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Platelet and plasma BDNF in lower respiratory tract infections of the adult

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Summary
Enhanced bronchial responsiveness during and following lower respiratory tract infections is a major clinical problem, but its pathogenesis is poorly understood. Brain-derived neurotrophic factor (BDNF), which can be released by platelets and leukocytes, has been identified as a mediator of bronchial hyperresponsiveness. It is unknown whether the release of BDNF is altered during lower respiratory tract infections of the adult. In this clinical pilot study, 16 patients (35–80 years old) with the diagnosis of an acute bacterial lower respiratory tract infection and elevated serum concentrations of c-reactive protein ($>100\,\mu g/ml$) and procalcitonin ($>0.1\,ng/ml$) were examined on admission to the hospital and 1 week after antibiotic treatment. Sixteen age- and sex-matched controls were examined in the same time period. BDNF concentrations in serum and platelets, but not in plasma, were markedly reduced in patients on the day of admission (median $<25\%$ of the controls). Analysis of the platelet marker serotonin (5-HT) suggested that the decrease of platelet BDNF is part of a non-specific release of platelet-derived mediators in this condition. Clinical improvement was accompanied by a restoration of serum and platelet BDNF concentrations which returned to control levels after 1 week of treatment. Cell culture experiments revealed that bacterial lipopolysaccharide (LPS) enhanced the release of BDNF by peripheral blood mononuclear cells of the patients at both time points. In conclusion, these data suggest that lower respiratory tract infections might be associated with an augmented release of BDNF by platelets and mononuclear cells.

Abbreviation: BDNF, brain-derived neurotrophic factor; LPS, lipopolysaccharide; LRTI, lower respiratory tract infection; CRP, c-reactive protein; PCT, procalcitonin; PBMC, peripheral blood mononuclear cells; 5-HT, 5-hydroxytryptamine, serotonin; TNF-alpha, tumor necrosis factor alpha

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Introduction

An exaggerated responsiveness of the airways during and following lower respiratory tract infections (LRTI) is a major clinical problem, particularly in patients with pre-existing airway diseases.[1] However, its pathogenesis is unclear and specific treatment options are not available. Although there is evidence that an increased reactivity of airway nerves contributes to bronchial hyperresponsiveness in this condition, the precise mechanisms are poorly understood.[2,3] Brain-derived neurotrophic factor (BDNF), a key regulator of neuronal plasticity and excitability,[4,5] has recently been identified as a mediator of enhanced neuronal reactivity in the airways.[6,7] BDNF is constitutively expressed in the airway epithelium and smooth muscle.[8,9] Enhanced BDNF secretion by epithelia and leukocytes[10,11] contributes to bronchial hyperresponsiveness in models of asthma.[12] There is increasing evidence that infections are associated with BDNF overexpression.[13–15] Therefore, BDNF has been suggested as a potential trigger of enhanced bronchial responsiveness in infected human airways.[14]

Large amounts of BDNF are stored in human platelets.[16] The difference between high BDNF concentrations in serum and very low BDNF concentrations in plasma[17] is attributable to a BDNF release from platelets during the clotting process.[16] Therefore, this difference between serum and plasma BDNF levels, corrected for the individual platelet count, represents an estimate for the individual amount of platelet BDNF.[17,18] BDNF is not produced by platelets or its precursors but actively taken up by platelets.[16] In addition, the wide range of platelet BDNF concentrations is not attributable to the individual age, body weight or height.[16,17] Recent studies demonstrating that platelet BDNF can change rapidly[19,20] confirmed the concept that platelets represent a highly dynamic repository of BDNF in human peripheral blood.

Since platelets release their mediators at the sites of inflammation,[21] it is likely that platelet-derived BDNF impacts on neuronal function in inflamed tissues. This hypothesis is supported by the finding that elevated concentrations of BDNF in platelets correlate with bronchial hyperresponsiveness in patients with asthma.[18] Although respiratory tract infections of the adult are associated with an exaggerated responsiveness of the airways, there is currently no information on platelet-derived or leukocyte-derived BDNF in this condition. This clinical pilot study, therefore, aimed to investigate BDNF concentrations in platelets and plasma, and the secretion of BDNF by mononuclear cells during acute LRTI of the adult.

