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Impaired neutrophil exocytosis in patients with severe pneumonia

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Abstract *Objective*: Polymorphonuclear neutrophils (PMN) are one of the major effector cells of pulmonary defence against bacterial infection. To determine whether neutrophil function is impaired in patients with severe pneumonia, we assessed the two main partial functions exocytosis and oxidative response (ROS production) in isolated neutrophils from the peripheral venous blood of pneumonia patients and healthy volunteers. In addition, pulmonary neutrophils and peripheral neutrophils were compared in pneumonia patients.
Patients and methods: Twenty-one patients with severe pneumonia were enrolled in the study. Eleven patients were mechanically ventilated, ten patients breathed spontaneously. For comparison, ten healthy adults were studied. The release of two markers of neutrophil exocytosis, lactoferrin and myeloperoxidase (MPO), with and without stimulation by phorbol-myristate-acetate (PMA), was determined using immunoluminometric assays. ROS production was quantified using luminol-enhanced chemiluminescence. In addition, the clinical severity of pneumonia was correlated to neutrophil exocytosis.
Results: With regard to blood neutrophils, both basal and PMA-stimulated exocytosis were significantly impaired in pneumonia patients compared to healthy volunteers (basal lactoferrin secretion in pneumonia patients: 0.25 ± 0.36 pg/PMN versus controls: 1.17 ± 0.78 pg/PMN, $p < 0.01$). In contrast, both basal and PMA-stimulated ROS production were increased in patients compared to controls (spontaneous chemiluminescence in pneumonia patients: 13.6×10^5 cpm versus controls: 5.5×10^5 cpm). In pneumonia patients, the pulmonary neutrophils released significantly more lactoferrin, MPO and ROS compared to blood neutrophils (basal lactoferrin secretion of pulmonary neutrophils: 1.19 ± 1.55 pg/PMN; $p < 0{,}01$). However, after stimulation with PMA the exocytosis of pulmonary and blood neutrophils was similar. The severity of pneumonia and prognostic indices like albumin were inversely correlated to the release of lactoferrin in blood neutrophils ($p < 0{,}05$).
Conclusion: In patients with severe pneumonia, the exocytosis of blood neutrophils was significantly impaired. In contrast to this, the oxidative response was increased. Impaired bone marrow maturation of neutrophils during severe infection, perhaps due to shortened maturation time, could explain these findings.

Introduction

Human polymorphonuclear neutrophils (PMN) are considered one of the major effector cells in normal host defence against bacterial and fungal infection [1]. This is illustrated by the severity of infections associated with neutropenia. Two major mechanisms are responsible for the neutrophils' bactericidal potential: 1) exocytosis of secretory granules and 2) activation of the oxidative response. Working synergistically, these two mechanisms develop a highly effective antibacterial potential [2].

Neutrophil secretory granules are traditionally classified into two major groups [1]. Primary (or azurophil) granules contain a variety of proteases, glycosidases, acid hydrolases and the enzymes myeloperoxidase (MPO) and elastase. MPO, the marker enzyme of these granules, acts via the MPO-halide system in cooperation with the neutrophil oxidative system as described below [3]. Lactoferrin from secondary granules is thought to play an essential role in the bactericidal power of the neutrophil [4]. Congenital neutrophil secondary granule deficiency results in severely depressed neutrophil chemotaxis and recurrent bacterial infections of the skin and deep tissues [3]. The oxidative response of neutrophils is catalyzed by a membrane-associated enzyme complex, the NADPH oxidase system, generating the oxygen metabolites hydroxyl radical (OH) and hydrogen peroxidase (H_2O_2). In the presence of myeloperoxidase, H_2O_2 reacts with the plasma chloride (Cl^-) to build the highly reactive metabolite hypochlorous acid (HOCl, [5]). The neutrophil oxidative response of pneumonia patients was shown to be increased in several studies [6, 7].

Although the exocytosis of lysosomal enzymes and other antibacterial proteins is an important partial function with regard to antibacterial defence [8], there are few studies quantifying the neutrophil exocytosis during infectious diseases, e. g. pneumonia. In non-infectious diseases such as asthma, neutrophils were found to release increased amounts of MPO [9]. To assess neutrophil exocytosis within the pulmonary compartment, the concentration of MPO, lactoferrin and other secretory products has been determined in bronchoalveolar lavage (BAL) fluid [6, 10, 11]. In pneumonia patients, MPO and lactoferrin were markedly increased [6]. This can be an indirect measure for an activation of pulmonary neutrophils in pneumonia. However, neutrophil exocytosis has not been directly compared to healthy controls.

