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Soluble complement receptor type 1 (CD35) in bronchoalveolar lavage of inflammatory lung diseases

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Soluble complement receptor type 1 (CD35) in bronchoalveolar lavage of inflammatory lung diseases. J. Hamacher, S. Sadallah, J.A. Schifferli, J. Villard, L.P. Nicod. ©ERS Journals Ltd 1998.

ABSTRACT: Complement receptor type 1 (CR1) (CD35; C3b/C4b receptor) is a transmembrane protein of many haematopoietic cells. Once cleaved, soluble complement receptor type 1 (sCR1) exerts opposite effects as a powerful inhibitor of complement. This study addressed both the question of whether sCR1 was found in bronchoalveolar lavage (BAL) of normals and patients with various inflammatory disease, and its possible origin.

In this retrospective study covering specimen and clinical data of 124 patients with acute and chronic inflammatory lung pathologies, BAL supernatants were analysed by enzyme-linked immunosorbent assay technique for sCR1. Correlations were made between the sCR1 levels obtained and the constituents of BAL. Human alveolar macrophages were cultivated in order to determine their secretory capacity of sCR1.

Alveolar macrophages from normal subjects were shown to release sCR1 *in vitro*. In addition, sCR1 was present in BAL of normal controls and was significantly increased in acute inflammatory lung diseases such as acute respiratory distress syndrome (ARDS), bacterial and *Pneumocystis carinii* pneumonia, as well as in chronic inflammatory diseases such as interstitial lung fibrosis and sarcoidosis. In BAL of ARDS, bacterial, and *P. carinii* pneumonia, there was a good correlation between sCR1 and the absolute neutrophil counts. In sarcoidosis, a correlation was found with BAL lymphocyte counts. Serum sCR1 was not increased in patients compared to controls.

Soluble complement receptor type 1 (sCR1) is found in the bronchoalveolar lavage in health as well as in acute and chronic inflammatory disease. Alveolar macrophages are capable of releasing sCR1 *in vitro* and may be the main physiological source of sCR1 in the alveoli. The good correlation between sCR1 and the absolute neutrophil or lymphocyte numbers in bronchoalveolar lavage of inflammatory diseases suggests a predominant role of leucocytes for the release of sCR1 in such conditions. The release of this inhibitor of complement may be crucial to control and reduce complement activation and thus prevent lung injury.

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Complement receptor type 1 (CR1; CD35, C3b/C4b receptor) is a transmembrane glycoprotein found on erythrocytes and most white blood cells [1, 2]. For polymorphonuclear leucocytes (PMNs) and monocytes, the expression of CR1 (5,000–40,000 receptors·cell^{-1}) depends on the cellular activation state: it is enhanced on PMNs after activation by agents such as formyl peptides, C5a, leukotriene B4, or tumour necrosis factor (TNF) [3]. On phagocytes, CR1 is mainly involved in the initial binding of particles coated with activated C3 (C3b), which are subsequently ingested [4]. In addition, CR1 serves as a cofactor for the degradation and inactivation of C3b by factor I [5]. Thus, CR1 is not only a cellular receptor, but also a potent inhibitor of both the classical and alternative pathway of complement activation. The suppression of alternative and classical pathway activation in whole serum at nanomolar concentrations led to the conclusion that sCR1 is more than 100 fold more effective than other soluble complement regulatory proteins, *e.g.* factor H, the physiological cofactor for the inactivation of C3b in plasma [4, 6].

In vitro-activated PMNs shed a soluble form of CR1 (sCR1) which corresponds to the extracellular portion of the molecule [6]. An identical soluble CR1 is found in serum [7, 8]. *In vivo* sCR1 was detected in the serum of severe combined immunodeficiency (SCID) mice populated with human peripheral blood leucocytes, indicating that the serum sCR1 derives from leucocytes [7].

Activation of the complement system is intended to protect our body from invading micro-organisms and other insults, but also has the potential to lyse our own cells [5]. Therefore, cells are protected from complement-induced cell lysis by a family of cell-membrane complement regulatory glycoproteins that down-regulate activation of homologous complement on their cell surface [5]. They include membrane cofactor protein (MCP; CD46), decay-accelerating factor (DAF; CD55), CD59 and CR1

(CD35). While MCP and DAF prevent activation of the classic and alternative complement pathways, CD59 prevents formation of complement membrane-attack complexes on the surface of host cells [5]. MCP, DAF and CD59 are found in the whole human respiratory tract epithelium from nose to alveoli, and are even increased in inflamed tissue. CR1 has not been visualized on cells of the respiratory tract [9].

The present study was undertaken to investigate the presence and origin of sCR1 in culture supernatant of alveolar macrophages which are known to express CR1 on their surface [10], in bronchoalveolar lavages (BALs) of normals and patients with inflammatory lung diseases.

