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Ultrastructural pathology of the alveolar type II pneumocytes of human donor lungs

Electron microscopy, stereology, and microanalysis

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Abstract Alveolar type II pneumocytes (PII) were studied in 12 human donor lungs perfused with modified Euro-Collins solution during single-lung transplantation (SLTx). While one lung was transplanted, the contralateral donor lung (cDL) was fixed at the time of SLTx for examination by electron microscopy, stereology, and microanalysis. Three groups were then formed: group A (n = 7), cDL without contusions, uneventful early postoperative course; group B (n = 3), cDL with contusions, uneventful early postoperative course; group C (n = 2), cDL without contusions, early postoperative respiratory dysfunction. The major findings were that the presence of contusions had no effect on PII ultrastructure and that intracellular surfactant-storing lamellar bodies of cDL in group C were characterized by a higher volume-to-surface ratio (VsR) and larger area per cell profile than group A. Correlation analysis based on pooled data (groups A and C) showed that ischaemic time had little effect on PII ultrastructure and bore no relationship to postoperative clinical variables. The duration of preoperative donor intubation had a pronounced influence on ultrastructure and postoperative clinical variables. The stereologically estimated amount of intracellular surfactant and mitochondrial VsR were the only ultrastructural parameters that were significantly associated with early postoperative oxygenation. Lamellar bodies were the only ultrastructural components found to have a significant relationship to postoperative intubation time. The ultrastructural integrity of type II pneumocytes of human donor lungs is an important determinant of early respiratory function following clinical lung transplantation.

Key words Lung transplantation pathology · Alveolar epithelium · Type II pneumocyte · Surfactant · Reperfusion injury

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Introduction

Lung transplantation has become a successful therapy in patients suffering from end-stage respiratory diseases [17]. In clinical lung transplantation, single-flush perfusion of the pulmonary artery with modified Euro-Collins solution (ECS) is the most usual way of preserving donor lungs [16, 19, 40]. Nevertheless, the duration of ischaemia is still limited to 6–8 h, and early graft dysfunction remains a significant and unpredictable problem [27]. Although in some cases, preservation injury has appeared to be the cause of primary graft failure, a common mechanism has not yet been established [33, 44]. Poor quality of the donor lung, pre-existing donor abnormalities, ischaemic injury, and events directly related to reperfusion have all been considered responsible for the immediate deterioration of pulmonary function following successful revascularization of the allograft, which is also referred to as "reimplantation response" or "reperfusion injury" [16, 44].

Recent experimental studies have demonstrated that alterations of pulmonary surfactant contribute to the reimplantation-related early respiratory dysfunction observed after lung transplantation [2, 20, 24, 37]. These findings are supported by the success of exogenous surfactant therapy in clinical treatment [32]. Since reperfusion injury is characterized by severe alveolar oedema [16], and oedema components have also been shown to impair alveolar surfactant [29], it is not clear whether

surfactant alterations are a cause or a consequence of reimplantation-related respiratory dysfunction. Since alveolar type II pneumocytes synthesize, store and secrete pulmonary surfactant [22], the hypothesis has been proposed that lung transplantation-related impairment of alveolar surfactant results from ischaemic injury to the alveolar type II pneumocytes [2, 20, 25]. However, no direct evidence supporting this hypothesis has been presented.

The object of this study was to characterize cellular alterations of human alveolar type II pneumocytes that might be present at the time of clinical transplantation in ECS-preserved donor lungs. Following previous studies [12, 31, 42], we examined the ultrastructure of the nontransplanted, contralateral donor lung in 12 cases of single-lung transplantations. Single donor lungs were allocated according to the clinicopathological diagnosis when the contralateral lung could not be allocated to a second recipient. Ultrastructural pathology was assessed by means of scanning and transmission electron microscopy (SEM, TEM) in conjunction with stereological methods, and by means of the microanalytical technique of electron spectroscopic imaging. On the basis of these quantitative data, we analysed potential relationships between ultrastructural findings and both donor-related variables and postoperative recipient-related variables. Special emphasis was put on the surfactant-storing lamellar bodies of alveolar type II pneumocytes.

Materials and methods

The study comprises 12 cases of clinical single-lung transplantation performed between January 1991 and January 1996 at Hanover Medical School. Preservation, and harvesting of the donor lungs was performed according to standard procedures using modified Euro-Collins solution (ECS) and prostacycline as an additive [40]. Left and right donor lungs were separated shortly before transplantation. While one donor lung was transplanted according to the techniques initially described by Cooper et al. [5], the contralateral donor lung was fixed at the time of transplantation as soon as the clinical procedure allowed. Fixation was performed by instillation via the airways to ensure rapid and uniform fixation of the whole organ for subsequent ultrastructural examination [3, 12, 13, 31]. Donor lungs were only used for investigation of lung structure on the understanding that they could not be made available for another suitable recipient by The Eurotransplant Foundation Centre, Leiden or they were excluded from transplantation by the explanting surgeon for clinical reasons.

The criteria for donor acceptance included an arterial oxygen tension (PO_2) of about 100 mmHg at an inspired oxygen fraction (FiO_2) of 0.3 and a positive end-expiratory pressure (PEEP) of 5 cmH_2O, resulting in a PO_2: FiO_2 ratio of 300 mmHg [40]. The duration of mechanical ventilation did not exceed 4 days, and radiographic evidence of pulmonary oedema, infection, or major lung trauma led to exclusion. In addition, fibreoptic bronchoscopy was performed routinely in all donors immediately before harvesting to exclude bronchopulmonary infection.