Earlier studies suggest that neutrophil exocytosis might be impaired in acute infection: the granule contents of MPO and lactoferrin were reduced during the first week of bacterial meningitis and pneumonia [12]. The normal peripheral neutrophil content of lactoferrin and MPO extracted with cetyl-trimethyl-ammonium-bromide was reported to be 4.4 pg/PMN and 3.9 pg/PMN, respectively [13]. Another study reported a minimal peripheral neutrophil content of 0.73 pg/PMN lactoferrin and 1.45 pg/PMN in the MPO of patients [12]. In ARDS, neutrophil chemotaxis was found to be depressed, whereas the basal lysosomal enzyme release of blood neutrophils related to their enzyme content was increased compared to healthy controls [14]. With regard to the important role of neutrophil exocytosis in pulmonary defence against bacterial infection, impairment of this function during pneumonia could lead to severe courses and worsened prognosis of the disease. Answers to the question of whether neutrophil function is impaired during pneumonia and whether this influences the outcome and prognosis of the disease might provide new arguments for the discussion concerning the therapeutic use of growth factors, such as granulocyte colony stimulating factor (G-CSF). To investigate whether exocytosis is affected during severe pneumonia, we compared the neutrophil function of pneumonia patients with that of healthy volunteers. We measured the quantity of both the basal and the stimulated release of marker proteins of the primary and secondary granules of neutrophils. In addition to neutrophil exocytosis, the oxidative response was assessed and peripheral neutrophils were compared with neutrophils from the pulmonary compartment.

Methods

Patients

Twenty-one patients with severe pneumonia were studied. Eleven of these were mechanically ventilated. For demographic data see Table 1. For the diagnosis of pneumonia the following criteria had to be present [15]:

1) A new or progressive infiltrate on chest X-ray suggestive of pneumonia and / or a microbiological finding of pathogenic bacteria in bronchial minilavage of more than 10^5 CFU/ml for non-treated patients and of 10^3 CFU/ml or more for patients pretreated with antibiotics.
2) A minimum of three of the following criteria: pulmonary symptoms (such as productive cough, chest pain, auscultatory finding), purulent tracheal secretions (> 70 % neutrophils in bronchial minilavage), positive blood culture or more than 7 % intracellular bacteria in Gram-stains of minilavage samples, fever or hypothermia, leukocytosis or leukopenia or immature neutrophil band forms in the blood count.

Bronchial minilavage was performed for diagnastic reasons on day 1 or 2 after the onset of clinical symptoms in hospital-acquired pneumonia (HAP) and on day 1 or 2 after admission in community-acquired pneumonia (CAP). Minilavage was performed on days 4–8 in five cases of pneumonia which did not respond to antibiotic treatment. Drug resistance or a change in the causative pathogen was established in these cases and antibiotic treatment was consequently altered according to the antibiogram. The underlying conditions were COPD ($n = 2$), cerebral insult with suspected aspiration ($n = 1$), convulsions ($n = 1$), sepsis ($n = 2$), aspiration

Table 1 Demographic data of 21 patients with severe pneumonia and 10 healthy controls. Patients were divided into two subgroups with hospital-acquired pneumonia and community-acquired pneumonia

	Group 1: hospital-acquired pneumonia	Group 2: community-acquired pneumonia	Group 3: healthy volunteers
Valid no.	14	7	10
Age	62 ± 11	63 ± 18	22 ± 34
Sex	10 male, 4 female	3 male, 4 female	5 male, 5 female
Quotient: pO_2/FIO_2	282 ± 115	271 ± 69	452 ± 31
CRP [mg/l]	134.9 ± 66	173 ± 100	2.7 ± 1.4
Leukocytes (G/l)	13.7 ± 3.3	25.3 ± 11.4	6.3 ± 2.1
% PMNs in minilavage	88.2 ± 15	87.7 ± 20	n. d.
PMN × 10^6/ml in minilavage	5.27 ± 1.3	4.93 ± 1.4	n. d.
Microbiological finding in minilavage > 10^5 cfu/ml non-treated patients, > 10^3 cfu/ml pretreated patients	*A. fumigatus* (n = 1), *S. aureus* (n = 1), *P. aeruginosa* (n = 3), none (n = 9)	*H. influenzae* (n = 1), *S. aureus* (n = 3), none (n = 3)	
Ventilated/non-ventilated	10 ventilated, 4 non-ventilated	1 ventilated, 6 non-ventilated	
Mortality	4	2	

