Altered Phenotype of Blood Dendritic Cells in Patients with Acute Pneumonia

Katharina Dreschler    Kai Bratke    Sebastian Petermann    Petra Thamm
Michael Kuepper    J. Christian Virchow    Marek Lommatzsch
Department of Pneumology, University of Rostock, Rostock, Germany

Abstract

Background: Dendritic cells (DCs) play a key role in the host defence against inhaled pathogens. However, the phenotype of blood DCs in patients with acute respiratory infections is unknown. Objective: To investigate the number and the expression of function-associated molecules of blood DCs in patients with acute infectious pneumonia. Methods: Sixteen patients with acute pneumonia and 19 controls without pneumonia were included in the study. The number as well as the expression of function-associated molecules of myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) were analysed in peripheral blood using four-colour flow cytometry. Results: Elevated concentrations of procalcitonin (median: 0.55 ng/ml) and the rapid response to antibiotic treatment suggested a bacterial origin of the pneumonia in the patients. Total mDC (median: 27% of the controls) and pDC counts (median: 53% of the controls) were markedly reduced in patients with pneumonia, as compared to the controls. Percentages of blood mDCs, but not pDCs, were negatively correlated with serum concentrations of C-reactive protein. Patients with pneumonia were characterised by a significantly increased expression of Fc gamma receptors (CD32 and CD64) on mDCs and the Toll-like receptor 9 (TLR9) on pDCs. Conclusions: Circulating DCs are markedly reduced in patients with pneumonia, and characterised by an up-regulation of molecules recognising pathogen-associated molecular patterns and opsonised antigens.

Introduction

Dendritic cells (DCs) are key regulators of human immunity [1]. As antigen-presenting cells, they initiate and control adaptive immune responses against infectious particles in the airways [2]. In addition, they have a variety of functions in the innate immune system, thus bridging the gap between innate and adaptive immunity [3]. Animal models suggest that the influx of DCs into the airways after exposure to infectious particles is an integral part of the early reaction of the pulmonary immune system [4]. After antigen uptake in the airways [5], DCs undergo a maturation process and migrate to the draining lymph nodes in order to present antigenic information to lymphocytes, which organise a specific inflammatory response against the encountered antigen [2].

There is now growing evidence from animal models that DCs are crucial players in the initiation and resolution of the pulmonary immune responses to pathogens [6]. Animal models showed that exposure to infectious particles results in an immediate influx of DCs into the...
airways [4] and an accelerated migration of antigen-load-
ed maturing DCs to the draining lymph nodes [7]. Sev-
eral functional studies using wild-type and genetically
engineered mice suggested that DCs play a central role in
the regulation of innate and adaptive immune responses
in lower respiratory tract infections [8–12].

In contrast to the animal models, there is very limited
information on the characteristics and function of DCs
in the lower respiratory tract infections of humans. There
are currently no histological studies examining DCs in
the airways or the lung parenchyma of patients with low-
er respiratory tract infections. Using a comprehensive
flow-cytometric method to analyse DCs in human bron-
choalveolar lavage fluid [13], we have recently shown that
there is an increase in bronchoalveolar lavage fluid plas-
macytoid DCs (pDCs) in patients with infectious pneu-
monia, and that this increase is abolished in patients with
concomitant immunosuppression [14]. There is evidence
that myeloid DCs (mDCs) and pDCs are decreased in the
peripheral blood and increased in the airways of patients
with acute viral respiratory infections, suggesting a re-
cruitment of DCs from peripheral blood to the site of vi-
ral respiratory infection [15, 16]. However, there are cur-
rently no data on the functional characteristics of blood
DC subsets in patients with acute respiratory infections.
It was the aim of this pilot study, therefore, to investigate
the expression of function-associated molecules on DC
subsets in the peripheral blood of patients with acute in-
fec-tious pneumonia, and to compare the results with a
control group of subjects without pneumonia.