Methods

Study design

This study was conducted at the University Hospital of Rostock (Germany). Patients who presented with cough, dyspnoea, or both (as the main symptom) at the medical emergency department were assessed for eligibility. The assessment at the emergency department included complete history, physical examination, measurement of body temperature, blood sampling for hematological analysis and blood chemistry (including c-reactive protein, CRP, and procalcitonin, PCT), capillary blood gases, and conventional chest radiography. Additional diagnostic procedures and the choice of the antibiotic regimen were left to the attending physician. The study was approved by the local ethics committee. All participants gave their written informed consent. Patients were examined on the day of admission to the hospital (''day 1''), and 1 week after antibiotic treatment (''day 8''). Inclusion criteria for the study were as follows: (1) onset of dyspnoea and/or cough within the last 7 days prior to admission, (2) radiological and clinical signs suggesting pneumonia, or purulent sputum in patients with chronic obstructive pulmonary disease, (3) CRP levels $>100\,\mu g/ml$ and PCT levels $>0.10\,ng/ml$ serum. Exclusion criteria were: (1) unstable clinical conditions requiring intubation and/or transfer to the intensive care unit, (2) other acute inflammatory diseases, (3) malignant diseases, (4) haemodialysis therapy, or (5) treatment with immunosuppressive drugs. In the same time period, age- and sex-matched volunteers were recruited as controls. Inclusion criteria for the controls were: (1) no signs of an acute infection, (2) no history of chronic inflammatory diseases, (3) c-reactive protein (CRP) concentrations $<5\,\mu g/ml$ and procalcitonin (PCT) levels $<0.10\,ng/ml$ serum. Exclusion criteria for controls were: (1) malignant diseases, (2) haemodialysis therapy, or (3) treatment with immunosuppressive drugs.

Blood parameters and cell culture

Blood was drawn from the cubital vein, into heparinised (plasma), additive-free (serum) and EDTA-containing (for cell separation and blood cell counts) containers, and placed on ice immediately. Heparinised (plasma) and additive-free (serum) containers were placed on ice for 60 min. Afterwards, serum and plasma samples were obtained by centrifugation for 15 min (2000*g*, 4 °C), and stored at −80 °C until measured.[17] Blood cell counts and serotonin (5-HT) were measured as described.[17] BDNF concentrations in serum, plasma and cell culture supernatants were measured using a commercial enzyme-linked immunosorbent assay (ELISA) following the manufacturers instructions (DuoSet BDNF ELISA, R&D Systems, Minneapolis, USA). All BDNF measurements were performed in duplicate. Serum samples were diluted 1:50, plasma samples were diluted 1:2 in phosphate buffered saline (PBS) for BDNF measurement. The detection limit was 4 pg BDNF/ml. Platelet BDNF content was calculated by subtracting plasma BDNF from serum BDNF, and dividing the result by the platelet count, as described.[17,18] Platelet 5-HT content was calculated accordingly. C-reactive protein (detection limit: $1\,\mu g/ml$ serum) was measured using Synchron LX 20 (Beckman Coulter, Krefeld, Germany), procalcitonin (detection limit: 0.06 ng/ml serum) was measured with the Kryptor PCT automated assay (Brahms, Hennigsdorf, Germany).[22] Monocyte-enriched PBMC were isolated for cell culture experiments as described,[18] and 2×10^6 cells/ml cultured in RPMI 1640 with 10% fetal calf serum, 100 U/ml penicillin, $100\,\mu g/ml$ streptomycin for 24 h. PBMC were stimulated with 50 ng/ml tumor necrosis factor alpha (TNF-alpha) or 100 ng/ml bacterial lipopolysaccharide (LPS)

(Sigma, Deisenhofen, Germany). Cell-free supernatants were aliquoted and stored at −80 °C until measured.

Statistical analysis

Data were analysed using SPSS (SPSS Inc., Chicago, IL, 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 was used for comparisons of patients at the two time points, and for the comparison of cell cultures with and without stimulation. Correlations were calculated using Spearman's correlation coefficient. *p*-values <0.05 were regarded as significant.

Results

Characteristics of the participants

Twenty-four patients with the clinical diagnosis of an acute bacterial LRTI were included in the study. Eight patients had to be excluded subsequently, due to concurrent cholecystitis (1 patient), newly diagnosed carcinoma (4 patients), death (1 patient), surgical treatment for empyema (1 patient), and a history of recurrent pneumonia in the middle lobe suggesting chronic recurrent infection (1 patient), resulting in a final cohort of 16 patients (Table 1). The majority (12 out of 16 patients) were smokers, with a median smoking history of 35 (range: 10–100) pack years (Table 1). Vital signs and the blood gas analysis on admission (day 1) are displayed in Table 2. All patients suffered from hypoxemia ($PaO_2 < 70$ mmHg) on admission, whereas only two patients displayed hypercapnia ($PaCO_2 > 45$ mmHg). None of the patients displayed acidosis (pH < 7.35) on admission. Tachycardia (> 100 beats/min) was present in 9 out of 16 patients (Table 2). Sixteen age- and sex-matched controls were recruited in the same time period. None of the controls reported any acute or chronic disease, and none of the controls was on regular medication. The majority of the controls (11 out of 16 controls) were smokers, with a median smoking history of 20 (range: 10–50) pack years.