(n = 3), surgery (n = 1), meningitis (n = 2), and none (n = 2) in patients with HAP. Ten cases of HAP were ventilator-associated, one patient had been ventilated temporarily. Three patients were known to abuse alcohol. The underlying conditions of CAP were immotile-cilia syndrome (n = 1), aspiration (n = 1), pulmonary carcinoma (n = 3) and none (n = 2). One patient with CAP had to be ventilated.

Estimation of severity of pneumonia

To estimate the severity of pneumonia in our patients, we used the modified clinical pulmonary infection score (CPIS) [16]. This score, with a range of 1–12 points, comprises six groups of clinical parameters such as (l) body temperature, (2) blood leukocyte count, (3) character and quantity of tracheal secretions, (4) bacterial culture of the bronchial secretions, (5) arterial oxygen tension / inspiratory fraction of oxygen (pO_2/FIO_2) and (6) chest X-ray. The quantity of tracheal secretions was not recorded in our study as this is difficult in non-intubated patients. The character of secretions was specified in minilavage samples: secretions were defined as purulent if they contained more than 70% neutrophils. We therefore used the CPIS with a range from 1 to 11 points. The scoring is shown in Table 2.

Table 2 The clinical pulmonary infection score (CPIS) for the diagnosis of pneumonia. The score takes into account clinical parameters for the estimation of severity of pneumonia

1. Temperature °C
> 36.5 and < 38.5 = 0 point
> 38.5 and < 38.9 = 1 point
> 39 and < 36 = 2 points

2. Blood-leukocytes/mm^3
> 4000 and < 11 000 = 0 point
< 4000 and > 11 000 = 1 point
+ Band forms = + 1 point

3. Character of tracheal secretions:
< 70% neutrophils = 0 point
> 70% neutrophils = 1 point

4. Oxygenation: pO_2/FIO_2 mmHg
> 240 or ARDS = 0 point
< 240 and no evidence for ARDS = 2 points

5. Pulmonary radiography
No infiltrate = 0 point
Diffuse (or patchy) infiltrate = 1 point
Localized infiltrate = 2 points

6. Culture of tracheal aspirate or lavage (semi-quantitative)
Pathogenic bacteria in culture < 10^3 = 0 point
Pathogenic bacteria in culture ≥ 10^3 = 1 point
Second pathogenic bacterium in culture ≥ 10^3 = + 1 point

Bronchoscopy

Bronchoscopy was performed according to standard conditions after local anesthesia in the segment affected most severely, otherwise in the right middle lobe [20].

Minilavage

Minilavage was performed during bronchoscopy in non-ventilated, and via suction catheter in ventilated, patients [16–18]. After instillation of 5 ml 0.9% NaCl, the lavage was immediately aspirated. Recovery was 60–120%. The minilavage was diluted to a final volume of 50 ml and vortexed until homogenous in appearance. To eliminate remaining mucus, the sample was filtrated through four layers of gauze. After centrifugation at 400 g for 10 min, the cell pellet was resuspended in 2 ml of phosphate-buffered saline (PBS). Total cell count was performed on a hemocytometer (Coulter Electronics Ltd). Cells were differentiated counting a minimum of 600 cells of a cytocentrifuge smear stained with Giemsa (Cyto-

spin II, Shandon). Viability was determined by trypan blue dye exclusion and the sample was diluted to a concentration of 10^6 viable cells/ml.

Blood

Blood was taken on day 1 or 2, analogous to the minilavage. Neutrophils from venous blood were isolated using Ficoll-Hypaque density gradient centrifugation at $800 \times g$ for 20 min [19]. Ten milliliters Histopaque (density 1.119, Sigma, St. Louis, Mo.) and 10 ml Ficoll (density 1.077, Wyeth Chemicals) together with 20 ml of heparinized blood diluted with an equal volume of PBS were used. The neutrophil-enriched layer was collected and washed twice with PBS at $400 \times g$ for 10 min. The lysis of contaminating erythrocytes was carried out using NaCl (concentration 0.2 % for 60 s. and then 1.6 %). The neutrophils were then suspended to a concentration of 10^6 cells/ml. The final preparation contained more than 98 % (range 94–99 %) neutrophils. These were 99 % viable as measured by trypan blue dye exclusion.

