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Compromised Antioxidant Status and Persistent Oxidative Stress in Lung Transplant Recipients

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Oxidative stress may be a key feature, and hence important determinant, of tissue injury and allograft rejection in lung transplant recipients. To investigate this, we determined the antioxidant status (urate, ascorbate, thiols and α-tocopherol) and lipid peroxidation status (malondialdehyde) in bronchoalveolar lavage (BAL) fluid and blood serum of 19 consecutive lung transplant recipients 2 weeks and 1, 2, 3, 6, and 12 months post-surgery. BAL fluid and blood samples from 23 control subjects and blood from 8 patients two days before transplantation were obtained for comparison. Before surgery, the antioxidant status of patients was poor as serum ascorbate and total thiol concentrations were significantly ($p < 0.05$) lower than control subjects. Two weeks post-surgery, ascorbate and total thiol concentrations were still low and urate concentrations had fallen compared to control subjects ($p < 0.01$). At this time, BAL fluid urate concentration was higher ($p < 0.01$), ascorbate concentration was lower ($p < 0.01$) and reduced glutathione concentrations were similar to control subjects. MDA, a product of lipid peroxidation, was higher ($p < 0.01$) in both BAL fluid and serum obtained from transplant patients compared to control subjects. During the first 12 months post-surgery, little improvement in antioxidant status or extent of lipid peroxidation was seen in transplant recipients. Regression analysis indicated no difference in serum or BAL fluid antioxidant status in patients with acute rejection compared to those without. In conclusion, lung transplant recipients have a compromised antioxidant status before surgery and it remains poor for at least the first year following the operation. In addition, these patients have elevated MDA concentrations in both their lung lining fluid and blood over most of this time. Oxidative stress is not, however, a sufficiently sensitive endpoint to predict tissue rejection in this group.

Keywords: Lung transplantation, antioxidants, tissue rejection, oxidative stress, neutrophils

INTRODUCTION

Lung transplantation is now an accepted therapy for end-stage lung disease.[1] Although considerable advancements have been made over the last decade, a number of complications still cause considerable morbidity in this patient group. Long term survival is restricted to 30–40% of recipients due to acute allograft rejection, which

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increases the risk of subsequent chronic rejection, i.e. obliterative bronchiolitis (OB).[2] In the early post-operative period, primary allograft failure, an extreme form of the reimplantation response, occurs in a number of cases resulting in increased pulmonary artery pressure, hypoxemia and pulmonary oedema.[3] These changes are caused by ischaemia-reperfusion injury of the lung allograft together with subsequent inflammatory reactions in the early post-operative period. Neutrophil activation results in the generation of reactive oxygen species (ROS), which contribute to tissue injury in the reperfusion syndrome.[4,5] Animal studies support this hypothesis since the use of ROS scavengers result in improved allograft function.[6–8]

Under normal circumstances, oxidative injury of the respiratory epithelium is minimised due to protective buffering by a thin layer of fluid, the epithelial lining fluid (ELF), which contains high concentrations of non-enzymatic, low molecular weight antioxidants.[9,10] The quantity and quality of this airways antioxidant network is an important determinant of the ability of the underlying respiratory epithelium to resist oxidative injury.[9–12] In certain circumstances, the antioxidant defence network in the airways is inadequate, or it becomes overwhelmed by the ensuing oxidative stress, and oxidation product generation increases.[13,14]

Knowledge of the antioxidant status in transplant patients is sparse, although it has been reported that ELF reduced glutathione status is low.[15] We hypothesised that oxidative stress may be a key feature, and hence an important determinant, of tissue injury and allograft rejection in lung transplant recipients. To investigate this, we examined the antioxidant and oxidative stress status of BAL fluid and blood serum of 19 lung transplant recipients on several occasions during the first year following surgery. BAL fluid and blood samples were obtained from 23 normal subjects and 8 patients before surgery for comparison.

