

Research

Open Access

Inducibility of the endogenous antibiotic peptide β -defensin 2 is impaired in patients with severe sepsisMalte Book¹, QiXing Chen^{1,2}, Lutz E Lehmann¹, Sven Klaschik¹, Stefan Weber¹, Jens-Christian Schewe¹, Markus Luepertz¹, Andreas Hoefft¹ and Frank Stuber¹¹Department of Anaesthesiology and Intensive Care Medicine, Rheinische-Friedrich-Wilhelms University Bonn, Sigmund-Freud-Str. 25, 53105 Bonn, Germany²Department of Anaesthesiology, School of Medicine, Zhejiang University, 388 Yuhang Tang Road, 310058 Hangzhou, People's Republic of ChinaCorresponding author: Malte Book, malte.book@ukb.uni-bonn.de

Received: 31 Jul 2006 Revisions requested: 1 Sep 2006 Revisions received: 8 Jan 2007 Accepted: 15 Feb 2007 Published: 15 Feb 2007

Critical Care 2007, **11**:R19 (doi:10.1186/cc5694)This article is online at: <http://ccforum.com/content/11/1/R19>© 2007 Book *et al.*; licensee BioMed Central Ltd.This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.**Abstract**

Introduction The potent endogenous antimicrobial peptide human β -defensin 2 (hBD2) is a crucial mediator of innate immunity. In addition to direct antimicrobial properties, different effects on immune cells have been described. In contrast to the well-documented epithelial β -defensin actions in local infections, little is known about the leukocyte-released hBD2 in systemic infectious disorders. This study investigated the basic expression levels and the *ex vivo* inducibility of hBD2 mRNA in peripheral whole blood cells from patients with severe sepsis in comparison to non-septic critically ill patients and healthy individuals.

Methods This investigation was a prospective case-control study performed at a surgical intensive care unit at a university hospital. A total of 34 individuals were tested: 16 patients with severe sepsis, 9 critically ill but non-septic patients, and 9 healthy individuals. Serial blood samples were drawn from septic patients, and singular samples were obtained from critically ill non-septic patients and healthy controls. hBD2

mRNA levels in peripheral white blood cells were quantified by real-time polymerase chain reaction in native peripheral blood cells and following *ex vivo* endotoxin stimulation. Defensin plasma levels were quantified by enzyme-linked immunosorbent assay.

Results Endotoxin-inducible hBD2 mRNA expression was significantly decreased in patients with severe sepsis compared to healthy controls and non-septic critically ill patients (0.02 versus 0.95 versus 0.52, $p < 0.05$, arbitrary units). hBD2 plasma levels in septic patients were significantly higher compared to healthy controls and critically ill non-septic patients (541 versus 339 versus 295 pg/ml, $p < 0.05$).

Conclusion In contrast to healthy individuals and critically ill non-septic patients, *ex vivo* inducibility of hBD2 in peripheral blood cells from septic patients is reduced. Impaired hBD2 inducibility may contribute to the complex immunological dysfunction in patients with severe sepsis.

Introduction

Endogenous antimicrobial peptides are widely distributed in various species [1,2]. They are part of the innate immune system and their genes are highly conserved throughout the animal and plant kingdoms. In humans, antimicrobial defensins are divided into α - and β -defensins according to their molecular structure. They display a broad antimicrobial effect against bacteria, fungi, mycobacteria, and coated viruses [2-5].

Defensins act by permeabilising microbial membranes. In addition, β -defensins are chemotactic for immature dendritic cells and memory T cells. They regulate cytokine production and adhesion-molecule expression, stimulate epithelial cell and fibroblast proliferation, and promote histamine release from mast cells [6,7].

To date, six human β -defensin genes have been characterised and located on chromosome 8. The epithelial human β -defensin 1 (*hBD1*) gene is constitutively expressed at low

AMV = avian myeloblastosis virus; ANOVA = analysis of variance; APACHE II = Acute Physiology and Chronic Health Evaluation II; BSA = bovine serum albumin; C_p = crossing point; hBD2 = human β -defensin 2; hHPRT = human hypoxanthine phosphoribosyl-transferase; HLA-DR = human leukocyte antigen-DR; ICU = intensive care unit; IL = interleukin; NF- κ B = nuclear factor-kappa B; PCR = polymerase chain reaction; PCT = procalcitonin; SOFA = Sepsis-related Organ Failure Assessment.

levels and slightly upregulated following stimulation [8]. In contrast, *hBD2*, *hBD3*, and *hBD4* gene expression is inducible mainly by various inflammatory stimuli in different cell types [9–12]. The recently described *hBD5* and *hBD6* represent epididymis-specific human defensins [13].

There is increasing evidence for the clinical relevance of defensins. Alpha- and β -defensins contribute to anti-HIV activity [14,15]. In newborns, respiratory tract β -defensin mRNA expression is upregulated in response to infection [16]. Moreover, a systemic release of β -defensins in infectious diseases has been reported [17]. Our own previous experiments detected *hBD2* mRNA expression in white blood cells following *ex vivo* stimulation by endotoxin [18]. In particular, systemic infection underlying syndromes such as severe sepsis challenges the immune system by constant activation of its adaptive and innate components. The responsiveness of the innate immune system, including expression of endogenous antibiotic peptides like β -defensins, contributes to the final resolution of the disease.

The present study investigated *hBD2* mRNA levels in native peripheral white blood cells as well as the *ex vivo* *hBD2* mRNA inducibility in patients with severe sepsis. Additionally, we determined *hBD2* protein plasma levels in patients. The hypothesis that *hBD2* expression is disturbed in patients with severe sepsis was tested.