Chemiluminescence

Reactive oxygen species (ROS) were quantified using chemiluminescence after amplification with luminol as a bystander substrate [20]. Luminol preferentially reacts with products of the MPO/H_2O_2 halide system, e. g. hypochlorous acid (OCl). Determination was performed in duplicate. The samples (100 µl) were incubated in 4 ml polystyrene tubes with 300 µl luminol (final concentration 50 µmol/l) and 100 µl PMA (final concentration 12 µmol/ml). Light emission was determined using the Autolumat LB 953 at 37 °C (Berthold, Germany). For calculation, we used 30-min integrals. The results were expressed as cpm (counts per min).

Quantification of neutrophil exocytosis

To quantify neutrophil exocytosis, 100,000 neutrophils were incubated in PBS on microtiter plates for 1 h at 37 °C [5, 9]. For stimulation, 100 µl containing 0.75 µg of phorbol-myristate-acetate (PMA) was added at the beginning of incubation. The total incubation volume was 300 µl. The incubation was stopped by centrifugation at 400 g for 10 min at a low temperature [9]. Two hundred microliters of the supernatant were carefully removed and stored at –20 °C until the assay for MPO and lactoferrin was performed. Storage never exceeded 2 months and a control measurement verified the proteins' stability. MPO and lactoferrin were then determined by immunoluminometric assay as previously described [21, 22]. The results were expressed in picogram of protein released per PMN (pg/PMN).

Statistics

Non-parametric statistics were used throughout the study. The Wilcoxon signed rank test was used for paired samples and the Mann-Whitney U test for independent samples. Correlations were made with the Spearman rank correlation. A value of p less than 0.05 was accepted as significant. The median was used as a marker of central tendency. Calculations were carried out with Statistica for Windows, version 5, 1997.

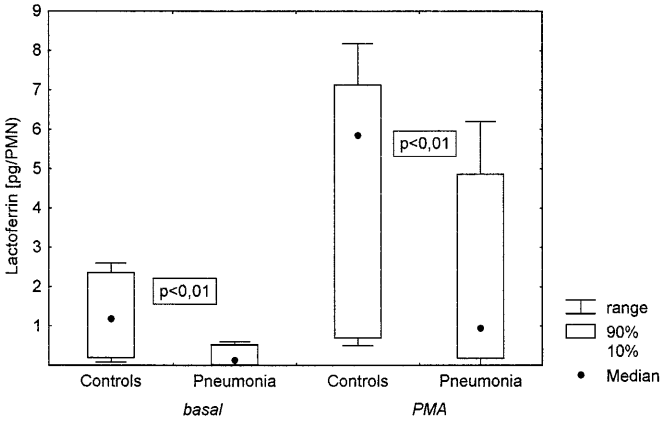

Fig. 1 Comparison of neutrophil-exocytosis of healthy volunteers and patients with pneumonia. 100,000 neutrophils isolated from peripheral venous blood were incubated at 37 °C for 1 h. For stimulation, 0.75 µg of phorbol-myristate-acetate (PMA) were added at the beginning of incubation. As a marker for exocytosis, lactoferrin (LF) was determined in the supernatant. Mean LF release per PMN in patients was significantly lower compared to healthy controls

Results

Neutrophil exocytosis in patients and healthy controls

Both the basal and PMA-stimulated exocytosis of blood neutrophils were decreased in patients with pneumonia compared to healthy volunteers (see Fig. 1). With regard to basal lactoferrin release, exocytosis was nearly 90 % lower in the patients (mean 0.25 ± 0.36 pg/PMN) than in controls (mean 1.17 ± 0.8 pg/PMN, $p < 0.01$). The complete data of MPO and lactoferrin release are shown in Table 3. In contrast to this, the luminol-enhanced chemiluminescence of neutrophils was higher in the patients than in the volunteers, both with regard to basal values and to those stimulated with PMA (basal chemiluminescence was $13.6 \pm 10.8 \times 10^5$ cpm for patients and $5.5 \pm 4.3 \times 10^5$ cpm for controls, $p < 0.03$).