After separation of the double lung bloc, the contralateral donor lung was fixed as described in detail elsewhere [13, 31]. Briefly, a 0.1 M cacodylate-buffered mixture of 1.5% glutaraldehyde and 1.5% paraformaldehyde was instilled via the airways at a pressure of 25 cmH_2O until the flow of the fixative ceased automatically. After storage in the same fixative for about 24 h at 8°C, a systematic random sampling procedure was performed to obtain an unbiased collection of about 20, 1-mm^3 blocks of tissue per lung for TEM. For SEM, about 5 additional 5 × 5 × 2 mm blocks of tissue were collected according to systematic random sampling. SEM samples were processed according to a modified OTOTO (*o*smium-*t*hiocarbohydrazide) method [21] as detailed recently [13, 31]. After critical-point drying, specimens were directly observed by means of a Zeiss DSM 960 with the omission of metal-sputtering. For TEM by means of an EM 10A or a CEM 902 (Zeiss, Oberkochen), the tissue blocks were processed according to an improved surfactant retention procedure described earlier [11]. Processing was standardized using an automated tissue processor Histomat (Bio-med, Theres). Briefly, after several buffer rinses, samples were postosmicated, stained en bloc with uranyl acetate, dehydrated through a graded series of ethanol and embedded in Araldite. En bloc staining with half-saturated, aqueous uranyl acetate solution for 18 h was shown to result in excellent ultrastructural preservation even of giant lamellar bodies in pathologically altered type II pneumocytes [34].

Stereological analysis of type II pneumocytes was performed as described recently [13]. Briefly, 5 blocks of tissue were studied in each case. One randomly orientated, ultrathin section per block was examined by meandering systematically over the whole section. Micrographs of alveolar type II pneumocyte profiles were recorded according to a random systematic subsampling scheme [23]. A primary magnification of 4,000× was chosen to ensure that the whole profile was recorded. The final magnification was determined to be 11,450× by means of a calibration grid. A multipurpose coherent test system was used for point and intersection counting (324 points, test line length d = 1 mm). A total of 813 type II pneumocyte profiles were analysed, with an average of 68 cell profiles per case. The following stereological variables, which have been shown previously to be the appropriate parameters for characterization of alveolar type II pneumocyte ultrastructure [13, 31], were determined: (1) the volume-to-surface (VsR) ratio of the cell (ce), the nucleus (nu), the mitochondria (mi), and the lamellar bodies (lb) as an estimate of size or swelling of the cell and its organelles, respectively; (2) the area (A) of the remaining cytoplasm (cy) per cell profile as an estimate of cytoplasmic swelling; and (3) the area (A) of the lamellar bodies (lb) per cell profile to estimate the amount of intracellular surfactant stored in type II pneumocytes. Volume densities calculated with reference to the cell volume have been excluded from analysis, since they are severely affected by cell swelling in experimental and human type II cells [13, 15].

Electron spectroscopic imaging (ESI) was used to check whether the phosphorus signal of lamellar bodies, an indicator of relative phospholipid content, was altered [11, 26]. Ultrathin sections about 40 nm in thickness were collected on bare 600 hexagonal copper grids. Computer-assisted phosphorus imaging was performed by means of a CEM 902 (Zeiss, Oberkochen) according to the three-window method as described previously [26]. This type of electron microscope is equipped with an integrated Castaing-Henry energy filter that allows for the selection of inelastically scattered electrons of a given energy loss [8].

Donor-related variables analysed included gender, age, weight, duration of mechanical ventilation, preoperative oxygen fraction of inspired air (FiO_2), preoperative PO_2: FiO_2 ratio, PCO_2, cause of death, presence of macroscopic lung contusion areas caused by thorax trauma, and duration of ischaemic storage. Recipient-related variables analysed were PO_2: FiO_2 ratios at 6, 12, and 24 h after transplantation, the duration of mechanical respiratory assistance, and the occurrence of early postoperative respiratory complications.

Unless stated otherwise, clinical data of individual lungs are given as discrete values. Data comprising several lungs are given as mean values ± SD. Stereological data referring to individual lungs are given as mean values ± SEM of 5 tissue blocks. Differences between individual lungs or between groups of lungs were tested for significance using a one-way ANOVA, given that normality and equal variance were not rejected at $P < 0.05$. Otherwise

Table 1 Donor-related clinical variables (*BTU* brain tumour, *HBT* head-brain trauma, *ICB* intracerebral bleeding)

Case no.	Group	Gender	Age (years)	Cause of death	Intubation time (h)	FiO_2 (%)	PO_2:FiO_2 (mmHg)	Ischaemic time (min)	Contusion areas
I	A	Female	31	BTU	28	0.5	654	294	No
II	A	Male	40	ICB	36	0.4	565	246	No
III	A	Female	41	ICB	13	0.4	273	215	No
IV	A	Male	24	HBT	70	0.6	327	240	No
V	A	Female	18	HBT	24	0.3	597	246	No
VI	A	Male	41	ICB	24	0.4	270	250	No
XI	A	Female	51	ICB	48	0.3	437	90	No
VII	B	Male	24	HBT	48	0.3	623	205	Yes
VIII	B	Male	18	HBT	33	0.3	660	181	Yes
IX	B	Male	17	HBT	57	0.5	368	225	Yes
X	C	Male	52	ICB	24	0.3	316	288	No
XII	C	Male	41	ICB	48	0.3	433	405	No

Table 2 Recipient-related clinical variables (*CI* cerebral insult, *EMPH* emphysema, *IF* idiopathic pulmonary fibrosis, *OB* obliterative bronchiolitis, *p.o.* post operationem, *ReTx* retransplantation, *RI* reperfusion injury, *TB* tuberculosis)

