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Potential role of Clara cell protein, an endogenous phospholipase A_2 inhibitor, in acute lung injury

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Potential role of Clara cell protein, an endogenous phospholipase A_2 inhibitor, in acute lung injury. P.G. Jorens, Y. Sibille, N.J. Goulding, F.J. van Overveld, A.G. Herman, L. Bossaert, W.A. De Backer, R. Lauwerys, R.J. Flower, A. Bernard. ©ERS Journals Ltd. 1995.

ABSTRACT: It is now recognized that epithelial cells lining airways and alveoli are capable of releasing various mediators, which have the potential to modulate local inflammatory reactions.

The amount of the 16 kDa Clara cell protein (CC16), an inhibitor of phospholipase A_2 activity produced by pulmonary epithelial cells, was measured by means of a sensitive immunoassay in the unconcentrated bronchoalveolar lavage fluid (BALF) of 13 control subjects, and in patients with acute lung injury (14 with the full-blown adult respiratory distress syndrome (ARDS); 21 after standard cardiopulmonary bypass surgery, a known risk factor for ARDS). The level of CC16 was compared with other markers of inflammation with a wide range of molecular weights: albumin (nephelometry); total protein (spectrophotometry); ß$_2$-microglobulin (latex immunoassay); cystatin C (latex immunoassay); α_1-antitrypsin (immunoradiometry), and lipocortin-1 (enzyme-linked immunosorbent assay (ELISA)).

The Clara cell protein (CC16) was detectable in all BALF, and significantly higher levels of this protein were observed in BALF from patients with acute lung injury. Changes in BALF Clara cell protein levels differed from those of α_2-macroglobulin and the natural phospholipase inhibitor lipocortin-1. Alpha$_2$-macroglobulin levels were not significantly enhanced in patients at risk for ARDS, but were increased in patients with ARDS; whereas, lipocortin 1 levels were not elevated in either group. Pretreatment of patients at risk for ARDS with high dose methylprednisolone did not alter the amount of Clara cell protein recovered in BALF. The mean CC16 level in BALF from patients with ARDS who died was significantly lower than from those who survived.

The data presented in this study suggest that pulmonary epithelial cells secrete a natural anti-inflammatory protein during acute lung injury, which might have a protective and immunosuppressive role.

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In the past, epithelial cells lining airways and the alveolar region were regarded as mainly passive, thereby functioning as barriers. It is now recognized that the pulmonary epithelium actively participates in many functions and is capable of releasing various mediators, which have the potential to modulate local inflammatory reactions [1].

Recently, a 16 kDa protein [2] has been found in the epithelium of the rat and human lung [3]. As it was first detected in pulmonary Clara cells, the nonciliated epithelial cells from the terminal bronchioles, this protein was named Clara cell (secretory) protein (CCSP or CC16) [3]. It has been proposed as a natural immunosuppressor [4].

The adult respiratory distress syndrome (ARDS) is an acute lung injury usually secondary to one or more major predisposing conditions, such as sepsis, trauma and extracorporeal circulation [5]. Despite the vast amount of accumulated data on inflammatory mediators released by macrophages and neutrophils during acute lung injury, as assessed by their determination in bronchoalveolar lavage fluid (BALF), there are still large gaps in our knowledge about the mediators involved in the development of acute lung injury. As there is now compelling experimental evidence that pulmonary epithelial cells can produce different mediators, which could modulate the inflammatory response during lung injury, we have determined concentrations of this epithelial cell-derived CC16 in the BALF of patients with ARDS and patients after cardiopulmonary bypass (CPB), a known risk factor for acute lung injury [5]. CC16 was determined by means of a sensitive assay for urinary protein 1, an

alpha-microprotein isolated from the urine of patients with renal tubular dysfunction, which has also been proven to be completely identical to CC16, based on size, subunit composition, amino acid sequence and its discovery in BALF [6]. The level of CC16 in BALF was compared with other markers of inflammation, with a wide range of molecular weights.

Materials and methods

Patient groups

In all cases, informed consent was obtained from the patients or their closest relatives in accordance with the revised Helsinki declaration of 1983. The study protocol and procedure were approved by the Ethics Committee of the University Hospital of Antwerp.