Comparison of systemic and pulmonary neutrophils

In unstimulated neutrophils isolated from minilavage, exocytosis was increased compared to the blood neutrophils of the same patient: mean spontaneous lactoferrin release from minilavage neutrophils was 1.19 ± 1.55 pg/PMN compared to 0.25 ± 0.36, $p < 0.02$ pg/PMN from blood neutrophils. In stimulated neutrophils, lactoferrin release was similar for blood and pulmonary neutrophils (2.02 ± 2.33 pg/PMN in minilavage versus 1.77 ± 2.02 pg/PMN in blood, p = n.s., Table 3). The spontaneous luminol-enhanced chemiluminescence (CL) of blood neutrophils was much lower than that of pulmonary neutrophils. After stimulation with PMA,

Table 3 Basal and PMA-stimulated lactoferrin release, MPO release and chemiluminescence by pulmonary and peripheral neutrophils of 21 patients with pneumonia and by peripheral neutrophils from healthy controls. Mean ± SD

	Blood neutrophils of healthy controls	Blood neutrophils of pneumonia patients	Pulmonary neutrophils of pneumonia patients
Basal lactoferrin release [pg/PMN]	1.17 ± 0.78	0.25 ± 0.36*	1.19 ± 1.55#
PMA-stimulated lactoferrin release [pg/PMN]	5.22 ± 2.42	1.77 ± 2.02*	2.02 ± 2.33
Basal MPO release [pg/PMN]	3.36 ± 1.26	1.57 ± 1.62	4.59 ± 6.07#
PMA-stimulated MPO release [pg/PMN]	9.51 ± 4.26	3.33 ± 4.73*	6.56 ± 8.31
Spontaneous chemiluminescence [cpm]	$5.5 \times 10^5 \pm 4.3 \times 10^5$	$13.6 \times 10^5 \pm 10.8 \times 10^5$*	$309 \times 10^5 \pm 10.6 \times 10^5$ #
PMA-stimulated chemiluminescence [cpm]	$452 \times 10^5 \pm 406 \times 10^5$	$2220 \times 10^5 \pm 3910 \times 10^5$*	$915 \times 10^5 \pm 1270 \times 10^5$

* $p < 0.03$ vs control; # $p < 0.05$ vs blood neutrophils of pneumonia patients

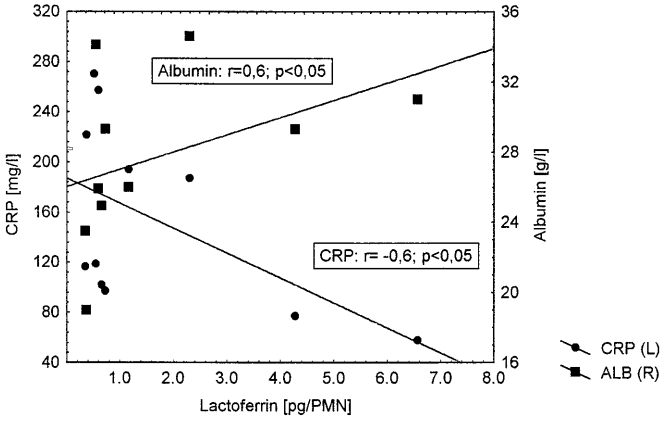

Fig. 2 Correlation of pulmonary neutrophil lactoferrin release in pneumonia patients with the concentration of C-reactive protein (CRP) and albumine in the blood of these patients. Pulmonary neutrophil exocytosis is decreased with an increase of CRP and with lower albumin levels

the ROS production of blood neutrophils increased to higher levels compared to pulmonary neutrophils (basal pulmonary CL: $309 \pm 1060 \times 10^5$ cpm, basal blood CL: $13.6 \pm 10.8 \times 10^5$ cpm, $p < 0{,}04$; PMA-stimulated: pulmonary CL = $915 \pm 1270 \times 10^5$ cpm, blood CL = $2220 \pm 3910 \times 10^5$ cpm, $p < 0.05$).

