Eur Respir J
1991, 4, 316–323

Lack of type II cells and emphysema in human lungs

C.J.M. Otto-Verberne, A.A.W. Ten Have-Opbroek, L.N.A. Willems, C. Franken, J.A. Kramps, J.H. Dijkman

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ABSTRACT: Ten surgically removed human lungs or lobes were studied, to assess the relationship between the abundance of type II alveolar epithelial cells and the degree of emphysema. Type II cell abundance (total number as well as percentage of the total parenchymal cell population) was determined in sections of randomly selected tissue samples of these lungs or lobes by using a type II cell specific antibody specific anti-lavage serum (SALS-Hu), which recognizes surfactant-associated proteins. In these tissue samples we also determined the degree of emphysema with the aid of a number of morphometric parameters, destructive index (DI), mean linear intercept (Lm in mm), and the number of normal alveolar attachments on (pre)terminal bronchioles (normal AA·mm^{-1}). We subsequently calculated the Spearman rank correlation coefficients (r_s) between the abundance of type II cells and parameters for emphysema. We found a significant negative correlation between the percentage of type II cells and DI at tissue sample level (r_s=-0.55; p=0.02). We also calculated correlation coefficients between the abundance of type II cells and the degree of small airways disease in (pre)terminal and respiratory bronchioles (SADscore), lung function, age and smoking habits. The results suggest a role for type II cells in the pathogenesis of emphysema.

Eur Respir J., 1991, 4, 316–323.

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Keywords: Pulmonary emphysema; pulmonary surfactant; type II alveolar epithelial cell.

Received: December 12, 1989; accepted after revision August 1, 1990.

Supported by grants from the Netherlands Asthma Foundation.

There are strong indications that elastolytic enzymes play an important role in the pathogenesis of pulmonary emphysema [1–3]. Therefore, many investigations concerning the pathogenesis of emphysema have been focused on the role of elastase inhibitors such as α_1-proteinase inhibitor and antileucoprotease (ALP) [4–8]. The latter is a potent inhibitor of granulocyte elastase [9, 10] produced in the lung by serous cells of bronchial submucosal glands and nonciliated epithelial cells of (pre)terminal and/or respiratory bronchioles [11]. Recent data in the literature suggest that pulmonary surfactant, a surface-active material secreted by type II alveolar epithelial cells [12], may influence the balance between elastases and elastase inhibitors in the lung parenchyma and possibly also at the bronchiolar level. The reported homology of a (part of) surfactant-associated protein B with proteinase inhibitor [13] indicates that surfactant may be able to inactivate elastolytic enzymes. It is also shown that surfactant enhances phagocytosis of bacteria by alveolar macrophages [14] and suppresses T-lymphocyte function [14, 15], which may result in a diminished inflammatory reaction to certain inhaled antigens and, thereby, in a reduced elastolytic enzyme burden of the lung. In addition, the surfactant lining layer may also influence the balance between elastases and elastase inhibitors by association with other elastase inhibitors, as reported for α_1-proteinase inhibitor [16].

Besides the synthesis and secretion of pulmonary surfactant, another important function of the type II cell (a cuboidal cell with a large, approximately round nucleus and microvilli at the alveolar surface) is its function as stem cell for type II and type I alveolar epithelial cells both in the prenatal and postnatal [17–21] and adult [22] lung. As a result of injuries such as those induced by exposure to toxic agents (for instance oxidants, noxious gases and cigarette smoke) [23–26], the type II cells, which are more resistant than the type I cells, begin to multiply and a number of these type II cells subsequently differentiate into type I cells. On the basis of the above mentioned functional/protective properties of the type II cell (capacity to proliferate and regenerate type I cells, and to provide a surfactant lining layer) we hypothesize that the type II cell may play

a role in preservation of the normal architecture of the lung and prevention of emphysema by providing conditions that protect the lung parenchyma from elastolytic enzyme-induced injuries. To the best of our knowledge, the functional state of the surfactant system in lungs of patients with emphysema has not been documented. In the present study, as a first step to assess a possible relationship between type II cells and parenchymal destruction, we determined the abundance of type II cells and the degree of emphysema and small airways disease in ten different human lungs or lobes, removed by surgery for local malignant processes. Subsequently, we calculated the Spearman rank correlation coefficients between the abundance of type II cells (total number as well as percentage of the total parenchymal cell population) and the standard morphometric parameters for emphysema, destructive index (DI), mean linear intercept (Lm in mm), number of normal alveolar attachments on preterminal/terminal (syn. = membranous) bronchioles (normal $AA \cdot mm^{-1}$), small airways disease (SADscore), lung function tests, age and smoking habits.

