ORIGINAL PAPERS

Adv. Clin. Exp. Med. 2003, **12**, 1, 23–31
ISSN 1230-025X

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Involvement of lipopolysaccharide-binding protein in spontaneous and lipopolysaccharide-induced production of tumor necrosis factor alpha by peripheral blood mononuclear cells: regulatory effects of lactoferrin

Udział białka wiążącego lipopolisacharyd (LPS) w spontanicznej i indukowanej LPS produkcji czynnika nekrozy nowotworów przez jednojądrzaste komórki krwi obwodowej: regulacyjne działanie laktoferyny

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Abstract

Background. Gram-negative bacterial sepsis contributes to high mortality and morbidity rates in the intensive care units. The lipopolysaccharide-binding protein (LBP) plays a major role in mediating effects of lipopolysaccharide (LPS) on the immune system. The aim of this study was to revaluate the role of LBP in LPS-induced production of tumor necrosis factor (TNF-α) in human peripheral mononuclear cell (PBMC) cultures and to investigate potential regulatory activity of lactoferrin (LF) in this model.
Objectives. The aim of this study was to verify the role of LBP in the TNF-α response of human PBMC to high and low LPS doses and to investigate the effects of bovine lactoferrin in the context of LBP-LPS interaction.
Material and methods. PBMC were isolated from 20 healthy donors, males and females, 24-54 years old. Two methodological approaches were applied. In the first one, PBMC were exposed to LPS and/or LF in culture medium containing 5% untreated autologous plasma or plasma pretreated with neutralizing dose of anti-LBP antibodies. In the second system, PBMC were exposed for 2 h to LPS, LF and/or LBP in a serum-free medium, followed by addition of 5% autologous plasma. The doses of LPS were 5 μg/mL or 100 ng/mL, LF-20 μg/mL and the concentrations of LBP ranged from 1.4 μg/mL to 20 pg/mL. TNF-α activity was determined in 24 h culture supernatants in a bioassay.
Results. We demonstrated that LBP had a role in eliciting TNF-α production by low and high LPS doses, that actions were, however, regulatory and not only confined to the accessory function in LPS-induced cytokine production. However, in low-responding blood donors LBP seemed to be necessary for LPS-induced TNF-α production. LF regulated TNF-α production in the absence of LBP and was able to replace the action of LBP in low-responding subjects. Addition of exogenous LBP in serum-free medium revealed that high LBP doses (1.4 and 0.3 μg/mL) did not restore normal TNF-α production observed in untreated cultures, but low doses of LBP (80-250 pg/mL) allowed to reconstitute high TNF-α production. Inclusion of LF to the experimental system partially restored lack of TNF-α induction at high LBP doses.
Conclusion. LBP appeared to be regulatory in mediating effects of both low and high LPS-doses and these actions were dependent on the immune reactivity of blood donors. LF, beside of its regulatory effects, in the absence of LBP, was able to replace the action of LBP. In addition, LF did not compete with LBP in LPS-induced TNF-α production as reported elsewhere. This study supports the notion that the response to LPS is regulated by more than one soluble serum mediator (**Adv. Clin. Exp. Med. 2003, 12, 1, 23–31**).

Key words: LBP, LPS, lactoferrin, PBMC, TNF-α.