Materials and methods

Subjects

One hundred and twenty four patients undergoing routine bronchoscopy were included in this retrospective study. They consisted of a control group of 17 patients who were investigated by bronchoscopy for persistent cough or for a pulmonary nodule, of 31 patients with untreated sarcoidosis, and of 27 patients with interstitial lung fibrosis (classified as idiopathic lung fibrosis in 24 patients, and associated with a lymphocytic alveolitis and an anti JO-1 autoantibody in three cases). Fifteen patients were investigated with adult respiratory distress syndrome (ARDS) defined by: 1) acute onset of respiratory failure; 2) rapid appearance of bilateral alveolar infiltrates on chest radiograph; 3) severe hypoxaemia with arterial oxygen tension (P_{a,O_2})/fractional concentration of oxygen in inspired gas (F_{I,O_2}) ratio <120; and 4) pulmonary wedge capillary pressure <15 mmHg. The last two groups consisted of 24 patients with *Pneumocystis carinii* pneumonia (23 human immunodeficiency virus (HIV)-infected and one cardiac transplant patient without HIV infection) and of 10 patients with bacterial pneumonia defined as the presence of a new infiltrate on chest radiograph with fever and purulent respiratory secretions, in addition to a positive culture (Š10^4 colony-forming units (cfu)·mL^{-1} in eight patients) obtained in the BAL, or a clinical and radiographic response to antibacterial antibiotics.

Bronchoalveolar lavage

Samples were collected according to published methods [11, 12]. Briefly, a fibreoptic bronchoscope was wedged into the segmental bronchus of one lobe and three to four aliquots of 50 mL sterile isotonic saline were instilled and recovered by gentle hand suction. The BAL fluid was pooled and processed within 1 h. After centrifugation (800 × g, 10 min), aliquots of the supernatant were stored at -20°C. Cell counts were determined in a Neubauer chamber. Differentials were made after cytocentrifugation on slides with May-Gruenwald-Giemsa colouration. Cell viability was assessed by Trypan blue exclusion.

Enzyme-linked immunosorbent assay (ELISA) for sCR1

For sCR1 in bronchoalveolar lavage fluid (BALF) and in serum, a sensitive ELISA sandwich assay using two anti-CR1 monoclonal antibodies (MAbs) that recognize different epitopes of CR1 was used as previously described [7], using an Amersham biotinylation kit (Amersham International, Buckinghamshire, UK). Polystyrene microwell plates (Nunc Maxisorp immunoplates, GIBCO BRL, Basel, Switzerland) were coated with 100 µL (0.3 µg·well^{-1}) in phosphate-buffered saline (PBS), pH 7.9) of purified 3D9 MAb and left overnight at room temperature. The plates were washed four times with PBS-Tween 0.05%, and unbound sites were saturated with 2% bovine serum albumin (BSA)-PBS-Tween for 1 h at 37°C. One hundred microlitres of the samples diluted in PBS-Tween were added, and the plates were incubated overnight at 4°C. The plates were then washed four times and subsequently incubated with 100 µL of biotinylated J3D3 MAb diluted at 1/3000 (stock concentration: 0.5 mg·mL^{-1}) for 3 h at 37°C. After washing the plates, 100 µL of peroxidase-conjugated Streptavidin (Jackson Immuno Research Labs, Inc., West Grove, PA, USA) diluted at 1/2,000 was added to each well and incubation continued for 1 h at 37°C. After washing, 100 µL of substrate at a concentration of 0.4 mg·mL^{-1} (orthophenyldiamine, in 50 mL citrate-phosphate buffer, pH 5, with 20 µL of H_2O_2) was added to each well. The reaction was stopped after 10 min with H_2SO_4, and absorbance was measured with a microplate reader (MR 600, Dynatech, 8423 Embrach, Switzerland) at 492 nm). The lower limit of detection in BALF was 0.02 ng·mL^{-1}. A standard of recombinant soluble CR1 was used (a kind gift of SmithKline Beecham Pharmaceuticals, King of Prussia, USA).

Stability testing of sCR1 in BALF supernatant

Immediately after BAL of two patients, the fluid was aliquoted and protease inhibitors (1 mM phenylmethylsulphonyl fluoride (PMSF), 10 mM ethylenediamine tetraacetic acid (EDTA), and 0.5 mM di-isopropylfluorophosphate (DFP)) were added to one aliquot, another receiving the same volume of saline 0.9% and being left at room temperature during 0, 10 and 100 h until measurement of sCR1 by ELISA. Two other BALF supernatant aliquots were exposed to room temperature for 0, 24 and 48 h and to 37°C for 12 h in order to determine stability at that temperature in BALF.

sCR1 measurement in purified human alveolar macrophages (AMs) supernatant with and without simulation by lipopolysaccharide (LPS)

