Cytokine 54 (2011) 144–148

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/issn/10434666

Effects of simvastatin on cytokines secretion from mononuclear cells from critically ill patients with acute kidney injury

Gabriela Laender Ferrari [a], Beata Marie Redublo Quinto [a,*], Kelly Cristina Batista de Souto Queiroz [a], Ilson Jorge Iizuka [b], Julio Cesar Martins Monte [a,b], Maria Aparecida Dalboni [a], Marcelino Souza Durão [a,b], Miguel Cendoroglo Neto [a,b], Oscar Fernando Pavão dos Santos [a,b], Marcelo Costa Batista [a,b]

[a] Nephrology Division, Department of Medicine, Federal University of São Paulo, Brazil
[b] IAEH/IEP – Hospital Israelita Albert Einstein/Instituto de Ensino e Pesquisa, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 9 June 2010
Received in revised form 1 February 2011
Accepted 3 February 2011
Available online 1 March 2011

Keywords:
Simvastatin
Inflammation
Cytokines
Sepsis
AKI

ABSTRACT

Purpose: To assess the *in vitro* effects of simvastatin on IL-10 and TNF-α secretion from peripheral blood mononuclear cells (PBMC) of critically ill patients with and without acute kidney injury (AKI).
Methods: PBMC were collected from 63 patients admitted to the intensive care unit (ICU) and from 20 healthy controls. Patients were divided in 3 subgroups: with AKI, with sepsis and without AKI and with AKI and sepsis. After isolation by ficoll-gradient centrifugation cells were incubated *in vitro* with LPS 1 ng/mL, simvastatin (10^{-8}M) and with LPS plus simvastatin for 24 h. TNF-α and IL-10 concentrations on cells surnatant were determined by ELISA.
Results: Cells isolated from critically ill patients showed a decreased spontaneous production of TNF-α and IL-10 compared to healthy controls (6.7 (0.2–12) *vs* 103 (64–257) pg/mL and (20 (13–58) *vs* 315 (105–510) pg/mL, respectively, $p < 0.05$). Under LPS-stimulus, IL-10 production remains lower in patients compared to healthy control (451 (176–850) *vs* 1150 (874–1521) pg/mL, $p < 0.05$) but TNF-α production was higher (641 (609–841) *vs* 406 (201–841) pg/mL, $p < 0.05$). The simultaneous incubation with LPS and simvastatin caused decreased IL-10 production in cells from patients compared to control (337 (135–626) *vs* 540 (345–871) pg/mL, $p < 0.05$) and increased TNF-α release (711 (619–832) *vs* 324 (155–355) pg/mL, $p < 0.05$). Comparison between subgroups showed that the results observed in TNF-α and IL-10 production by PBMC from critically ill patients was independent of AKI occurrence.
Conclusions: The PBMC treatment with simvastatin resulted in attenuation on pro-inflammatory cytokine spontaneous production that was no longer observed when these cells were submitted to a second inflammatory stimulus. Our study shows an imbalance between pro and anti-inflammatory cytokine production in PBMC from critically ill patients regardless the presence of AKI.

1. Introduction

The systemic inflammatory response (SIRS) to severe infection remains the leading cause of death among critically ill patients [1]. Despite extensive research, over more than two decades, the mortality rates may reach more than 70% [2–4]. This inflammatory state is characterized by the overproduction of host inflammatory cytokines, such as TNF-α, IL-6, IL-8 and the activation of plasma protein cascade systems. Although numerous studies have attempted to develop therapeutic approaches on the field, no effective improvement on patient's outcome have been achieved.

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) have antihypercholesterolemic effects by catalyzing the formation of mevalonate from acetyl-CoA, resulting in positive effects on lipid profile [5,6]. Recent studies have demonstrated that statins have several nonlipid properties called pleiotropic effects. These pleiotropic effects include anti-inflammatory actions, modulation of endothelial nitric oxide synthase (eNOS) resulting in improvement of endothelial and microvascular function [7]. These effects might account for the observed benefits of statins in patients with any inflammatory disorder [8,9]. A report of Ando et al. suggested that pretreatment

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; AKI, acute kidney injury; APACHE II, acute physiology and chronic healthy evaluation II; CRP, C-reactive protein; ELISA, enzyme linked immunosorbent assay; ICU, intensive care unit; LPS, lipopolissacharide; PBMC, peripheral blood mononuclear cells; SIRS, systemic inflammatory response.