Case no.	Group	Gender	Age (years)	Diagnosis	PO_2:FiO_2 6 h p.o. (mmHg)	PO_2:FiO_2 24 h p.o. (mmHg)	Intubation time [h]	Early respiratory complications	Current status (postoperative day)	Cause of ReTx/death
I	A	Female	35	IF	253	329	29	No	Alive	–
II	A	Male	65	IF	245	373	31.5	No	Deceased (124)	TB
III	A	Male	53	IF	290	427	32.5	No	Alive	–
IV	A	Male	46	IF	175	178	240	No	Alive	–
V	A	Female	52	IF	345	417	28.5	No	Alive	–
VI	A	Female	48	OB	178	265	159	No	Alive	–
XI	A	Male	49	IF	210	360	288	No	Deceased (12)	CI
VII	B	Male	54	IF	387	373	42	No	Alive	–
VIII	B	Female	42	EMPH	393	317	720	Yes	Alive	–
IX	B	Female	54	EMPH	523	507	43	No	Alive	–
X	C	Male	26	IF	262	249	239	Yes	ReTx (512)	OB
XII	C	Female	53	IF	452	233	408	Yes	ReTx (17)	RI

the nonparametric Kruskal-Wallis ANOVA on ranks was performed. Differences between individual lungs were analysed on the basis of the discrete values obtained from the corresponding tissue blocks. Differences between groups of lungs were analysed on the basis of the corresponding mean values or medians depending on the statistical test used. Correlation between variables was tested by means of Pearson's product moment correlation, given that normality was not rejected at $P < 0.05$. Otherwise the nonparametric Spearman rank order correlation was used. $P < 0.05$ was considered to be statistically significant. To look for multiple influences of donor-related functional and ultrastructural parameters on postoperative dependent variables, which were logarithmically transformed, stepwise multiple regression models were developed. Models were accepted if normality and equal variance were not rejected at $P < 0.05$. Statistical analyses, computation of regression curves, and graphic presentations were performed using the software programs SigmaStat 2.0 and SigmaPlot 3.0 (Jandel Scientific, Erkrath, Germany).

Results

Clinical data

In 4 female and 8 male multiorgan donors, the lungs were accepted for transplantation in patients requiring single lung replacement (SLTx) for end-stage idiopathic pulmonary fibrosis ($n = 9$), emphysema ($n = 2$), or obliterative bronchiolitis ($n = 1$). Donor-related variables are shown in Table 1. The causes of donor death were intracerebral bleeding ($n = 6$), head-brain trauma ($n = 5$), and brain tumour ($n = 1$). In all cases, the explanting surgeon noted good to excellent pulmonary arterial perfusion with ECS. The average duration of cold ischaemia of the transplanted lung was 240 min (median: 243 min, range: 90–405 min). For clinical reasons, a slightly longer average duration of cold ischaemia of 284 min (median: 275 min, range: 121–458 min) was noted in the nontransplanted lung. While in cases I–V, X, XI and XII the clinical status of the donors was considered unremarkable, in cases VII–IX limited lung contusions of the nontransplanted lung caused by chest trauma were seen by roentgenographic and/or macroscopic inspection. Therefore, these 3 lungs were excluded from transplantation, and consequently from the analysis of correlation between ultrastructure and postoperative parameters in this study.

Recipient-related variables are shown in Table 2. In cases I–III, V, VII and IX, postoperative oxygenation, which is considered to be the most sensitive indicator of

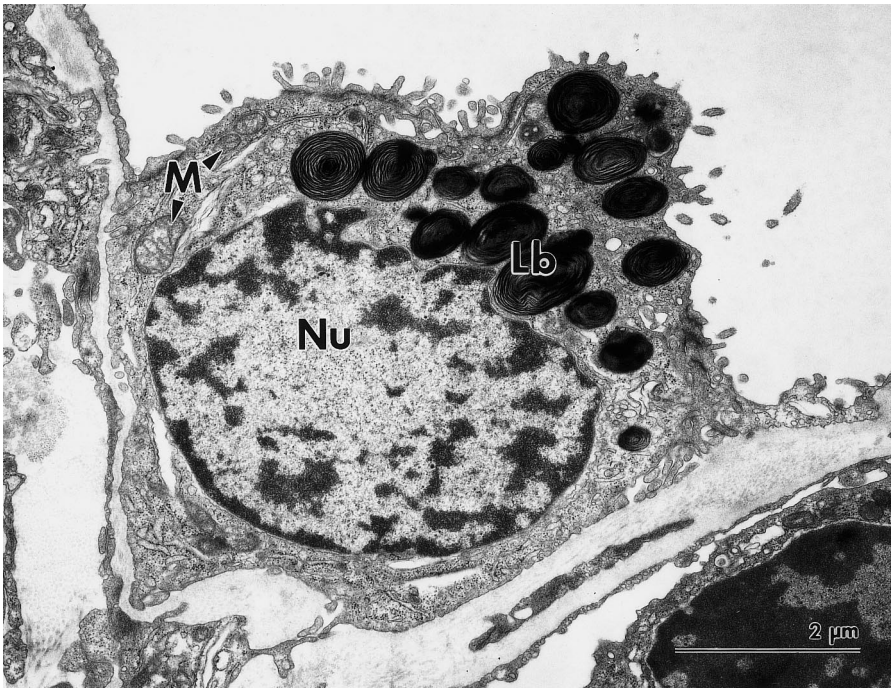


Fig. 1 TEM micrograph of alveolar type II pneumocyte showing normal appearance of cytoplasm, nucleus (*Nu*), mitochondria (*M*), and lamellar bodies (*Lb*). Case II

lung function [19], was excellent in terms of a steady increase in or a constantly high level of the PO_2: FiO_2 ratio recorded during the first 24 h following transplantation. These patients required mechanical ventilation for an average of only 34.4 h (median: 32, range: 28.5–43). One patient (case XI) though he had excellent postoperative lung function, died 12 days after transplantation of a cerebral insult. In another 2 patients (cases VIII and X), appropriate initial PO_2: FiO_2 ratios were achieved. In both cases, however, prolonged mechanical ventilation was necessary (720 and 239 h, respectively, after surgery), because ARDS developed in case VIII and because of a considerable decrease in the oxygenation capacity at about 36 h after surgery in case X. In 2 patients (cases IV and VI), PO_2: FiO_2 ratios were constantly low throughout the early postoperative period. These 2 cases were recently shown to be characterized by a significantly higher degree of damage to the alveolar type I pneumocytes than the others [42]. Both patients required mechanical ventilatory support for 240 and 159 h, respectively. In case XII, severe reperfusion injury developed within the first 24 h after surgery, with a continuous decrease in the PO_2: FiO_2 ratio and development of pulmonary oedema. In this patient, retransplantation had to be performed 17 days after the initial SLTx.