METHODS

Subjects

Nineteen consecutive patients (mean age 41.3 ± 10.9 years; 10 female, 9 male) undergoing single lung ($n = 10$), bilateral lung ($n = 4$), or heart and lung ($n = 5$) transplantation were studied during the period from September 1994 to February 1996 (Table I). One patient underwent single-lung re-transplantation early in the post-operative period. Samples from the same patients have been analysed for inflammatory mediators and these data have been reported separately.[16] Donors and recipients were matched for cytomegalovirus (CMV) serological status. All organs were harvested in a similar fashion. Surgical procedures and immunosuppressive therapy were performed as earlier described.[17,18] Oral cyclosporine A immunosupression was monitored by serum levels, and doses adjusted at three months to achieve serum levels of $> 300\,\mu g/L$, and at 6 months to achieve $> 200\,\mu g/L$, respectively (radioimmunoassay for whole blood,

TABLE I Clinical data of transplanted patients

Pre-op diagnosis	*n* (gender)	Surgical procedure	Previous smoker	Mean age (range)
Emphysematous disease	9 (6f/3m)	single-lung	Yes	48 (43–53)
Idiopathic pulmonary fibrosis	1 (f)	single-lung	No	51
Eisenmenger's syndrome	3 (1f/2m)	heart-lung	No	31 (19–40)
Primary pulmonary hypertension	3 (1f/2m)	bilateral-lung	No	33 (25–43)
Primary pulmonary hypertension	2 (1f/1m)	heart-lung	No	33 (21–44)
Cystic fibrosis	1 (m)	bilateral lung	No	25

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Cyclotrac; Incstar, Stillwater, MN, USA). Azathioprine was given orally and adjusted to obtain a white blood cell count of $3–5 \times 10^9$/L. Episodes of acute rejection were treated with methylprednisolone (1 gm i.v.) for three days, and no rejection episode was resistant to steroid treatment during the time of the study. The study design was approved by the Ethics Committee of the University of Göteborg, and all subjects gave consent after both a written and oral explanation. Samples from 23 normal subjects (mean age 23 ± 5.4 years; 10 female, 13 male) were used as controls. They were healthy, non-smoking, asymptomatic volunteers, with no previous medical history of asthma, or any other pulmonary disease, including respiratory infection 6 weeks prior to the investigation. None of these volunteers was on any form of dietary supplementation or anti-inflammatory medication.

Post-operative follow-up

Surveillance bronchoscopy with transbronchial biopsy (TBB) and BAL were performed at 2 weeks and 1, 2, 3, 6 and 12 months after surgery. Additional TBB and BAL were performed approximately 4 weeks after augmented immunosuppressive treatment of rejection episodes, and whenever indicated by clinical parameters such as dyspnea, hypoxemia, decline in FEV_1 values, radiographic infiltrate or unexplained fever. Fibreoptic bronchoscopy was performed transorally with local anaesthesia and intravenous propofol sedation. Supplemental 100% oxygen was delivered nasally at a rate of 4–5 L/min with blood oxygen saturation continually monitored with an Ohmeda pulse oximeter (Ohmeda, Louisville, Kentucky).

The histopathological diagnosis of rejection was based on assessment of TBB and BAL samples. The evaluation of rejection followed the recommendations of the International Working Formulation for Classification and Grading of Pulmonary Rejection Study Group.[19] BAL analysis included direct microscopy for CMV inclusion bodies, *Pneumocystis carinii* (PCP), fungi and mycobacteria. In addition, immunocytochemistry techniques for PCP, CMV, and *Legionella pneumophilia* in BAL fluid and/or TBB were applied routinely. Cultures for bacteria including legionella and mycobacteria, fungi and virus were performed, and the presence of CMV and respiratory syncytial virus (RSV) genome was investigated by polymerase chain reaction (PCR) amplification.

Collection of Samples

All bronchoscopies were performed between 8:30 and 10:30 a.m. A full description of this procedure is given in our previous paper.[16] BAL fluid was strained to remove mucus aggregates and the recovered volume recorded. The filtered aspirate was then separated into a number of 10 mL aliquots. Metaphosphoric acid (16%) was added to those aliquots intended for ascorbate analysis as it helps prevent ascorbate loss during sample processing and storage. Samples were then rapidly frozen in liquid nitrogen and stored at −80°C prior to transportation on dry ice to London for antioxidant/MDA analysis.