AMs were recovered by *ex vivo* lavage of surgical lung specimens with 0.9% NaCl. The aspirated cell population averaged 88% macrophages, 10% lymphocytes and 2% PMNs as assessed by May-Gruenwald-Giemsa slides. The cells were washed three times with PBS, and 1×10^6 cells·mL^{-1} were adhered for 60 min in RPMI 1640 (Kibbutz Bit Hae Mek, Israel) supplemented with 10% heat-inactivated foetal calf serum, penicillin 50 U·mL^{-1}, streptomycin 50 µg·mL^{-1}, and glutamine 1% (all from GIBCO, Paisley, UK). The dishes were rinsed three times in order to remove all nonadherent cells. The adhered AMs were then cultured at 37°C with 5% CO_2 in the presence or absence of LPS (1 µg·mL^{-1} *Escherichia coli* O55;B5, Difco laboratories, Detroit, MI, USA) for time courses extended to 48 h.

ELISA for free and protease inhibitor complexed PMN elastase

BALF concentration of free PMN elastase and PMN elastase complexed with protease inhibitor (abbreviated as "PMN elastase") was measured by sandwich ELISA (PMN ELASTASE® Kit, Merck Immunoassay, Darmstadt, Germany), with a lower limit of detection of 0.33 $\mu g \cdot mL^{-1}$. Sample dilutions ranging 1:5 to 1:500 were necessary for the BALF fluid supernatants studied.

Protein assay

Total protein in BALF was measured using Biuret technique based on the absorbance of Coomassie Blue to proteins, as described previously [13].

Correlation of results of the P. carinii *pneumonia group with the alveolar-arterial oxygen difference (*$P_{A\text{-}a,O_2}$*)*

The alveolar-arterial oxygen difference was calculated by the simplified formula:

$$P_{A\text{-}a,O_2}\ (kPa) = 19.5 - 1.2 \times P_{CO_2} - P_{O_2}$$

where P_{CO_2} is partial pressure of carbon dioxide and P_{O_2} is partial pressure of oxygen.

Statistical analysis

Data were analysed using the SYSTAT for Windows® software package (SYSTAT Inc., Evanston, IL, USA), using Pearson correlation for the relationship analyses between sCR1, cytokine, protein, and "PMN elastase" levels and cell count. Probabilities were adjusted by the Bonferroni method for multiple comparisons of variables. Due to not normally distributed variables, the Kruskal-Wallis test was used for the analysis of significant differences of a variable between three or more independent groups; if significant, Mann-Whitney test was used when differences between two groups were analysed. The Wilcoxon matched-pairs signed ranks test was used for matched pairs of variables. In all tests, a p-value ð0.05 was considered as significant. All descriptive statistical results were expressed as mean (SD).

Results

sCR1 production by AMs in vitro

Purified AMs stimulated by the culture conditions alone released high concentrations of sCR1 into culture supernatant. This release reached 0.14±0.12 $ng \cdot mL^{-1}$ sCR1 (mean ±SD) after 24 h and 0.25±0.24 $ng \cdot mL^{-1}$ after 48 h (fig. 1). This release was not increased by the presence of LPS (p=1.0; Wilcoxon test). The viability of AMs after 48 h was always >80% determined by Trypan blue exclusion.

sCR1 in BALF of various lung disease

sCR1 was present in BALF supernatant of all subjects tested. Differences between the diagnostic groups were significant (Kruskal-Wallis test; p<0.0005). Compared to controls, sCR1 levels were significantly increased in BALF of all acute and chronic inflammatory diseases tested (p< 0.005, Mann-Whitney test), with highest levels in bacterial pneumonia, followed by the ARDS group (fig. 2). In bacterial pneumonia, the mean BAL sCR1 content is about 300 times higher and in ARDS 60 times higher than in controls. In interstitial lung fibrosis, sarcoidosis, and *P. carinii* pneumonia it is about eight, 12 and seven times higher compared to the same controls.

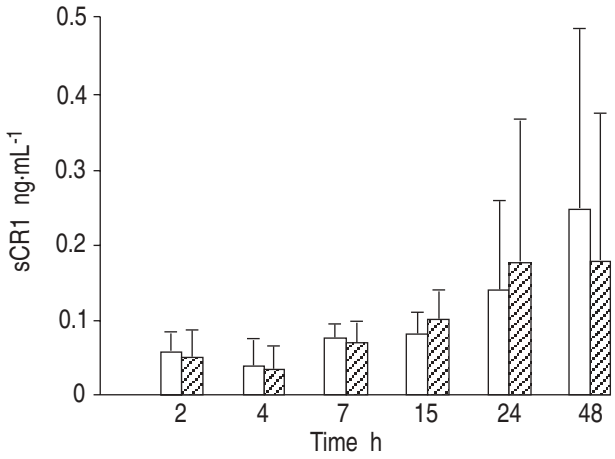

Fig. 1. – Release of soluble complement receptor type 1 (sCR1) by human alveolar macrophages in culture in the presence or absence of lipopolysaccharide (LPS). Results are expressed as mean±SD at different time points. No differences were found between the two conditions tested by Wilcoxon test (p=0.10). ▭ : without LPS; ▨ : with LPS.