* Corresponding author. Address: Rua Pedro de Toledo, 781, 14° Andar, CEP 04039-032, São Paulo-SP, Brazil. Tel.: +55 11 5084 6836x26; fax: +55 11 5573 9652.
E-mail address: marie@nefro.epm.br (B.M. Redublo Quinto).

1043-4666 © 2011 Elsevier Ltd. Open access under the Elsevier OA license.
doi:10.1016/j.cyto.2011.02.005

with statins improves survival in a murine model of sepsis [10]. The translation of such findings to clinical settings shows that statin therapy may reduce mortality in patients with bacteremia [11]. In addition, *in vitro* and *in vivo* studies have demonstrated that simvastatin has anti-inflammatory effect in patients with pre-dialytic chronic kidney disease, and may play an important role in counteracting the mechanisms involved in pathogenesis of inflammation [12].

Acute kidney injury (AKI) is a frequent complication in critically ill patients and has been associated with an excess risk of hospital mortality that ranges rates of 50–80% [13]. There are evidences showing the involvement of intrarenal inflammation in ischemia–reperfusion injury using *in vitro* and *in vivo* models, which makes this scenario an interesting target for the development of new strategies for AKI management [14]. Although some evidences suggested that statins reduce morbidity related to SIRS, the effects of statin treatment in critically ill patients with AKI have not been properly explored so far. Therefore, this study was conducted to assess the *in vitro* effects of simvastatin on IL-10 and TNF-α secretion from peripheral blood mononuclear cells (PBMC) of critically ill patients with and without AKI.

2. Materials and methods

In this study we evaluated the secretion of IL-10 and TNF-α by cultured human peripheral mononuclear blood cell's (PBMC's) isolated from healthy subjects and critically ill patients.

2.1. Patients and healthy volunteers

The study population consisted of 63 patients admitted to the intensive care unit (ICU) of Hospital Israelita Albert Einstein, São Paulo, Brazil. Acute Physiology and Chronic Healthy Evaluation II (APACHE II) was routinely used as severity of disease score [15]. All patients were enrolled into this study under informed consent guidelines approved by the Investigation Review Boards of the Federal University of São Paulo and Albert Einstein Hospital. The control group ($n = 20$) included healthy adult volunteers. Patients who were <18 years of age were not included. Baseline demographic and clinical data were obtained from the patients hospital records. For further analysis, critically ill patients were sub-divided on those who developed AKI ($N = 20$), a group who developed sepsis without AKI ($N = 22$) and a third group who had sepsis with AKI ($N = 21$). Patients with AKI were defined by the Acute Kidney Injury Network (AKIN) criteria. The diagnostic criteria for acute kidney injury is defined by an abrupt (within 48 h) reduction in kidney function, currently defined as an absolute increase in serum creatinine of more than or equal to 0.3 mg/dl ($\geqslant$26.4 μmol/l), a percentage increase in serum creatinine of more than or equal to 50% (1.5-fold from baseline), or a reduction in urine output (documented oliguria of less than 0.5 ml/kg per hour for more than 6 h) [16].

Patients with SIRS were defined by the occurrence of at least two of the following criteria: (a) a temperature of >38 or < 36 °C; (b) an increased heart rate of >90 beats/min; (c) tachypnea, and (d) altered white blood cells count of >12,000,<4000 cells/mm^3, or the presence of >10% immature neutrophils [1]. Patients with sepsis were stratified by the same clinical response described above plus the occurrence of an infectious focus [1].

The exclusion criteria included patients with end-stage renal disease, renal transplanted patients, and previous participation in this study.

2.2. Blood sampling

Forty mL of peripheral blood was collected from patients in the first 48 h of ICU admittance and healthy volunteer's samples was collected as well in sterile heparinized syringes and immediately transferred to the laboratory for cell isolation procedure.

2.3. Biochemical methods

Renal function was evaluated by serum urea, creatinine, sodium and potassium using standard auto-analyzer techniques [17–20].

The C-reactive protein (CRP) was measured by immune turbidimetry technique [21] and leukocytes were automatically counted at CELL DYN 3200 (ABBOTT).