**Patients and Methods**

**Participants**

Patients with acute pneumonia were recruited in the medical
clinic of the University of Rostock (Rostock, Germany), using
the following inclusion criteria: (1) age older than 18 years, (2) onset
of symptoms of an acute lower respiratory tract infection within
the last 72 h, (3) clinical signs of pneumonia, (4) infiltrates in the
chest X-ray compatible with pneumonia and (5) C-reactive pro-
tein (CRP) concentrations higher than 50 mg/l (normal range:
<5 mg/l). Exclusion criteria for patients with pneumonia were:
(1) any current malignant disease, (2) any other concurrent infec-
tion, (3) treatment with systemic corticosteroids or other immu-
nosuppressant drugs in the last 4 weeks prior to inclusion in the study, (4) inability
to give informed consent, (5) CRP concentrations higher than
5 mg/l and (6) haemodialysis therapy. The study was approved by
the local ethics committee of Rostock. All participants gave their
written informed consent.

**Study Design**

Patients who presented with a cough or dyspnoea or both were
assessed for eligibility. The assessment in the emergency depart-
ment included a complete medical history, a physical examina-
tion, measurement of body temperature, heart rate and blood
pressure, blood sampling and conventional chest radiography.
Additional diagnostic procedures and the choice of the antibiotic
regimen were left to the attending physician. Patients were exam-
ined on the day of admission to the hospital (‘day 1’). Those pa-
tients who were still in hospital after 5 days of antibiotic treatment
were re-examined at this time point (‘day 5’). Controls were re-
cruited in the same time period, and assessed at one time point.

**Blood Parameters, Flow Cytometry and Cytokine
Measurements**

Blood was drawn from the cubital vein, into heparinised (plas-
ma; for CRP measurements), additive-free (serum; for the mea-
surement of procalcitonin, PCT) and EDTA-containing (for flow
cytometry and blood cell counts) containers. Blood cell counts,
CRP (detection limit: 1 mg/l plasma) and PCT (detection limit:
0.06 ng/ml serum) were measured as described [17]. Blood DCs
were analysed with four-colour flow cytometry as described
[13, 14, 18], using the antibody panel detailed in the online supple-
mentary table E1 (see www.karger.com/doi/10.1159/000328406),
and the gating strategy shown in figure 1. To identify DC subsets,
CD3, CD14, CD16, CD19, CD20, and CD56 negative/dim cells
(lin$^{neg/dim}$) were gated using a commercial lineage cocktail with
antibodies against all of these antigens (fig. 1). Among these
lin$^{neg/dim}$ cells, distinct populations of CD123+HLA-DR+ cells
(plasmacytoid DC) and CD11c+HLA-DR+ cells (myeloid DC)
were identified (fig. 1). Surface molecules were quantified in his-
togram plots using appropriate isotype control antibodies to dis-
criminate between specific and non-specific antibody staining
(see online suppl. fig. E1 and E2). For the measurement of each
DC marker, a total of 150,000 blood leucocytes were counted. A
minimum of 100 DCs were measured for each marker. In most
cases, 300–500 DCs were counted. Dead cells are excluded in the
initial gating step, which excludes highly fluorescent lineage-pos-
itive and highly autofluorescent cells such as dead cells [13, 14, 19].
Interferon-gamma (IFN-γ) (R&D Systems, Minneapolis, Minn.,
USA; detection limit: 5 pg/ml) and neopterin (IBL International,
Hamburg, Germany; detection limit: 4 nmol/l) were measured as described
[17].