Patients with ARDS. Fourteen patients (10 males and 4 females) aged 28–78 (53±4) yrs (mean±SEM) had established ARDS according to the classical criteria [5, 7]: a history of a predisposing illness; sudden onset of dyspnoea; severe hypoxaemia; widespread diffuse lung infiltrates on chest radiograph without evidence of left ventricular failure (normal cardiothoracic index on chest radiography, no history of heart failure); and a pulmonary capillary wedge pressure <15 mmHg. All patients had scores greater than 2.5 according to the ARDS classification proposed by MURRAY *et al*. [7]. All patients underwent lavage within 12 h, once the diagnosis of ARDS was established and their clinical status was stable enough (intubation, ventilation) to allow bronchoscopy and lavage. Predisposing factors were: CPB (n=8); hypovolaemic shock with excessive transfusion (n=3); postcardiac arrest coma (n=1); disseminated intravascular coagulation (n=1); and parathion poisoning (n=1). Eight of the 14 patients died; five with ARDS observed after CPB and three with other predisposing factors.

Control subjects. Thirteen nonsmoking subjects (5 males and 8 females) aged 26–72 (45±4) yrs were referred to the out-patient clinic for elective bronchoscopy. All subjects had a normal macroscopic view of the airways at bronchoscopy. The cytology of the BALF was normal and culture of the BALF was negative as well as the subsequent clinical investigations (normal chest radiograph, normal spirometric results, normal lung volume). No major diseases were found, and the patients did not take any medication.

Patients at risk for ARDS. Thirty four postcoronary bypass surgery patients underwent bronchoalveolar lavage (BAL) 4 h after the end of the extracorporeal circulation. Twenty one male patients (aged 44–75 (59±2) yrs) underwent standard CPB, and 13 male patients received 30 $mg \cdot kg^{-1}$ methylprednisolone (MP) (Solumedrol®, Upjohn Ltd, Crawley, UK) intravenously after induction of anaesthesia in an attempt to suppress BALF signs of lung inflammation. The preoperative care and medication, general anaesthesia, priming solution, type of extracorporeal support system, and postoperative care were standard in all patients, and have been described previously [8]. Briefly, anaesthesia with endotracheal intubation and balanced administration of premedications and transfusions was uniform in all cases (lorazepam, fentanyl, isoflurane, diazepam, pancuronium, cephazolin, and lidoflazine). CPB equipment, consisting of a Bentley membrane silicone oxygenator (System CM50; Baxter Bentley Lab., Irvine, CA, USA) was uniformly employed. The pump-oxygenator system was primed with 1,800 mL of crystalloid solution (Plasma-Lyte A®; Baxter, Lessines, Belgium) and 400 mL of human albumin 20% (Merieux, Brussels, Belgium). Anticoagulation was obtained by 300 $U \cdot kg^{-1}$ heparin. All patients were cooled at 28°C. Myocardial protection was achieved during the intermittent cross-clamp technique. Fifteen minutes after decannulation, heparin was neutralized with protamine sulphate in a 1:1 ratio. None of the patients included in this group developed ARDS afterwards. None of these patients had a history of left ventricular failure (normal cardiothoracic index on chest radiography, no history of heart failure, pulmonary capillary wedge pressure <15 mmHg). Bacterial cultures both of the initial bronchial aspirate and the lavage fluid were negative for all ARDS and postsurgical patients. At the time of lavage, all patients (ARDS and patients at risk) were afebrile.

Bronchoalveolar lavage (BAL)

ARDS and postsurgical patients were still sedated and mechanically-ventilated at the moment of the lavage. A flexible bronchoscope (Olympus, type P20Dn, outer diameter 5 mm, inner diameter 2 mm) was passed through the endotracheal tube of the ventilated patients after preoxygenation ($F_{I,O_2}=1$). In control subjects, bronchoscopy was performed by a standard procedure after premedication with diazepam/atropine and local anaesthesia with lidocaine. After wedging into the right middle lobe, three successive 50 mL aliquots of 0.9% saline were instilled and immediately aspirated. The aspirate of the first aliquot was discarded to prevent bronchial contamination [9], and the recovered fluid of the second and third aliquots were pooled and kept at 4°C. Blood tinged lavages were not used. After gauze filtration, the lavage fluid was immediately centrifuged at 500×g for 10 min, and aliquots of the cell-free supernatant were stored at -25°C until analysis. The cells of the pellet were resuspended in 10 mL of Dulbecco's phosphate buffered saline and counted with a Coulter counter; cell differentiation was performed on cytospin preparations, stained according to May-Grünwald Giemsa. A minimum of 300 cells was examined.