After admission, patients received standard medical care (oxygen delivery and inhalation therapy) and were treated with antibiotics for at least 7 days. One patient who initially received cefuroxime was put on vancomycin (on day 3) due to methicillin-resistant staphylococcus aureus (MRSA) in a sputum culture (Table 1). Sputum cultures from 4 patients yielded positive results in 2 cases, and blood cultures from 5 patients gave 2 positive results. All 16 patients showed a significant improvement in clinical and laboratory parameters after 1 week of treatment, and were discharged after a median hospital stay of 9.5 days (Table 1). Patients displayed high concentrations of CRP and PCT, and elevated leukocyte counts on admission (day 1). All parameters decreased significantly after 1 week of antibiotic treatment (day 8), but remained significantly elevated compared to the controls (Table 3). Platelet counts were indistinguishable between patients on day 1 and controls, but increased significantly after 1 week of treatment (Table 3).

BDNF and 5-HT concentrations in serum, platelets and plasma

Compared to the control group, serum BDNF (median: 24% of the controls) and 5-HT concentrations (median: 21% of the controls) were significantly lower in patients on day 1. This difference was even more pronounced when evaluating platelet BDNF (median: 23% of the controls) and 5-HT levels

Table 1 Patient characteristics.

Patient no.	Gender	Age	Pack years	Diagnosis	Antibiotic treatment	Hospital stay (days)
1	Male	64	40	Pneumonia	Cefuroxime	8
2	Female	35	0	Pneumonia	Sultamicillin	8
3	Male	75	30	Pneumonia	Cefuroxime	10
4	Female	80	40	AECOPD	Sultamicillin	10
5	Male	39	20	Pneumonia	Moxifloxacin	10
6	Male	52	40	Pneumonia	Levofloxacin	16
7	Female	43	20	Pneumonia	Moxifloxacin	14
8	Female	80	0	Pneumonia	Cefuroxime	9
9	Male	55	30	AECOPD	Cefuroxime/ vancomycin	17
10	Male	68	30	AECOPD	Ceftazidime	12
11	Male	75	45	AECOPD	Sultamicillin	8
12	Male	80	100	Pneumonia	Sultamicillin	8
13	Male	64	40	Pneumonia	Levofloxacin	11
14	Male	65	0	Pneumonia	Moxifloxacin	8
15	Male	65	10	Pneumonia	Levofloxacin	8
16	Male	78	0	Pneumonia	Cefuroxime	9

The table displays the age and gender of the patients, the smoking history (in pack years), the clinical diagnosis (AECOPD: acute exacerbation of chronic obstructive pulmonary disease), the antibiotic treatment during the study period, and the length of the hospital stay.

Table 2 Vital signs and blood gas analysis on admission.

	Median	Minimum	Maximum
Temperature (°C)	38.0	36.7	40.2
Heart rate (bpm)	108	76	127
Systolic BP (mmHg)	128	96	174
Diastolic BP (mmHg)	70	49	100
pH	7.47	7.41	7.60
PaO_2 (mmHg)	52.6	42.9	64.7
$PaCO_2$ (mmHg)	34.6	27.8	57.9

The table displays the body temperature, heart rate (bpm: beats per minute), blood pressure (BP), and blood gases (while breathing ambient air) of the 16 patients on admission (day 1).

Table 3 Cells and inflammatory markers.

	Controls	Patients on day 1	Patients on day 8
Platelets (10^6/ml blood)	231 (143–310)	231 (111–491)	295 (209–594)*
Leukocytes (10^6/ml blood)	5.9 (3.3–7.9)	13.7 (9.2–30.0)*	8.6 (4.5–14.6)*
CRP (μg/ml serum)	<1	201 (110–493)*	50 (12–262)*
PCT (ng/ml serum)	0.07 (0.06–0.10)	0.29 (0.13–14.40)*	0.15 (0.06–0.73)*

The table displays the median (range) of platelet and leukocyte counts in peripheral blood as well serum concentrations of c-reactive protein (CRP) and procalcitonin (PCT), of the controls ($n = 16$) and of the patients ($n = 16$) at both time points. Significant differences compared to the control group ($p<0.05$) are marked with an asterisk.