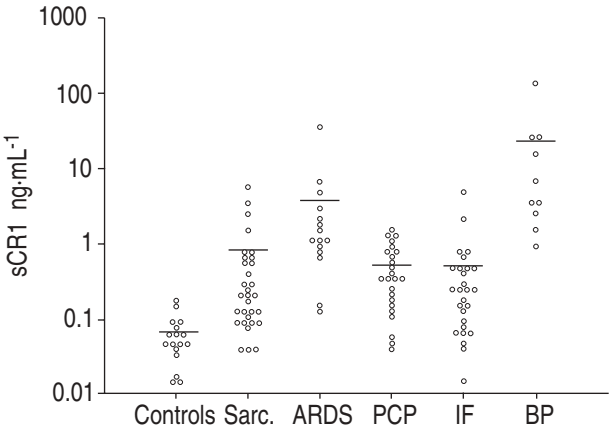

Fig. 2. – Levels of sCR1 in bronchoalveolar lavage. sCR1 levels differ significantly between the groups (p<0.0005, Kruskal-Wallis test). Compared to controls, sCR1 is significantly increased in every disease group (p<0.001 between controls and every disease group, Mann-Whitney test). A significant difference between the ARDS and the bacterial pneumonia groups is present (p=0.015). The differences between the sarcoidosis, PCP, and idiopathic pulmonary fibrosis group are nonsignificant. Bars represent mean values. sCR1: soluble complement receptor type 1; Sarc.: sarcoidosis; ARDS: acute respiratory distress syndrome; PCP: *Pneumocystis carinii* pneumonia; IF: interstitial fibrosis; BP: bacterial pneumonia.

sCR1 stability in BALF

Aliquots of BAL supernatants were stored at room temperature for 100 h or at 37°C for 12 h with presence or absence of protease inhibitors. With and without enzyme

Table 1. – Cell count distribution "PMN elastase" and protein content in bronchoalveolar lavage fluid or supernatant of the various subjects studied

Diagnosis	Subjects	sCR1	Cell count	PMN	Macrophages	Lymphocytes	"PMN elastase"	Protein
	n	ng·mL^{-1}	×10^4·mL^{-1}	%	%	%	µg·mL^{-1}	µg·mL^{-1}
Controls	17	0.07±0.04	19.5±11.2	1.6±1.0	85.5±8.1	12.0±8.0	2.6±2.4	46±23
Sarcoidosis	31	0.69±1.28‡	41.1±25.3‡	6.2±9.7	50.4±20.9‡	41.5±21.7‡	10.8±11.1*	430±733‡
IF	27	0.55±1.04‡	47.2±51.1*	18.4±23.8‡	51.1±24.4‡	25.4±22.3	28.5±37.6‡	212±332‡
ARDS	15	4.20±2.4‡	44.5±11.5	64.3±29.8‡	29.0±23.7‡	5.3±4.6*	91.1±60.8‡	1101±1974‡
PCP	24	0.54±0.46‡	29.1±15.7*	18.9±24.8‡	55.3±23.1*	23.5±15.3*	16.2±14.7‡	233±225‡
BP	10	21.30±38.00‡	206.0±230.0‡	84.5±12.1‡	9.8±7.3‡	5.1±4.7*	565.0±626.0‡	776±949‡

Values are means±SD. All parameters showed significant differences concerning diagnostic groups (Kruskal-Wallis test, p<0.0005). *: p<0.05; ‡: p<0.005, *versus* controls (Mann-Whitney test). "PMN elastase": free protease inhibitor complexed polymorphonuclear leucocyte elastase. For further definitions see legend to figure 2.

inhibitor, the aliquots remained stable. The difference of sCR1 was always ð10%, suggesting that sCR1 is stable in the BAL and is not rapidly degraded by enzymes which might be present (data not shown).

sCR1, various cells, "PMN elastase", and protein in BAL

The cell count and distribution found in these various pathologies are given as well as the content of protein and the "PMN elastase" (table 1). All categories (cell count; PMN percentage; macrophages percentage; lymphocytes percentage; "PMN elastase"; protein) analysed in table 1 showed significant differences concerning diagnostic groups (Kruskal-Wallis test, p<0.0005). As expected, bacterial pneumonia and ARDS showed the highest PMN numbers, "PMN elastase", and protein levels in BAL, whereas in interstitial lung fibrosis, sarcoidosis and *P. carinii* pneumonia the "PMN elastase" and protein levels found in BAL are of intermediate amount and quite similar.

sCR1 correlations with various cells and cell viability in BAL in the pooled data

A significant correlation was found between sCR1 and absolute PMN count (r=0.89, p<0.005; n=124; Pearson test, probability corrected for five comparisons (Bonferroni's inequality correction)) in the pooled data, suggesting a close relationship between sCR1 and PMN count in BAL.