Owing to the differences in donor status (contusion versus free of contusion) and in early postoperative outcome (without versus with decline in early postoperative oxygenation), individual cases were assigned to the following groups: group A, both donor lungs free of contusion and early postoperative course without decline in oxygenation (cases I–VI, XI); group B, contralateral donor lung with contusion and early postoperative course without decline in oxygenation (cases VII–IX); group C, both donor lungs free of contusion, but with decline in early postoperative oxygenation (cases X, XII).

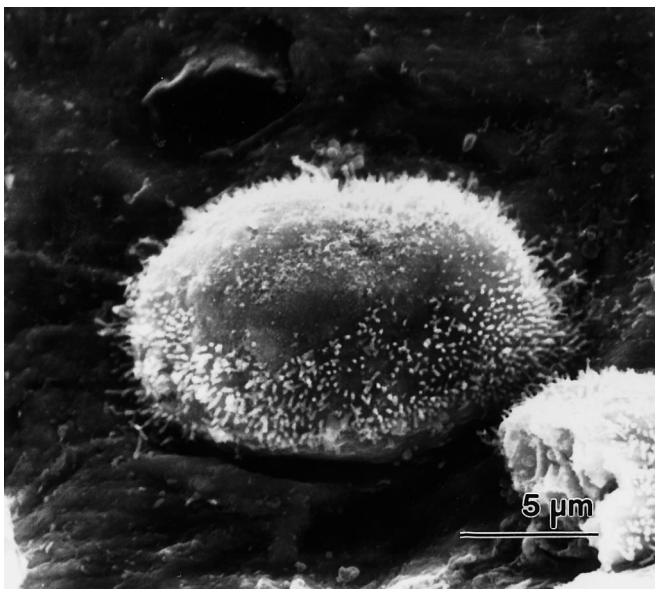


Fig. 2 SEM micrograph of alveolar type II pneumocyte showing normal external appearance of an individual cell surrounded by type I pneumocytes. Surface exhibits a bald-looking central patch, which is surrounded by a marginal zone studded with short microvilli. Case XII

Ultrastructural data

On qualitative examination, the alveolar type II pneumocytes of the donor lungs of all three groups usually

Table 3 Stereological data characterizing the alveolar type II pneumocytes of the contralateral donor lungs (*VsR* volume-to-surface ratio, *A* area per cell profile, *ce* cell, *cy* remaining cytoplasm, *nu* nucleus, *mi* mitochondria, *lb* lamellar bodies)

Case no.	Group	VsR, ce (μm)	A, cy (μm^2)	VsR, nu (nm)	VsR, mi (nm)	VsR, lb (nm)	A, lb (μm^2)
I	A	1.04±0.07	4.77±0.32	761±76	111±8	159±7	0.84±0.16
II	A	1.15±0.11	7.04±0.87	839±164	119±9	157±16	0.93±0.13
III	A	1.21±0.14	6.02±0.95	835±137	102±6	190±7	1.67±0.12
IV	A	1.39±0.13	9.03±1.63	965±151	129±7	163±7	0.92±0.20
V	A	1.14±0.12	6.99±0.92	763±82	113±15	170±13	1.03±0.20
VI	A	1.18±0.13	5.88±1.06	654±48	108±4	172±13	0.79±0.13
XI	A	1.11±0.19	6.88±1.91	760±83	131±8	193±10	0.96±0.22
VII	B	1.30±0.12	8.26±1.13	989±141	123±10	185±13	0.78±0.14
VIII	B	1.17±0.02	6.18±0.22	792±42	102±7	176±15	0.96±0.10
IX	B	1.39±0.09	7.84±1.02	821±45	121±8	170±12	0.64±0.08
X	C	1.16±0.07	6.28±0.21	614±57	95±9	230±18	1.72±0.30
XII	C	1.52±0.09	7.86±0.60	824±105	118±6	248±20	2.57±0.43

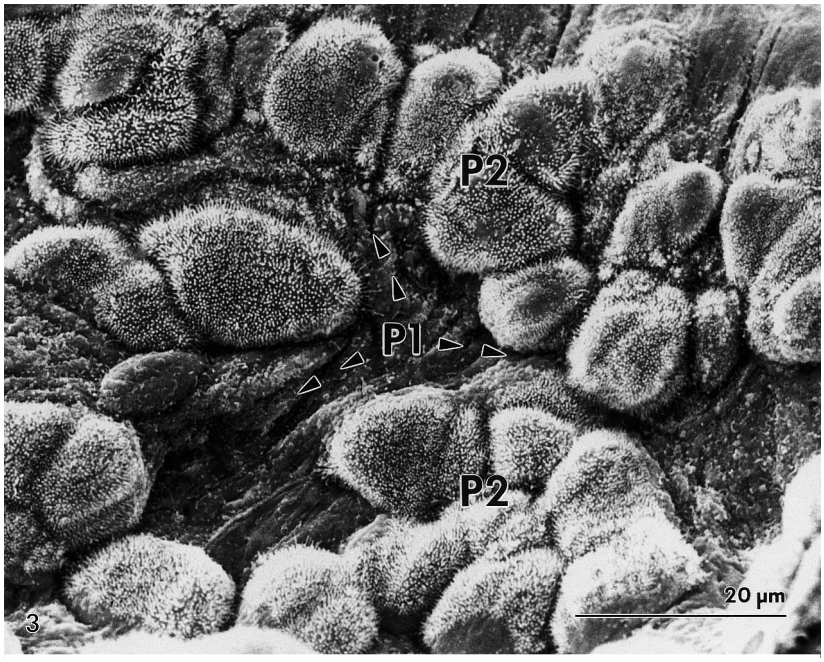


Fig. 3 SEM micrograph of an alveolar wall composed of groups of alveolar type II pneumocytes (*P2*) with smaller component of type I pneumocytes (*P1*). Case VI