Materials and methods

Patients and controls

This study was performed according to the ethical standards stated in the 1964 Declaration of Helsinki. After approval by the local ethics committee and receipt of the written informed consent of either the patient or a close relative, 16 patients treated on a surgical intensive care unit (ICU) at a university hospital with the diagnosis of severe sepsis were included in this prospective case-control study. The diagnosis of severe sepsis met the criteria of the American College of Chest Physician/Society of Critical Care Medicine Consensus Conference Committee [19]. Exclusion criteria were (a) lack of informed consent, (b) age younger than 18 years, and (c) pre-existing immunological or haematological diseases. Whole blood samples were drawn on the day of diagnosis (day 1) and on the third and fifth days of severe sepsis. A fourth blood sample was drawn after recovery from severe sepsis at ICU discharge in survivors or at imminent death in the case of non-survivors (day X).

In addition, two control groups were included: nine non-septic critically ill ICU patients who were in need of intensive care and who were without any signs of infection (blood samples were drawn once during the ICU treatment) and nine healthy volunteers (blood samples were drawn once). All patients and volunteers were of German Caucasian origin.

Blood culture and RNA isolation

Whole blood was co-cultured for four hours with 500 pg/ml lipopolysaccharide contained in the Milenia[®] *ex vivo* stimulation kit (Milenia Biotec, Hohe Str. 4–8, 61231 Bad Nauheim, Germany) at 37°C and 5% CO₂. After incubation, the blood was centrifuged at 1,500 *g* for five minutes. The supernatant was stored at -70°C for further analysis. Total RNA was extracted from whole blood by means of a QIAamp[®] RNA Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and then dissolved in diethylpyrocarbonate-treated water and stored at -70°C until further analysis.

Basic *hBD2* mRNA levels were investigated using Paxgene[®] Blood RNA System tubes (PreAnalytiX; Qiagen GmbH, Hilden, Germany). For this analysis, 2.5 ml of whole blood was drawn in Paxgene[®] tubes and treated as indicated in the manufacturer's instructions. By this method, intracellular RNA was stabilised until further analysis. RNA isolation was performed using the Paxgene[®] kit according to the manufacturer's instructions.

cDNA preparation

cDNA was produced as polymerase chain reaction (PCR) template using 1st Strand cDNA Synthesis Kit for RT-PCR[®] (avian myeloblastosis virus [AMV]) (Roche Diagnostics, Sandhofer Str. 116, 68305, Mannheim, Germany). The reaction mixture contained 8.2 μ l (approximately 500 ng) of total RNA, 5 mM MgCl₂, 1 mM dNTP, 3.2 μ g of random primer p(dN)₆, 50 units of RNase inhibitor, 20 units of AMV reverse transcriptase, and 1 \times reaction buffer in a total volume of 20 μ l. The reaction was incubated at 25°C for 10 minutes, 42°C for 60 minutes, and 99°C for 5 minutes and then cooled to 4°C for 5 minutes.

Real-time PCR

The PCR was performed on a LightCycler[®] instrument (Roche Diagnostics). For the amplification of *hBD2*, the reaction mixture included 10 μ l of cDNA, 1 μ M each primer (forward and reverse), 0.15 μ M each hybridisation probe (labelled with fluorescein and LC-Red640; TIB MOLBIOL GmbH, Berlin, Germany), and 1 \times Lightcycler FastStart Master^{PLUS} Mix (Roche Diagnostics) in a total volume of 20 μ l. For detection of the housekeeping gene *hHPRT* (human hypoxanthine phosphoribosyl-transferase), the 20 μ l of reaction mixture consisted of 2 μ l of cDNA, 2 μ l of reaction mix for *hHPRT* (Roche), and 12 μ l of ddH₂O in 1 \times Lightcycler FastStart Master^{PLUS} Mix (Roche Diagnostics). The sequences of primers and hybridisation probes specific for *hBD2* measurement were as follows: forward primer: 5'-CTGATGCCTCTTCCAGGTGT-3'; reverse primer: 5'-GGAGCCCTTCTGAATCCG-3'; probes: 5'-GGTATAAACAAATTGGCACCTGTGGTC-FL and 5'-LC Red640 CCCTGGAACAAATGCTGCAAAA-PH.

The PCRs for *hBD2* and *hHPRT* were conducted in separate capillaries as duplicates. The reaction was performed as

follows: initial denaturation at 95°C for 10 minutes followed by 45 cycles of 95°C for 5 seconds, 55°C for 8 seconds, and 72°C for 10 seconds. The reaction was then cooled at 40°C for 30 seconds. Fluorescence was monitored at the end of each 55°C incubation and detected in channel F2/F1. The crossing point (C_p) of each reaction was analysed by the method of second derivative maximum algorithm (C_p was defined as cycle number at detection threshold).

Relative quantification analysis

The expression level of *hBD2* mRNA in each sample was analysed by LightCycler Relative Quantification Software (Roche Diagnostics). The principles and workflows have been described previously [20]. In summary, the quantity of a target (*hBD2*) and a reference (*hHPRT*) gene is a function of the PCR efficiency and the sample C_p and does not require a standard curve in each LightCycler analysis run for its determination. C_p value is most reliably proportional to the initial template concentration. Differences in PCR efficiency result from different primers as well as hybridisation probes and can be corrected by the software. Results are expressed as the target/reference ratio of each sample normalised by the target/reference ratio of the calibrator. The calibrator is included in every run and its ratio is set to a value of 1. This normalisation provides a constant calibrator point between PCR runs.

$$\text{Normalised ratio} = \frac{\text{conc.target (sample)}}{\text{con.reference (sample)}} \cdot \frac{\text{conc.target (calibrator)}}{\text{conc.reference (calibrator)}}$$

$$\text{Normalised ratio} = E_T^{C_p(T)(C)} \cdot C_p(T)(S) \times E_R^{C_p(R)(S)} \cdot C_p(R)(C),$$

where E = efficiency of PCR amplification, T = target gene, R = reference gene, S = unknown sample, and C = calibrator.