No correlations were found between sCR1 and macrophage counts in the pooled data (r=0.01; p=1.0; n= 124; Pearson test, probability corrected for five comparisons (Bonferroni's inequality correction)). The same was true for the lymphocytes (r=0.4, p=1.0; n=124; Pearson test, probability corrected for five comparisons (Bonferroni's inequality correction)).

There was also no correlation between cell viability (trypan exclusion) and sCR1 in BAL (r=0.04; p=1; n=124, Pearson test, probability corrected for five comparisons (Bonferroni's inequality correction)).

sCR1 correlations with BAL cells and "PMN elastase" in the different diagnostic groups

The different diagnostic groups were analysed for correlations with BAL sCR1 levels according to their cell prevalence in BAL. "PMN elastase" correlations were only performed where PMNs were predominant.

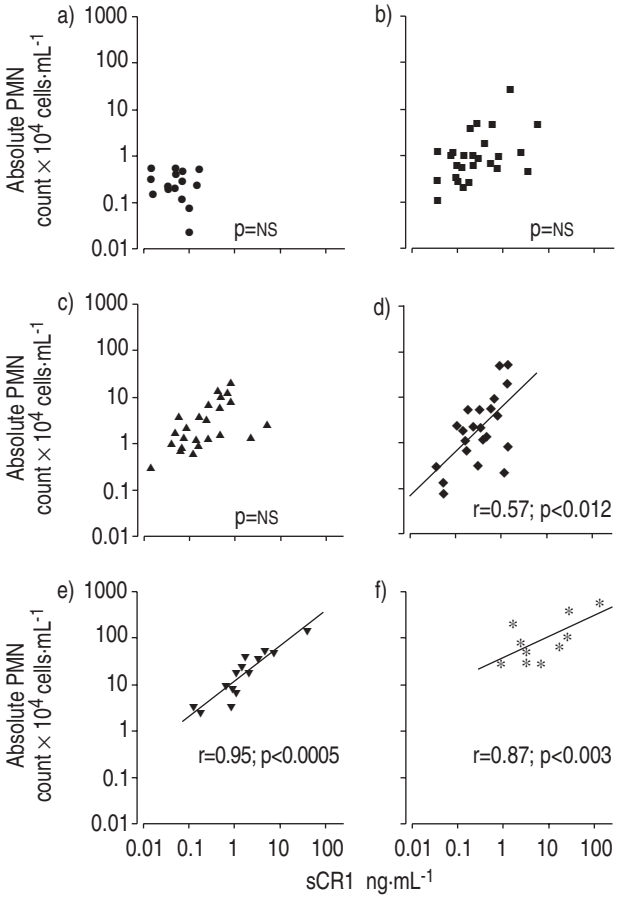

Fig. 3. – Relationship between the absolute BAL PMN count and the BAL sCR1 level. Correlations between the absolute BAL PMN count and the BAL sCR1 level are found in both pneumonia groups and in acute respiratory distress syndrome (ARDS) by Bonferroni-adjusted Pearson test. a) controls; b) sarcoidosis; c) interstitial fibrosis; d) *Pneumocystis carinii* pneumonia; e) ARDS; f) bacterial pneumonia; NS: non-significant; PMN: polymorphonuclear leucocyte; BAL: bronchoalveolar lavage; sCR1: soluble complement receptor type 1.

As summarized in figure 3, a correlation was found between BAL SCR1 and absolute PMN count in bacterial and *Pneumocystis carinii* pneumonia as well as in ARDS.

In the control group, no correlation was found between sCR1 and absolute macrophage, lymphocyte, or PMN cell count (r=-0.36, p=1; r=0.002, p=1; and r=-0.07, p=1, respectively; Pearson test, probability corrected for four comparisons (Bonferroni's inequality correction); one correlation see later). Due to the missing correlations of

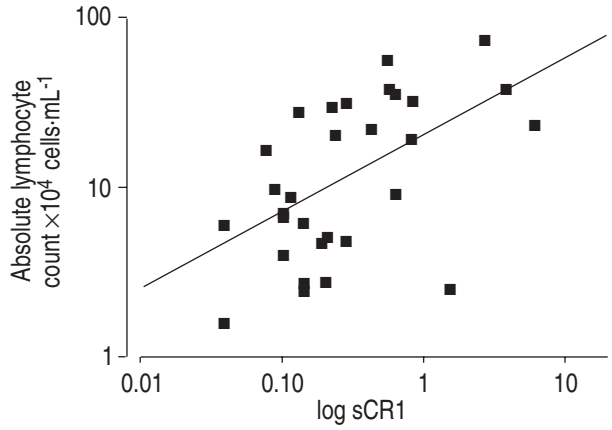

Fig. 4. – Relationship between the absolute BAL lymphocyte count and the BAL sCR1 level. There is a significant correlation in the sarcoidosis group by Bonferroni-adjusted Pearson test (r=0.41, p<0.044). sCR1: soluble complement receptor type 1; BAL: bronchoalveolar lavage.

sCR1 with macrophages in the overall data as well as in controls, which is the group with highest macrophages percentage, there were no more correlations analysed between those two parameters.