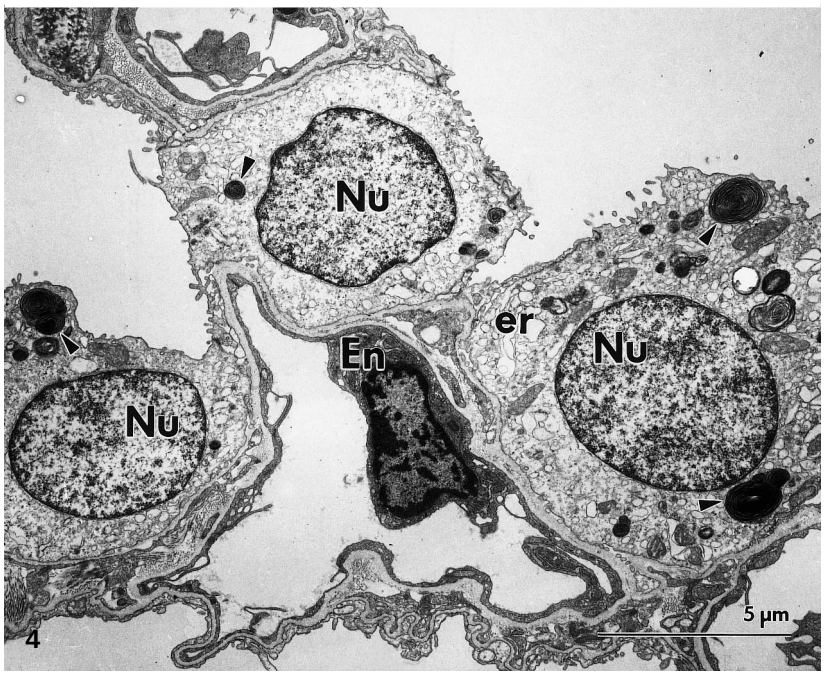
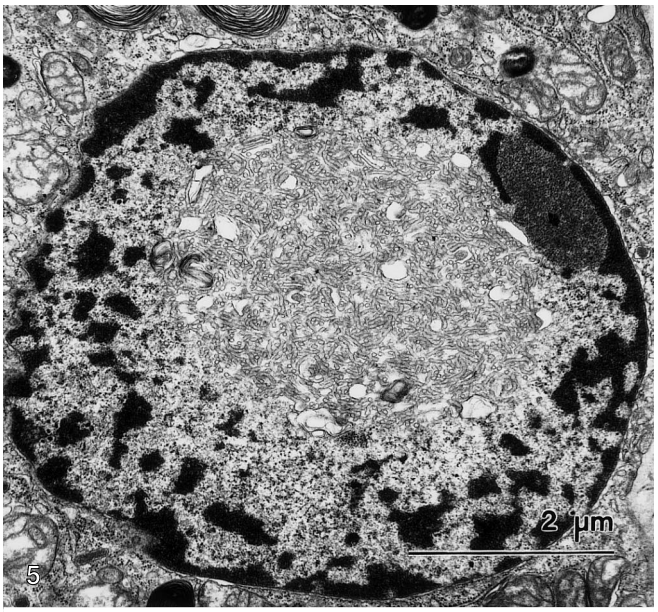


Fig. 4 TEM micrograph of a group of three alveolar type II pneumocytes, which are characterized by swelling of nuclei (*Nu*), dilatation of endoplasmic reticulum (*er*), clearing of cytoplasm, and the presence of only a few lamellar bodies (*arrowheads*). Note the integrity of the adjacent air–blood barrier (*En* capillary endothelium). Case IV



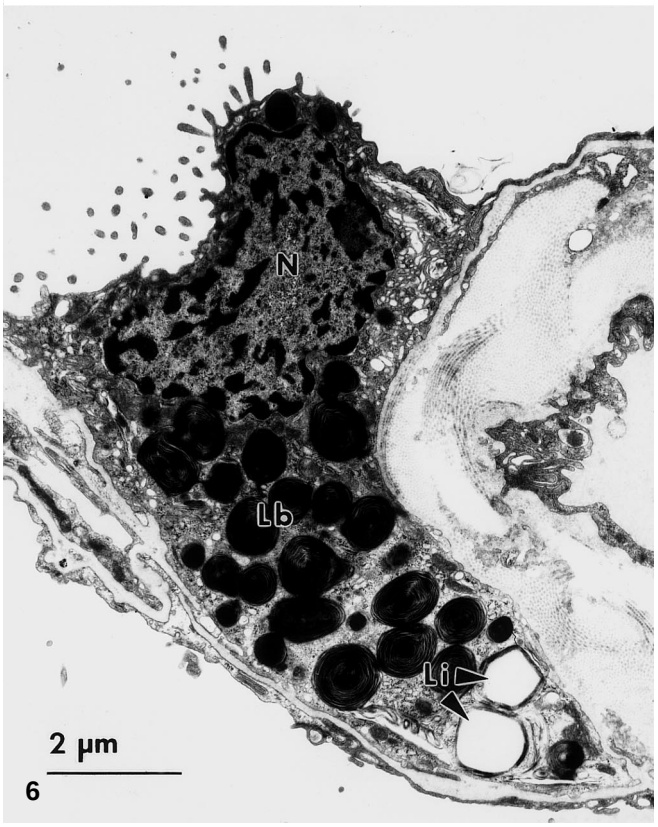


Fig. 5 TEM micrograph of the nucleus of an alveolar type II pneumocyte exhibiting tubular inclusions. Case IV

Fig. 6 TEM micrograph of alveolar type II pneumocyte densely filled with lamellar bodies (*Lb*), and characterized by the presence of homogeneous lipid bodies (*Li*). (*N* nucleus). Case XII