2.4. Cell isolation procedure

For isolation of mononuclear cells, 40 mL of blood were collected and PBMC's were isolated by Ficoll-gradient centrifugation (Histopaque-1077, Sigma–Aldrich Co.) from blood that was withdrawn into tubes containing heparin as coagulant. Cells were washed in NaCl 0.9% solution, counted and cultured in RPMI 1640 medium (Sigma–Aldrich Co.) supplemented with 50% of fetal bovine serum and antibiotics in 6-well plates at 37 °C in an atmosphere containing 5% CO_2. Cell viability was assessed by Trypan Blue method.

The cells were incubated with lipopolysaccharide (LPS) from *Escherichia coli* (Sigma–Aldrich Co.) at a final concentration of 1 ng/ml and simvastatin (Sigma–Aldrich Co.) for 24 h. The PBMC were incubated in the following conditions: only RPMI 1640 medium, medium with LPS, medium with simvastatin 10^{-8} M and medium with LPS and simvastatin 10^{-8} M simultaneously.

2.5. Dose effects of simvastatin on cytokines release by PBMC

To determine optimal simvastatin concentration on cytokines release by PBMC, cells isolated from healthy subjects ($N = 5$) as described above, were incubated with increasing doses of simvastatin (10^{-10}, 10^{-8} and 10^{-6} M) for 24 h. Previously, simvastatin was diluted and activated as followed: in a 1.5-mL tube, 5 mg sinvastatin (Sigma) were dissolved in 190 μL DMSO and 810 μL Milli-Q water. NaOH, 0.1 M, was added to the tube, which was then heated at 70 °C for 2 h (activation protocol) [12]. This solution was serially diluted with DMEN medium, in order to obtain a medium with 10^{-10}, 10^{-8} and 10^{-6} M simvastatin. The PBMCs were incubated in the following conditions: only RPMI 1640 medium and medium with increasing concentrations of simvastatin (10^{-10}, 10^{-8} and 10^{-6} M). Concentrations of TNF-α and IL-10 were determined in the 24 h culture supernatants of PBMCs. In this preliminary experimental protocol, our data indicates that simvastatin had no dose-dependent effect (Fig. 1) and, therefore, 10^{-8} M concentration was chosen to carry out the study. Under LPS stimulating condition simvastatin either had no dose-dependent effect (data not shown).

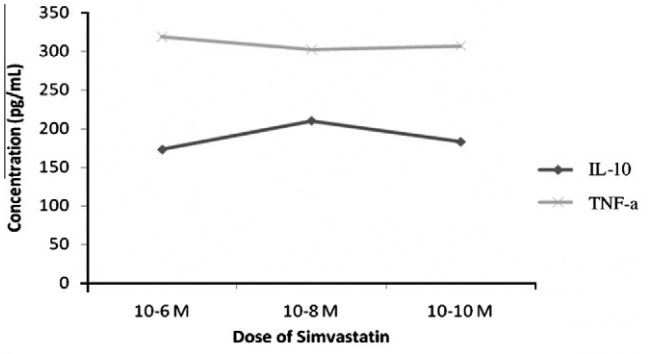


Fig. 1. Dose–response effect os simvastatin on PBMC's cytokines release.

Table 1
Baseline characteristics of healthy controls and critically ill patients stratified by the occurrence of acute kidney injury (AKI), sepsis, and AKI related to sepsis.

	Healthy controls ($N = 20$)	AKI ($N = 20$)	Sepsis ($N = 22$)	AKI + sepsis ($N = 21$)	Total of patients ($N = 63$)	p
Male sex (%)	65	55	50	71	67	NS
Age (years)	64 ± 14	64 ± 16	65 ± 20	63 ± 18	64 ± 17	NS
Creatinine (mg/dL)	#	2.0 ± 1.5	1.0 ± 1.1	2.3 ± 1.6*	1.7 ± 1.5	0.05
Urea (mg/dL)	#	59 ± 31	46 ± 26	90 ± 50*	65 ± 41	0.017
Sodium (mg/dL)	#	137 ± 5	137 ± 3	137 ± 5	137 ± 5	NS
Potassium (mg/dL)	#	4.6 ± 0.7	4.4 ± 0.4	4.5 ± 0.6	4.5 ± 0.6	NS
CRP (mg/dL)	#	7.0 ± 5.4	7.9 ± 9.8	7.5 ± 5.7	7.6 ± 7.6	NS
Total leukocyte count	#	10,500 ± 5627	8327 ± 3863	13,298 ± 9482*	10,674 ± 6932	0.055
APACHE score	#	22 ± 10	19 ± 6	21 ± 7	21 ± 8	NS
Mortality (%)	#	25	9	47*	27	0.017

NS: Not significant.
Data not evaluated.
* $p < 0.05$, AKI + sepsis *vs* sepsis.