Assay of CC16

The concentration of CC16 in BALF was determined by a sensitive immunoassay relying on the agglutination of latex particles [10]. Detailed descriptions of this

immunoassay have been published recently in its application to urinary protein-1 [6], and its detection in BALF [11].

The analytical recovery (measured over a period of 2–3 days) of CC16 from BALF averaged 94% (SD 9.3%) when CC16 was added to samples of BALF. CC16 from different BALFs showed a complete identity with the protein-1 (α-microprotein) isolated from the urine of patients with renal tubular dysfunction [6].

Western blot analysis

To further check the specificity of the CC16 assay, a Western blot analysis was performed on pooled BALF samples (n=5 for each patient group). The samples were concentrated 20 times by lyophilization prior to the analysis. They were subjected to electrophoresis on polyacrylamide gel (20%) in the presence of 0.1% sodium dodecyl sulphate and electrophoretically blotted on nitrocellulose membranes using the Phasystem (Pharmacia-LKB Biotechnology AB, Uppsala, Sweden). The membranes were then incubated overnight with a blocking buffer (5% w/v nonfat dry milk in TRIS buffered saline containing 0.02% Tween (TTBS)). They were then incubated at room temperature for 1 h with a rabbit polyclonal antibody against CC16 (anti-protein-1 antibody; Dakopatts, Glostrup, Denmark; dilution 1/1,000). The bands were revealed using the Amplified Alkaline Phosphatase Immuno-Blot assay kit of Bio-Rad (Bio-Rad Laboratories, Hercules, CA, USA). Briefly, after three washings with TTBS, the membranes were incubated for 1 h with a 1/3,000 dilution of a biotinylated goat anti-rabbit antibody. They were then washed twice with TTBS and incubated for 1 h with a streptavidin-biotinylated alkaline phosphatase complex. Colour development was performed by using a solution of nitro-blue tetrazonium/5-bromo-4-chloro-3-indolyl phosphate.

Assay of other proteins

Albumin was determined by immune nephelometry. Total protein concentration in BALF was measured by the Bio-Rad protein assay (Bio-Rad, Richmond, CA, USA). Beta$_2$-microglobulin and the cysteine proteinase inhibitor, cystatin C, were measured by latex immunoassay [12]. Alpha$_2$-macroglobulin and α_1-antitrypsin were determined by an immunoradiometric assay as described previously [13].

Free BALF lipocortin-1 was measured by a sandwich enzyme-linked immunosorbent assay (ELISA; detection limit 500 pg·mL^{-1}) as described previously [14].

Statistical analysis

Data are presented as the mean±SEM or as the geometric mean with the range in parenthesis. One-way analysis of variance (ANOVA) followed by a two-tailed Student's t-test with the Bonferroni correction for multiple comparisons was used to assess differences between groups. Statistical significance was assumed when the p-value was less than 0.05.

Results

General characteristics of the BALF

The characteristics of the BALF are summarized in table 1. There was no significant difference in volume of BALF recovered from the four groups.

The percentage of neutrophils recovered and the concentrations of albumin and total protein were significantly higher in the patients studied after CPB and the ARDS group as compared to the control subjects. Pretreatment with MP did not alter the total protein concentration or percentage of neutrophils in the patient group after CPB, as described previously [8].

Concentrations of different proteins (table 1)

The concentrations of β_2-microglobulin, α_1-antitrypsin and α_2-macroglobulin in the BALF of control subjects were well within the range reported previously [11, 12]. In patients at risk for ARDS with no MP-pretreatment, there was a small but significant increase in the level of β_2-microglobulin and α_1-antitrypsin, but not of α_2-macroglobulin as compared to control patients. MP pretreatment caused no significant change in cystatine C or α_2-macroglobulin levels as compared to non-MP-pretreated patients. High levels of β_2-microglobulin, α_1-antitrypsin and α_2-macroglobulin were found in the BALF of patients with full-blown ARDS, again comparable with those reported previously in ARDS patients [15], reflecting both an important inflammatory reaction and increased protein permeability of the normal alveolar capillary membrane.

Concentrations of CC16

Western blot of rabbit anti-CC16 antibody tested against pooled BAL samples (n=5) from all patient groups and against purified CC16 showed that the antibody used is specific and recognizes only the CC16 band (fig. 1).