Biochemical Determinations

Reduced (GSH) and oxidised (GSSG) glutathione concentrations were determined using the enzyme recycling method described by Tietze,[20] adapted for use on a microplate reader as reported previously.[21] The high-pressure liquid chromatography (HPLC) determination of urate and ascorbate was based on the method of Iriyama and colleagues[22] and has been reported recently.[21] Plasma sulfhydryls were assayed by the method of Ellman[23] in which 25 μL of plasma was added to 975 μL of 0.2 mol/L Na_2HPO_4, 2 mmol/L ethylenediaminetetraacetic acid (EDTA) pH 9.0, and 20 μL of 5,5′-dithio-bis(2-nitrobenzoic) acid (10 mmol/L in 0.05 mol/L phosphate buffer, pH 7.0). Absorbance at 412 nm

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was determined on an LKB Ultraspec II spectrophotometer against an appropriate blank and the concentration of sulfhydryls calculated using the equation $c = A/\varepsilon \times d$ where A = absorbance, ε = the extinction coefficient (13,600) and d = the pathlength (1 cm).

The HPLC determination of malondialdehyde was based on the method of Chirico[24] with the modifications described previously.[21] Total protein concentration was determined by taking a 10 µL sample of bronchoalveolar lavage and combining it with 200 µL of bicinchoninic acid solution (made up of 50 mL of bicinchoninic acid and 1 mL of 4% $CuSO_4$ solution), incubating them at 37°C for 30 min, and then measuring the absorbance at 560 nm. Protein measurements were standardised against human serum albumin.

Statistical Evaluation

Results are expressed as medians (25th percentile, 75th percentile). Due to lack of normal distribution in the data, non-parametric statistical analysis was performed throughout the study. Comparison of antioxidant/oxidative stress data between patients and controls was by Mann Whitney U test. Analysis of patient antioxidant changes over the first 12 months post-transplant was by Kruskal–Wallis ANOVA followed by Dunn's test for multiple comparisons. For calculation of possible covariation between the variables studied, the longitudinal time factors, as well as the repeated measures in the same subject, were adjusted for by use of random coefficient regression analysis.[25] The analysis allowed separate evaluation of the impact of rejection and infection, respectively, on the variables studied. A p value < 0.05 was considered statistically significant.

RESULTS

Antioxidant Status of Transplant Patients

Pre-surgery antioxidant status of transplant recipients is poor. Serum ascorbate [0.07(0.0, 1.8) µmol/L median, 25th percentile and 75th percentile values] and total thiols [297(234, 396) µmol/L] were significantly less than control values which were 37.8(20.8, 64.0) µmol/L and 642(517, 798) µmol/L, respectively. Urate concentrations did not differ from control values [239.3(109, 604) versus 270.9(196.8, 358.4) µmol/L] at this time. Two weeks post-surgery, however, serum urate concentrations had also fallen [81.4(66.3, 113.6) µmol/L] while serum ascorbate [2.6(0.3, 7.8) µmol/L], and total thiol concentrations [239(139, 293) µmol/L] remained low (Figure 1). At this time, BAL fluid ascorbate concentration was significantly lower [0.0(0.00, 0.10) versus 0.53(0.35, 0.75) µmol/L; $p < 0.01$], urate concentration was significantly higher [6.03(2.34, 8.60) versus 1.09(0.64, 1.99) µmol/L; $p < 0.01$] and reduced glutathione concentration was unchanged [0.69(0.29, 0.99) versus 0.47(0.27, 0.71) µmol/L] in transplant recipients compared to control subjects (Figure 2). Serum and BAL fluid MDA concentrations were higher ($p < 0.01$) in transplant recipients 2 weeks post-surgery than in control subjects [0.80(0.53, 1.46) versus 0.37(0.31, 0.40) µmol/L] and [5.8(5.0, 20.5) versus 3.1(2.7, 4.0) nmol/L; $p < 0.01$], respectively (Figure 3). BAL fluid protein concentration was also significantly higher in transplant recipients [0.33(0.24, 0.78) versus 0.14(0.08, 0.19) mg/mL; $p < 0.01$] at this time.