Patients and material

Lung materials. Permission for the use of human lung tissue was given by the Committee for Medical Ethics of the School of Medicine at the University of Leiden. Human lung tissue was obtained from 8 male and 2 female patients undergoing thoracotomy for a localized malignant pulmonary process. Three patients were lifelong nonsmokers (table 1). As in our earlier study [8], patients having a condition known to influence lung function or morphology, other than chronic bronchitis or emphysema, were eliminated from the study.

Antisera. Specific anti-lavage serum (SALS-Hu) and preimmunization serum (PS) applied in the present study are the same antisera as used in our study on the role of the type II cell in human lung development [27]. The latter report mentions our antiserum preparation procedures in detail. In short, SALS-Hu has been prepared by injecting a rabbit with a surfactant-enriched pellet fraction of lavage material from adult human lung, and absorbing the immune serum with human serum and cross-reactive organs. Immunoblotting showed that this antiserum recognizes surfactant-associated protein A (SP-A) in human lung and lavage fractions [27]. Immunocytochemistry has revealed that SALS-Hu is a lung specific antiserum that recognizes type II alveolar epithelial cells, but no bronchial epithelial cells (including Clara cells) [27, 28].

Methods

Tissue processing. Within one hour after surgical removal the lung specimens were processed by inflation *via* the bronchial tree with Bouin's fixative to a distending pressure of 25 cm fixative during 4 h using balloon-tipped catheters. Subsequently, the specimens were sliced and tissue samples were collected randomly from each slice and processed to paraplast.

Immunostaining. Tissue sections (*ca* 6 μm) obtained from the selected tissue samples were incubated with SALS or PS, diluted from 1:200 up to 1:600 with 0.05 M Tris-HCl buffer (pH 7.6) containing 1% bovine serum albumin. The primary antibody was applied to the sections for an overnight incubation, followed by a 45 min incubation with biotinylated swine rabbit anti-immunoglobulin G (IgG) (Dakopatts Denmark, absorbed with human IgG), diluted 1:500 in Tris-HCl

Table 1. – Characteristics of the 10 lungs or lobes

Subject no.	Age yrs	Smoking habits	Resected area	Type II %	Type II n	Nuclei n	DI %	Lm mm	nAA mm	SADscore (pre)term. br.	SADscore resp. br.	FEV_1 %pred
1	68	0	LUL	11	215	1873	20	0.35	6.5	28	18	77
2	49	0	RLL	6	112	1923	14	0.34	7.1	26	22	83
3	59	0	LLL	11	225	2057	32	0.39	7.7	22	16	109
4	43	27	LLL	12	442	3572	9	0.25	8.3	20	19	84
5	47	78	LUL	9	176	1978	15	0.39	8.3	20	28	66
6	58	55	LUL	8	254	3208	43	0.31	6.9	29	35	45
7	52	24	LUL	6	178	3414	34	0.30	4.5	34	37	61
8	65	-	LL	9	166	1856	41	0.32	7.9	17	21	98
9	60	63	RLL	10	247	2514	45	0.35	7.4	30	41	62
10	62	9	RUL	13	304	2488	19	0.33	6.4	16	14	104
mean	56			9.5	231.9	2488.3	27.2	0.333	7.10	24.2	25.1	79.0
±SD	8			2.4	91.4	674.5	13.3	0.042	1.14	6.0	9.5	21.0