Correlation of neutrophil function and clinical severity

To define the correlation between the parameters of disease severity and neutrophil function, clinical data such as arterial oxygen and carbondioxide tension, the quotient of arterial oxygen tension and inspiratory fraction of oxygen (respiratory quotient, paO_2/FIO_2), C-reactive protein (CRP), leukocyte count and albumin concentration were recorded. The severity of pneumonia was quantified using the clinical pulmonary infection score (CPIS). In addition, ventilated and non-ventilated patients were compared. With regard to neutrophil exocytosis and chemiluminescence, there were no significant differences between ventilated and non-ventilated patients or between CAP and HAP. The albumin concentration was significantly lower in ventilated patients ($p < 0.01$).

In the group of ventilated patients, the arterial carbon dioxide tension ($paCO_2$) was inversely correlated to the lactoferrin release of pulmonary neutrophils: neutrophil exocytosis was decreased with elevated $paCO_2$ ($p < 0.01$, $r = -0.85$) and with an increase of C-reactive protein and was decreased with lower albumin concentration (Fig. 2). In peripheral neutrophils, the stimulated release of lactoferrin correlated with the respiratory quotient: a lower quotient, indicating worse pulmonary function, coincided with lowered exocytosis ($r = 0.74$ $p < 0.01$). The stimulated lactoferrin secretion of blood neutrophils was inversely correlated with blood leukocyte count ($r = -0.62$ $p < 0.04$) and with the severity of pneumonia: the more CPIS points the patient had, the lower was his neutrophil release of lactoferrin.

Discussion

The main finding of our study was that neutrophil exocytosis in patients with severe pneumonia is significantly impaired: basal as well as stimulated exocytosis was decreased in these patients compared to healthy controls. In contrast to this, the oxidative response was increased. One possible explanation for the decrease of neutrophil exocytosis in pneumonia patients is an impaired neutrophil maturation. Hansen et al. studied the changes in intraneutrophilic concentrations of the three antibacterial proteins lysozyme, lactoferrin and MPO during acute bacterial infection. They found that the intraneutrophilic concentrations of all three proteins decreased to less than 50 % during the first week of infection. Nadir values coincided with maximal toxic granulation of the neutrophils [12]. The authors suggested that neutrophilic granulocytes are deficient during early bacterial infection. To provide the high requirement of neutro-

phils during infection, marrow maturation time is shortened [1] and might be insufficient. This is illustrated by the presence of immature neutrophil band forms in the blood count of these patients. Deficient neutrophil maturation could explain our finding of a decreased capacity for exocytosis in pneumonia.

Another possible explanation is the fact that neutrophils are "end-stage cells". Primary and secondary granules containing MPO and lactoferrin are synthesized mainly during the promyelocytic and myelocytic stages of marrow maturation [1, 23). In consequence, the neutrophil granule contents are exhausted if exocytosis has taken place and the capacity for further secretion is diminished. Our previous findings of high levels of MPO and lactoferrin in the BAL fluid of pneumonia patients and other studies reporting increased serum levels of MPO and lactoferrin in patients with pneumonia, meningitis or septicemia point to an activation of neutrophil exocytosis during acute infection [6, 12, 13]. Neutrophil activation during bacterial infection itself may therefore explain the incapability to increase exocytosis further.

The recruitment of activated cells to the lung, as the site of inflammation, offers another explanation for our results. The effect of pulmonary neutrophil activation is illustrated by the higher level of basal exocytosis in the pulmonary compartment. Animal models have shown that neutrophil sequestration in the lung increases in acute lung inflammation [24]. The idea of the compartimentalization of activated neutrophils to the lung has been supported in a previous study, reporting higher chemiluminescence in prepulmonary neutrophils than in postpulmonary neutrophils [21]. These findings can explain lowered spontaneous exocytosis in the blood of pneumonia patients – however, after stimulation with PMA, the exocytosis of pulmonary and peripheral neutrophils is similar, showing that the capacity for exocytosis is similar in the two compartments. Therefore the decrease of PMA stimulated exocytosis in the blood of pneumonia patients is not completely due to the sequestration of activated cells to the lung, but rather due to the differential effects of pro- and anti-inflammatory cytokines in the systemic and pulmonary compartments during inflammation. In addition, pathogens themselves can directly induce neutrophil activation in the lung, thus giving an additional explanation for the higher basal levels both of exocytosis and of the release of reactive oxygen species with regard to pulmonary neutrophils.

