G. Monneret, B. Laroche, J. Bienvenu

Procalcitonin Is Not Produced by Circulating Blood Cells

Summary: A large number of clinical studies has described procalcitonin (ProCT) as a marker of bacterial infection and a good predictor of disease severity and antibiotherapy efficacy. Nevertheless, the mechanism of ProCT synthesis remains unclear. The aim of this study was to demonstrate potential ProCT production by peripheral blood mononuclear cells as is the case for cytokines involved in sepsis. In a whole blood model, LPS (10 μg/ml) stimulation on blood samples from healthy volunteers (n = 14) was tested. Early (TNF-α and IL1-β) and late (IL-6 and IL-8) cytokines were produced in large amounts in contrast to the absence of ProCT. Additional experiments with nitric oxide or detection of intra-cellular ProCT (cell lysis, flow cytometry) had negative results. It was concluded that ProCT is not produced in this model. Data are still needed to investigate the cellular origin of ProCT in order to better define its clinical usefulness.

Introduction

Since the first description of high serum procalcitonin (ProCT) concentrations in patients with sepsis [1], a large number of clinical studies has confirmed ProCT as a specific marker of bacterial infection and a good predictor of disease severity and antibiotherapy efficacy [references in 2]. Nevertheless, the cellular origin and the mechanism of ProCT release into the blood are not yet documented. ProCT synthesis seems to be closely dependent on the cytokines tumor necrosis factor (TNF) and interleukin (IL)-6, which are considered as early markers of bacterial invasion and are produced mainly by circulating blood cells [3–4]. This prompts us to examine whether peripheral blood mononuclear cells (PBMC) could be a potential source for ProCT synthesis. In order to investigate this hypothesis, we used the whole blood (WB) model initially described by *De Groote* and colleagues [5].

Materials and Methods

Cell preparation: Blood samples from 14 healthy volunteers from our laboratory were collected into heparinized tubes (Endotube ET, Chromogenix AB, Sweden). WB was diluted 1/10 in RPMI 1640 (GIBCO BRL, Scotland) complemented with glutamine 2 mM. Lipopolysaccharide (LPS, *Salmonella enteritidis*, Sigma, USA) was added at a final concentration of 10 μg/ml. Sodium nitroprussiate (NITRIATE, Larguenon Int., France) was added at a final concentration of 10^{-5}, 10^{-6} and 10^{-7} M. Plates were incubated at 37°C in a 5% CO_2 atmosphere. For each sample, a WB aliquot was incubated in RPMI only as a negative control. Cell viability assessed using the blue trypan exclusion method gave results for all of more than 90%.

Cytokines and ProCT assays: After a 24-h and 48-h incubation, WB samples were centrifuged (1,500 rpm, 10 min) and supernatants were immediately stored at -80°C until cytokine and ProCT measurement. TNF-α and IL-8 were quantified using Medgenix EASIA kits (Biosource, Belgium); IL-6 and IL1-β with Immunotech kits (Immunotech, France) and ProCT with an immunoluminometric assay (B.R.A.H.M.S. Diagnostica, Germany). Furthermore, after centrifugation cells were resuspended in 0.5 ml of the wash solution used for ProCT measurement and then lysed (three cycles of freezing/thawing/sonication) to release potential trapped intra-cellular ProCT.

Flow cytometry: Brefeldin A (final concentration: 50 μg/l, Sigma) was added 4 h before the end of incubation to block protein transport in the Golgi apparatus [6]. After incubation, leukocytes were stained with PC5-conjugated anti-human CD45 (Immunotech). The Intraprep kit (Coulter, USA) was then used for cell fixation and permeabilization. Intracellular ProCT was stained with FITC-conjugated anti-human calcitonin and katacalcin (gift from Dr. *Struck*, B.R.A.H.M.S.). Samples were analysed on a Coulter EPICS XL flow cytometer. Negative controls (without LPS) were used to define a threshold (about the first decade on a log scale) between positive signals and non-specific fluorescence.

Results and Discussion

The aim of our work was to demonstrate potential ProCT production by PBMC, as is the case for cytokines involved in sepsis. We therefore used a whole blood model known to be a good reflection of pro-inflammatory cytokine response [5, 7]. Cell stimulation was obtained by using LPS only, to provide conditions similar to sepsis. The importance of cytokine release illustrates the validity of the model; as shown in Table 1, early (IL1-β and TNF-α) and late cytokines (IL-6 and IL-8) were produced in large amounts in contrast to the absence of ProCT in supernatants.

Several reasons could explain this negative result. First, we tested the effect of a longer incubation time; in five additional experiments, ProCT results in supernatants remained negative after a 48-h stimulation. Another hypothesis is that a mediator was missing in this model.

Received: 6 July 1998/Revision accepted: 24 September 1998

G. Monneret, B. Laroche, J. Bienvenu, Ph. D., Immunology Laboratory, Centre Hospitalier Lyon-Sud, F-69495 Pierre-Bénite cedex, France.