In this experiment, the coefficient file was created by PCR amplification of *hBD2* and *hHPRT* as the housekeeping gene in a series of diluted cDNA (relative standard curve) in triplicates. Data of real-time PCR, including calibrator and samples, were imported into the Relative Quantification Software and analysed with the Fit Coefficients File. Finally, the normalised ratios were calculated. These ratios directly reflect the expression level of *hBD2* mRNA.

hBD2 plasma protein quantification

Twenty micrograms of *hBD2* polypeptides was diluted in acetic acid to form the 1 µg/µl stock solution and then adjusted to 10 mM Tris/0.5% bovine serum albumin (BSA)/0.05% Tween-20 to obtain serial concentrations of the *hBD2* standard: 2,000 pg/ml, 1,000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, and 62.5 pg/ml. Samples were diluted in 1:4 dilution buffer 10 mM Tris/0.5% BSA/0.05% Tween-20. Coating of the standards and samples was performed in a 96-well plate with 100 µl of phosphate-buffered saline coating buffer at 4°C overnight.

Thereafter, the plates were blocked with 300 µl of 5% non-fat bovine milk blocking buffer at 37°C for 2 hours. The goat polyclonal β-defensin 2 antibody (Abcam plc, 332 Cambridge Science Park, Milton Road, Cambridge, UK) was diluted to 0.5 µg/ml with 5% non-fat bovine milk antibody dilution buffer. One hundred microlitres was applied to each well. After additional washing, the peroxidase-conjugated rabbit anti-goat immunoglobulin G antibody (1:1,200) (Sigma-Aldrich Chemie GmbH, Eschenstrasse 5, 82024 Taufkirchen, Germany) was applied to the wells. Plates were covered and incubated at 37°C for two hours. Washing was followed by the addition of 100 µl of ready-to-use tetramethylbenzidine substrate to each well. The plate was then covered and incubated at room temperature for 0.5 hours. One hundred microlitres of stop solution was added to each well. Absorbance was measured at 405 nm using a microtiter plate spectrophotometer followed by an endpoint measurement within one hour.

Human leukocyte antigen-DR quantification on circulating monocytes

Flow cytometric human leukocyte antigen-DR (HLA-DR) quantification was performed according to the method of Docke and colleagues [21]. In brief, this new method quantifies the number of molecules per monocyte and allows direct comparisons between laboratories.

Whole blood cell counts

Leukocyte and monocyte cell counts in whole blood were quantified routinely by standardised clinical biochemical methods.

Statistical analysis

Significance levels between groups were examined using the Kruskal-Wallis test with the Dunn multiple comparison test and Mann-Whitney *U* test where indicated. A *p* value of less than 0.05 was regarded as statistically significant. The time course of the Sepsis-related Organ Failure Assessment (SOFA) scores was analysed by two-way analysis of variance (ANOVA) with repeated measures and Bonferroni *post hoc* analysis. Two-way ANOVA with repeated measures was also used for the time course of *hBD2* plasma levels. In contrast, the non-gaussian distribution of *ex vivo* inducible defensin mRNA expression was analysed by the Kruskal-Wallis test. Correlation of the scores with *hBD2* inducibility was tested using the Spearman test. Statistical power calculations were performed using an open-access statistical web page [22].

Results

Sixteen patients with severe sepsis were included in this study. Eight of these patients died from sepsis-induced organ failure. In addition, nine critically ill but non-septic ICU patients and nine healthy volunteers were included. Table 1 shows demographic and clinical data of the patients. Acute Physiology and Chronic Health Evaluation II (APACHE II) and Simplified Acute Physiology Score II scores differed between

Table 1**Demographic and clinical data of critically ill non-septic patients and patients with severe sepsis**

| | Critically ill non-septic (<i>n</i> = 9) | Severe sepsis (<i>n</i> = 16) | <i>p</i> |
|---|---|--------------------------------|----------|
| Median age (years) | 68 | 55 | > 0.05 |
| Median APACHE II score at inclusion | 12 | 29 | < 0.05 |
| Median SAPS II score at inclusion | 27 | 60 | < 0.05 |
| Mechanically ventilated at inclusion (<i>n</i>) | 5 | 16 | |
| Vasopressor treatment at inclusion (<i>n</i>) | 0 | 13 | |
| Median IL-6 plasma levels (ng/l) | 18 | 72 | < 0.05 |
| Median procalcitonin plasma levels (µg/l) | 0.19 | 2.01 | < 0.05 |
| Antibiotic treatment at inclusion (<i>n</i>) | 7 | 16 | |

Statistical differences were calculated by Mann-Whitney test. APACHE II, Acute Physiology and Chronic Health Evaluation II; IL-6, interleukin-6; SAPS II, Simplified Acute Physiology Score II.

critically ill non-septic patients and patients with severe sepsis ($p < 0.05$, Mann-Whitney test), whereas age did not ($p > 0.05$, Mann-Whitney test). Underlying diseases for severe sepsis were necrotising fasciitis ($n = 2$; at inclusion, both showed clinical signs of additional pulmonary infection), faecal peritonitis ($n = 8$), and pneumonia ($n = 6$). Finally, all patients with severe sepsis suffered from abdominal or pulmonary infection.

Eight of the nine critically ill non-septic patients were in the perioperative period after trauma, abdominal or pharyngeal cancer, or aortic aneurysm rupture with a prolonged postoperative recovery. All of these patients except one were treated with perioperative antibiotic prophylaxis. One patient from this control group suffered from abacterial pancreatitis without antibiotic therapy. None of these patients showed clinically or laboratory signs of infection.