Smoking habits: cigarettes per day; LL: left lung; LUL: left upper lobe; LLL: left lower lobe; RUL: right upper lobe; RLL: right lower lobe; % type II: percentage type II cells of total lung parenchymal cell population; n Type II: total number of type II cells counted; n Nuclei: total number of nuclei counted; DI: destructive index; Lm: mean linear intercept; $nAA \cdot mm^{-1}$: number of normal alveolar attachments$\cdot mm^{-1}$ of circumference of (pre)terminal bronchioles; SADscore: pathological score for small airways disease in (pre)terminal and respiratory bronchioles (br.); FEV_1: forced expiratory volume in one second; %pred: percentage of predicted value.

buffer, and a 45 min incubation with avidin alkaline phosphatase (Dakopatts, Denmark), diluted 1:180 in Tris-HCl buffer (ABComplex AP method). Each incubation was preceded and followed by rinsing with three changes of 0.05 M Tris-HCl buffer (pH 7.6) for 10–15 min. The sections were then stained for alkaline phosphatase, for which the following substrate was used. Two ml naphthol AS-MX phosphate (sodium salt; Sigma, St. Louis, USA) was mixed with 0.2 M Tris-HCl buffer to a total volume of 40 ml (pH 8.6). Just before incubation of the sections, this solution was mixed with a freshly made solution of 30 mg Fast Blue RR salt (British Drug House Ltd, Poole, UK) in 10 ml aqua dest. After 30–90 min of incubation at room temperature (until the blue staining was considered to be optimal), the enzyme reaction was stopped by washing with tap water for 10 min. The sections were postfixed with 4% formalin for 10 min, rinsed in distilled water and counterstained with 0.1% nuclear fast red for about 1 min. Finally, after two more washes (tap water, 10 min, followed by aqua dest), the sections were air-dried, and mounted in DePex (British Drug House Ltd, Poole, UK). Photography on Kodak FX film was performed with a Leitz Dialux, model 20 EB microscope, using a red filter.

Morphometry

Abundance of type II cells. For determination of the abundance of type II cells in immunostained tissue sections, we used a 100× objective and a 8× eyepiece containing a counting grid covering 1.25 mm^2 of the tissue section. Examination was performed without any prior knowledge of data concerning patients' age, smoking habits, lung function tests or morphological parameters for the degree of emphysema. Fields showing airways, respiratory bronchioles, arteries or veins, large areas of connective tissue or lymphocytic infiltrations or artifacts were excluded. Type II cells were identified by the specific blue colour of their cytoplasm, surrounding a (routinely stained) red nucleus. In each section the number of type II cells and the number of red stained nuclei of the total parenchymal cell population were counted for 25 randomly selected microscopic fields. From each lung tissue sample we examined five tissue sections (at least 20 μm apart) and three randomly selected tissue samples were examined from each surgical specimen. The abundance of type II cells was expressed both in absolute (= counted) number and in percentage of the total population of lung parenchymal cells, the latter being calculated by assuming that each parenchymal cell had one nucleus. The number of parenchymal cells is the total number of parenchymal cells minus the number of type II cells. Alveolar macrophages, when recognized by pigmentation and internalized surfactant material, were not counted as parenchymal cells.

Parameters for emphysema and small airways disease. Morphometric data concerning parenchymal destruction, *i.e.* destructive index (DI) and number of normal alveolar attachments·mm^{-1} (normal AA·mm^{-1}), and small airways disease (SADscore) were obtained as described in a former study [8]. The mean linear intercept (Lm) was determined in tissue sections of all tissue samples lacking evidence of atelectasis, according to DUNNILL [29]. The dimensional data were corrected for tissue shrinkage after fixation [30]. The mean shrinkage factor was found to be 0.81.

Pulmonary function studies. Lung function tests were performed preoperatively, according to standardized methods [31]. These included inspiratory vital capacity (VC), forced expiratory volume in one second (FEV_1) and maximum expiratory flow at 50% of the forced vital capacity (MEF_{50}), obtained by a dry rolling seal spirometer (Mijnhardt Volugraph). Residual volume (RV) was measured by the closed circuit helium equilibration method [31] using the same type of spirometer. Small airways dysfunction was judged from the single-breath nitrogen test [32], by using the slope of phase III ($\Delta N_2 \cdot l^{-1}$). The transfer factor for carbon monoxide (Kco) per litre effective alveolar volume was determined by the single breath-holding method (Morgan Transfer Test) and corrected for the haemoglobin concentration [31]. All lung function variables, except for the ΔN_2, were expressed as percentage of the predicted value [31].