Antioxidant Status of Patients during the First 12 Months Post-transplant

No significant improvement in antioxidant status (serum or BAL fluid) was observed in lung transplant recipients over the first 12 months post-surgery (Table II). One month post-transplant, BAL fluid protein concentration returned to control levels, suggesting that pulmonary microvascular permeability had returned to normal. The concentrations of GSSG and MDA increased during the first 6 months post-transplant, although neither change was significant. In particular, GSSG levels increased from 1% to 13%

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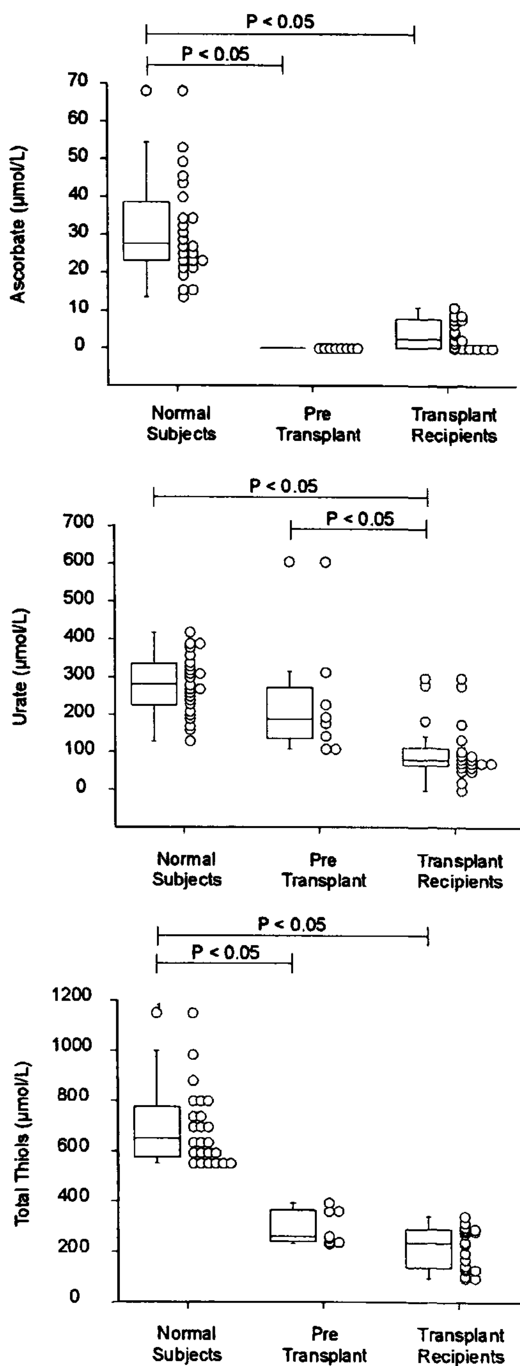

FIGURE 1 Serum antioxidant concentrations (ascorbate, urate and total thiols) in healthy control subjects, lung transplant patients 2 days before surgery and lung transplant recipients 2 weeks post-surgery. Data are illustrated both as individual values and as medians with inter-quartile ranges. Groups were compared by analysis of variance followed by a Mann–Whitney U-test for nonparametric data.