Based on a pilot time-response protocol (2, 6, 12, 24, 30 h) were cells isolated from critically ill patients raised maximum production of TNF-α after 24 h of incubation with LPS; all assays were carried out during this period (data not shown).

2.6. Measurement of TNF-α and IL-10

Concentrations of TNF-α and IL-10 were determined in the 24 h culture supernatants of PBMCs using commercially available enzyme linked immunosorbent assay (ELISA) kits (BD OptEIA). The results were normalized to the PBMC concentration of 1×10^6 cells and expressed in mg/dL. All analysis was performed according to the manufacturer's protocols. Sensitivities for the TNF-α and IL-10 ELISAs were 2 pg/mL. The average intra assay coefficient of variation for TNF-α and IL-10 were ⩽4.5% and ⩽7.1% respectively.

TNF-α/IL-10 log ratio was expressed as surrogate of inflammatory modulation.

2.7. Statistic analysis

Data were expressed as interquartile ranges (IQR) as appropriate. Comparisons among groups were made with ANOVA plus Bonferroni. To evaluate cytokine production into the group we performed the one-way analysis of variance. X^2 or Ficher's exact test were used for categorical variables. A subgroup analysis was performed to compare critically ill patients stratified by the occurrence of AKI, sepsis and AKI related to sepsis. All the analysis was performed using SPSS 16.0. p values of less than 0.05 were considered statistically significant.

3. Results

The patients' baseline characteristics and their laboratory parameters are shown in Table 1. Patients with AKI related to sepsis had higher serum urea levels and total leukocyte counts compared to septic subjects ($p < 0.05$). The control group, comprised of healthy subjects, was not tested for the laboratory parameters and presented a mean age of 64 years and 65% of male sex (Table 1). Diagnosis at admission in ICU is reported in Table 2.

Table 2
Diagnosis at ICU admission of critically ill patients.

Diagnosis at ICU admission	Total ($n = 63$)
Cardiovascular (%)	5 (7.9)
Respiratory (%)	1 (1.6)
Gastrointestinal/hepatic (%)	8 (12.7)
Metabolic (%)	1 (1.6)
Trauma (%)	1 (1.6)
Surgical (%)	4 (6.3)
Sepsis (%)	
Respiratory sepsis	22 (34.9)
Abdominal sepsis	9 (14.3)
Sepsis	7 (11.1)
Gastrointestinal sepsis	3 (4.8)
Urinary sepsis	2 (3.2)

The priming of PBMCs TNF-α production in critically ill patients appears to be a specific effect since IL-10 production is not enhanced by LPS stimulation.

Compared to healthy controls, critically ill patients showed a decreased spontaneous production of TNF-α and IL-10 (6.7 (0.2–12) *vs* 103 (64–257) pg/mL and (20 (13–58) *vs* 315 (105–510) pg/mL, respectively, $p < 0.05$) in PBMC. With LPS stimulation, IL-10 production remained lower in patients compared to healthy controls (451 (176–850) *vs* 1150 (874–1521) pg/mL, $p < 0.05$), but TNF-α production was 1.3-fold higher compared to healthy control (641 (609–841) *vs* 406 (201–841) pg/mL, $p < 0.05$).

Occurrence of AKI does not lead to differences in cytokine production between subgroups of critically ill patients.

The comparison between subgroups showed that the results observed in TNF-α and IL-10 production by PBMC from critically ill patients was independent of occurrence of AKI (Fig. 2).

Simvastatin treatment reduces the TNF-α/IL-10 ratio in PBMC of critically ill patients compared to healthy control (Table 3).