Statistical analysis

Abundance of type II cells. For each tissue section, the percentage of type II cells was calculated as described above. From the data of the five sections the mean values of each tissue sample were computed. The patient mean values were calculated from the data of three tissue samples in each case.

Other variables. Calculation of tissue sample mean values and patient mean values of data concerning DI, SADscore, and normal AA·mm^{-1} were described previously [8]. The patient mean values concerning Lm were computed as mean of Lm values determined for all tissue samples examined for each lung or lobe.

Correlations between variables for patient level and (when appropriate) for tissue sample level were calculated using the Spearman rank correlation coefficient (r_s). A one-sample t-test was performed to assess whether r_s was significant, using a significance level of 0.05.

Results

Type II cells showed a pronounced cytoplasmic (dark) staining (fig. 1a) after immunoincubations with SALS-Hu according to the ABComplex AP-method. The clear colour-contrast of the alkaline phosphatase reaction (dark blue label) enabled the differential counting of type II cells in the present study. Immunoreactivity to SALS-Hu indicates that surfactant associated protein A (SP-A) is present in the cells [27]. Macrophages (fig. 1a) that internalized pulmonary surfactant stained faintly by SALS-Hu, but could

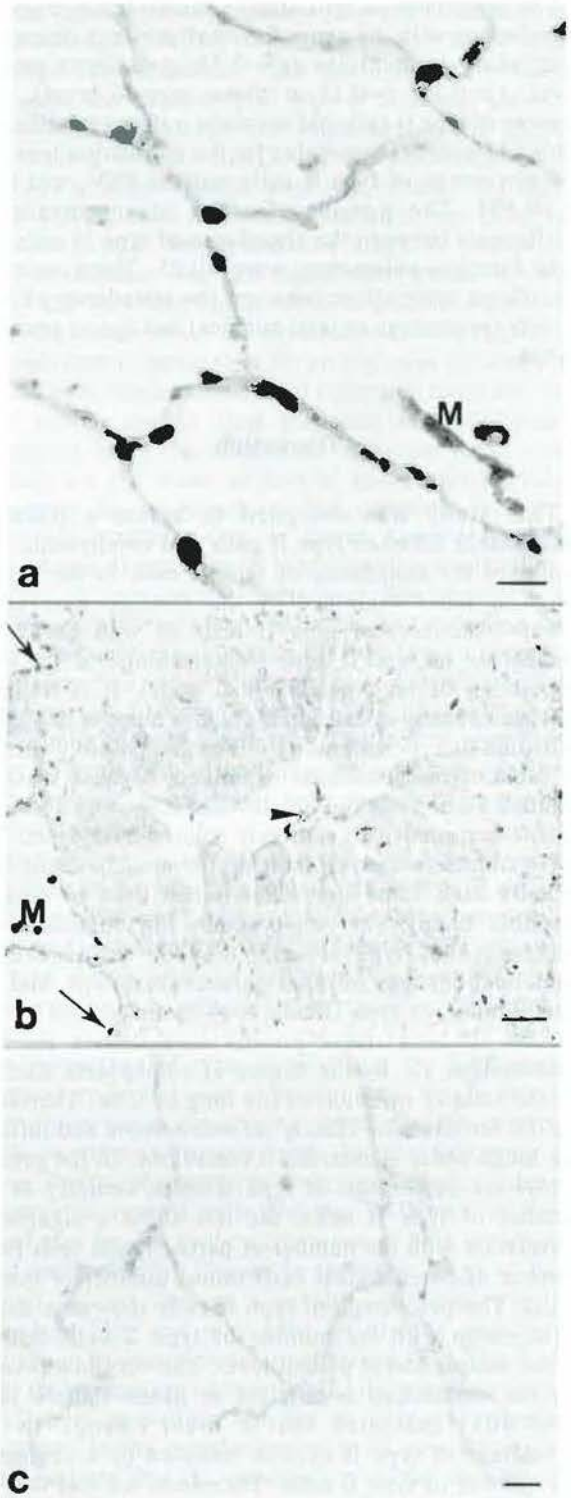

Fig. 1. – Tissue sections of human lung immunoincubated (ABComplex AP-method) with SALS-Hu (a and b) or PS (c). a) Type II cells exhibit a pronounced dark (= brilliant blue) stained cytoplasm, which enables differential counting. Alveolar macrophages (M) usually contain a yellow/brown pigmentation and often show some reactivity to SALS-Hu. Bar is 6.2 mm. b) Bronchial epithelium is not stained by SALS-Hu. Some specific staining of surfactant containing material can sometimes be seen in the bronchial lumen (arrowhead). Specifically stained type II cells (arrows) are present in the lining of surrounding airspaces, in which macrophages (M) may also be present. Bar is 5.4 mm. c) No specific staining is visible in the lung parenchyma after application of PS. Bar is 4.6 mm. SALS: specific anti-lavage serum; PS: preimmunization serum.

usually be easily distinguished from type II cells by their size, their yellow/brown pigmentation, their shape and frequently also by their localization. Bronchial epithelium was not stained by SALS-Hu (fig. 1b), as has been reported previously [27, 28]. Incidentally, some little patches of staining are visible at the luminal surface, probably due to retention of surfactant material/conjugate between epithelial ruffles. Endogenous activity of alkaline phosphatase, which is present in type II cells, was completely abolished by the Bouin fixation, as has been described previously [33]. No staining was detectable after immunoincubations with PS instead of SALS-Hu, see figure 1c.