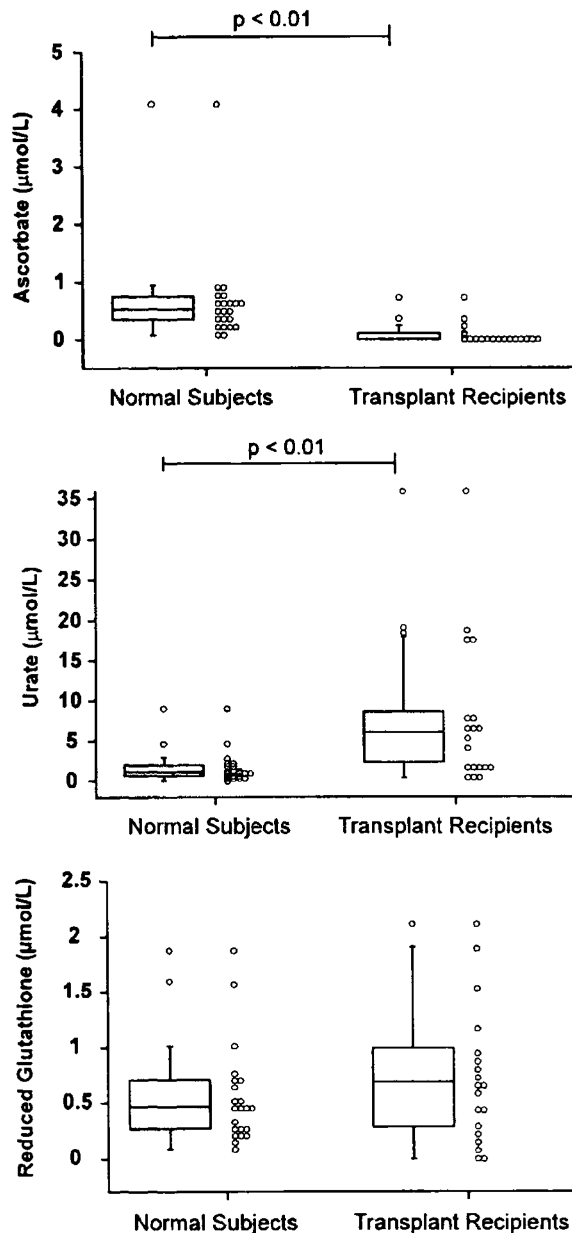

FIGURE 2 Bronchoalveolar lavage (BAL) fluid antioxidant concentrations (ascorbate, urate and reduced glutathione) in healthy controls and lung transplant recipients 2 weeks post surgery. Data are illustrated both as individual values and as medians with inter-quartile ranges. Groups were compared by Mann–Whitney U-test for nonparametric data.

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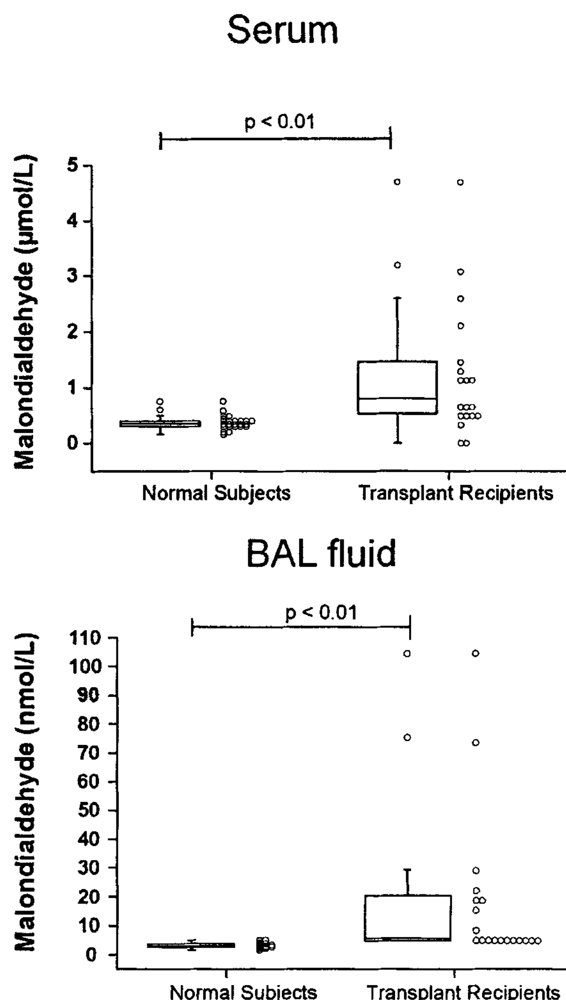

FIGURE 3 Serum and BAL fluid malondialdehyde (MDA) concentrations in healthy controls and lung transplant recipients 2 weeks post-surgery. Data are illustrated both as individual values and as medians with inter-quartile ranges. Groups were compared by Mann–Whitney *U*-test for non-parametric data.

of the total glutathione pool during this period (Table II).

Impact of Chemotherapy Treatment

Renal function of the transplant patients fell over the duration of the study, probably as a result of Cyclosporine A nephrotoxicity. No patient however developed renal failure. Initial median serum creatinine values were 91 (IQR 67, 115) compared to 131 (96, 166; $p < 0.01$) in the final observation period. Use of Azathioprine fell with time (initial median dose was 75 mg (IQR 50, 100) and the final dose was 50 mg (18, 82 mg; $p < 0.01$).