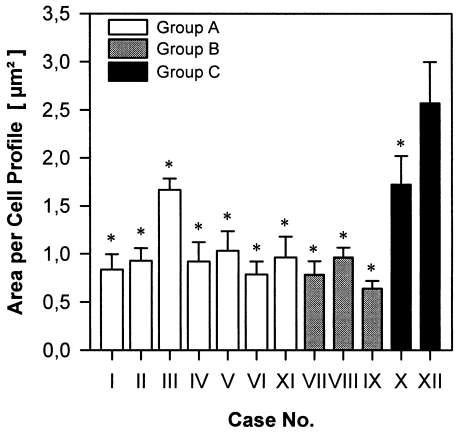


Fig. 7 Stereological estimates of the amount of intracellular surfactant stored in lamellar bodies. In comparison with contusion-free normal lungs (group A) the presence of contusions (group B) did not affect the area of lamellar bodies per cell profile. Contralateral donor lungs whose transplanted twin lung developed postoperative respiratory complications (group C), however, showed the highest amounts of intracellular surfactant. In case XII, a patient who developed severe reperfusion injury within the first 24 h after transplantation, the amount of intracellular surfactant was significantly different from all other cases (one-way ANOVA on ranks; $*P < 0.05$). *Bars* represent mean values (+SEM) obtained by analysis of five systematic random samples per case

had largely normal ultrastructural appearances, as seen by TEM (Fig. 1) and SEM (Fig. 2), neither of which revealed severe damage. In fine detail, however, interindividual variations in subcellular structures were observed by TEM and confirmed by stereology (Table 3). In some cases (IV, V, VI) type II pneumocytes were seen to constitute groups or rows of several closely adjacent cells, indicative of a previous episode of type II cell proliferation (Figs. 3, 4), but no signs of ongoing proliferation were observed. In cases IV and X, type II pneumocytes (1 of 50 and 2 of 77 cells, respectively), which exhibited membranous tubular inclusions in the nucleus were seen (Fig. 5). In case IV swelling of the cisternae of the endoplasmatic reticulum was noted, which was accompanied by clearing of the cytoplasm (Fig. 4). Consequently, the cytoplasmic area per cell profile was highest in case IV (see Table 3). In cases IV, VII, IX, and XI swelling of mitochondria and focal destruction of mitochondrial cristae were seen. In these cases, mitochondrial volume-to-surface ratios ranged between 121 and 131 nm, while in the other cases they were between 95 and 119 nm. While lamellar bodies of alveolar type II pneumocytes in groups A and B predominantly showed a single lamellation centre, lamellar bodies with two or three lamellation centres surrounded by a common bounding membrane were frequently seen in group C. Moreover, while homogeneous lipid bodies were almost completely absent in groups A and B, they were regularly observed in case XII (Fig. 6). In cases X and XII, which together constitute group C, lamellar bodies were larger than in the other cases. Their volume-to-surface ratios amounted to 230 and 248 µm, respectively, while they ranged between 157 and 193 µm in the other cases (Table 3). Notably, post hoc multiple comparison revealed that in case XII the area of lamellar bodies per cell profile was significantly different than in all other

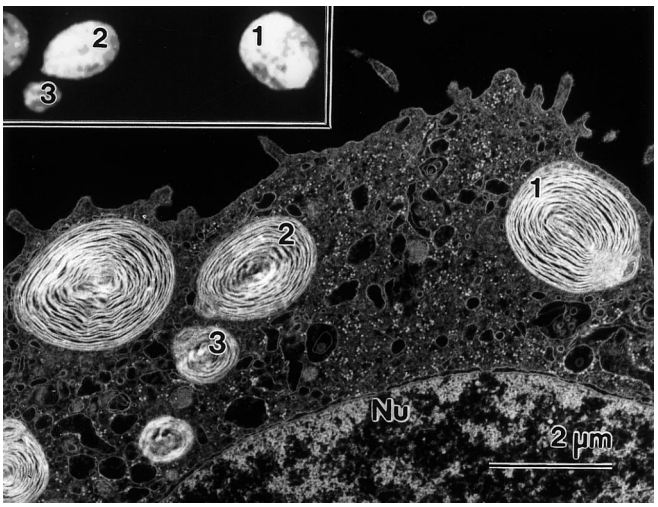


Fig. 8 Electron micrograph obtained by ESI using inelastically scattered electrons of ΔE=155±10 eV just beyond the energy loss edge of phosphorus (132 eV). Owing to the use of inelastically scattered electrons for imaging, such structures as lamellar bodies (*1*, *2*, *3*), ribosomes and nucleus (*Nu*), which in the conventional TEM stain darkly, have a bright appearance. *Inset* shows phosphorus-enhanced image obtained by computer-assisted ESI analysis. Lamellar bodies (*1*, *2*, *3*) exhibit the highest phosphorus intensities

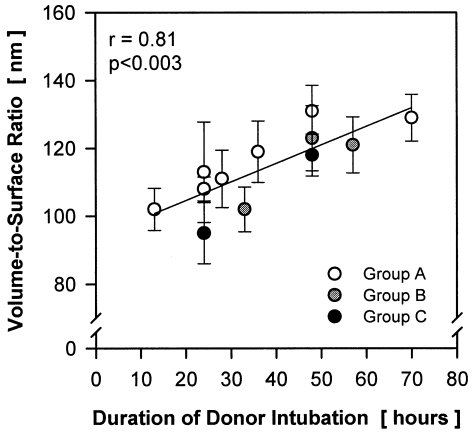


Fig. 9 Volume-to-surface ratios of mitochondria, a stereological estimate of mitochondrial swelling, increases with the time of donor intubation. It appears to be independent of the absence (group A) or presence of contusions (group B) or the development of postoperative respiratory complications (group C). Each *point* represents the mean value (± SD) in an individual case, obtained by analysis of five systematic random samples per lung

cases (Fig. 7). In the cases studied, computer-assisted phosphorus imaging by ESI revealed the normal image of highest signals obtained over lamellar bodies (Fig. 8).