Streszczenie

Wprowadzenie. Sepsa wywołana zakażeniami przez bakterie Gram-ujemne przyczynia się do wysokiej śmiertelności i powikłań na oddziałach intensywnej terapii. Białko wiążące lipopolisacharyd (LBP) odgrywa główną rolę w mediowaniu efektów LPS na układ immunologiczny. W pracy oceniono rolę LBP w wytwarzaniu czynnika nekrozy nowotworów (TNF-α) indukowanego LPS, w ludzkich komórkach jednojądrzastych z krwi obwodowej (PBMC) oraz zbadano regulatorowe działanie laktoferyny (LF) w tym modelu.
Cel pracy. Weryfikacja roli LBP w wytwarzaniu TNF-α przez ludzkie PBMC w odpowiedzi na wysokie i niskie dawki LPS i zbadanie efektów działania bydlęcej LF w kontekście interakcji LBP-LPS.
Materiał i metody. PBMC izolowano od 20 zdrowych dawców, kobiet i mężczyzn w wieku 24–54 lat. Zastosowano dwa modele doświadczalne. W pierwszym, PBMC eksponowano na LPS i/lub LF w płynie hodowlanym zawierającym 5% nietraktowane, autologiczne osocze lub osocze traktowane neutralizującą dawką przeciwciał anty-LBP. W drugim systemie PBMC eksponowano przez 2 godziny na LPS, LF i/lub LBP w medium hodowlanym pozbawionym surowicy, następnie dodawano 5% autologicznego osocza. LPS użyto w dawkach 5 μg/ml lub 100 ng/ml, LF – 20 μg/ml, a stężenia LBP w przedziale 1,4–20 pg/ml. Aktywność TNF-α oznaczano w supernatantach znad 24-godzinnej hodowli stosując metodę biologiczną.
Wyniki. LBP odgrywała rolę w zwiększeniu wytwarzania TNF-α indukowanej niską i wysoką dawką LPS. Działanie to było jednakże regulatorowe i nieograniczone jedynie do funkcji pomocniczej w indukcji wydzielania TNF-α przez LPS. U dawców słabo reagujących na LPS, LBP wydawało się jednak niezbędne do indukcji TNF-α przez LPS. LF regulowała wydzielanie TNF-α w nieobecności LBP i była zdolna do zastąpienia działania LBP u dawców słabo reagujących na LPS. Dodatek LBP w medium hodowlanym bez surowicy ujawnił, że wysokie dawki LBP (1,4 i 0,3 μg/ml) nie doprowadziły do odnowy produkcji TNF-α obserwowanej w hodowlach nietraktowanych, ale niskie dawki LBP (80-250 pg/ml) przywracały wysoką produkcję TNF-α. Dodatek LF do systemu doświadczalnego częściowo przywracał zdolność do indukcji TNF-α przy wysokich stężeniach LBP.
Wnioski. LBP okazało się regulatorowe w indukcji wydzielania TNF-α przez małą i dużą dawkę LPS i ta właściwość zależała od immunologicznej reaktywności dawców krwi. LF, oprócz jej regulatorowych efektów, w nieobecności LBP była zdolna do zastąpienia działania LBP. LF ponadto nie współzawodniczyła z LBP w wytwarzaniu TNF-α indukowanej LPS. Opisane wyniki są zgodne z opinią, że odpowiedź cytokinowa na LPS jest regulowana przez więcej niż jeden czynnik surowicy krwi (**Adv. Clin. Exp. Med. 2003, 12, 1, 23–31**).

Słowa kluczowe: białko wiążące lipopolisacharyd, lipopolisacharyd, laktoferyna, jednojądrzaste komórki krwi obwodowej, czynnik nekrozy nowotworów α.

Mortality and morbidity, resulting from gram negative bacteria sepsis, still remain unresolved problems in intensive care units. Numerous research efforts provided insight into mechanism of lipopolysaccharide-induced septic shock in experimental animals and humans [for review see 1].

It is generally accepted that recognition of LPS by cells is accomplished via CD14 receptors [2]. Since CD14 is devoid of cytoplasmatic domain, thus unable to transmit activating signals, it is postulated that it cooperates with toll-like receptors for delivery of such signals [3]. CD14 exists also in a soluble form (sCD14) enabling cells, which lack CD14, to respond to LPS [4]. Although a critical role of CD14 in initiating response to LPS has been demonstrated in several models [5, 6], involvement of other receptors, such as CD11c/CD18 [7], moesin [8] and a 70 kDa protein [9] was also suggested. Surprisingly, mice lacking CD14 and TLR-4 were shown to be resistant to gram negative sepsis [10] indicating that other mechanisms of LPS recognition are operating. In addition, higher concentrations of LPS may not require participation of CD14 [11, 12].

LBP is a 65-kDa protein present in blood at concentration of 2-20 μg/mL [2], produced by liver and lung. It was shown that the LBP gene is transcriptionally activated during the acute phase response [13] and LBP levels may reach concentration up to 200 μg/mL [14]. The protein, originally isolated from rabbits [15] strongly enhances binding of LPS monomers from aggregates to CD14 [16] resulting in increase of sensitivity of cells to LPS [17–19]. LBP was also shown to accelerate and augment phagocytosis of *Escherichia coli* by alveolar macrophages [19]. Also, it was demonstrated that a second role of LBP is to remove excess of LPS into HDL fraction which results in LPS neutralization [2], so that the actual role of LBP is to adequately stimulate the immune system with LPS.