Lee et al. studied neutrophil desensitization in normal volunteers after pretreatment with formylmethionyl-leucyl-phenylalanine (fMLP). They could demonstrate a marked reduction of neutrophil aggregation, whereas neutrophil exocytosis was only slightly influenced by prior activation and ROS production was not altered. Thus, desensitization does not explain our findings [25]. Finally, PMN apoptosis in infected tissue could influence neutrophil function. However, Matute-Bello et al. found no increase in PMN apoptosis in the BAL of ARDS patients [26]. Moreover, G-CSF, which was shown to inhibit neutrophil apoptosis, increases MPO and lactoferrin release in healthy volunteers (unpublished observation). Therefore it seems improbable that the impairment of neutrophil function documented in our study is due to increased apoptosis.

Impaired neutrophil exocytosis correlated with the clinical severity of pneumonia, indicated by lowered respiratory quotient, increased $paCO_2$, elevated leukocyte count and CRP concentration, lower albumin concentration and higher CPIS score [21]. This illustrates that either exocytosis is further impaired with worsened clinical condition or that a greater reduction of neutrophil exocytosis results in a worsened clinical condition. As the direct comparison of pulmonary neutrophils from pneumonia patients with those of healthy controls was impossible, the correlation of pulmonary neutrophil exocytosis with clinical parameters is valuable evidence of the impairment of pulmonary neutrophils during infectious disease. Thus, impaired pulmonary neutrophil exocytosis may indicate a worse prognosis. Other studies hint in a similar direction: Donnelly et al. found significantly higher plasma elastase concentrations within hours after multiple trauma in those patients who subsequently developed ARDS [28]. Elastase is released from the primary granules of neutrophils, which may thus exhaust their stock of proteins.

In contrast to exocytosis, the oxidative response is increased in the neutrophils of pneumonia patients. This is in keeping with other publications: an increased respiratory burst of peripheral neutrophils compared to healthy controls was reported in AIDS [29], sepsis [30], ARDS [31, 32] and pneumonia [7]. In a recent study we found increased oxidative activity in BAL samples of pneumonia patients, which was mainly due to the recruitment of activated neutrophils and was maintained even in severely immunocompromised patients [20]. The NADPH oxidase system lies dormant in unstimulated neutrophils, but triggered cells rapidly activate the enzyme system [5]. Therefore the capacity of the oxidative enzyme system is not limited the way exocytosis is. In the pulmonary compartment of pneumonia patients the oxidative burst is further increased compared to peripheral neutrophils. This is in agreement with other studies [33]. The role of bacterial infection as an activator of oxidative response is illustrated by the comparison of pneumonia patients and patients with respiratory failure due to cardiac pulmonary edema: chemiluminescence was significantly increased in pneumonia patients [21].

Both the morbidity and mortality of pneumonia is increased in patients with severe underlying conditions. This is highlighted by the fact that only 4 of our 21 patients did not have additional severe diseases. These may influence neutrophil function, as has been shown

with regard to alcohol or other infections [1]. Thus it can not be ruled out that the impairment of neutrophil function is due to some extent, to the underlying disease or to the stress caused by the severe illness and is not only a consequence of the severe pneumonia. This aspect deserves further studies in homogenous patient groups "at risk" of developing bacterial pneumonia.

As the deterioration of the patients' clinical condition correlates with a decrease of exocytosis, the stimulation of both bone marrow production and neutrophil functions with growth factors such as G-CSF or GM-CSF could be a possible therapy [34]. In a recent study in healthy volunteers, we could demonstrate an increased release of the anti-inflammatory cytokines soluble TNF α receptor and interleukin β receptor antagonist and an increase of the neutrophil exocytosis of lactoferrin and MPO [35]. In patients with severe pneumonia, G-CSF did not decrease the neutrophil granule content, but increased the PMA-stimulated exocytosis in relation to basal release (unpublished observation).

In summary, our study demonstrates that neutrophil degranulation is impaired in the blood of pneumonia patients compared to that of healthy controls. Two explanations are at hand for the decrease of neutrophil exocytosis in pneumonia: exhaustion of the granule contents as a result of exocytosis during infection, on the one hand, and impaired marrow maturation with decreased filling of the granule contents on the other. It is likely that both mechanisms play a role in the pathogenesis of impaired neutrophil exocytosis in pneumonia patients. Whether the impairment of neutrophil exocytosis in the pulmonary and systemic compartments is a prognostic factor for the outcome of pneumonia remains to be studied.

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