A search for potential relationships with donor-related variables (Table 4), correlation analysis, and ANOVA revealed no significant relationships between any of the stereological parameters and gender, age, cause of death, preoperative PO_2: FiO_2 ratio, PCO_2, or the presence of lung contusions. While lamellar body size (VsR,lb) was positively correlated with ischaemic time, it was inversely related to the fraction of inspired oxygen (FiO_2). The donor-related variable that had the most prominent effect on the ultrastructure of the alveolar type II pneumocytes was the duration of donor intubation. There were highly significant relationships between intubation time and swelling of the cytoplasm and of the mitochondria (Fig. 9), respectively. In addition, significant positive correlations were observed between intubation time and swelling of cells and of nuclei (Table 4).

A search for potential relationships with recipient-related postoperative variables (Table 5), correlation analysis, and ANOVA revealed no significant correlations between any of the clinical variables and any of the stereological parameters characterizing the swelling of cells, cytoplasm, nucleus, and mitochondria. However, significant positive relationships were seen between the area of lamellar bodies, an indicator of the amount of stored surfactant, and the PO_2: FiO_2 ratios determined at 6 and 12 h after transplantation. The volume-to-surface ratio of lamellar bodies, indicative of the mean lamellar body size, correlated with the duration of postoperative intubation. Although group C consists of only 2 cases, it is interesting to see that both stereological parameters achieved considerably higher values in this group (patients with early respiratory complications) than in

Table 4 Summary of correlation analysis[a] between the pooled stereological and donor-related variables (groups A and C)

Clinical/stereological variables	VsR, ce (µm)	A, cy (µm²)	VsR, nu (nm)	VsR, mi (nm)	VsR, lb (nm)	A, lb (µm²)
Gender (male vs female)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Age	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Weight	n.s.	n.s.	n.s.	r=0.69 P<0.05	n.s.	n.s.
Cause of death (ICB vs HBT)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Intubation time	r=0.65 P<0.05	r=0.82 P<0.01	r=0.62 P<0.05	r=0.81 P<0.01	n.s.	n.s.
Inspired oxygen fraction, FiO_2	n.s.	n.s.	n.s.	n.s.	r=–0.69 P<0.05	n.s.
PO_2:FiO_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
PCO_2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ischaemic time	n.s.	n.s.	n.s.	n.s.	r=0.62 P<0.05	n.s.
Contusion (yes vs no)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[a] P<0.05 is considered significant, and the corresponding coefficient of correlation r is given. P>0.05 is considered not significant (*n.s.*)

Table 5 Summary of correlation analysis between the pooled stereological and recipient-related variables (groups A and C)

[a] $P<0.05$ is considered significant, and the corresponding coefficient of correlation r is given. $P>0.05$ is considered not significant (*n.s.*)
[b] Note that these P-values are based on comparison of 7 lungs (group A) against 2 lungs (group B)

Clinical/stereological variables	VsR, ce (μm)	A, cy (μm²)	VsR, nu (nm)	VsR, mi (nm)	VsR, lb (nm)	A, lb (μm²)
PO_2:FiO_2 6 h p.o.	n.s.	n.s.	n.s.	n.s.	n.s.	r=0.78 P<0.01
PO_2:FiO_2 12 h p.o.	n.s	n.s	n.s	n.s	n.s	r=0.68 P<0.05
PO_2:FiO_2 24 h p.o.	n.s	n.s	n.s	n.s	n.s	n.s
Intubation time	n.s	n.s	n.s	n.s	r=0.73 P<0.05	n.s
Early complications (group A vs C)	n.s	n.s	n.s	n.s	P<0.001[b]	P=0.005[b]

Table 6 Summary of multiple stepwise regression procedure for postoperative dependent variables[a] (groups A and C)

[a] Independent variables offered were: donor intubation time, transplant ischaemia, volume-to-surface ratios (*VsR*) of nucleus (*nu*), mitochondria (*mi*), and lamellar bodies (*lb*), and area (*A*) of cytoplasm (*cy*) and lamellar bodies (*lb*)

Dependent variable (log10)	Step	Variable entered	Adjusted r^2	r^2 change	F	P-value
PO_2:FiO_2, 6 h p.o.	1	A, lb	0.525	0.585	9.854	0.016
PO_2:FiO_2, 24 h p.o.	1	Donor intubation time	0.357	0.437	5.435	<0.001
	2	VsR, mi	0.828	0.434	20.206	0.004
Intubation time, recipient	1	VsR, lb	0.318	0.403	4.726	0.012
	2	Donor intubation time	0.662	0.344	8.138	0.008
	3	A, lb	0.826	0.145	6.654	0.049

group A (patients characterized by an uneventful early postoperative course).

The relationship between ultrastructural features of the alveolar type II pneumocytes of human donor lungs and the postoperative clinical course is further supported by the results of stepwise multiple regression analysis (Table 6). The following parameters were offered as independent variables: donor intubation time, ischaemic time, volume-to-surface ratios of nucleus, mitochondria and lamellar bodies, and cytoplasmic and lamellar body area per cell. We now summarize most interesting points. (1) The area of lamellar bodies was the only parameter to be significantly related to the PO_2: FiO_2 ratio achieved at 6 h after surgery. (2) the PO_2: FiO_2 ratio at 24 h after surgery was related to the mitochondrial volume-to-surface ratio, an indicator of mitochondrial swelling, and to donor intubation time. (3) The duration of postoperative intubation could be predicted by a linear combination of both volume-to-surface ratio and area of lamellar bodies together with donor intubation time. Notably, the duration of ischaemia did not influence the variables in the early postoperative period.

Discussion

After the clinical introduction of single- and double-lung transplantation (SLTx, DLTx) in the mid-1980s, a total of 3194 SLTx and 1845 DLTx had been reported to the Registry of the International Society for Heart and Lung Transplantation by February 1996 [17]. The 1-year actuarial survival of all registered SLTx was about 70%. Recently, survival has improved, as shown by comparison of the 3-year periods of 1988–1991 and 1992–1995 [17]. At Hanover Medical School, a 1-year survival rate of 84% after SLTx (n = 40) has been reported [16]. Despite these encouraging results, lung transplantation continues to be plagued by early and late postoperative complications [4, 27].

