body weight, height, total cholesterol or low-density lipoprotein cholesterol, or plasma vitamin E concentration (Table 1).

Study power
The study power was calculated on the basis of expected changes in cytotoxicity, cell membrane 4-HNE and plasma MDA at two levels (90% and 80%) for sample size \( n = 11 \), with \( \alpha \) (threshold \( P \) value) = 0.05 (two-sided). The mean differences between groups required to achieve these power levels (90% and 80%) were 0.39 and 0.45% LD release for cytotoxicity, 0.99 and 1.15 \( \mu \text{mol/g} \) of protein for membrane 4-HNE, and 72.1 and 62.3 \( \mu \text{mol/mmol of cholesterol} \) for plasma MDA respectively, demonstrating a study power of > 90%.

Endothelial cell cytotoxicity and markers of peroxidation
Absolute mean (S.E.M.) levels of cytotoxicity and markers of membrane peroxidation in the two groups are summarized in Table 2. The percentage of endothelial cells killed after exposure to serum from patients with CF, based upon release of LD, was significantly lower than that of cells exposed to serum from controls (LD release: 1.8% (0.29%) and 3.0% (0.33%) respectively; mean difference −1.2% [95% confidence intervals (CI) −1.9% to −0.1%]; \( P < 0.05 \)). There was no significant difference in the amount of MDA in the membranes of endothelial cells exposed to serum from patients compared with that in cells exposed to serum from controls [17.3 (4.7) and 19.9 (4.4) \( \mu \text{mol/g of protein} \) respectively]. The 4-HNE content of membranes of endothelial cells exposed to serum from patients was significantly lower than that in cells exposed to serum from controls [0.75 (0.38) and 3.41 (1.05) \( \mu \text{mol/g of protein} \) respectively; mean difference −2.66 (95% CI −5.10 to −0.22) \( \mu \text{mol/g} \); \( P < 0.05 \)]. No lipid hydroperoxides were detected in any of the membrane samples.

Plasma markers of oxidative stress
Absolute mean (S.E.M.) levels of markers of plasma oxidative stress and endothelial function in the two groups are summarized in Table 3. The concentration of lipid-standardized MDA in plasma of CF patients was higher than in controls [317 (67) and 150 (20) \( \mu \text{mol/mmol of cholesterol} \) respectively; mean difference 167 (95% CI 93–241) \( \mu \text{mol/mmol} \); \( P < 0.05 \)]. Patients with CF had higher plasma levels of protein carbonyls than controls [1.01 (0.26) and 0.59 (0.15) \( \mu \text{mol/g of protein} \) respectively; mean difference 0.42 (95% CI 0.12–0.72) \( \mu \text{mol/g} \); \( P < 0.05 \)]. Plasma levels of total protein thiols were not significantly different between the two groups [CF

### Table 1 Characteristics of patients with CF and controls
Values are means (S.E.M.) for \( n = 11 \) in each group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CF patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>8/3</td>
<td>8/3</td>
</tr>
<tr>
<td>Age (years)</td>
<td>25 (1.9)</td>
<td>25.4 (1.3)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.0 (4.2)</td>
<td>63.5 (2.9)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160 (9.5)</td>
<td>170 (1.8)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>3.9 (0.41)</td>
<td>4.3 (0.30)</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol (mmol/l)</td>
<td>2.18 (0.36)</td>
<td>2.48 (0.48)</td>
</tr>
<tr>
<td>Vitamin E (nmol/mmol of cholesterol)</td>
<td>5008 (1800)</td>
<td>4832 (1516)</td>
</tr>
</tbody>
</table>

### Table 2 Cytotoxicity and membrane markers of peroxidation in endothelial cells exposed to serum from patients with CF and from controls
Values are means (S.E.M.) for \( n = 11 \) in each group. Significance of differences: *\( P < 0.05 \) compared with controls. ND, not detected.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CF patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD release (%)</td>
<td>1.8 (0.29)*</td>
<td>3.0 (0.33)</td>
</tr>
<tr>
<td>MDA (\mu mol/g of protein)</td>
<td>17.3 (4.7)</td>
<td>19.9 (4.4)</td>
</tr>
<tr>
<td>4-HNE (\mu mol/g of protein)</td>
<td>0.75 (0.38)*</td>
<td>3.41 (1.05)</td>
</tr>
<tr>
<td>Hydroperoxides</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

The two groups (CF patients and controls) were made using the Mann–Whitney rank sum test. Absolute values are summarized in Tables 1–3, and are expressed as means (S.E.M.). A \( P \) value of \( \leq 0.05 \) was considered statistically significant.
patients, 4.42 (0.84) μmol/g of protein; controls, 4.67 (0.78) μmol/g].