Table 1: Procalcitonin (ProCT) and cytokines values in supernatants after LPS stimulation (initial protocol: 24 h incubation, mean ± SEM) and results of additional experiments.

Initial protocol	N	ProCT (µg/l)	TNFα (pg/ml)	IL1β (pg/ml)	IL6 (pg/ml)	IL8 (pg/ml)
Negative control	8	< 0.1	< 3	< 2	< 4	< 1
LPS	8	< 0.1	6,340 ± 250	3,520 ± 380	12,300 ± 550	2,480 ± 510
Additional experiments						
LPS / 48 h incubation	5	< 0.1				
LPS + sodium nitroprussiate (24 h incubation)	5	< 0.1				
LPS / cell lysate (24 h incubation)	6	< 0.1				
LPS / Flow cytometry + brefeldin A (24 h incubation)	5	No significant intra-cellular fluorescence				

Mainly produced by endothelial cells, nitric oxide (NO) is involved in septic shock. Furthermore, nitrite/nitrate (NO terminal metabolites) correlates with severity or ProCT levels in sepsis [8]. We therefore studied the effect of a NO donor (sodium nitroprussiate) at different concentrations with LPS, but all ProCT values remained below the detection limit of the test. We also considered that ProCT could be trapped in the cells. Nevertheless, ProCT measurement after cell lysis showed negative results. Finally, even when we measured cytokine levels as high as 10^3 and 10^4 pg/ml, the absence of ProCT could be due to a simple analytical problem of the detection limit of the assay (about 10^2 pg/ml). Brefeldin A is used for intracellular cytokine detection by flow cytometry to stop protein transport in the Golgi apparatus. This is a means to increase a low fluorescence signal in cases where there is a small amount of protein, as for Th2 cytokines such as IL4 or IL5 [7]. In this context, we also investigated the value of intra-cellular ProCT using brefeldin A in flow cytometry experiments. As previously, we have been unable to detect any ProCT. Taken together, these data allow the conclusion that ProCT is not produced in this model. Even if this is not a definitive experiment and if a potential ProCT synthesis by PBMC remains possible, we do not believe that this could be the main mechanism/site of ProCT production. Additional data are still needed to investigate the cellular origin of ProCT in order to better define its clinical usefulness.

References

1. **Assicot, M., Gendrel, D., Carsin, H., Raymond, J., Guilbaud, J., Bohuon, C.:** High serum procalcitonin concentrations in patients with sepsis and infection. Lancet 341 (1993) 515–518.
2. **Chiesa, C., Panero, A., Rossi, N., Stegagno, M., De Guisti, M., Osborn, J. F., Pacifico, L.:** Reliability of procalcitonin for the diagnosis of sepsis in critically ill neonates. Clin. Infect. Dis. 26 (1998) 664–672.
3. **Dandona, P., Nix, D., Wilson, M. F., Aljada, A., Love, J., Assicot, M., Bohuon, C.:** Procalcitonin increase after endotoxin injection in normal subjects. J. Clin. Endocrinol. Metab. 79 (1994) 1605–1608.
4. **Zeni, F., Viallon, A., Assicot, M., Tardy, B., Vindimian, M., Page, Y., Lafond, P., Bertrand, J. C., Bohuon, C.:** Serum procalcitonin in sepsis: relation to severity and cytokines (TNF, IL6, IL8). 34th ICAAC, Orlando 1994.
5. **De Groote, D., Zangerle, P. F., Gevaert, Y., Fassotte, M. F., Beguin, Y., Noizat-Pirenne, F., Pirenne, J., Gathy, R., Lopez, M., Dehart, I., Igot, D., Baudrihaye, M., Delacroix, D., Franchimont, P.:** Direct stimulation of cytokines (IL-1β, TNF-α, IL-6, GM-CSF) in whole blood. I. Comparison with isolated PBMC stimulation. Cytokine 4 (1992) 239–247.
6. **Schauer, U., Jung, T., Krug, N., Frew, A.:** Measurement of intracellular cytokines. Immunol. Today 17 (1996) 305–306.
7. **Bienvenu, J., Doche, C., Gutowski, M. C., Lenoble, M., Lepape, A., Perdrix, J. P.:** Production of proinflammatory cytokines involved in the TH1/TH2 balance is modulated by pentoxifylline. J. Cardiovasc. Pharmacol. 25 (1995) S80–S84.
8. **De Werra, I., Jaccard, C., Corradin, S. B., Chiolero, R., Yersin, B., Gallati, H., Assicot, M., Bohuon, C., Baumgartner, J. D., Glauser, M. P., Heumann, D.:** Cytokines, nitrite/nitrate, soluble tumor necrosis factor receptors, and procalcitonin concentrations: comparisons in patients with septic shock, cardiogenic shock, and bacterial pneumonia. Crit. Care Med. 25 (1997) 607–613.