In the sarcoidosis group, a significant correlation of sCR1 was found with absolute lymphocyte count (r=0.41, p<0.044; Pearson test, probability corrected for two comparisons (Bonferroni's inequality correction) (fig. 4).

In the interstitial lung fibrosis group, sCR1 did not correlate with the absolute PMN or the absolute lymphocyte count (r=0.06, p=1; and r=0.24, p<0.67, respectively; Pearson test, probabilities corrected for three comparisons (Bonferroni's inequality correction)).

In *P. carinii* pneumonia, sCR1 correlated with the absolute PMN count and with "PMN elastase", (r=0.57, p< 0.012; and r=0.81, p<0.0005, respectively, Pearson test, probabilities corrected for four comparisons (Bonferroni's inequality correction) (fig. 5)).

In the ARDS group, sCR1 correlated with the absolute PMN count and with the "PMN elastase", but not with protein (r=0.95, p<0.0005; and r=0.60, p<0.037, respectively; Pearson test, probabilities corrected for three comparisons (Bonferroni's inequality correction)).

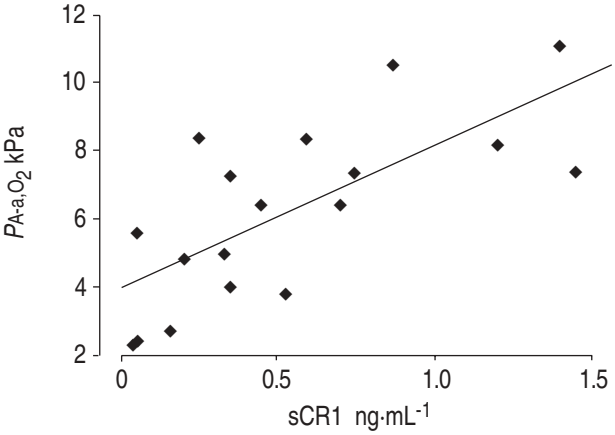

Fig. 5. – Relationship between the alveolar-arterial oxygen difference ($P_{A\text{-}a,O_2}$) and the BAL sCR1 levels in *Pneumocystis carinii* pneumonia. A linear correlation is found by Bonferroni-adjusted Pearson test (n=18; r=0.71, p<0.004). For definitions, see legend to figure 4.

In the bacterial pneumonia group, sCR1 correlated with the absolute PMN count, whereas the correlation with "PMN elastase" was only nearly significant (r=0.87, p<0.003; and r=0.74, p<0.067, respectively; Pearson test, probabilities corrected for three comparisons (Bonferroni's inequality correction)).

sCR1 correlations with protein in BAL of various lung diseases

The correlations of sCR1 with protein in BAL were investigated in the different diagnostic groups in order to examine whether or not a plasma leakage was the main source of sCR1 in BAL. The only significant correlation found between the two parameters was in the bacterial pneumonia group (r=0.97, p<0.005; Bonferroni-corrected Pearson test). In ARDS, a disease classically associated with plasma leakage into the alveoli, no correlation was found between BAL sCR1 and BAL protein (r=0.10, p=1; Bonferroni-corrected Pearson test). The same was true in the *P. carinii*, sarcoidosis, interstitial fibrosis, and the control group (r=0.32, p<0.89; r=0.20, p<0.90, r=0.39, p<0.15; and r=0.13, p=1, respectively; Bonferroni-corrected Pearson tests).

sCR1 levels in serum compared to BALF

The normal level of sCR1 in serum is 31.4±7.8 ng·mL^{-1} (mean±SD; n=31) [7, 14]. The concentrations of sCR1 in available sera were studied in most pathological groups and were not shown to be significantly changed compared to normal controls (table 2). The mean serum level of all samples studied (n=29) was 40.0±25.7 ng·mL^{-1} with higher mean values in pathological states such as sarcoidosis. However, the values are dispersed in each group and more samples would be needed to infirm or confirm a real difference compared to normal levels.

In the 29 paired samples of patients, there was no correlation between the serum sCR1 and BAL sCR1 (r=0.10, p=1; n=29; Bonferroni-corrected Pearson test), suggesting that BAL sCR1 originates from the local cells.

The correlation between sCR1 present in BAL and the protein content was determined. A correlation was found only in bacterial pneumonia (see above), implying that in this condition some of the sCR1 concentration found in BAL might be due to exudation of plasma through vascular leaks. However, while the protein content of the BAL is 100 fold lower than the normal concentration of the proteins in plasma, the sCR1 in BAL reached 20% of the plasma values despite the high molecular weight of sCR1. This requires a local production of sCR1 in the alveoli, *e.g.* from leucocytes.