Antioxidant Status of Patients in the Presence of Acute Tissue Rejection

When the antioxidant status of these patients was compared during their first period of acute rejection and a period of no rejection, no differences in BAL fluid ascorbate, urate or reduced glutathione concentrations or serum ascorbate, urate or total thiols concentrations were found (Table III). Likewise, neither MDA levels in serum or BAL fluid nor GSSG and protein concentrations in BAL fluid were found to differ during periods of acute rejection (Table III). A random coefficient regression analysis, performed to look at the effect of rejection, infection and time post-transplant on BAL fluid antioxidant status and MDA concentrations in the same individual, revealed no significant differences in these biochemical indices with any of these parameters (data not shown). Moreover, total and differential cell counts did not differ between periods of rejection and non-rejection (Table III).

DISCUSSION

The findings of this study indicate that lung transplant patients have poor antioxidant status before surgery and that this does not improve for at least 12 months post-surgery. Pre-surgery serum ascorbate and thiol concentrations were low compared to control subjects while post-surgery, deficiencies of all the major water-soluble antioxidants in blood were found. At this time, BAL fluid ascorbate concentrations were also low compared to control subjects. Pre-surgery, these antioxidant deficiencies are likely due to a combination of poor nutrition and inflammation in the diseased lung while post-surgery, extensive neutrophil activation in the transplanted lung(s)

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TABLE II Blood and BAL fluid antioxidant status over the first 12 months post-transplant

	Antioxidant	Months post-transplant					
		0.5 ($n=19$)	1 ($n=17$)	2 ($n=15$)	3 ($n=13$)	6 ($n=13$)	12 ($n=9$)
Blood serum	Ascorbate	2.59 (0.30, 7.75)	3.91 (0.28, 5.99)	3.10 (0.72, 5.16)	4.90 (4.22, 9.10)	6.75 (3.13, 8.57)	5.98 (3.32, 8.36)
	Urate	81.4 (66.3, 113.6)	80.6 (61.3, 92.9)	76.8 (54.7, 85.6)	78.4 (69.0, 97.3)	70.9 (54.9, 84.3)	77.7 (74.7, 96.6)
	Thiols	239 (139, 293)	186 (131, 243)	242 (188, 304)	216 (195, 263)	262 (238, 276)	255 (233, 271)
	MDA	0.80 (0.53, 1.46)	1.15 (0.81, 1.31)	1.24 (0.84, 1.62)	1.34 (0.88, 1.69)	0.96 (0.88, 1.20)	1.16 (0.93, 1.62)
BAL fluid	Ascorbate	0.0 (0.0, 0.1)	0.13 (0.0, 0.29)	0.06 (0.00, 0.22)	0.00 (0.00, 0.11)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)
	Urate	6.03 (2.34, 8.60)	2.40 (1.97, 7.08)	4.68 (2.00, 7.85)	3.11 (2.08, 5.57)	2.78 (1.52, 3.87)	3.53 (2.89, 4.65)
	GSSG	0.01 (0.01, 0.26)	0.12 (0.01, 0.18)	0.12 (0.01, 0.22)	0.13 (0.01, 0.36)	0.01 (0.01, 0.12)	0.01 (0.01, 0.31)
	GSH	0.69 (0.29, 0.99)	0.79 (0.33, 0.92)	0.79 (0.39, 1.46)	0.83 (0.60, 1.16)	1.03 (0.57, 1.41)	1.07 (0.51, 2.04)
	Thiols	3.30 (1.65, 8.85)	2.03 (0.30, 2.85)	3.45 (0.60, 6.30)	2.25 (0.30, 4.65)	2.70 (0.00, 4.20)	0.75 (4.50, 4.50)
	Protein	0.33 (0.24, 0.78)	0.18 (0.14, 0.24)	0.24 (0.11, 0.51)	0.23 (0.11, 0.37)	0.22 (0.16, 0.28)	0.32 (0.29, 0.38))
	MDA	5.8 (0.0, 20.5)	19.0 (6.8, 38.2)	14.8 (10.8, 42.4)	10.8 (5.0, 26.7)	16.3 (6.0, 31.6)	5.0 (5.0, 31.8)

Data shown as medians (25th percentile, 75th percentile). All data expressed as μmol/L with the exception of BAL fluid protein (mg/mL) and MDA (nmol/L). Statistics were done using Kruskal–Wallis ANOVA (for non-parametric data) with Dunn's test for multiple comparisons.