The concentration of CC16 was significantly higher ($p<0.05$) in the BALF from patients after CPB and those with established ARDS as compared to control subjects (table 2 and fig. 2). Pretreatment with MP did not significantly alter the levels of CC16 in BALF of patients at risk for ARDS (table 2).

When values of CC16 in BALF were expressed in relation to those of albumin (table 2), a significant increase was only present in both groups after CPB and not the ARDS group. CC16 represented 0.95±0.19% (mean±SEM) of the total protein content of BALF after CPB, as opposed to 0.36±0.07 and 0.33±0.10% in normal control patients and patients with ARDS, respectively.

Table 1. – Bronchoalveolar lavage characteristics

	Controls (n=13)	CPB surgery No MP-pretreatment (n=21)	CPB surgery MP-pretreatment (n=13)	ARDS (n=14)
Volume recovered mL	41±5	48±4	45±4	49±5
Total cell count $\times 10^6$	7.6±1.3	12.8±2.2	6.3±1.1	18.9±3.8*
PMN %	1.5±0.5	5.7±2.0*	5.1±1.4*	46.5±5.8*
Protein $\mu g \cdot mL^{-1}$	54 (24–152)	148 (52–518)*	107 (29–418)*	330 (75–1837)*
Albumin $\mu g \cdot mL^{-1}$	28 (9–91)	57 (19–94)*	49 (10–202)*	161 (43–761)*
β_2-microglobulin $\mu g \cdot L^{-1}$	40 (14–123)	103 (34–300)*	59 (9–202)	133 (29–750)*
Cystatin C $\mu g \cdot L^{-1}$	4 (2–16)	9 (2–66)	7 (1–36)	9 (2–46)
α_1-antitrypsin $\mu g \cdot L^{-1}$	0.42 (0.10–3.16)	1.6 (0.2–8.8)*	1.0 (0.01–7.80)	19.8 (1.8–200)*
α_2-macroglobulin $\mu g \cdot L^{-1}$	0.17 (0.02–3.15)	0.28 (0.04–2.30)	0.29 (0.03–5.08)	3.2 (0.24–20)*

Data are presented as mean±SEM or geometric mean and range in brackets. *: significantly different from normal controls, p<0.05. PMN: polymorphonuclear neutrophils; CPB: cardiopulmonary bypass; ARDS: adult respiratory distress syndrome; MP: methylprednisolone.

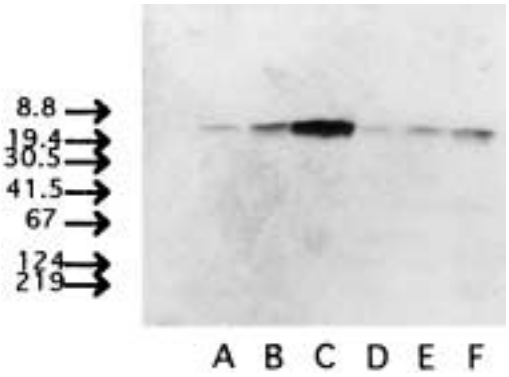

Fig. 1. – Westen blot of rabbit anti-CC16 antibody tested against pooled BALF samples (n=5) from patients after coronary bypass (lanes A and E), with the adult respiratory distress syndrome (lane D), control healthy subjects (lanes B and F) and against purified CC16 (500 $mg \cdot L^{-1}$, lane C). The immunoreactive bands were detected with a biotinylated goat anti-rabbit antibody and the formation of a streptavidin-biotinylated alkaline phosphatase complex. Molecular weight standards are indicated to the left. CC16: Clara cell protein; BALF: bronchoalveolar lavage fluid.

The rise in CC16 was not accompanied by an increase in free BALF lipocortin-1 levels, which did not differ significantly between all groups investigated (table 2). No correlation was observed between any characteristic of the BALF and the amount of CC16 observed.