In vivo endothelial function

Absolute mean (S.E.M.) values for measures of endothelial function in the two groups are summarized in Table 3. Basal FBF was not significantly different in patients with CF compared with controls [4.3 (1.0) and 2.6 (0.5) ml min⁻¹ 100 ml⁻¹ respectively]. Endothelium-dependent vasodilation induced by acetylcholine infusion (assessed as area under the curve) was not significantly different in patients with CF compared with controls [32.0 (8.7) and 32.7 (7.3) arbitrary units of area respectively]. Endothelium-independent vasodilation induced by sodium nitroprusside infusion was higher in patients with CF than in controls [34.1 (2.5) and 23.1 (2.5) arbitrary units of area respectively], but this did not reach statistical significance (P < 0.06).

DISCUSSION

In the present study, we have shown that patients with CF do not have impaired endothelial function. Basal FBF and the response to sodium nitroprusside (endothelium-independent response) showed a trend to be increased in CF patients, although these differences did not reach statistical significance. At the same time, cultured human endothelial cells exposed to serum from patients with CF experienced less cell death and oxidative stress than cells exposed to serum from controls. These findings are despite the presence of increased systemic oxidative stress. We have previously demonstrated evidence for increased systemic oxidative stress in patients with CF [20]. The degree of stress was increased still further during periods of acute respiratory exacerbation. While these present findings are unexpected, considered in the context of the increased oxidative stress found in CF patients, they are consistent with the absence of atherosclerotic disease processes in such patients [11,12].

The ability of endothelial cells to generate NO in response to a receptor-dependent agonist such as acetylcholine has become a key marker of vascular health [21]. Synthesized from the amino acid L-arginine in a reaction catalysed by NO synthase (NOS), NO is one of the most potent endogenous vasodilators known. Endothelial function may be assessed in vivo on the basis of changes in FBF in response to infusion of both endothelium-dependent (e.g. acetylcholine) and -independent (e.g. sodium nitroprusside) vasodilators into the brachial artery. Impaired endothelial-dependent arterial vasodilation is a well-documented finding associated with many of the established risk factors for atherosclerotic disease, such as diabetes, hypercholesterolaemia, hypertension and hyperhomocystinaemia. Although this reduction in the capacity of endothelial cells to generate NO is likely to exacerbate the severity of injurious events such as vasospasm and thrombosis, this pathogenic alteration may also be a key feature in the initiation of atherosclerotic change.

The decision to examine cell responses at 6 h following a 1 h exposure to serum was based upon the work of Block [22] and pilot studies within our own department; these suggested consistent membrane lipid peroxidation by 1 h, with the maximum response being present by 6 h. In the present study, the extent of membrane lipid peroxidation was assessed through measurement of three end-products of this process, lipid hydroperoxides, MDA and 4-HNE. Since lipid hydroperoxides are unstable compounds that are readily detoxified by glutathione peroxidase or decompose to alkanals (e.g. MDA) or hydroxyalkenals (e.g. 4-HNE), it is not surprising that none could be detected in the cell membranes. In keeping with trends apparent from the cytotoxicity limb of the study, levels of membrane MDA (although non-significantly) and 4-HNE were greater in the endothelial monolayers exposed to control serum than in those exposed to serum from CF patients. The decreased oxidative stress found in cells exposed to serum from CF patients could not be explained in terms of the lipid-phase antioxidant vitamin E, the serum levels of which were similar in the two groups. Cycles of lipid peroxidation within the plasma membrane, initiated by free radicals or other reactive oxygen species and propagated by the production of lipid peroxides (LOO⁻), are known to damage cultured endothelial cells, reducing viability [23]. A consistent body of evidence would appear to support the potential for increased oxidative damage to important biomolecules in CF; various authors have documented heightened plasma levels of lipid [7] and DNA-derived [9] markers.