(median: 10% of the controls). In contrast, plasma concentrations of BDNF and 5-HT did not differ between controls and patients on day 1 (Fig. 1). After 1 week of treatment, serum and platelet BDNF concentrations were significantly increased and no longer different to the concentrations measured in controls. Although there was a significant increase of platelet 5-HT levels after 1 week of treatment, serum and platelet 5-HT concentrations were still significantly lower compared to the control group at this time point (Fig. 1).

Association with clinical parameters

Vital signs (temperature, heart rate, blood pressure) were not significantly correlated with BDNF levels in serum, platelets or plasma on admission (data not shown). There were non-significant trends ($p>0.05$) to negative correlations between platelet BDNF levels and CRP ($r = -0.29$) or PCT levels ($r = -0.13$) on admission. $PaCO_2$ values and PaO_2 values did not correlate significantly with BDNF concentrations in peripheral blood. In contrast, pH values on admission were negatively correlated with BDNF concentrations in serum ($r = -0.60$, $p<0.05$), platelets ($r = -0.53$, $p<0.05$) and plasma ($r = -0.74$, $p<0.01$).

BDNF secretion by peripheral blood mononuclear cells

There were no significant differences in BDNF concentrations in cell culture supernatants of unstimulated PBMC between controls (median: 13.1 pg/ml), patients on day 1 (median: 14.6 pg/ml) and patients on day 8 (median: 20.6 pg/ml). Stimulation with TNF-alpha or LPS increased BDNF concentrations significantly (Fig. 2). There were no significant differences in the TNF-alpha- or LPS-induced increase in BDNF concentrations between controls and patients at both time points (Fig. 2).

Discussion

Platelets play a critical role in inflammatory processes of the lung. They release potent mediators into the local microenvironment, mainly because of intravascular activation, but also because of transmigration into inflamed parenchyma.[21,23,24] This pilot study is the first to demonstrate that platelet BDNF is markedly reduced in patients with an acute LRTI. In addition, we show that platelet BDNF concentrations recover after only 1 week of successful treatment. It cannot be completely excluded that the medication of the patients could have interfered with the BDNF release or with the BDNF measurements. However, since the medication was almost identical on days 1 and 8 of the study it appears highly unlikely that the medication accounted for the differences in BDNF platelet concentrations. Therefore, the changes of BDNF platelet concentrations appear to be specifically attributable to the LRTI. The reduction of platelet BDNF during the acute LRTI could either be due to a decreased uptake or an increased release of BDNF by platelets.[16] Previous studies suggested an enhanced release of platelet-derived mediators during acute infections.[25,26] In addition, there is evidence indicating BDNF overexpression in infected organs.[13–15] Therefore, we hypothesised that low