The level of CC16 in BALF of ARDS patients who died (n=8; 441±100 $\mu g \cdot L^{-1}$, (mean±SEM); geometric mean 359 $\mu g \cdot L^{-1}$) was significantly lower (p=0.03) than in patients who survived (n=6; 1,164±308 $\mu g \cdot L^{-1}$ (mean± SEM); geometric mean 913 $\mu g \cdot L^{-1}$) (table 3 and fig. 3). This was unique as the mean level of the other proteins in BALF of patients who died did not differ significantly from the levels observed in ARDS patients who survived (table 3). The level of CC16 in BALF of those patients in whom ARDS developed after CPB (n=8; 707±140 $\mu g \cdot L^{-1}$ (mean±SEM); geometric mean 585

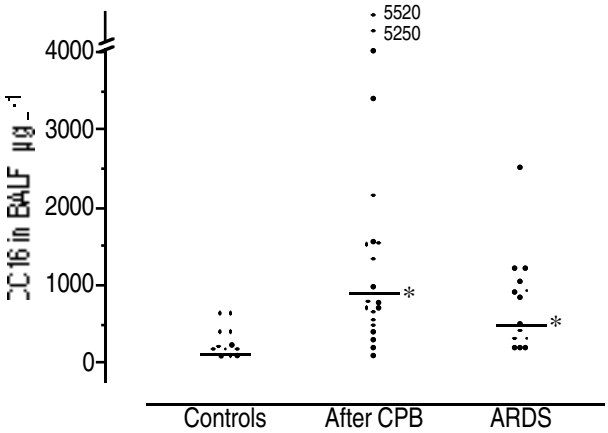

Fig. 2. – Clara cell protein (CC16) in the bronchoalveolar lavage fluid (BALF) of nonsmoking control patients (n=13), patients at risk for ARDS lavaged after CPB (n=21) and patients with ARDS (n=14). Horizontal bars represent the geometric mean. ARDS: adult respiratory distress syndrome; CPB: cardiopulmonary bypass. *: significantly different from controls, p<0.05.

Table 2. – Clara cell protein concentrations in bronchoalveolar lavage fluid

	Controls (n=13)	CPB surgery No MP-pretreatment (n=21)	CPB surgery MP-pretreatment (n=13)	ARDS (n=14)
CC16 $\mu g \cdot L^{-1}$	161 (70–605)	943 (111–5520)*	558 (128–4940)*	544 (159–2500)*
Lipocortin-1 $\mu g \cdot L^{-1}$	7.3 (1.8–133.8)	8.3 (0.9–71.5)	7.7 (1.3–50.3)	8.9 (2.1–37.5)
CC16/albumin ratio %	0.55 (0.08–1.91)	1.61 (0.26–5.64)*	1.15 (0.23–12.05)	0.39 (0.04–7.52)
CC16/protein ratio %	0.28 (0.05–0.79)	0.7 (0.12–3.2)*	0.52 (0.09–6.1)	0.17 (0.02–1.50)

Data are presented as geometric mean and range in brackets. CC16: Clara cell protein. *: significantly different from normal controls, p<0.05. For further abbreviations see legend to table 1.

Table 3. – Bronchoalveolar lavage characteristics of ARDS patients depending on their outcome

	Outcome	
	Died (n=8)	Survived (n=6)
Volume recovered mL	49±5	47±9
PMN %	45.1±8.5	41.6±7.9
Albumin $\mu g \cdot mL^{-1}$	161 (43–761)	132 (71–555)
Protein $\mu g \cdot mL^{-1}$	338 (75–1836)	279 (167–954)
β_2-microglobulin $\mu g \cdot L^{-1}$	125 (33–750)	131 (29–438)
Cystatin C $\mu g \cdot L^{-1}$	7 (2–46)	16 (4–46)
α_1-antitrypsin $\mu g \cdot L^{-1}$	12.8 (2.2–200.0)	27.5 (1.8–200.0)
α_2-macroglobulin $\mu g \cdot L^{-1}$	3.2 (0.6–20.0)	2.5 (0.24–20.0)
Lipocortin-1 $\mu g \cdot L^{-1}$	7.8 (2.1–37.5)	9.4 (3.6–29.9)
CC16 $\mu g \cdot L^{-1}$	359 (159–915)	913 (179–2500)*

Data are presented as mean±SEM or geometric mean and range in brackets. *: significantly different from normal controls,