**Statistical Analysis**

Data were analysed using SPSS (SPSS Inc., Chicago, Ill., USA).
Most parameters were non-normally distributed; therefore, the
Mann-Whitney U test was chosen for the comparison of patients
and controls. The Wilcoxon signed-rank test for paired samples
was used for comparisons of patients with pneumonia at the 2
time points. Correlations were calculated using the Spearman
correlation coefficient; p values <0.05 were regarded as statisti-
cally significant.
Results

Characteristics of the Participants

Sixteen patients with acute pneumonia and 19 controls were recruited based on our inclusion and exclusion criteria. Subject characteristics are shown in table 1. Vital signs and laboratory parameters of the participants are detailed in table 2. On day 1, the temperature, heart rate, respiratory rate, leukocyte counts and concentrations of CRP and PCT were significantly higher in patients with pneumonia compared to the controls. PCT levels were >0.25 ng/ml in 13 patients (82%), between 0.1 and 0.25 ng/ml in 1 patient (6%) and <0.1 ng/ml in 2 patients (12%) on day 1. Median CRP and PCT concentrations decreased significantly on day 5, but remained elevated compared to the controls (table 2).

Participants' chronic diseases, chronic medication and the antibiotics used during the study period are supplied in the online supplement (see online suppl. tables E2–E4). There was no difference regarding the age, gender, height, weight or smoking status between controls and patients (table 1). The prevalence of arterial hypertension, coronary heart disease and diabetes in both groups is shown in table 1. None of the participants suffered from COPD (online suppl. table E3). Of the 16 patients with pneumonia, 10 patients were discharged from the hospital within...
4 days after admission. Hence, 6 patients with pneumonia were re-examined on day 5 in our hospital.

**Presence of DCs in Blood and Correlation with Markers of Inflammation**

Percentages and total numbers of blood DCs are shown in figure 2. Both pDCs and mDCs were decreased in patients with pneumonia at both time points (day 1 and day 5) compared to the controls. The median decrease was more pronounced in mDCs (27% of controls) than in pDCs (53% of controls) at day 1. Of the 6 patients who were re-examined after 5 days of antibiotic treatment, there were no significant differences between pDC and mDC counts between day 1 and day 5 (fig. 2). There was a negative correlation between CRP concentrations and the percentage of blood mDCs in patients with pneumonia on day 1 (fig. 3). The correlation between CRP concentrations and blood pDCs did not reach statistical significance (fig. 3). Negative correlations between PCT concentrations and the total number of pDCs and mDCs in patients on day 1 were not significant.

**Surface and Intracellular Molecules in Blood pDCs and mDCs**

The expression of function-associated molecules on DCs is detailed in table 3. There was a decrease in the CD40 expression and an increase in the expression of Toll-like receptor 9 (TLR9) on pDCs (fig. 4) in patients with pneumonia on day 1 (table 3). There was an increased expression of CD32 and CD64 on mDCs (fig. 4), and a decreased expression of CD86 in patients with pneumonia on day 1 (table 3). Owing to the low number of patients with pneumonia on day 5 with pDC and mDC concentrations that could be analysed, a statistical evaluation of function-associated molecules was not performed at this time point (table 3).

**Concentration of IFN-γ and Neopterin in Serum**

To elucidate a possible mechanism leading to the phenotype of the mDCs, we measured concentrations of IFN-γ in serum, one of the regulators of the expression of Fc gamma receptors on human blood mDCs. Concentrations of IFN-γ and Neopterin in Serum.
Fig. 2. Presence of pDCs and mDCs in blood. The percentage of DCs among all leucocytes in blood (a, b) and the total number of DCs in peripheral blood (c, d) in controls (white) and patients with pneumonia on day 1 (dark grey) and day 5 (light grey). Box plot graphs display the median (line within the box), interquartile range (edges of the box) and the range of all values less distant than 1.5 interquartile ranges from the upper or lower quartile (vertical lines). Outliers (all values more distant than 1.5 interquartile ranges from the upper or lower quartile) were omitted in the graphs.

Fig. 3. Association of blood DC counts with CRP levels. a, b Correlation between CRP plasma concentrations and the percentage of DCs among all blood leucocytes. c, d Total number of DCs in peripheral blood. Each dot represents 1 patient, the line is the regression line calculated with SPSS. r = Spearman’s rank correlation coefficient; p = significance of the correlation.
trations of IFN-γ were below the detection limit (5 pg/ml) in all controls and in the majority of patients with pneumonia (data not shown). Therefore, we analysed serum concentrations of neopterin, a more stable protein regulated by IFN-γ [20]. Neopterin concentrations in patients with pneumonia on day 1 were significantly higher than in the controls (fig. 4).