TABLE III Blood and BAL fluid antioxidant and oxidation marker concentrations during a non-rejection and acute rejection period in lung transplant patients

	No rejection	Rejection
Blood Serum		
Ascorbate (μmol/L)	3.14 (0.22, 5.46)	4.26 (1.07, 6.97)
Urate (μmol/L)	75.08 (60.15, 89.17)	74.06 (59.46, 99.47)
Thiols (μmol/L)	195.0 (134.3, 266.3)	222.4 (183.3, 625)
MDA (μmol/L)	0.90 (0.61, 1.20)	1.24 (0.92, 1.36)
Bronchoalveolar lavage fluid		
Ascorbate (μmol/L)	0.06 (0.00, 0.40)	0.06 (0.00, 0.30)
Urate (μmol/L)	2.67 (1.95, 4.82)	3.31 (1.66, 5.25)
GSH (μmol/L)	0.96 (0.69, 1.42)	0.94 (0.50, 1.54)
GSSG (μmol/L)	0.24 (0.01, 0.32)	0.01 (0.01, 0.20)
Protein (mg/mL)	0.23 (0.17, 0.42)	0.21(0.13, 0.31)
MDA (nmol/L)	16.3 (5.0, 45.5)	15.4 (5.0, 30.0)
Cell No. ($*10^6$)	0.21 (0.02, 0.4)	0.27 (0.05, 0.49)
Neutrophils (%)	5.5 (0.0, 12.0)	7.5 (0.0, 35.5)
Eosinophils (%)	0.0 (0.0, 1.2)	0.01 (0.0, 0.8)
Lymphocytes (%)	3.8 (0.8, 8.8)	6.0 (0.0, 15.8)
Macrophages (%)	87 (73, 100)	75 (38, 100)

Data represent the first period of non-rejection and acute rejection for each patient ($n=16$). One individual was excluded on the basis that they did not experience rejection and two other patients were excluded as they were rejecting throughout the study. Results are expressed as median and 25th percentile, 75th percentile. Statistics were performed using a Mann–Whitney U test.

along with prolonged chemotherapy and inadequate nutrition are the most likely causes for these findings.

Post-surgery antioxidant status was examined, in the first instance, 2 weeks after the operation to circumvent any surgery-related effects on antioxidant defences. At this time, serum urate, ascorbate and total thiol concentrations were all much lower in transplant recipients than in normal subjects. It should be noted that due to practical considerations, the control subjects recruited were younger (23.0 ± 5.4 year) than the

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patients examined (41.3 ± 10.9 year). This age difference may explain some, but not all, of the antioxidant differences between these groups, especially as some of the transplant recipients had serum ascorbate levels in the scorbutic range (< 10 μmol/L). Likewise, although accurate measurement of any solute concentration in diluted lung lining fluid is fraught with problems,[26] the precautions taken in the present study (use of standardised lavage procedure by a minimum number of personnel; careful handling and storage of BAL fluid samples; reporting the antioxidant concentrations per ml recovered lavage fluid) all helped to minimise experimental variability. A full discussion of these problems and measures that can be taken to minimise them is given in Kelly *et al.*[27]

Of the 19 patients studied, only 2 were taking regular vitamin supplements. One cystic fibrosis patient took a multivitamin preparation while another patient took vitamins A and D. Previously, Schorah and colleagues[28] have reported low plasma ascorbate concentrations in critically ill patients. Interestingly, they found that vitamin C status was associated with the severity of illness and that provision of additional vitamin C through parenteral nutrition did not improve plasma ascorbate levels. From these findings, Schorah and colleagues concluded that the heightened acute phase response in critically ill patients is responsible for the sustained low plasma ascorbic acid levels. Hence, the marked differences in antioxidant status between patients and controls observed in the present study are probably due to both an increased requirement for antioxidants by transplant patients, as they experience increased oxidative stress in their airways[16] and because they have insufficient dietary intake of antioxidants.

We were surprised to find that the 8 transplant patients examined before surgery had serum ascorbate levels in the scorbutic range. As blood ascorbate status did not improve post-surgery it was therefore not surprising that BAL fluid ascorbate levels were also very low 2 weeks post-surgery. It was not appropriate to carry out bronchoscopy on patients before surgery but we feel that it is likely that their ELF ascorbate concentrations were also low at this time. Ascorbate scavenges a variety of reactive oxygen species, including superoxide, peroxyl radicals, hydrogen peroxide, hypochlorous acid and singlet oxygen.[29–31] Since activated inflammatory cells release most of these species they are likely to be present in increased quantities in the lung allograft in the early post-operative period. As a result, ascorbate may be consumed in the ELF as a first line of defence against oxidative insult, thus protecting the underlying epithelial cells from damage. The 9 patients with emphysema had previously smoked while the other 10 patients had never smoked. Comparison of the antioxidant status of these two groups revealed no differences, suggesting that past smoking history was not responsible for the low ascorbate status.