None of the critically ill patients was treated with hydrocortisone. In contrast, 11 patients with severe sepsis were medicated with low-dose hydrocortisone (3 mg/kg body weight per day) at at least one measuring point. All patients with sepsis were treated according to guidelines issued by the Surviving Sepsis Campaign [23].

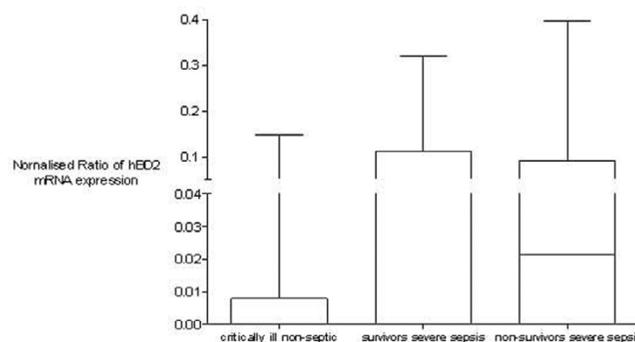
SOFA score was determined at every time point of blood drawing in the included patients, and APACHE II score was calculated at inclusion. The score differences between the patient groups are illustrated in Table 1. Neither the hBD2 inducibility nor the protein levels showed correlations with APACHE II or SOFA scores ($p > 0.05$, Spearman test; data not shown). hBD2 plasma levels did not show a correlation with the Horowitz quotient, thrombocyte count, creatinin levels, or the need of use of vasopressors ($p > 0.05$, Spearman test; data not shown).

SOFA scores in survivors of severe sepsis were decreased at day five and the last sampling day compared to non-survivors

($p < 0.05$, two-way ANOVA with repeated measures and Bonferroni *post hoc* analysis; data not shown).

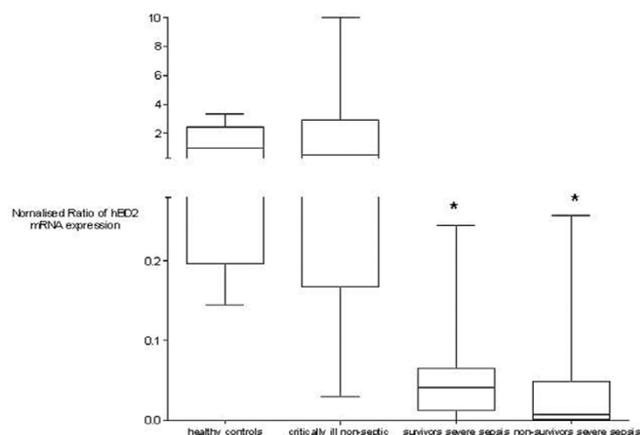
Basic hBD2 mRNA expression was not detectable in peripheral blood cells from healthy controls. The basic hBD2 mRNA expression in survivors and non-survivors of severe sepsis and critically ill patients was normalised to the leukocyte count of every blood sample and showed no differences ($p > 0.05$, Kruskal-Wallis test with the Dunn multiple comparison test; Figure 1).

In contrast, hBD2 mRNA was detectable in *ex vivo* stimulated cultured whole blood. Endotoxin stimulation (4 hours, 0.5 ng/ml) induced hBD2 mRNA expression in all groups and led to low inducibility in patients with severe sepsis. Figure 2 indicates the inducible mRNA expression normalised to leukocyte count at all measured time points. The inducibility in patients with severe sepsis was significantly decreased compared to both other groups ($p < 0.05$, Kruskal-Wallis test with the Dunn

Figure 1

Basic human β -defensin 2 (hBD2) mRNA expression normalised to leukocyte count in critically ill non-septic patients and survivors and non-survivors of severe sepsis shows no differences. No basic mRNA expression was detected in healthy controls ($p < 0.05$, Kruskal-Wallis test with the Dunn multiple comparison test). Data are presented as box-and-whisker plots.

Figure 2

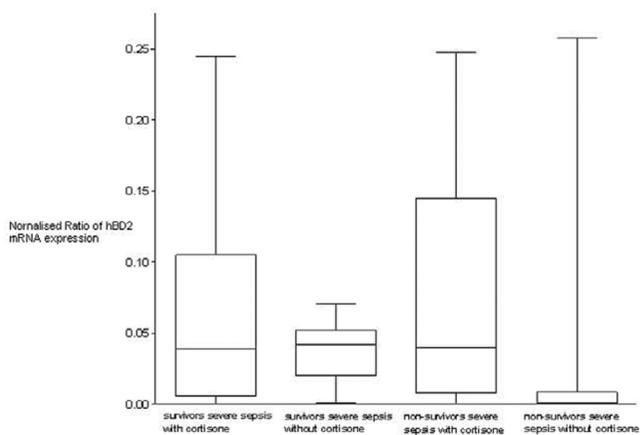


Ex vivo human β -defensin 2 (hBD2) inducibility in healthy controls, critically ill non-septic patients, and survivors and non-survivors of severe sepsis. Inducible hBD2 mRNA expression normalised to leukocyte count is decreased in survivors and non-survivors of severe sepsis compared to healthy controls and critically ill non-septic patients ($*p < 0.05$, Kruskal-Wallis test with the Dunn multiple comparison test). Data are presented as box-and-whisker plots.

multiple comparison test) without differences between survivors and non-survivors of severe sepsis. Despite the limited number of patients, the statistical power of the comparison of hBD2 mRNA inducibility between patients with severe sepsis and controls was 0.95. Hydrocortisone treatment did not impair the leukocyte count-normalised hBD2 mRNA inducibility in patients with severe sepsis ($p > 0.05$, Kruskal-Wallis test with the Dunn multiple comparison test; Figure 3).