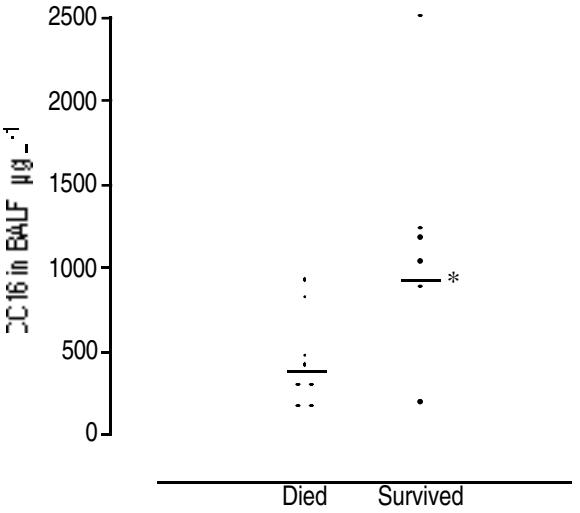

Fig. 3. – Clara cell protein (CC16) levels in the bronchoalveolar lavage fluid (BALF) of ARDS patients who survived and those who died. Horizontal bars represent the geometric mean. *: significantly different from those who died, $p<0.05$.

$\mu g \cdot L^{-1}$) was not different from the level observed in those patients with other predisposing factors (n= 6; 888±350 $\mu g \cdot L^{-1}$ (mean±SEM); geometric mean 610 $\mu g \cdot L^{-1}$).

Incubation of CC16 with BALF of ARDS patients did not result in a significant loss of activity of CC16 in the assay (data not shown).

Discussion

The data presented here show that levels of CC16 well above the detection limit of the assay are found in the BALF of control subjects, patients at risk for lung injury and with ARDS. The levels were significantly higher in those at risk for or with ARDS, as compared to normal controls. Its concentration averaged 0.95% of the total protein level in the BALF after CPB, whilst being approximatively 0.35% in BALF both from ARDS and control subjects.

It is unlikely that the high levels of CC16 found in the various patient groups can be explained by plasma transudation. Indeed, CC16 levels in BALF from control patients are a factor 40 higher than those in plasma, even without taking the dilution by the BALF procedure into account [1, 11]. Also, the probability that the intravascular secretion of CC16 from the urogenital tract could account for or significantly contribute to the respiratory production of CC16 is very low. Moreover, the degree of plasma transudation was minimal in patients after CPB, as revealed by a limited increase of albumin BALF levels. Since CC16 is, therefore, unlikely to derive from serum, it seems reasonable to assume that the marked increase in patients at risk for ARDS reflects an increased local pulmonary production of CC16.

When expressed as its ratio to albumin or total protein, BALF CC16 of ARDS patients tends to be decreased as compared to normals. This decrease, however, did not reach significance. An increased destruction by proteases known to be present in ARDS can be excluded, since incubation of CC16 with BALF from ARDS patients did not result in breakdown of the protein (data not shown).

Only a few cell types could have been responsible for the production and secretion of this CC16 protein. By immunohistochemistry, it has been found in the secretory granules of bronchiolar Clara cells [16]. Human and rat CC16 is homologous to rabbit uteroglobin, and presumably derived from the same ancestral gene [17]. This uteroglobin is a hormonally-regulated, homodimeric, secretory protein, and was first exemplified in the endometrium of the rabbit during early pregnancy [18]. It is also expressed by epithelial cells of other rabbit organs, such as the oviduct, the male genital tract and the digestive tract [19]. It has several biological properties, including potent inhibition of phospholipase A_2 (PLA_2) [19]. It has been shown that uteroglobin is also expressed by tracheobronchial epithelial cells [20], and alveolar type II cells [21]. The physiological role of uteroglobin has, however, not been clarified. By *in situ* hybridization of rabbit lung with a uteroglobin complementary deoxyribonucleic acid (cDNA) probe, intense labelling was found in ciliated and bronchiolar cells of the epithelium of bronchi and bronchioles [22], as well as in alveolar epithelium. Similarities between rabbit uteroglobin and CC16 were first proposed on the basis of immunological cross-reactivity between these proteins [23], and confirmed by the cloning both of the human [24] and rat cDNA [25], their identical distribution [3], and amino acid sequence similarity [6]. CC16 has been identified in and purified from rat [20], and human lung lavage [23, 24]. It has also been demonstrated in nonciliated columnar cells in large and small bronchi, as well as in bronchioles [26].