Lactoferrin (LF) belongs to the family of proteins involved in iron metabolism and represents a very important element of the innate defense system. The protein is abundant in secretory fluids of mammals [20] and can be found in secondary granules of circulating neutrophils [21]. The serum level of LF upon infection [22] or clinical insult [21] is significantly elevated. LF presents a wide array of antibacterial actions, both direct [23] and indirect [24], as well as protective properties in experimental endotoxemia [25, 26]. Inhibition of proinflammatory cytokines [25] and free radicals [27] may account for those actions. The protective action of LF in endotoxemia may also derive from

the ability of LF to bind LPS and lipid A [28, 29]. Recently, Elass-Rochard et al. [30] showed that LF may interfere with LPS/CD14 interaction by competition of LF with LBP. The consequences of such interaction in terms of cytokine production were, however, not studied.

Majority of investigations on the role of LBP in mediation of LPS effects has been performed in the mouse model or cell lines and the studies in the human model are scant. Recently we demonstrated regulatory effects of bovine lactoferrin, given orally, on several immune parameters of healthy individuals and patients subjected to surgery [31, 32]. The actions of LF were differential and dependent on immune status of individuals. The aim of this study was to verify the role of LBP in the TNF-α response of human PBMC to high and low LPS doses and to investigate the effects of bovine lactoferrin in the context of LBP-LPS interaction.

Material and methods

Lactoferrin, LPS and other reagents

Low endotoxin bovine milk lactoferrin (< 4.4 EU/mg, < 25% iron saturated) and bacterial endotoxin (*Escherichia coli*, Serotype 0111:B4, 3×10^6 EU/mg) were purchased from Sigma Chemical Company, MO, USA. Human LBP and rabbit antiserum to human LBP were purchased from Biometrics GmbH, Germany. All chemicals were of analytical grade.

Isolation of PBMC and plasma

Venous blood (20 mL) was taken from healthy donors, males and females, 24–54 years old, into heparinized syringes. The blood donors gave their written consent to donate blood. After a short time sedimentation of blood cells (5 min), 1 mL of plasma was removed for cell culture (see below). Blood was diluted 2 x with PBS and centrifuged on a Ficoll-uroproline gradient (1.077 g/mL). The cells from the interphase were 80% lymphocytes and 20% monocytes, as determined microscopically by staining the cell smears. The cells were washed twice with PBS and resuspended in 1640 RPMI medium containing 0.05 M glutamine, sodium pyruvate and 2 mercaptoethanol, at concentration of 10^6/mL.

Cell culture and cytokine induction

In the first model, PBMC (3 x 10^6/well/2 mL in 24 well plates) were exposed to the action of LF and/or LPS in a culture medium containing 5% autologous plasma or plasma pretreated for 1 hour at 37°C with neutralizing concentration of rabbit antiserum to human LBP anti LBP antibody (dilution 1/100, for 1 h at 37°C). LF was used at concentration 20 μg/mL and LPS (Sigma, *E. coli* serotype 0111:B4, lot-4130) at doses 5 μg and 100 ng/mL. In the second model, PBMC (2 x 10^6/well/2 mL), were exposed for 2 h to LPS, LF and/or LBP in a serum-free medium (1640 RPMI, glutamine, sodium pyruvate, 2 ME – containing 0.1% BSA, Miles Laboratories, LTD, code 81003-3), followed by addition of 5% autologous plasma. The doses of LPS was 5 μg/mL, LF – 20 μg/mL and the concentration of LBP ranged from 1.4 μg/mL to 20 pg/mL. After an overnight culture in a cell culture incubator the supernatants were harvested and assayed for the TNF-α activity in a bioassay [33].