The study of lung transplantation pathology has revealed some important clues to the reasons for postoperative complications, particularly those that develop later than the 1st day after transplantation [27, 28, 30]. On the basis of chest radiography, reperfusion oedema was reported to have been present within the first 3 days in 144 of 148 lungs transplanted at Washington University School of Medicine, St. Louis, between June 1991 and September 1993 [1]. Little is known, however, about clinical lung pathology related to the early graft dysfunction apparent shortly after transplantation [33, 44]. On the basis of a number of recent experimental studies, however, Novick et al. [25] pointed out that the integrity of type II pneumocytes is an important factor in pulmonary ischaemia-reperfusion injury.

Our investigation is the first clinicopathological study of the ultrastructure of alveolar type II pneumocytes that has yielded direct evidence to support the hypothesis that intracellular surfactant alterations are involved in the development of reimplantation-related respiratory failure. Since transplanted and nontransplanted donor lungs were exposed to identical influences until SLTx and fixation by airway instillation, respectively, the quality of each transplanted donor organ at the time of transplantation can be inferred from the ultrastructural characteristics of the corresponding nontransplanted donor lung. Thus, we can argue that if any significant correlation between ul-

trastructural parameters and postoperative variables is seen, the parameter in question can be considered to be a potential cause, and not a mere consequence, of reimplantation-related events.

It must be borne in mind that only 12 cases have been examined. Our main findings are interesting nonetheless. The duration of ischaemic storage of donor lungs failed to show any significant relationship with ultrastructural or postoperative clinical variables. The duration of preoperative donor intubation was the clinical donor-related variable that interfered most strongly with the type II pneumocyte ultrastructure and with postoperative clinical variables. The stereologically estimated amount of surfactant per cell and the mitochondrial volume-to-surface ratio were the only ultrastructural parameters that were significantly associated with early postoperative oxygenation. The intracellular surfactant-storing lamellar bodies were the only ultrastructural components that appeared to be significantly related to the postoperative intubation time.

In a retrospective clinical study of lung transplant oedema, radiographic lung scores did not correlate with lung ischaemic times [1]. Correspondingly, an effect of ischaemic time on the amount of intracellular surfactant cannot be inferred from our data, in agreement with experimental ultrastructural observations in dogs [10, 14] and bronchoalveolar lavage (BAL) studies in rabbit [39] and canine lungs [6, 9, 41]. In contrast, alterations of BAL surfactant have been reported to increase in severity with ischaemic time in the rat [2], findings that have been commented critically [9]. In the present study of human donor lungs, we observed a significant increase in mean lamellar body size with ischaemia, which was not seen in our study of canine lungs [14]. These discrepancies may be due to species differences, as was indicated in the study of human and canine bronchial epithelium [31], although we cannot rule out a neurogenic effect, since all human lung donors died of cerebral causes. In the rat, increased intracranial pressure has been shown to result in early alterations of lung ultrastructure associated with severe haemorrhagic intraalveolar oedema [18], changes that were not seen in the human lungs studied.

Our data indicate that type II pneumocytes were affected by the duration of mechanical respiratory assistance, which is in line with the general experience of the negative effects of prolonged mechanical ventilation [25]. Swelling of cells and organelles other than lamellar bodies increased with intubation time. The mean size of lamellar bodies decreased with increasing inspired oxygen fraction. This has also been observed by increasing the time of isolated perfusion and ventilation rat lungs [34]. These observations are in line with reports of adverse effects of mechanical ventilation or high oxygen concentrations on surfactant and respiratory function [15, 38].

The determination of intracellular surfactant by morphometry was shown to correlate well with biochemical measurements [43]. Our microanalytical study using electron spectroscopic imaging [26] indicates that the phospholipid content of the lamellar bodies was not decreased in the human donor lungs studied. The amount of lamellar bodies per cell correlated with the early postoperative oxygenation achieved after SLTx. Although it was the only significant predictor of early postoperative oxygenation, it accounted for only about 50% (adjusted r^2) of the variation observed. As was shown in previous studies of human donor lungs [12, 42], pre-existing damage to type I pneumocytes also correlated with early postoperative oxygenation. Both parameters together account for about 73% of the variation in oxygenation achieved 6 h after SLTx in the cases studied (H. Fehrenbach, unpublished work).

Notably, both cases presenting with a decline in early postoperative oxygenation were characterized by the highest values for both mean size and amount of surfactant-stored in lamellar bodies. While, on first glance, this may appear to be a paradox, it must be taken into account that the presence of higher amounts of intracellular surfactant and/or larger lamellar bodies may indicate that the intracellular metabolism and/or secretion of surfactant stored in are disturbed. In an experimental ARDS model of endotoxin-induced respiratory distress, type II pneumocytes of rat lungs were shown to form giant lamellar bodies and the volume-to-surface ratio was significantly increased [34]. These effects on intracellular surfactant have recently been shown to be paralleled by alterations in secreted alveolar surfactant [35]. Although the aetiology of ARDS is considered to be multifactorial, the bulk of the evidence supports a role of surfactant alterations in the development of ARDS [24]. In this respect, the unusual presence of homogeneous lipid bodies in the type II pneumocytes of case XII may be a further indication that intracellular lipid metabolism has been altered. Notably, homogeneous lipid bodies of human type II pneumocytes have been shown by immunoelectron microscopy to be a site of cyclooxygenase activity [7]. Together with experimental evidence of a central role of this arachidonic acid-converting enzyme in the development of endotoxin-induced respiratory distress [36], the high frequency of lipid bodies might be an indicator of the involvement of arachidonic acid metabolites in the development of ischaemia-reperfusion injury or in the type II pneumocytes' response to this.

In conclusion, our study of clinical single-lung transplantations indicates that variations observed in the early postoperative outcome rely at least in part on variations in the ultrastructure of alveolar type II pneumocytes already present at the time of transplantation. The findings provide direct evidence supporting the hypothesis that alterations to type II pneumocytes are a source of donor lung-related early postoperative respiratory dysfunction.

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