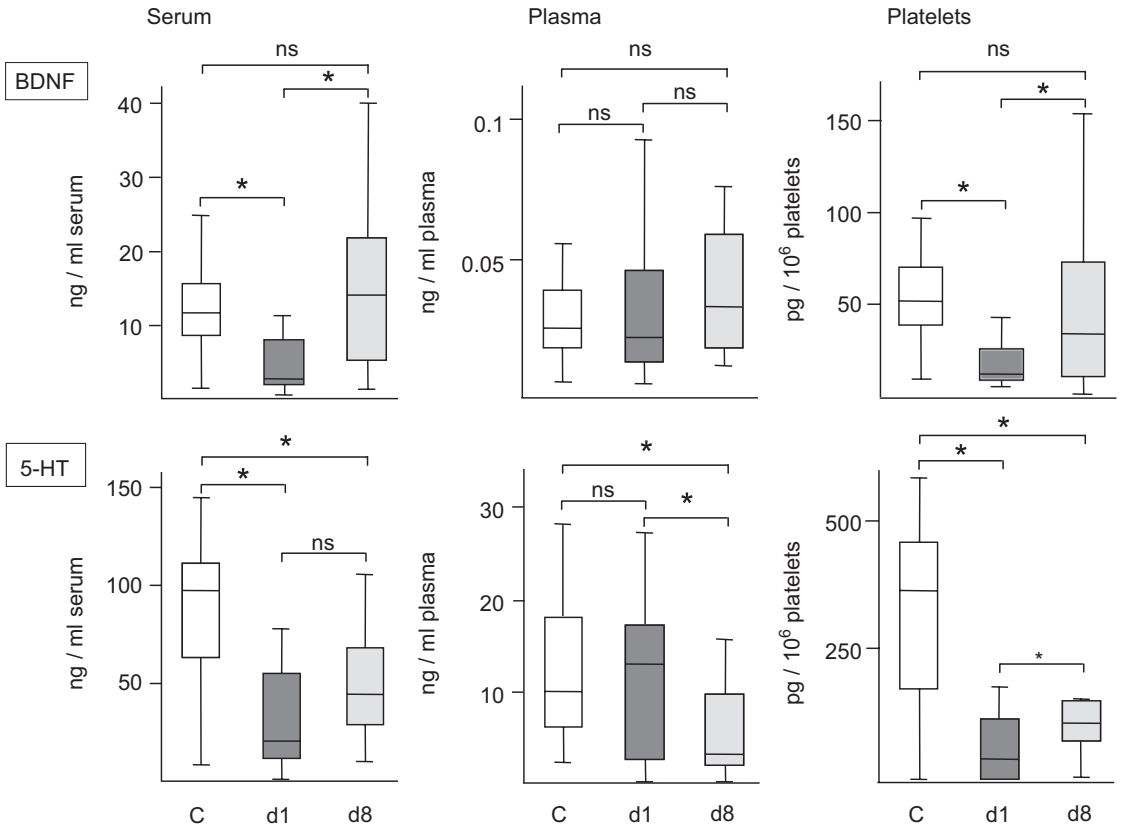

Figure 1 BDNF and 5-HT concentrations in serum, plasma and platelets. Shown are the concentrations of BDNF (upper panel) and 5-HT (lower panel) in serum, plasma and platelets, of the following groups: control group (**C**, white boxes), patients on the day of admission to the hospital (**d1**, dark grey boxes), and the same patients after 1 week of antibiotic treatment (**d8**, light grey boxes). Asterisks mark significant differences ($p<0.05$) between groups or time points; ns denotes not significant ($p>0.05$). Boxplot 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).

platelet BDNF concentrations are due to an enhanced release, rather than a decreased uptake of BDNF in this condition.

To test this, we examined the platelet marker serotonin (5-HT), which is known to be released from human platelets during bacterial infections.[25,26] The decreases of 5-HT and BDNF platelet concentrations during the acute LRTI (day 1) were strikingly similar. Thus, it is likely that the reduction of platelet BDNF is indeed reflecting an enhanced release of BDNF in this condition. Of note, BDNF and 5-HT are stored in different granules of the platelet. While 5-HT is stored in platelet dense-core granules, BDNF is most likely localised in platelet alpha-granules.[17–20] Therefore, the parallel decrease of BDNF and 5-HT platelet concentrations suggests a rather non-specific release of platelet mediators during an acute LRTI. Notably, platelet BDNF and 5-HT significantly increased after 1 week of treatment. This finding suggests that platelet BDNF and 5-HT stores recover after clinical improvement. However, it appears that there are differences in the time course. After 1 week of treatment, platelet BDNF returned to control levels, whereas platelet 5-HT was still markedly decreased. This could be attributable to the storage of BDNF and 5-HT in different platelet granules, which differ considerably in physiology and function.[27,28] In contrast to platelet concentrations, plasma levels of BDNF and 5-HT remained unaltered during the acute LRTI. In the literature, a very short plasma half-life of BDNF (<1 min)[29] and 5-HT (<2 min)[30] has been reported. Therefore, our observations would be compatible with a rapid clearance of platelet-derived BDNF and 5-HT from the circulation in this condition.

However, the actual site of the BDNF release by platelets is yet unclear. Platelets may release BDNF within the pulmonary circulation. This would result in low BDNF levels in circulating platelets, as observed in our study. A rapid clearance of platelet-derived BDNF from the plasma (either by enzymatic degradation or by yet unknown transport mechanisms into the perfused tissue) would explain the unchanged BDNF plasma levels during the LRTI (see above). However, platelets have also been postulated to transmigrate into the inflamed lung parenchyma, either alone (by active diapedesis) or in conjunction with leukocytes (in platelet-leukocyte aggregates).[21,23,24] In this scenario, BDNF would be directly released from transmigrating platelets into the lung parenchyma. The pulmonary sequestration of mature platelets and the infection-related thrombopoiesis in the bone marrow would be an alternative explanation for the low platelet BDNF concentrations measured: since BDNF