Urate is an oxidised purine base with considerable free radical scavenging activities. It can directly scavenge hydroxyl radicals, peroxyl radicals, singlet oxygen and myleperoxidase-derived hypochlorous acid.[32,33] BAL fluid urate concentrations were increased in transplant recipients. This may represent an adaptive response to increased oxidative stress and ascorbate deficiency in these patients. BAL fluid reduced glutathione concentrations were found to be similar in transplant recipients and control subjects. This finding differs from that reported recently by Baz *et al.*[15] These investigators found decreased ELF glutathione levels in transplant recipients, especially those without acute rejection, compared to control subjects. The cause of these different findings is not currently clear as the control subjects and patients were of similar age ranges in both studies, although the control subjects in the Baz study had a higher glutathione concentration than the control subjects in the present study.

Having established that lung transplant recipients have marked deficiencies in various antioxidants both before and following surgery, we

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next examined if these deficiencies resolved with time. Patients were monitored for the first 12 months post-surgery, with blood and BAL fluid samples taken at regular intervals. No significant improvement, in either serum or BAL fluid antioxidant status, was observed over the first year post-transplant. This surprising finding is probably explained by a combination of events including an inappropriately low intake of dietary antioxidants and decreased production of others such as reduced glutathione. Moreover, as high numbers of neutrophils were observed in BAL fluid from transplant recipients (15.8% compared to 3–5% in normal subjects), the ensuing oxidative stress would increase the utilisation of, and hence requirement for, antioxidants by transplant recipients. Furthermore, as neutrophil numbers correlated positively with lung injury, while microvascular permeability correlated with BAL fluid MDA concentration (data not shown), these data support the contention that these neutrophils were activated, and hence consuming antioxidant defences in the lungs of transplant recipients.

It has previously been demonstrated that chemotherapy itself can result in increased oxidative stress. Cyclosporine A has been reported to increase lipid peroxide production[34] and nephrotoxicity.[35] In the present study, although MDA levels were elevated 2 weeks post-surgery compared to control subjects, no further increase in MDA was seen over the next 54 weeks suggesting that cyclosporine A did not increasing lipid peroxidation in these patients. Moreover, those patients with increased plasma MDA levels tended to be those with increased BAL fluid levels suggesting that the source may have been oxidative reactions in the lung (data not shown). The immunospressant Azathioprine has also been reported to increase oxidative stress yet, as already mentioned, no further increases in MDA were seen over the first year post-transplant. This may be due, in part, to the decreased use of Azathiopriene with time (initial median dose was 75 mg (IQR 50,100) and the final dose was 50 mg (18,82 mg; $p < 0.01$). We cannot of course, rule out the possibility that increased aldehyde formation did occur in these patients but was also accompanied by increased clearance. We feel however that this is the less likely explanation, given that renal function of the transplant patients was further compromised towards the end of the study.

A further goal of this investigation was to establish the levels of antioxidants and oxidation products in lung transplant recipients during tissue rejection. This prospective, longitudinal analysis demonstrated that there was no significant change in antioxidant status (both serum and BAL fluid) of lung transplant recipients when they experienced an episode of acute rejection when the confounding factors of time and repeated measures in the same individual were taken into account. This finding, for reduced glutathione, differs from that of Baz *et al.*[15] who reported that ELF reduced glutathione concentration increases significantly during acute rejection. The reason for this discrepancy is unknown, however, the already severely compromised antioxidant status of the patients may form part of the explanation.

In summary, lung transplant recipients have marked perturbations in both, blood and ELF antioxidant status. This is likely due to both insufficient intakes of dietary antioxidants and increased antioxidant requirements owing to the elevated oxidative stress resulting from pulmonary inflammation. Further work is required to determine whether antioxidant supplementation regimes can be employed to rectify this situation, as these may help increase allograft viability.

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