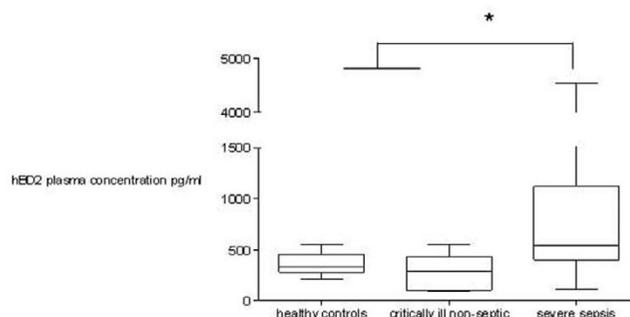
In addition, hBD2 protein concentration was quantified in plasma at all included time points. hBD2 plasma concentra-

Figure 3



Ex vivo human β -defensin 2 (hBD2) inducibility in patients with severe sepsis. Inducible hBD2 mRNA expression normalised to leukocyte count shows no differences in cortisone-treated or non-cortisone-treated patients ($p > 0.05$, Kruskal-Wallis test with the Dunn multiple comparison test). Data are presented as box-and-whisker plots.

Figure 4



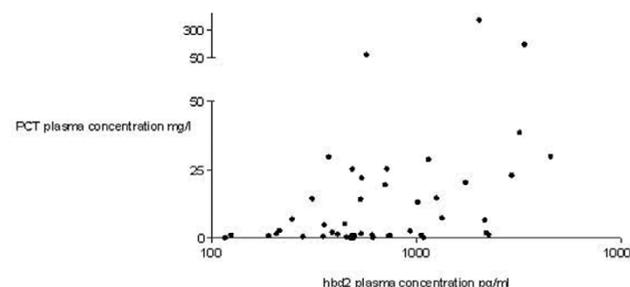
Human β -defensin 2 (hBD2) plasma protein concentration in healthy controls, critically ill non-septic patients, and patients with severe sepsis. Plasma concentration in healthy controls and critically ill non-septic patients was significantly lower compared to patients with severe sepsis ($*p < 0.05$, Kruskal-Wallis test with the Dunn multiple comparison test). Data are presented as box-and-whisker plots.

tions in non-septic critically ill patients and healthy controls were significantly lower compared to patients with severe sepsis ($p < 0.05$, Kruskal-Wallis test with the Dunn multiple comparison test; Figure 4). The comparison of hBD2 plasma levels reached statistical significance at a power of 0.98. No differences were detected between survivors and non-survivors of severe sepsis.

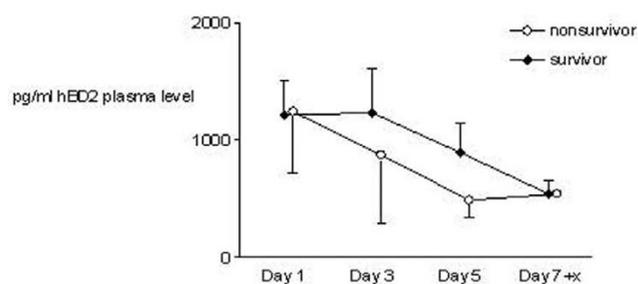
hBD2 protein levels showed no correlation with interleukin (IL)-6 plasma levels in septic patients ($p > 0.05$, correlation coefficient $r = -0.041$, Spearman test; data not shown). In contrast, procalcitonin (PCT) plasma levels and hBD2 protein plasma levels showed a positive correlation in patients with severe sepsis ($p < 0.005$, correlation coefficient $r = 0.4203$, Spearman test; Figure 5).

The time course of hBD2 plasma protein concentration in patients with severe sepsis did not differ significantly between survivors and non-survivors, however it showed considerable variation between survivors and non-survivors ($p > 0.05$, two-way ANOVA with repeated measures; Figure 6).

Figure 5



Human β -defensin 2 (hBD2) plasma protein and procalcitonin (PCT) levels showed a significant correlation in patients with severe sepsis ($p < 0.005$, Spearman test).

Figure 6

Human β -defensin 2 (hBD2) plasma protein concentration at different time points in patients with severe sepsis. Time course of hBD2 plasma protein concentration in survivors and non-survivors of severe sepsis showed no statistical differences ($p > 0.05$, two-way analysis of variance with repeated measures). Data are presented as mean \pm standard error of the mean.

HLA-DR quantification was performed in patients with severe sepsis and non-septic critically ill patients. HLA-DR molecules on circulating monocytes per cell in non-septic critically ill patients were significantly higher compared to patients with severe sepsis ($p < 0.05$, Mann-Whitney U test; data not shown).

Discussion

The present investigation shows the novel finding of impaired *hBD2* gene inducibility in peripheral cells and elevated plasma protein concentration in patients with severe sepsis compared to non-septic critically ill patients and healthy controls. The meaning of β -defensins for the defence of infections is based on well-described antimicrobial activities [24,25]. In addition, β -defensins induce prostaglandin D_2 production, degranulate mast cells, and present chemotactic activities on CCR6-positive dendritic cells [6,26]. In mice, additional immunomodulatory effects have been reported [6,27,28]. These data indicate their involvement in innate immunity. These reported effects suggest regulatory or mediatory defensin functions. The role of antibiotic peptides in the pathogenesis of Crohn's colitis, cystic fibrosis, and panbronchiolitis has been described clearly. An effective defence related to levels and inducibility of defensins has been reported [17,29-33].