Uteroglobin has been proposed to function as an antiinflammatory agent, based on its ability to inhibit the activation of PLA_2, an enzyme involved in the production of prostaglandins and other eicosanoids [27], to inhibit thrombin-induced platelet aggregation [28], and

human and rabbit phagocyte chemotaxis [29]. Very little is known about the real function of uteroglobin or CC16 in the lung. Two authors [21, 30] have recently proposed the hypothesis that uteroglobin may have a vital role in increasing the half-life of surfactant by preventing its hydrolysis by PLA_2 and by reducing the level of eicosanoids through PLA_2 inhibition. Alterations of the endogenous surfactant system (abnormal surface tension properties, alterations in surfactant handling, alteration of phospholipid composition, altered metabolism or surfactant inactivation by phospholipases in BALF) contribute substantially to the lung dysfunction in patients with ARDS [31]. Although the hypothesis of a protective role of CC16 *in vitro* has not yet been proved, it would underline our observation of high levels of CC16 in patients at risk for ARDS who did not develop this fulminant acute lung injury and in patients with ARDS who did survive. ARDS is observed after CPB, but its incidence is not higher than 1% [5, 32].

The approximately sixfold increase of mean BALF CC16 levels in patients at risk (after CPB) as opposed to control patients was specific as compared to many other markers of inflammation. In this patient group, there was a nonsignificant change in α_2-macroglobulin, reflecting minimal inflammation [15], and a significant but very moderate increase in β_2-microglobulin and α_1-antitrypsin. Moreover, the rise in CC16 was unique as compared to two other important anti-inflammatory mediators, the cysteine proteinase inhibitor, cystatine C, and lipocortin-1. The glucocorticoid-inducible lipocortin-1 is also a potent inhibitor of PLA_2. Synthetic peptides corresponding to the region of highest similarity between human lipocortin-1 and rabbit uteroglobin inhibit PLA_2, and there is some evidence of anti-inflammatory activity [33, 34].

We are not aware of any study on the determination of CC16 in acute lung injury in humans, and only scanty data are available about the pharmacological modulation of its secretion both *in vivo* and *in vitro*. Classically, ARDS is defined as a typical injury of the alveolocapillary membrane. Although we cannot rule out the possibility that alveolar cells could contribute to the production of CC16, it is reasonable to assume that it is produced mainly by airways [26], in particular bronchiolar epithelial cells. It is, therefore, interesting to postulate that in patients after CPB, at risk for ARDS, not only the alveolar compartment but also the conducting airways are involved.

HACKETT *et al*. [35] have reported that CC16 gene expression was similar in the lungs of control and lipopolysaccharide- or hyperoxia-treated rats, despite marked changes in the histology of the lungs and, hence, the induction of pulmonary inflammation. However, they did not measure the concentration of the protein but only gene-expression. Glucocorticoids induced the uteroglobin gene in rabbit lung explants [36]. We did not observe a higher content of CC16 in BALF of patients at risk for ARDS pretreated with MP. However, this was not surprising in this model, as we have previously published that pretreatment with MP does not prevent pulmonary neutrophil infiltration or albumin transudation after CPB [8]. We also did not observe an increase in BALF lipocortin-1, a PLA_2 inhibitor produced by mononuclear cells, which is known to be increased in mononuclear cells but not in the plasma after corticoid-treatment in humans [14]. These results corroborate the observation that treatment *in vivo* with a high dose of MP is not as effective in preventing lung injury as expected from *in vitro* experiments.

BERNARD and co-workers [11] have reported a small but significant reduction of CC16 in BALF from smokers or patients with chronic obstructive pulmonary disease and lung cancer. Our control patients did not smoke or have any of these underlying disorders, which can therefore not explain a lower CC16 level in the control subjects as compared to the other groups.

Finally, data represented in a study by VOLOVITZ *et al*. [37] indicate that a uteroglobin-like protein was detected in tracheobronchial secretion of children with a mean value of 176 $\mu g \cdot L^{-1}$, comparable to the amounts measured in our study. During acute respiratory illness, the concentration of this protein decreased, which was inversely correlated with the concentration of the leukotriene C_4.

Identification and isolation of markers in BALF might facilitate the study of specific cells in physiological and pathological conditions. The present study suggests that CC16 in BALF may provide a sensitive and specific biochemical marker to assess the role of the epithelium in acute lung injury. Although it remains to be established precisely which epithelial cells secrete CC16 in the respiratory tract, and to elucidate the mechanisms regulating this secretion, we launch the hypothesis that epithelium-derived CC16 might play an important role as a natural anti-inflammatory agent during processes of acute lung injury. Our data warrant a further study to explore whether BALF CC16 levels can discriminate between those patients at risk who do or do not develop ARDS.

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