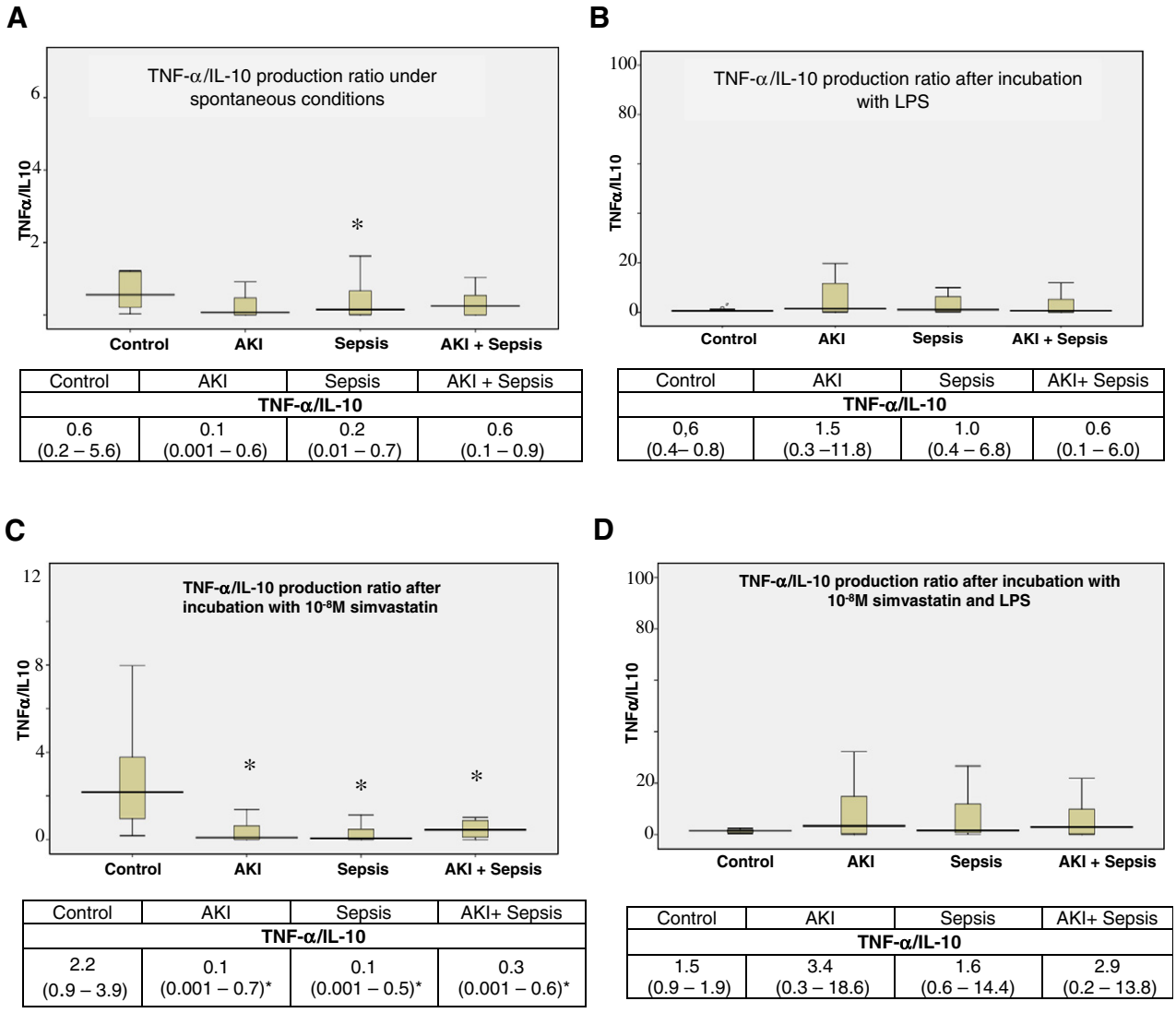
In simvastatin-treated PBMC isolated from critically ill patients, the production of TNF-α and IL-10 remained lower than that of the healthy control group (1.7 ± 1.3 *vs* 2.4 ± 0.2; and 1.4 ± 0.6 *vs* 2.1 ± 0.4 pg/mL, respectively, $p < 0.05$). The decrease in TNF-α/IL-10 ratio observed with simvastatin treatment was consistent in all subgroups (Fig. 2C).

Effect of simultaneous incubation of PBMC with LPS and 10^{-8} M simvastatin on priming of PBMC and TNF-α production in critically ill patients.