Table 1 presents relevant data concerning patients' age, smoking habits, FEV_1, resected area and the patient mean values of morphometric data concerning the number of parenchymal cells, abundance of type II cells and the degree of emphysema. The number of red stained nuclei (= total number of parenchymal cells) and the number of type II cells (= red nuclei surrounded by brilliant blue cytoplasm) are expressed as total cell number counted for each patient (lung or lobe), which means that these numbers were obtained by adding the number of cells counted in three tissue samples, five sections each (thus covering 1.25 $mm^2 \times 25 \times 5 \times 3 = 469$ mm^2). The abundance of type II cells is also expressed as percentage (% type II) of the total population of lung parenchymal cells. Lung function tests revealed that five of the ten patients had a $FEV_1 \leq 80\%$ predicted. The mean FEV_1 of the patients was 79.0% predicted (range 45–109%), the mean VC was 91.9% (range 80–105%), and mean RV was 119.3% predicted (range 67–154%).

Table 2. – Spearman rank correlation coefficients (r_s) with p-values

	% Type II		n Type II		
	r_s	p	r_s	p	n
Patient level					
n Type II	0.65	0.04			10
Npar	-0.15	0.68	0.56	0.09	10
Tissue sample level					
n Type II	0.74	<0.001			30
Npar	-0.12	0.52	0.47	0.01	30

% Type II: percentage of type II cells; n Type II: number of type II cells; Npar: total number of parenchymal cells minus the number of type II cells; n: number of patients or tissue samples examined.

Table 2 shows that there is a strong relationship between the number of type II cells and the percentage of type II cells (r_s=0.65, p=0.04 at patient level; r_s=0.74, p<0.001 at tissue sample level), but not between the number of parenchynmal cells (total number of parenchymal cells minus number of type II cells) and the percentage of type II cells. The number of type II cells shows a significant correlation with the number of parenchymal cells at tissue sample level (r_s=0.47; p=0.01), but not at patient level. These findings

indicate that in the lungs or lobes examined a change in percentage of type II cells reflects a change in the number of type II cells rather than a (nonspecific) change in number of other types of cells in the lung parenchyma. However, all correlation coefficients are given both for the percentage of type II cells and for the number of type II cells.