Rubbo et al. [24] have illustrated the ability of NO to terminate cycles of lipid peroxidation within low-density lipoprotein molecules and act as a cytoprotectant. The apparent protective, or decreased pro-oxidative, effect of serum from CF patients towards human endothelial monolayers in culture thus may be linked to NO generation. The patients in this study demonstrated a normal ability to produce and respond to endogenous NO in response to acetylcholine infusion, and to exogenous NO in response to nitroprusside infusion. Basal NO synthesis within endothelial cells is catalysed by the constitutive form of NOS, but a second inductible form of the enzyme can be detected following exposure to pro-inflammatory cytokines such as tumour necrosis factor-α or interferon-γ, which are elevated in CF [2,25]. It is possible that exposure to serum from CF patients induces increased activity of both inducible NOS and superoxide dismutase within endothelial cells, resulting in heightened levels of NO, which may protect against oxidative damage to vital biomolecules.
damage in vitro and prevent pro-atherogenic endothelial dysfunction in vivo. This would be consistent with a report documenting elevated serum levels of NO in a sample of subjects with CF [26]. Insights into the cellular and molecular basis of vessel disease have highlighted the role of cytotoxic cytokines in the inflammatory process, likely to be directed towards chronic endothelial injury [10]. Reduced endothelial cytotoxicity and a trend suggestive of decreased membrane peroxidation following exposure to serum from CF patients thus appear counter-intuitive.

The improved viability of cells exposed to serum from patients may also be explained in terms of VEGF. VEGF is a potent angiogenic agent expressed by epithelial cells in the lung, and Meyer et al. [27] have demonstrated a decreasing concentration gradient from the epithelial bronchoalveolar surface fluid to serum. McCollery et al. [28] demonstrated increased vascular angiogenesis in tissue and increased VEGF in serum from patients with CF. This increased serum VEGF, probably related to chronic inflammation, would enhance the survival and health of cultured cells exposed to serum from subjects with CF. A functional relationship between VEGF and NO has been proposed by Hood et al. [29], who demonstrated that exposure of cultured human umbilical vein endothelial cells to VEGF resulted in a dose-dependent increase in intracellular endothelial cell NOS mRNA and protein and a related increase in NO release.

As well as having chronic infection, patients with CF are different from healthy controls in that they are treated chronically with antibiotics. Inflammation and infection, bacterial and viral, have long been considered to be atherogenic. While the causal relationship has not been proven, large ongoing intervention studies are addressing the subject, raising the possibility that antibiotics may play a preventive role in atherogenesis. Preliminary evidence from clinical trials, reviewed by Meier [30], suggests that treatment with new macrolide antibiotics may improve outcome after ischaemic events, and that tetracyclines or quinolines may be useful in primary prevention. In addition to possibly reducing atherosclerosis by treating infection, certain antibiotics have a direct beneficial effect upon the cellular response to oxidative stress. In vitro treatment with ampicillin increases cellular hypoxic tolerance in hippocampal slices, while antimycin A prevents cell death in cells exposed to organic hydroperoxides [31].

The results of the present study are in keeping with the findings of post-mortem studies, which suggest a decreased prevalence of atherosclerotic disease within the CF population. It is possible that the absence of atherosclerotic involvement in the presence of increased systemic oxidative stress may be mediated through a number of mechanisms acting in isolation or in concert. These include cytokine modulation of cellular inducible NOS and superoxide dismutase activity, increased levels of VEGF, and a direct and/or indirect effect of chronic antibiotic treatment. This hypothesis requires further investigation in a larger study, specifically designed to assess changes in cellular NO synthesis and/or antioxidant status.

ACKNOWLEDGMENTS

We thank The Irish Lung Foundation for support.

REFERENCES

6 Andersen, D. H. (1938) Cystic fibrosis of the pancreas...
Anti-atherosclerotic processes in cystic fibrosis


Received 3 April 2001/29 May 2001: accepted 9 July 2001