Taking the whole group of critically ill patients, it was observed that simultaneous incubation with LPS and simvastatin caused a decrease in IL-10 PBMC's production compared to healthy control cells (337 (135–626) *vs* 540 (345–871) pg/mL, $p < 0.05$) and increased TNF-α (711 (619–832) *vs* 324 (155–355) pg/mL, $p < 0.05$).

4. Discussion

In the present study we found a significant reduction in spontaneous release of IL-10 and TNF-α by PBMC isolated from critically ill patients compared to healthy control. These results suggest a preactivation of cells from critically ill patients that resulted in a decreased spontaneous IL-10 and TNF-α production. In fact, studies have shown a down regulation of nuclear factor-kappa B (NF-κB) in PBMC from sepsis patients, due to an imbalance between its active (p65p50) and inactive (p50p50) forms and to a weak cytoplasmic expression of its inhibitor (IκBα) [22].



Control	AKI	Sepsis	AKI + Sepsis
TNF-α/IL-10			
0.6 (0.2 – 5.6)	0.1 (0.001 – 0.6)	0.2 (0.01 – 0.7)	0.6 (0.1 – 0.9)

Control	AKI	Sepsis	AKI+ Sepsis
TNF-α/IL-10			
0,6 (0.4– 0.8)	1.5 (0.3 –11.8)	1.0 (0.4 – 6.8)	0.6 (0.1 – 6.0)

Control	AKI	Sepsis	AKI+ Sepsis
TNF-α/IL-10			
2.2 (0.9 – 3.9)	0.1 (0.001 – 0.7)*	0.1 (0.001 – 0.5)*	0.3 (0.001 – 0.6)*

Control	AKI	Sepsis	AKI+ Sepsis
TNF-α/IL-10			
1.5 (0.9 – 1.9)	3.4 (0.3 – 18.6)	1.6 (0.6 – 14.4)	2.9 (0.2 – 13.8)

Fig. 2. Comparison of TNF-α/IL-10 production ratio between group control and critically ill patients stratified in AKI, sepsis, and AKI related to sepsis.

Table 3
Comparison of TNF-α/IL-10 ratio between group control and critically ill patients.

	TNF-α/IL-10 (IQR)	
	Control ($N = 20$)	Critically ill patients ($N = 63$)
Spontaneous	0.6 (0.2–5.6)	0.16 (0.0001–0.7)
LPS	0.6 (0.4–0.8)	1.2 (0.2–7.1)*
S8	2.2 (0.9–3.9)	0.3 (0.001–0.5)*
S8 + LPS	1.5 (0.9–1.9)	2.4 (0.2–11.9)*

* $p < 0.05$, compared to control. Data are expressed as medians and interquartile ranges (brackets).

Asmis et al. demonstrated increased IL-10 and TNF-α serum levels but decreased monocyte IL-10 production in hemodialysis patients compared to healthy subjects [23]. In agreement with our findings, these authors attributed the observed reduction in cytokine secretion to monocyte preactivation. Moreover, decreased IL-10 production in our patients was observed independent of AKI occurrence, suggesting that this effect could be related to the inflammatory response dysfunction of critically ill patients *per se*. In fact, Van Deuren et al. demonstrated that patients with meningococcal infection also have reduced levels of IL-8, IL-10, IL-12 and INF-γ [24].

We observed upon exposure of cells to LPS, a high TNF-α production associated with low IL-10 production, suggesting an unopposed pro-inflammatory state in critically ill patients. PBMCs from critically ill patients seemed to be in a primed state. The occurrence of AKI did not reflect a different pattern in the secretion of pro-inflammatory cytokines. It is well known that TNF-α plays an important role in acute diseases. In the course of sepsis, there is a biphasic inflammatory response characterized by an initial hyperinflammatory phase changing to a hypoinflammatory state [25]. This hyporeactivity was observed in monocytes/macrophages after tolerance to endotoxin was induced *in vivo* and *in vitro* by LPS it self [25]. Thus, the differences in magnitude of pro-inflammatory cytokine production between TNF-α and IL-10, observed in our results, are likely to be influenced by the type and severity of disease, as well as the mononuclear cell functionality.