The elevated plasma levels of hBD2 in patients with severe sepsis indicate a higher activity of inflammation compared to non-septic individuals. Proinflammatory cytokines such as IL-1 and tumour necrosis factor induce hBD2 gene expression in alveolar macrophages and monocyte-derived epidermis cells (IL-1) [10,12]. These proinflammatory cytokines, which are frequently elevated in severe sepsis, are potentially involved in the upregulation of systemic hBD2 release in sepsis as well. The decreased hBD2 inducibility in peripheral blood cells was not associated with decreased plasma levels, suggesting that peripheral blood cells do not represent the exclusive source of released hBD2 protein *in vivo*.

The hBD2 plasma concentration in healthy controls agrees with findings from other investigations [17,34]. It should be taken into account that circulating endothelial cells or reticuloendothelial cells also represent a possible source of hBD2 [35]. The results for hBD2 mRNA inducibility and the basic protein plasma levels showed no significant differences between healthy controls and critically ill non-septic patients. Median PCT levels were in normal range, indicating a lack of systemic infection, whereas a median IL-6 of 18 ng/l (normal is below 15 ng/l) suggested minor systemic inflammatory activation in the non-septic critically ill patient group. For gene activation of hBD2 and IL-6, the transcription factor nuclear factor-kappa B (NF- κ B) is crucial. The low IL-6 levels in the critically ill non-septic group provide a hint for, but are not proof of, low NF- κ B activation in this group. This minor activation showed no influence on hBD2 inducibility or protein levels compared to healthy controls. Only the systemic infection in the severe septic patient group led to changes in gene inducibility and plasma levels. These results underline a specific impact of systemic infections on *hBD2* gene expression and plasma levels.

The decreased hBD2 mRNA inducibility in peripheral blood cells of patients with severe sepsis could mirror a serious inhibition of innate immune function. But given that the detected plasma concentrations were lower than required for bactericidal/antiviral activity, antimicrobial peptides may not exert their antimicrobial effects via the bloodstream [36-38]. However, innate immunity may be impaired not only due to the lack of direct antimicrobial activity but because of limited immunomodulating effects of defensins.

This immunological imbalance occurring in severe sepsis can be monitored, among other ways, by HLA-DR quantification on circulating monocytes. In this manner, the immune competence of monocytes can be assessed. It is well established that monocytes with diminished HLA-DR expression are inhibited in some of their main tasks (for example, antigen presentation and mediator production) [39,40]. Indeed, the investigated patients with severe sepsis showed signs of immunodepression by decreased HLA-DR expression on circulating monocytes. This finding underlines that sepsis may contribute to the impaired hBD2 inducibility as reported in the present investigation.

In this investigation, hydrocortisone treatment did not impair hBD2 inducibility in patients with severe sepsis. However, at the present time, there are no consistent data on the influence of steroid medication on hBD2 inducibility [41-43].

An individual's age can modulate immune function. Activities of cellular components of innate immunity are impaired at different levels [44-46]. To date, no data assessing antimicrobial peptide gene expression in the elderly have been collected. However, in insects, antimicrobial peptide gene expression

increases with age [47]. The median age of the control group was significantly lower compared to both other groups. There was no significant difference of the median age between the critically ill and the septic patients. Therefore, the differences between the critically ill and the septic patients concerning hBD2 mRNA inducibility and plasma levels cannot be explained by differences in age.

Conclusion

hBD2 inducibility in leukocytes from patients with severe sepsis is decreased. This special part of innate immunity is influenced by severe sepsis. The downregulation of inducibility may contribute to the complex immunological imbalance occurring in patients with severe sepsis.

The importance of plasmatic hBD2 for patients with severe sepsis is unclear. In particular, knowledge of the interaction with mediators and effectors of the immune system is scarce but of prime importance. To date, the antimicrobial and immunomodulatory activities of hBD2 have been tested only in *ex vivo* settings with limited numbers of additional co-factors. However, *in vivo*, hBD2 is an integral component of a set of effectors that function together in the innate immune line of defence.

Key messages

- *Ex vivo* endotoxin hBD2 inducibility in leukocytes was decreased in patients with severe sepsis compared to healthy controls and critically ill non-septic patients.
- hBD2 plasma levels were elevated in the severe sepsis group compared to both other groups.
- hBD2 inducibility and plasma levels showed no differences between survivors and non-survivors of severe sepsis.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MB participated in the coordination and design of the study and performed the statistical analysis. QC participated in the design of the study and worked on *ex vivo* gene inducibility and protein quantification. LEL participated in the statistical analysis, planning of the study, and selection of patients and helped to draft the manuscript. SK participated in the coordination of the study and the protein quantification. SW participated in *ex vivo* stimulations and the design of the study. J-CS participated in the coordination of the study and in generating the manuscript. ML participated in the design and coordination of the study. AH participated in revising the manuscript and in the design of the study. FS initiated the study and gave major advice for the design of the study and the methods used. All authors read and approved the final manuscript.

Acknowledgements

The authors thank Angelika Zoons for excellent technical assistance with hBD2 enzyme-linked immunosorbent assay. This study received financial support from the German Research Foundation (BO 1929/1-1).