Discussion

This pilot study is the first to investigate the phenotype of blood DCs during an acute respiratory infection in humans. We demonstrated that blood DCs are markedly reduced during acute infectious pneumonia and characterised by an up-regulation of receptors recognising pathogen-associated molecular patterns and opsonised antigens. Herewith, our study provides important new information on the characteristics of DCs in acute respiratory infections in humans.

Although we did not perform a microbiological examination of blood or sputum specimens in this study, there were two findings which suggested a bacterial origin of the infection in the patients examined. Firstly, the majority of patients (79%) displayed PCT concentrations >0.25 ng/ml. This cut-off has been proposed to identify patients with a bacterial community-acquired pneumonia eligible for antibiotic treatment [21, 22]. Secondly, all patients included in the study responded well to antibiotic treatment. In fact, the clinical success of the antibiotic treatment led to a discharge of 63% of the patients from the hospital within 4 days after admission. Thus, it appears likely that the large majority of the patients examined in our study had a bacterial respiratory infection. It has previously been shown that patients with a severe viral respiratory infection display a strong decrease in blood mDCs and pDCs [15]. Thus, the marked reduction of blood mDCs and pDCs found in our study suggests that a decrease in both blood DC subsets may be a general phenomenon in acute human respiratory infections.

It might be speculated that reduced blood DC counts reflect an increased influx of DCs into the inflamed lung. Indeed, there is evidence suggesting that decreased blood DC counts can be due to a pulmonary recruitment of DCs in acute inflammatory reactions of the lung. The influx of DCs into the airways following allergen challenge in patients with allergic asthma [13, 23, 24] is accompanied by a concomitant decrease of blood DCs [13, 25, 26]. Acute cigarette-smoke exposure is associated with an influx of mDCs into the airways and a concomitant decrease in blood mDCs [18]. However, another possible explanation for the decrease of circulating DCs during acute pneumonia is an apoptosis of these cells. The strong reduction of DCs in the spleen of patients with sepsis and in animal models of sepsis has been attributed to an apoptosis of this cell type during acute systemic infections [27, 28].

We have previously shown an increase in pDCs, but not mDCs, in the bronchoalveolar lavage fluid of patients with pneumonia [14]. In contrast, in our study, there was a reduction of both pDCs and mDCs in peripheral blood. There are several possible explanations for this discrepancy. Firstly, as discussed above, the reduction in circulating mDCs might not only reflect a pulmonary recruit-
ment of these cells, but also an apoptosis of mDCs during acute pneumonia [27, 28]. Secondly, the differences in the design of the previous (onset of symptoms up to 2 weeks prior to inclusion) and current (onset of symptoms up to 3 days prior to inclusion) studies may have detected changes in pulmonary DC recruitment in the course of the disease (such as an early mDC and a late pDC recruitment into the airways). Finally, there might be a compartmentalization of recruited pDCs and mDCs in the lung. In fact, DC characteristics differ between the airway lumen and sub-epithelial layers, suggesting different mechanisms of DC recruitment [29]. Therefore, further studies in patients with lower respiratory tract infections and in animal models are needed to resolve this issue. In addition, the contribution of blood monocytes to the number of DCs in the lung must be explored in further studies, since blood monocytes can differentiate to mDCs within the tissues, especially in acute inflammatory settings [30, 31].

It has been well established that an increased expression of CD40 and CD86 can indicate a more mature phenotype of DCs [32]. Therefore, the reduced expression of the co-stimulatory molecules CD40 and CD86 on blood DCs in patients with acute pneumonia might reflect a less mature phenotype of circulating DCs in this condition. One explanation for this finding might be an increased efflux of more mature DCs into the lung and/or an increased influx of less mature DCs from the bone marrow.