Our study population consisted mainly of patients with SIRS and sepsis. In this case, the exacerbation of pro-inflammatory response with LPS exposure could be explained by the preactivation state of PBMC isolated from critically ill patients. On the other hand, our results showed a decreased anti-inflammatory response, since with LPS stimulus IL-10 production remained reduced compared to healthy subjects. In fact, Walley et al. suggested that the balance between inflammatory mediators is related to severity

and mortality in a murine sepsis model. In this study, less severe sepsis was associated with higher anti-inflammatory mediator expression [26].

IL-10 seems to play a pivotal position in the regulatory mechanism that induces LPS desensitization, and it is essential for induction but not for maintenance of LPS hyporesponsiveness [25]. On the other hand, TNF-α induces IL-10 synthesis and increases the binding of this cytokine to polymorphonuclear leukocytes by increasing the mobilization of IL-10 receptors from the intracellular granules to the cell membrane [27]. Taken together, our findings suggest that pro-inflammatory cytokine synthesis was not able to modulate IL-10 production indicating that the acute inflammatory processes in critically ill patients could be associated with a impairment in the signaling pathway of cytokine production in SIRS.

In the present study, we also showed that treatment with simvastatin led to a decrease in IL-10 and TNF-α production compared with control, regardless of the presence of AKI. Moreover, the simultaneous addition of LPS and simvastatin did not result in a decrease in the inflammatory response of critically ill patients compared to healthy subjects. Erikstrup et al., in studying 30 young healthy males that received an injection of the bacterial cell wall product endotoxin to induce systemic inflammation, demonstrated that short-term treatment with simvastatin did not influence circulating cytokine levels during endotoxemia [28]. In addition, in a recent study of 295 blunt-injured adults with hemorrhagic shock, Neal et al. demonstrated that the previous use of statins was related with higher in-hospital morbidity [29]. In this multicenter prospective cohort study, preinjury statin use was independently associated with the development of multiple organ failure syndromes. Therefore, similar to what we showed with the additional LPS stimulus, Neal et al. suggested that the protective effect of HMG-CoA reductase inhibitor may be lost in the severe clinical setting, and that exacerbation of the inflammatory response might have been involved in their findings [29].

5. Conclusions

In conclusion, our study shows an imbalance between pro- and anti-inflammatory cytokine productions in mononuclear cells from critically ill patients, regardless of the presence of AKI. The treatment of these cells with simvastatin resulted in attenuation of spontaneous pro-inflammatory cytokine production which was no longer observed when these cells were submitted to a second inflammatory stimulus. Further studies are necessary to understand the effects of statin use on the inflammatory response triggered in the acute disease state.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

This study was supported by Fundação de Amparo à pesquisa do estado de São Paulo (FAPESP) and Instituto de Ensino e Pesquisa do Hospital Israelita Albert Einstein.