If a serum protein content of 50 g·L^{-1} is assumed, the relation of sCR1/protein in BAL divided by sCR1/protein in serum (the "sCR1 concentration ratio") ranges 0.5–73. This means that, when referred to the protein concentrations in BAL or serum, the concentration of sCR1 in BAL is enhanced compared to sCR1 in serum in all groups. The mean enhancement varies between 1.6 and 15.5 (table 2), *e.g.* in the two patients from the bacterial pneumonia group this "sCR1 concentration ratio" was calculated to be 14 and 16. the "sCR1 concentration ratios" of more than

Table 2. – sCR1 in paired samples of BAL and serum in different disease groups

Group	Subjects tested n	BAL sCR1 ng·mL^{-1}	serum sCR1 ng·mL^{-1}	sCR1 BAL/ serum ratio	sCR1 BAL/BAL protein ÷ sCR1 serum/serum protein#
Controls	2	0.04±0.03	50.8±3.1	0.01±0.01	1.6±1.5
Sarcoidosis	4	0.46±0.24	67.5±26.0	0.06±0.03	2.6±2.3
IF	14	0.45±0.60	39.5±26.1	0.02±0.02	9.2±19.0
ARDS	0*	ND	ND	ND	ND
PCP	7	0.75±0.44	21.0±12.2	0.04±0.03	6.0±3.7
BP	2	9.80±9.20	45.2±29.5	0.20±0.08	15.5±1.0

Values are means±SD. #: assuming an amount of serum protein of 50 g·L^{-1} in each patient; *: no sera available; ND: not determined; BAL: bronchoalveolar lavage. For further definitions, see legend to figure 2.

10 fold in the two pneumonia patients and reaching in one patient more than 70 fold strongly suggest again that the sCR1 found in BAL is not only the result of serum leakage.

sCR1 and P. carinii *pneumonia severity*

In *P. carinii* pneumonia, a correlation was found between sCR1 and the worsening of the $P_{A\text{-}a,O_2}$ measured in each case, as shown in figure 5.

Discussion

This study demonstrates significant amounts of sCR1 in BALFs of normal individuals. Higher amounts of sCR1 were found in BALFs of several inflammatory lung diseases. *In vitro*, AMs release sCR1. They might be a physiological source of the sCR1 found in BAL, whereas in conditions where PMNs predominate in the alveoli, the PMNs may be the most important producers. In these conditions, sCR1 correlated with the BAL neutrophil count as well as with "PMN elastase", underlining the potential role of the activated PMNs in the release of sCR1. The highest sCR1 levels in BAL were found in bacterial pneumonia, followed by ARDS. In sarcoidosis, interstitial lung fibrosis and *P. carinii* pneumonia, the BAL sCR1 levels were much lower, but still differed significantly from normal controls. The BAL levels of this most potent complement inhibitor seemed to be independent of the sCR1 blood levels and, in BAL of bacterial pneumonia patients, reached nearly the range of blood levels, favouring the hypothesis of local alveolar release of sCR1.

Complement is a proteolytic cascade system that interacts with cells and is present in blood plasma and other body fluids of all vertebrates. It induces and mediates an inflammatory response by producing peptides with chemotactic or anaphylactic activity and by interacting with specific cellular complement receptors [15], thereby enhancing the innate and specific immune responses [4]. The former is mediated by products of C3 and C5 activation that cause changes in vascular permeability (C3a, C5a), leucocyte adhesion, migration, the stimulation of cytokine synthesis such as interleukin-6 by C5a [15, 16], and direct membrane damage (C5b-9) [4, 15].

PMNs play a key role in acute inflammatory diseases. Their recruitment at inflamed sites is followed by activation of these cells with release of proteolytic enzymes and complement proteins C3a and C5a [15, 16]. The observation that a regulator of complement activation, *i.e.* CR1, is also released by PMNs is thus of great interest since this release will have an opposite effect by reducing further complement activation and shifting locally the balance in favour of complement inhibition [17].

Pneumocytes type II, AMs and fibroblasts synthesize and release several complement components [18–21]. Complement plays an important role in the defence against pulmonary infections, for the clearance of *Streptococcus pneumoniae* or *Pseudomonas aeruginosa* in models of bacterial pneumonia [22–24]. CR1 as well as the complement receptor and leucocyte adhesion molecule CR3 (CD11b/CD18; Mac-1) have been shown to be crucial for the phagocytosis of organisms such as *Mycobacterium tuberculosis* and *Legionella pneumophila* [25, 26].

sCR1 accelerates the decay of the C3/C5 convertase complexes and participates in the degradation of C3b and C4b [4, 6]. *In-vivo* studies in the rat have shown that sCR1 is able to reduce infarct size after induced myocardial ischaemia [6], local and remote tissue damage after intestinal ischaemia reperfusion [27], and tissue injury following intradermal injection of immune complexes [28]. sCR1 has been shown to attenuate the complement-mediated hyperacute rejection in animal models of xenograft [29, 30]. sCR1 protects against lung and dermal injury in different models of complement-dependent acute inflammatory injury [31]. sCR1 in plasma is thought to be released by leucocytes, probably similarly to selecting, with which it shares many structural features [7]. *In vitro* results show its release by monocytes, lymphocytes, and PMNs [7].