Table 3 shows the Spearman rank correlation coefficients between the abundance of type II cells (percentage as well as number) and other variables at patient level, and table 4 at tissue sample level. The percentage of type II cells showed a significant negative correlation with DI at tissue sample level (r_s=-0.55; p=0.02), although this correlation was not significant at patient level. The correlations between the percentage of type II cells and other parameters for parenchymal destruction (Lm and normal AA·mm^{-1}) were not significant. The number of type II cells did not, either at patient level or at tissue sample level, show significant correlations with the morphometric parameters for emphysema.

Table 3. – Spearman rank correlation coefficients (r_s) with p-values between the percentage of type II cells (% Type II), the number of type II cells (n Type II) and other variables at patient level

	% Type II		n Type II		
	r_s	p	r_s	p	n
% DI	-0.26	0.47	0.02	0.96	10
Lm	0.12	0.75	-0.28	0.42	10
normal AA	0.19	0.60	-0.02	0.96	10
SAD membr. br.	-0.53	0.12	-0.03	0.93	10
SAD resp. br.	-0.73	0.02	-0.24	0.51	10
FEV_1	0.62	0.05	0.05	0.88	10
ΔN_2	-0.49	0.18	<0.001	1.00	9
Kco	0.35	0.35	-0.08	0.83	9
MEF_{50}	0.27	0.47	0.37	0.33	9
RV	-0.16	0.65	0.50	0.14	10
VC	0.24	0.51	0.39	0.26	10
Age	0.35	0.33	-0.03	0.93	10
PY	-0.03	0.93	0.36	0.35	9

FEV_1: forced expiratory volume in one second; ΔN_2: single-breath nitrogen test; Kco: transfer factor for carbon monoxide; MEF_{50}: maximum expiratory flow at 50% of the forced vital capacity; RV: residual volume; VC: vital capacity. For further abbreviations see legend to table 1.

Table 4. – Spearman rank correlation coefficients (r_s) with p-values between the percentage of type II cells (% Type II), the number of type II cells (n Type II) and other morphological variables at tissue sample level

	% Type II		n Type II		
	r_s	p	r_s	p	n
% DI	-0.55	0.02	-0.37	0.14	17
Lm	0.18	0.36	-0.28	0.14	29
normal AA	0.35	0.12	0.09	0.70	21
SAD membr. br.	-0.12	0.57	0.08	0.70	24
SAD resp. br.	-0.31	0.11	-0.12	0.53	28

See legend to table 1 for abbreviations.

The percentage of type II cells showed clear negative correlations with the score for small airways disease in respiratory bronchioles (r_s=-0.73; p=0.02 at patient level; r_s=-0.31; p=0.11 at tissue sample level). The number of type II cells did not show a strong relationship with SADscore. The p-value for the correlation between the percentage of type II cells and the FEV_1 was 0.05 (r_s=0.62). The p-values for the other correlation coefficients between the abundance of type II cells and lung function parameters were >0.05. There were no significant correlations between the abundance of type II cells (percentage or total number) and age or smoking habits.

Discussion

This study was designed to assess a possible relationship between type II cells and emphysema. We indicated the abundance of type II cells in the human lungs or lobes examined by the absolute number (= counted number) of type II cells as well as by the percentage of type II cells (= percentage of the total population of lung parenchymal cells). It is unlikely that we counted an artificially higher number of type II cells due to hypertrophic type II cells, possibly present in some of the specimens examined, because we only counted a cell as a type II cell when it showed a nuclear profile surrounded by antibody stained cytoplasm. The two parameters used to indicate the abundance of type II cells have some limitations when used to study a possible change in emphysema. For instance, the percentage of type II cells may be influenced by numerical changes of other parenchymal cells, and the total number of type II cells may be influenced by the amount of lung tissue present in a certain field of examination, *i.e.* by the degree of emphysema itself or by the state of inflation of the lung or lobe. Therefore, we did not examine clearly inflamed tissue and inflated the lungs under standardized conditions. In the present study, the percentage of type II cells, contrary to the number of type II cells, did not show a significant correlation with the number of parenchymal cells (total number of parenchymal cells minus number of type II cells). The percentage of type II cells showed a strong relationship with the number of type II cells both at tissue sample and at patient level. The very low p-value for the correlation coefficient at tissue sample level (p<0.001) indicates that a local change in the percentage of type II cells is reflected by a change in the number of type II cells. Therefore, we feel that the percentage of type II cells may be the most suitable and reliable parameter to establish a possible change in abundance of type II cells in emphysematous lungs. It seems reasonable to assume that the percentage of type II cells, compared to the absolute number of type II cells, is less influenced by possible differences in compliance among the lungs.