TLR9, which is exclusively expressed by human B cells and pDCs, is a specialised receptor for the recognition of pathogenic nucleic acids such as bacterial or viral DNA [33]. Animals deficient in TLR9 on pDCs display significantly increased mortality associated with a strong increase in the pulmonary bacterial load, and fail to generate an effective Th1 cytokine response following bacterial administration [10]. Thus, the observed increase in the expression of TLR9 on pDCs of patients with pneumonia might be an important means to augment antibacterial immunity in pneumonia.

Activation of the Fc gamma receptors I (CD64) and II (CD32) triggers a wide variety of effector functions including phagocytosis of antibody-antigen complexes, antibody-dependent cytotoxicity and the release of pro-inflammatory mediators [34]. In human blood, the expression of these receptors is weaker in DCs than in monocytes [30, 35]. Accordingly, DCs cause less cellular

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**Fig. 4.** Fc gamma receptor expression on mDCs and cytokines in blood. Expression of the Fc gamma receptors CD32 and CD64 on blood mDCs (a, b) and concentration of neopterin in serum and the expression of TLR9 on pDCs (c, d) of controls (white) and patients with pneumonia on day 1 (dark grey). Box plot graphs display the median (line within the box), interquartile range (edges of the box) and the range of all values less distant than 1.5 interquartile ranges from the upper or lower quartile (vertical lines). Outliers (all values more distant than 1.5 interquartile ranges from the upper or lower quartile) were omitted in the graphs.
toxicity following antibody-dependent phagocytosis than monocytes [36]. However, DCs are more efficient than monocytes with respect to T cell activation following an antibody-dependent internalisation of pathogens. This led to the hypothesis that CD64- and CD32-mediated phagocytosis augments the T cell stimulatory potential of DCs, rather than representing simple phagocytic activity [36]. Thus, the increased expression of CD64 and CD32 on blood DCs in patients with pneumonia might reflect an enhanced potential of mDCs to process pathogens and to induce T cell responses against these pathogens.

The current concept of DC maturation suggests that immature DCs have a strong antigen uptake capacity and a weak capacity to present antigens, whereas maturing DCs lose their antigen uptake capacity and gain the ability to present antigens to lymphocytes. The up-regulation of CD32, CD64 and TLR9 identified in our study (suggesting an improved ability to take up antigens and opsonised antigens) would be compatible with a more immature phenotype of DCs and with the observed reduction of co-stimulatory molecules CD86 and CD40 (which are important for antigen presentation). Thus, the up-regulation of CD32, CD64 and TLR9 on blood DCs could simply reflect an immature phenotype of these cells. However, other surface molecules found on immature DCs such as CCR5 and several TLRs (TLR 1, 2, 4) were not up-regulated on the DCs. In addition, we have recently shown in patients with Hymenoptera venom allergy that a reduced expression of CD40 and CD86 (suggesting a more immature phenotype) is not necessarily associated with an increased expression of CD32, CD64 or TLR9 on blood DCs [37]. Thus, we hypothesise that the observed pattern of DC surface molecule expression may reflect a specific change in acute infectious pneumonia.

IFN-γ, a central mediator of innate and adaptive immune responses against pathogens [38], has been shown to regulate the expression of Fc gamma receptors on human blood mDCs [36]. We measured neopterin, a marker of systemic IFN-γ release in humans [20]. Serum neopterin concentrations were increased in patients with acute pneumonia, suggesting that IFN-γ may contribute to the observed up-regulation of Fc gamma receptors on blood mDCs during pneumonia. Thus, we speculate that enhanced systemic IFN-γ levels during a pulmonary infection could lead to an up-regulation of Fc gamma receptors on blood mDCs, and consequently to an enhanced potential of these mDCs to process pathogens and to induce T cell responses against the encountered antigens.

In conclusion, we describe for the first time the phenotype of circulating DCs in patients with acute infectious pneumonia. These data provide important new information on the pathophysiology of the adaptive immune system in human respiratory tract infections.

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References