AMs, the predominant immune cells in the healthy individual's alveolar space and important immunoregulators [10, 32], are known to express CR1 on their surface [10]. Our data demonstrate the release of sCR1 by AMs *in vitro*. Being the predominant cell expressing CR1 in normal alveoli, AMs may be its major source in normal subjects. Other local sources such as alveolar or airway epithelial cells appear unlikely according to the findings of VARSANO *et al.* [9].

We found the highest sCR1 levels in BAL of bacterial pneumonia. The good correlation of sCR1 with neutrophil counts suggests that they might be the source of sCR1. The sCR1 found in the alveoli might protect the lung from tissue injury. The correlation found between the protein content of the alveoli and sCR1 in the alveoli may imply that some sCR1 comes from the vascular compartment and thus is not exclusively produced locally. However, despite a ~100 fold lower protein content due to the dilution of the alveolar lining fluid during BAL procedure [33], its content of sCR1 is close to that measured in serum, which suggests a local production of sCR1 in this condition. However, our data do not allow to determine its

biological effect, nor if a lack of sCR1 may favour tissue necrosis or lung abscesses.

In *P. carinii* pneumonia, the lowest sCR1 values of the investigated diseases are found. In this condition, activation of the complement system has been postulated with the augmented BAL C3 levels found [34]. Nevertheless, compared to bacterial pneumonia, the mean sCR1 value is 40 times lower. There is, however, a significant correlation in *P. carinii* pneumonia between sCR1 and BAL PMN counts. A significant correlation is also found between sCR1 and the patient's $P_{A\text{-}a,O_2}$ as an index of severity of the disease. An inverse correlation between $P_{A\text{-}a,O_2}$ and sCR1 was thus not found. Nevertheless, an imbalance between the activity of complement and its inhibitors in the most severe clinical manifestation cannot be ruled out as a factor increasing lung injury [35].

The second highest levels were found in ARDS, with a mean BAL sCR1 level five times lower than in bacterial pneumonia. As in *P. carinii* pneumonia, sCR1 correlates with both the PMN count and "PMN elastase" in BAL. In ARDS, complement is activated [36, 37], but not in a predictive way [38]. In a rat model of ARDS induced by LPS and platelet-activating factor, high dose of human sCR1 prevented lung oedema, alveolar neutrophilic infiltration, decreased BAL cell count and protein changes [39]. sCR1 also attenuated C3 and membrane attack complex deposition to lung vessels. It did not decrease lung myeloperoxidase activity, a marker of neutrophil infiltration in the lung interstitium [39], which is complement independent [40]. These experiments and our finding may justify pursuing the study of the potential protection that sCR1 could provide in ARDS.

There is also evidence of a role of complement in pulmonary sarcoidosis [34, 41]. Increases of C3a levels in BAL [34] and of complement synthesis by AMs have been observed [19, 42]. Compared to normal controls, PETTERSEN *et al*. [43] found a higher expression of CR1 and CR3 and phagocytosis of AMs in patients with sarcoidosis stages I to III according to SILTZBACH [44]. In sarcoidosis, sCR1 correlated with the BAL lymphocyte count, suggesting a lymphocyte-dependent sCR1 production in this disease. These findings strengthen a role of complement in sarcoidosis that is independent of PMNs.

Our data do not elucidate the small amount of evidence of a pathogenic role of complement in interstitial lung fibrosis. Increased numbers of lung fibroblasts with high-affinity C1q receptors are found in fibrotic lung disease and may play a pathogenic role. Compared to fibroblasts with low affinity C1q receptors they grow faster, synthesize more collagen in response to TGF-β and are less inhibited by interferon γ [45].

In conclusion, soluble complement receptor type 1, a very potent complement inhibitor, can be found in the bronchoalveolar lavage in health as well as in acute and chronic inflammatory disease. This important physiological complement inhibitor found in the respiratory tract in soluble form, contrarily to the epithelium-bound complement regulatory proteins membrane cofactor protein, decay-accelerating factor and CD59 [9], may be important to prevent lung injury in several acute and chronic inflammations. The observation that soluble complement receptor type 1 could be measured in bronchoalveolar lavage with a monoclonal antibody known to recognise the C3b/C4b binding sites suggests that the molecule is functional [46, 47]. Whether this complement receptor type 1 release is sufficient to inhibit complement activation *in vivo* remains to be determined.

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