For the assessment of parenchymal destruction we used standard morphometric parameters for emphysema, *i.e.* DI, Lm and normal AA·mm^{-1}. Earlier studies had already shown that in human specimens the Lm is a

much less sensitive parameter then the DI and the normal AA·mm^{-1} [34, 35, 38]. In the present study we calculated the Spearman rank correlation coefficients between the abundance of type II cells and the above mentioned parameters for emphysema. The negative correlation between the DI and the percentage of type II cells at tissue sample level was found to be significant and indicates that lower number of type II cells are present in more emphysematous lungs. At patient level this correlation was not significant, which is in line with the fact that emphysema is present focally. All correlations between the abundance of type II cells and morphometric parameters for emphysema showed lower p-values at tissue sample level compared to patient level. We assume that in these predominantly mild emphysematous lungs the calculation of patient mean values, which are the mean of several tissue sample values, obscures the relationships. We conclude that in the microenvironment (*i.e.* at tissue sample level) the abundance of type II cells is negatively correlated to the degree of parenchymal destruction. The remaining correlation coefficients (both at patient and at tissue sample level) between the abundance of type II cells (expressed as percentage or as total number) and morphometric parameters for emphysema (DI, Lm and normal AA·mm^{-1}), are not significant. The lack of significance may be explained by the low number of lung specimens examined or the relatively mild degrees of emphysema in our materials.

In the present study we also calculated the correlation coefficients between the abundance of type II cells and the degree of small airways disease, lung function, age and smoking habits to find out whether the abundance of type II cells was also influenced by these parameters. The results, *i.e.* significant correlations between the percentage of type II cells and the FEV_1 and between the percentage of type II cells and the SADscore in respiratory bronchioles at patient level, are in line with our observations concerning lower number of type II cells in emphysematous lungs for the following reasons. Firstly, the negative correlation between the percentage of type II cells and the SADscore at patient level may be due to the occurrence of both emphysema and small airways disease in the same lungs or lobes. Small airways disease is usually associated with emphysema [36] and may even be directly involved in causation of centrilobular emphysema [37]. The finding that the percentage of type II cells did correlate with the SADscore at patient but not at tissue sample level (contrary to the correlations with DI, see above), may also indicate that the relationship of the abundance of type II cells to the degree of small airways disease is more circumstantial or indirect than the correlation with parenchymal destruction. Secondly, the FEV_1 is found to be negatively correlated with the severity of emphysema in most clinicopathological studies [35, 38–40]. In conclusion, the correlation coefficients presented in this study indicate that a decrease of the percentage of type II cells goes along with an increase in destruction of lung parenchyma. This conclusion is based on the significantly negative correlation between the percentage of type II cells and the DI at tissue sample level, but as far as all other correlations calculated reach a value of importance they are in line with the conclusion that lower numbers of type II cells are present in more emphysematous lungs.