References

1. Lehrer RI, Ganz T: **Antimicrobial peptides in mammalian and insect host defence.** *Curr Opin Immunol* 1999, **11**:23-27.
2. Boman HG: **Antibacterial peptides: basic facts and emerging concepts.** *J Intern Med* 2003, **254**:197-215.
3. Miyasaki KT, Bodeau AL, Selsted ME, Ganz T, Lehrer RI: **Killing of oral, gram-negative, facultative bacteria by the rabbit defensin, NP-1.** *Oral Microbiol Immunol* 1990, **5**:315-319.
4. Porro GA, Lee JH, de Azavedo J, Crandall I, Whitehead T, Tullis E, Ganz T, Liu M, Slutsky AS, Zhang H: **Direct and indirect bacterial killing functions of neutrophil defensins in lung explants.** *Am J Physiol Lung Cell Mol Physiol* 2001, **281**:L1240-L1247.
5. Thevissen K, Francois IE, Takemoto JY, Ferket KK, Meert EM, Cammue BP: **DmAMP1, an antifungal plant defensin from dahlia (*Dahlia merckii*), interacts with sphingolipids from *Saccharomyces cerevisiae*.** *FEMS Microbiol Lett* 2003, **226**:169-173.
6. Yang D, Chertov O, Bykovskaia SN, Chen Q, Buffo MJ, Shogan J, Anderson M, Schroder JM, Wang JM, Howard OM, et al.: **Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6.** *Science* 1999, **286**:525-528.
7. Yang D, Biragyn A, Kwak LW, Oppenheim JJ: **Mammalian defensins in immunity: more than just microbicidal.** *Trends Immunol* 2002, **23**:291-296.
8. Bajaj-Elliott M, Fedeli P, Smith GV, Domizio P, Maher L, Ali RS, Quinn AG, Farthing MJ: **Modulation of host antimicrobial peptide (beta-defensins 1 and 2) expression during gastritis.** *Gut* 2002, **51**:356-361.
9. Garcia JR, Krause A, Schulz S, Rodriguez-Jimenez FJ, Kluever E, Adermann K, Forssmann U, Frimpong-Boateng A, Bals R, Forssmann WG: **Human beta-defensin 4: a novel inducible peptide with a specific salt-sensitive spectrum of antimicrobial activity.** *FASEB J* 2001, **15**:1819-1821.
10. Liu L, Roberts AA, Ganz T: **By IL-1 signaling, monocyte-derived cells dramatically enhance the epidermal antimicrobial response to lipopolysaccharide.** *J Immunol* 2003, **170**:575-580.
11. Sorensen OE, Cowland JB, Theilgaard-Monch K, Liu L, Ganz T, Borregaard N: **Wound healing and expression of antimicrobial peptides/polypeptides in human keratinocytes, a consequence of common growth factors.** *J Immunol* 2003, **170**:5583-5589.
12. Tsutsumi-Ishii Y, Nagaoka I: **Modulation of human beta-defensin-2 transcription in pulmonary epithelial cells by lipopolysaccharide-stimulated mononuclear phagocytes via proinflammatory cytokine production.** *J Immunol* 2003, **170**:4226-4236.
13. Yamaguchi Y, Nagase T, Makita R, Fukuhara S, Tomita T, Tomimaga T, Kurihara H, Ouchi Y: **Identification of multiple novel epididymis-specific beta-defensin isoforms in humans and mice.** *J Immunol* 2002, **169**:2516-2523.
14. Quinones-Mateu ME, Lederman MM, Feng Z, Chakraborty B, Weber J, Rangel HR, Marotta ML, Mirza M, Jiang B, Kiser P, et al.: **Human epithelial beta-defensins 2 and 3 inhibit HIV-1 replication.** *AIDS* 2003, **17**:F39-F48.
15. Zhang L, Yu W, He T, Yu J, Caffrey RE, Dalmasso EA, Fu S, Pham T, Mei J, Ho JJ, et al.: **Contribution of human alpha-defensin 1, 2, and 3 to the anti-HIV-1 activity of CD8 antiviral factor.** *Science* 2002, **298**:995-1000.
16. Schaller-Bals S, Schulze A, Bals R: **Increased levels of antimicrobial peptides in tracheal aspirates of newborn infants during infection.** *Am J Respir Crit Care Med* 2002, **165**:992-995.
17. Hiratsuka T, Mukae H, Iiboshi H, Ashitani J, Nabeshima K, Minematsu T, Chino N, Ihi T, Kohno S, Nakazato M: **Increased concentrations of human beta-defensins in plasma and bronchoalveolar lavage fluid of patients with diffuse panbronchiolitis.** *Thorax* 2003, **58**:425-430.