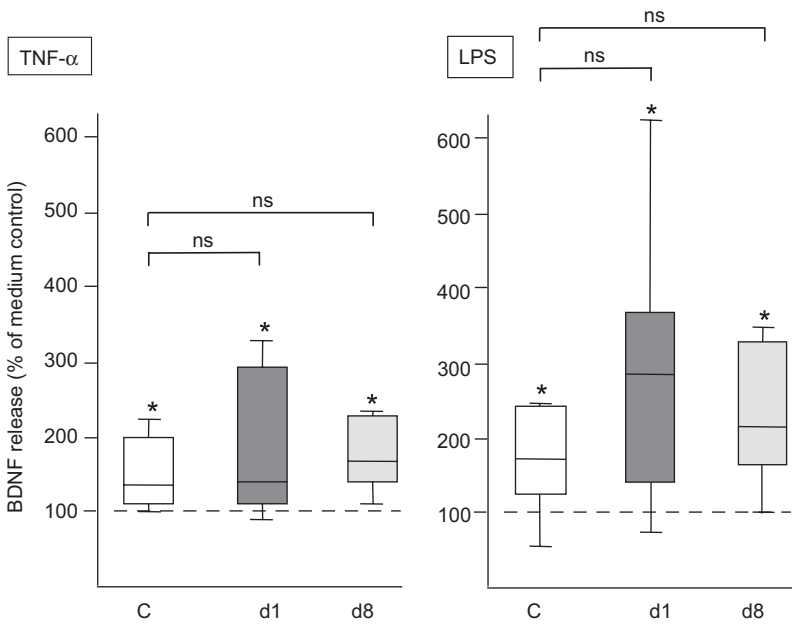

Figure 2 Release of BDNF by cultured mononuclear cells. Monocyte-enriched PBMC were cultured and stimulated with TNF-alpha (left panel) or LPS (right panel) for 24 h. Boxplots display BDNF concentrations in supernatants after stimulation, in percent of the corresponding medium control, of the following groups: control group (**C**, white boxes), patients on the day of admission to the hospital (**d1**, dark grey boxes), and the same patients after 1 week of antibiotic treatment (**d8**, light grey boxes). Significant differences ($p<0.05$) compared to the corresponding medium control are marked with asterisks. The BDNF release did not significantly differ between the groups or time points (ns denotes not significant). Boxplot details are described in Fig. 1.

is not produced in megakaryocytes but actively taken up by circulating platelets,[16] an enhanced percentage of newly released platelets should result in low median platelet BDNF levels.

The underlying cause of the BDNF release by platelets is speculative. Due to the non-specific character of the release, immunological mechanisms leading to an activation and degranulation of platelets appear to be likely.[25] Indeed, the release of BDNF by platelets has been shown to be stimulated by a variety of agonists which play a role in inflammatory processes.[16] An altered acid-base balance might influence this BDNF release since we found an association between pH values and platelet BDNF concentrations. On the other hand, a contribution of LRTI-associated mood disturbances to reduced platelet BDNF concentrations appears unlikely. Mood disorders are characterised by a specific decrease in BDNF production. A non-specific release of BDNF and other mediators by platelets does not comply with current concepts of mood disorders.[31]

The amount of BDNF secreted by monocytes, which represent the major source of BDNF among leukocytes,[32] is lower than the amount of BDNF secreted by platelets. Platelets in 1 ml of human blood can release more than 10 ng of BDNF,[16] whereas monocytes in 1 ml of human blood release less than 1 ng of BDNF.[32] Nevertheless, monocytes might contribute to a sustained local release of BDNF. We, therefore, measured BDNF secretion of monocyte-enriched PBMC of patients and controls. We found no differences in the releasibility of BDNF from mononuclear cells between patients and controls. However, bacterial LPS significantly enhanced the release of BDNF, which was consistent with previous data from an animal model.[33] Therefore, LPS might augment the secretion of BDNF by infiltrating mononuclear cells in infected airways.

In conclusion, our data suggest that lower respiratory tract infections might be associated with an augmented release of BDNF by platelets, as part of a general release of platelet-derived mediators in this condition. Mononuclear cells stimulated with bacterial LPS could further enhance local BDNF concentrations. Because BDNF causes neuronal hyperexcitability, we hypothesise that an increased BDNF secretion by platelets and leukocytes might contribute to an exaggerated responsiveness of the airways in respiratory tract infections. Further clinical and experimental studies which include bronchoalveolar lavage, lung biopsies and lung function tests are needed to test this hypothesis.

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