References

[1] 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(6): 864–74.
[2] Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med 2003;348:138–50.
[3] Riedemann NC, Guo RF, Ward PA. Novel strategies for the treatment of sepsis. Nat Med 2003;9:517–24.
[4] Martins PS, Kallas EG, Cendoroglo Neto C, Dlaboni MA, Blecher S, Salomão R. Upregulation of reactive oxygen species generation and phagocytosis, and increased apoptosis in human neutrophils during severe sepsis and septic shock. Shock 2003;20:208–12.
[5] Heeshen C, Hamm CW, Laufs U, Snapinn S, Bohm M, White HD. Withdrawal of statins increases event rates in patients with acute coronary syndromes. Circulation 2002;105:1446–52.
[6] Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, Macfarlane PW, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med 1995;333:1301–7.
[7] Laufs U, Liao JK. Post-transcriptional regulation of endothelial nitric oxide synthase mRNA stability by Rho GTPase. J Biol Chem 1998;273:24266–71.
[8] Gupta R, Plantinga LC, Fink NE, Melamed ML, Coresh J, Fox CS, et al. Statin use and hospitalization for sepsis in patients with chronic kidney disease. JAMA 2007;297:1455–64.
[9] Albert MA, Danielson E, Rifai N, Ridker PM. Effect of statin therapy on C-reactive protein levels. JAMA 2001;286:64–70.
[10] Ando H, Takamura T, Ota T, Nagai Y, Kobayashi K. Cerivastatin improves survival of mice with lipopolysaccharide-induced sepsis. J Pharmacol Exp Ther 2000;294:1043–6.
[11] Laippis AP, Kan VL, Rochester CG, Simon GL. The effect of statins on mortality in patients with bacteremia. Clin Infect Dis 2001;33:1352–7.
[12] Panichi V, Paoletti S, Mantuano E, Manca-Rizza G, Filippi C, Santi S, et al. *In vivo* and *in vitro* effects of simvastatin on inflammatory markers in pre-dialysis patients. Nephrol Dial Transplant 2006;21:337–44.
[13] Welten GMJM, Chonchol M, Schouten O, Hoeks S, Bax JJ, Van Domburg RT, et al. Statin use is associated with early recovery of kidney injury after vascular surgery and improved long-term outcome. Nephrol Dial Transplant 2008;23: 3867–73.
[14] Sharyo S, Yokota-Ikeda N, Mori M, Kumagai K, Uchida K, Ito K, et al. Pravastatin improves renal ischemia-reperfusion injury by inhibiting the mevalonate pathway. Kidney Int 2008;74:577–84.
[15] Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. Crit Care Med 1985;13:818–29.
[16] Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, et al. The Acute Kidney Injure Network. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Crit Care 2007;11:R31.
[17] Tabacco A, Meiattini F, Moda E, Tarli P. Simplified enzymic colorimetric serum urea nitrogen determination. Clin Chem 1979;25:336–7.
[18] Jaffe M, Hoppe Selyer's Z. Ueber den Niederschlag, welchen Pikrinsäure innormalem Harn erzeugt und über eine neue Reaction desKreatinins. Physiol Chem 1886;10:391–400.
[19] Fujihara CK, Michellazzo SM, de Nucci G, Zatz R. Sodium excess aggravates hypertension and renal parenchymal injury in rats with chronic NO inhibition. Am J Physiol 1994;266:F697–705.
[20] Kunau RT, Stein JH. Disorders of hypo and hyperkalemia. Clinic Nephrol 1977;7:173.
[21] Kimberly MM, Caudill SP, Vesper HW, Monsell EA, Miller WG, Rej R, et al. Standardization of high-sensitivity immunoassays for measurement of C-reactive protein; II: two approaches for assessing commutability of a reference material. Clin Chem 2009;55(2):342–50.
[22] Adib-Conquy M, Adrie C, Moine P, Asehnoune K, Fitting C, Pinsky MR, et al. NF-B expression in mononuclear cells of patients with sepsis resembles that observed in lipopolisaccharide tolerance. Am J Respir Crit Care Med 2000;162:1877–83.
[23] Asmis R, Stevens J, Begley JG, Grimes B, Zant GV, Fantil P. The isoflavone genistein inhibits LPS-stimulated TNF-α, but not IL-6 expression in monocytes from hemodialysis patients and healthy subjects. Clinic Nephrol 2006;65: 267–75.
[24] Van Deuren M, Van der Ven-Jongekrijg H, Demacker PNM, Bartelink AK, van Dalen R, Sauerwein RW, et al. Differential expression of proinflammatory cytokines and their inhibitors during the course of meningococcal infections. J Infect Dis 1994;169:157–61.
[25] Randown F, Syrbe U, Meisel C, Krausch D, Zuchermann H, Platzer C, et al. Mechanism of Endotoxin desensitization: Involvement of Interleukin 10 and Transforming Growth Factor β. J Exp Med 1995;181:1887–92.
[26] Walley KR, Lukacs NW, Standiford TJ, Strieter RM, Kunkel SL. Balance of inflammatory cytokines related to severity and mortality of murine sepsis. Infect Immun 1996;64:4733–8.
[27] Elbim C, Reglier H, Fay M, Delarche C, Andrieu V, El Benna J, et al. Intracellular pool of IL-10 receptors in specific granules of human neutrophils: differential mobilization by proinflammatory mediators. J Immunol 2001;166:5201–7.
[28] Erikstrup C, Ullum H, Pedersen BK. Short-term simvastatin treatment has no effect on plasma cytokine response in a human *in vivo* model of low-grade inflammation. Clinic Exp Immunol 2006;144:94–100.
[29] Neal DM, Cushieri J, Rosengart MR, Alarcon LH, Moore EE, Maier RV, et al. Preinjury statin use is associated with a higher risk of multiple organ failure after injury: a propensity score adjusted analysis. J Trauma 2009;67:476–84.