18. Fang XM, Shu Q, Chen QX, Book M, Sahl HG, Hoefft A, Stuber F: **Differential expression of alpha- and beta-defensins in human peripheral blood.** *Eur J Clin Invest* 2003, **33**:82-87.
19. **American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis.** *Crit Care Med* 1992, **20**:864-874.
20. Sagner G, Goldstein C: **Principles, workflows and advantages of the new LightCycler Relative Quantification Software.** *Biochemica* 2001, **3**:15-17.
21. Docke WD, Hoflich C, Davis KA, Rottgers K, Meisel C, Kiefer P, Weber SU, Hedwig-Geissing M, Kreuzfelder E, Tschentscher P, et al.: **Monitoring temporary immunodepression by flow cytometric measurement of monocytic HLA-DR expression: a multicenter standardized study.** *Clin Chem* 2005, **51**:2341-2347.
22. **Researcher's toolkit, Statistical Power Calculator, Averages, Two Samples.** DSS Research web site [http://www.dssresearch.com/Toolkit/SpCalc/Power_A2.Asp]
23. Dellinger RP, Carlet JM, Masur H, Gerlach H, Calandra T, Cohen J, Gea-Banacloche J, Keh D, Marshall JC, Parker MM, et al.: **Surviving sepsis campaign guidelines for management of severe sepsis and septic shock.** *Crit Care Med* 2004, **32**:858-873.
24. Boman HG: **Gene-encoded peptide antibiotics and the concept of innate immunity: an update review.** *Scand J Immunol* 1998, **48**:15-25.
25. Zasloff M: **Antimicrobial peptides of multicellular organisms.** *Nature* 2002, **415**:389-395.
26. Niyonsaba F, Someya A, Hirata M, Ogawa H, Nagaoka I: **Evaluation of the effects of peptide antibiotics human beta-defensins-1/-2 and LL-37 on histamine release and prostaglandin D(2) production from mast cells.** *Eur J Immunol* 2001, **31**:1066-1075.
27. Biragyn A, Ruffini PA, Leifer CA, Klyushnenkova E, Shakhov A, Chertov O, Shirakawa AK, Farber JM, Segal DM, Oppenheim JJ, et al.: **Toll-like receptor 4-dependent activation of dendritic cells by beta-defensin 2.** *Science* 2002, **298**:1025-1029.
28. Biragyn A, Belyakov IM, Chow YH, Dimitrov DS, Berzofsky JA, Kwak LW: **DNA vaccines encoding human immunodeficiency virus-1 glycoprotein 120 fusions with proinflammatory chemoattractants induce systemic and mucosal immune responses.** *Blood* 2002, **100**:1153-1159.
29. Ashitani J, Mukae H, Hiratsuka T, Nakazato M, Kumamoto K, Matsukura S: **Plasma and BAL fluid concentrations of antimicrobial peptides in patients with Mycobacterium avium-intracellulare infection.** *Chest* 2001, **119**:1131-1137.
30. Beisswenger C, Kandler K, Hess C, Garn H, Felgentreff K, Wegmann M, Renz H, Vogelmeier C, Bals R: **Allergic airway inflammation inhibits pulmonary antibacterial host defense.** *J Immunol* 2006, **177**:1833-1837.
31. Dauletbaev N, Gropp R, Frye M, Loitsch S, Wagner TO, Bargon J: **Expression of human beta defensin (HBD-1 and HBD-2) mRNA in nasal epithelia of adult cystic fibrosis patients, healthy individuals, and individuals with acute cold.** *Respiration* 2002, **69**:46-51.
32. Schmid M, Fellermann K, Wehkamp J, Herrlinger K, Stange EF: **[The Role of defensins in the pathogenesis of chronic-inflammatory bowel disease].** *Z Gastroenterol* 2004, **42**:333-338.
33. Smith JJ, Travis SM, Greenberg EP, Welsh MJ: **Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid.** *Cell* 1996, **85**:229-236.
34. Isomoto H, Mukae H, Ishimoto H, Nishi Y, Wen CY, Wada A, Ohnita K, Hirayama T, Nakazato M, Kohno S: **High concentrations of human beta-defensin 2 in gastric juice of patients with helicobacter pylori infection.** *World J Gastroenterol* 2005, **11**:4782-4787.
35. Mutunga M, Fulton B, Bullock R, Batchelor A, Gascoigne A, Gillespie JJ, Baudouin SV: **Circulating endothelial cells in patients with septic shock.** *Am J Respir Crit Care Med* 2001, **163**:195-200.
36. Sahly H, Schubert S, Harder J, Kleine M, Sandvang D, Ullmann U, Schroder JM, Podschun R: **Activity of human β -defensins 2 and 3 against ESBL-producing klebsiella strains.** *J Antimicrob Chemother* 2006, **57**:562-565.
37. Singh PK, Tack BF, McCray PB Jr, Welsh MJ: **Synergistic and additive killing by antimicrobial factors found in human airway surface liquid.** *Am J Physiol Lung Cell Mol Physiol* 2000, **279**:L799-L805.
38. Sun L, Finnegan CM, Kish-Catalone T, Blumenthal R, Garzino-Demo P, La Terra Maggiore GM, Berrone S, Kleinman C, Wu Z, Abdelwahab S, et al.: **Human beta-defensins suppress human immunodeficiency virus infection: potential role in mucosal protection.** *J Virol* 2005, **79**:14318-14329.
39. Pitton C, Fitting C, van Deuren M, van der Meer JW, Cavallion JM: **Different regulation of TNF alpha and IL-1ra synthesis in LPS-tolerant human monocytes.** *Prog Clin Biol Res* 1995, **392**:523-528.
40. Wolk K, Docke W, von Baehr V, Volk H, Sabat R: **Comparison of monocyte functions after LPS- or IL-10-induced reorientation: importance in clinical immunoparalysis.** *Pathobiology* 1999, **67**:253-256.
41. Duits LA, Rademaker M, Ravensbergen B, Van Sterkenburg MA, van Strijen E, Hiemstra PS, Nibbering PH: **Inhibition of hBD-3, but not hBD-1 and hBD-2, mRNA expression by corticosteroids.** *Biochem Biophys Res Commun* 2001, **280**:522-525.
42. Meyer JE, Harder J, Gorogh T, Weise JB, Schubert S, Janssen D, Maune S: **Human beta-defensin-2 in oral cancer with opportunistic Candida infection.** *Anticancer Res* 2004, **24**:1025-1030.
43. Terai K, Sano Y, Kawasaki S, Endo K, Adachi W, Hiratsuka T, Ihiboshi H, Nakazato M, Kinoshita S: **Effects of dexamethasone and cyclosporin A on human beta-defensin in corneal epithelial cells.** *Exp Eye Res* 2004, **79**:175-180.
44. Fulop T, Larbi A, Douziech N, Fortin C, Guerard KP, Lesur O, Khalil A, Dupuis G: **Signal transduction and functional changes in neutrophils with aging.** *Aging Cell* 2004, **3**:217-226.
45. Lloberas J, Celada A: **Effect of aging on macrophage function.** *Exp Gerontol* 2002, **37**:1325-1331.
46. Plowden J, Renshaw-Hoelscher M, Engleman C, Katz J, Sambhara S: **Innate immunity in aging: impact on macrophage function.** *Aging Cell* 2004, **3**:161-167.
47. Zerofsky M, Harel E, Silverman N, Tatar M: **Aging of the innate immune response in Drosophila melanogaster.** *Aging Cell* 2005, **4**:103-108.