The decreased abundance of type II cells in emphysematous lungs is in line with the hypothesized protective effect of the type II cell and its secretory product pulmonary surfactant when we assume that emphysematous lungs may have been exposed not once but repetitively and/or incessantly to injurious agents for a long period of time. This assumption is consistent with a recent report [41] mentioning that daily (but not a single) exposure to nicotine resulted in emphysema in rabbits given a threshold dose of elastase. We hypothesize that, under these conditions, the normal rate of proliferation and differentiation of type II cells cannot maintain a sufficient grade of regeneration, possibly also resulting in an inadequate production/formation of the surfactant lining layer. Then a situation may arise in which emphysema is prone to develop because (a part of) the protective capacity of the lung to injuries leading to emphysema is being reduced. The literature provides some data which support the hypothesis that the protective mechanisms of surfactant may (finally) fail or be impaired due to elastolytic enzyme activity. Exposure of the lung to elastase was found to result in type II cell proliferation [42, 43], suggesting that type I or type II cells can be damaged by elastolytic enzymes. *In vitro* studies showed that neutrophilic elastase is capable of cleaving surfactant-associated proteins resulting in a reduced absorbtion rate of surfactant to an air/fluid interface [44], suggesting that elastolytic enzymes may be able to interfere with surfactant function *in vivo*. Furthermore, SANDERSON *et al.* [45] showed that elastase is capable of removing the surfactant lining layer from the pores of Kohn resulting in a significant increase in stress on the elastin skeleton of the alveolar septa and a significant enlargement of the pores. A lower yield of surfactant is reported for human cigarette smokers [46] and for rabbits with experimentally (pancreatic elastase) induced emphysema [47].

The mean percentage of type II cells (9.5±2.4%) as determined in the lungs or lobes in this study, was much lower than the value (15.9±0.8%) reported by CRAPO *et al.* [48]. This difference in percentage of type II cells may be caused by the presence of mild to moderate emphysema in the lungs we examined, whereas the lungs used by CRAPO *et al.* [48] were obtained from autopsy cases and lacked any clinical or radiological signs of respiratory diseases. However, we cannot exclude that the above mentioned difference in percentage of type II cells between the two groups is also affected by differences in techniques used (morphometry at light microscopic level *versus* electron microscopic level), differences in smoking habits (the study of Crapo *et al.* was performed with lungs from subjects with predominantly unknown smoking habits) or by the presence of coin lesions in all subjects of the present study.

In conclusion, we can say that the negative correlations observed between the abundance of type II cells and the degree of emphysema support our hypothesis concerning a possible protective role of the type II cell and its secretory product, pulmonary surfactant, in the pathogenesis of emphysema. Further investigation of underlying mechanisms, for instance in animal models, is required and planned to test this preliminary conclusion.

Acknowledgements: The authors thank J. Hermans from the Dept of Medical Statistics, University of Leiden for contributing to the statistical evaluation of the data, E.C.P. De Vries for technical assistance and J. Lens for preparing the photographs.

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Absence de cellules de type II et emphysème dans les poumons humains. C.J.M. Otto-Verberne, A.A.W. Ten Have-Opbroek, L.N.A. Willems, C. Franken, J.A. Kramps, J.H. Dijkman.
RÉSUMÉ: Nous avons étudié dix lobes ou poumons humains après résection chirurgicale, pour apprécier les relations entre l'abondance des cellules épithéliales alvéolaires de type II et le degré d'emphysème. L'abondance des cellules de type II (nombre total, ainsi que pourcentage de la population cellulaire parenchymateuse totale) a été déterminée sur des sections d'échantillons tissulaires sélectionnés au hasard dans ces poumons ou lobes, grâce à un anticorps spécifique des cellules de type II (SALS-Hu) qui reconnaît les protéines associées au surfactant. Dans ces échantillons tissulaires, nous avons déterminé également le degré d'emphysème à l'aide d'un certain nombre de paramètres morphométriques (index de destruction = DI, intercept linéaire moyen = Lm en mm, et nombre d'attaches alvéolaires normales sur les bronchioles (pré)terminales = normal AA·mm^{-1}). Nous avons calculé ensuite les coefficients de corrélation de rang de Spearman (r_s) entre l'abondance des cellules de type II et les paramètres emphysémateux. Nous avons trouvé une corrélation négative significative entre le pourcentage de cellules de type II et le DI au niveau des échantillons tissulaires (r_s=-0.55; p=0.02). Nous avons calculé également les coefficients de corrélations entre l'abondance des cellules de type II et le degré d'atteinte des petites voies aériennes dans les bronchioles (pré)terminales et respiratoires (SADscore), la fonction pulmonaire, l'âge et les habitudes tabagiques. Nous concluons que ces résultats suggèrent une intervention des cellules de type II dans la pathogénie de l'emphysème.
Eur Respir J., 1991, 4, 316–323.