Determination of TNF-α activity [33]

For determination of TNF-α activity the indicator clone WEHI 164.13 was used. The cells were washed 3 times with Hanks' solution and resuspended in RPMI 1640, supplemented with 10% FCS, glutamine and antibiotics at a concentration of 2×10^5/mL. The cells were then distributed into 96-well, flat-bottom plates (2×10^4/well). Serially diluted plasma samples were prepared on separate plates and transferred to microtiter plates containing WEHI 164.13 indicator cells. The medium contained in addition 1 μg/mL of actinomycin D to increase sensivity of the assay. After an overnight incubation the survival of cells was determined using MTT colorimetric assay [10]. The results of TNF-α activity are presented in pg/mL where 10 picograms of TNF-α correspond to 1 unit of activity when tested a recombinant human TNF-α (kindly delivered by Prof. W. Stec, Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Łódź, Poland) [8]. 1 unit of TNF-α was calculated as an inverse dilution of a given plasma sample where 50% survival of WEHI 164.13 cells took place.

Colorimetric determination of cell proliferation/death

The assay was performed according to Hansen [34]. Briefly, MTT solution, 5 mg/mL in 0.9% NaCl, was added in a volume of 25 μL/well and incubated for 2–4 hours. Then, 100 μL of a lysing buffer was added (20% SDS, 50% DMF, pH 4.7). After an overnight incubation at 37°C the optical density (OD) was measured using ELISA reader Dynatech 5000, at the wavelength of 550 nm and reference wavelength of 630 nm.

Results

Effects of LBP neutralization on TNF-α production by PBMC cultures and regulatory action of LF

The effects of LBP neutralization with anti-LBP antibodies on cytokine production by PBMC cultures, in the presence of LPS or/and BLF are presented in Table 1. The experiments were performed on 13 blood donors differing in their ability to produce TNF-α spontaneously and upon LPS induction. The donors are listed according to their increasing ability to produce LPS-induced TNF-α. In these experiments a low LPS dose (100 ng/mL) was used. In the normal culture conditions LPS stimulated TNF-α production except of donors 5, 11, 12 and 13. However, in these donors and in donor 8, the inactivation of LBP resulted in an increase of LPS-induced cytokine production. The regulatory effect of LF was observed in 1 and 9 (downregulation) and donors 2 and 3 (upregulation) in the normal culture conditions. The majority of donors were resistant to regulatory action of LF in the normal culture conditions, however, in the absence of LBP, LF behave differently. For example, in donor 1, LF apparently replaced the action of LBP (reconstitution of LPS-induced response), in two cases [4 and 8] LF reduced LPS-induced TNF-α to the background levels. On the other hand, in donors 5, 11 and 13 LF was downregulatory and in donor 10 upregulatory. Donors 6 and 7 appeared to be resistant to regulatory action of LF in both experimental conditions. In the Table 2, the results of a similar study are presented using a high dose of LPS (5 μg/mL) as TNF-α inducer. It is of interest that in donor 2 inactivation of LBP caused very significant reduction in the ability of LPS to induce TNF-α, whereas LF was able to reconstitute the cytokine production to the levels observed in the cultures non treated with anti-LBP antibody. The actions of LF were up- (donor 2) or down-regulatory (donor 1) with respect to inducible TNF-α production. In the case of donor 4, LF stimulated the spontaneous TNF-α production whereas in donor 7 that type of cytokine response was inhibited. Removal of LBP resulted in reconstitution of anti-LPS response (donor 2) and decreases of the LPS-induced TNF-α production (donors 1, 4 and 3).

The effects of egzogenous LBP on TNF-α production

To directly assess significance of LBP in LPS-induced TNF-α production the decreasing doses of LBP were added to the plasma-free cultures (Tab. 3). After 2 h the cultures were supplemented with 5% autologous plasma. Although the TNF-α values registered in unmanipulated PBMC cultures from that donor were as following (pg/mL): medium only – 717 pg, LPS – 11.144, BLF – 110, LPS/BLF – 13 165, the spontaneous TNF-α production in the plasma-free experimental system was1498 pg/mL and the action of LPS was inhibitory (208 pg/mL). Addition of high LBP doses (1.4 and 0.3 μg/mL) were inhibitory with respect to LPS-dependent TNF-α production, whereas the dose range 80 ng – 250 pg/mL increased the ability of cells to produce TNF-α to the levels observed in control cultures containing from the beginning 5% plasma (11.144 pg/mL). BLF, added at a dose 20 μg/mL changed the dose response at high LBP concentration (1.4 and 0.3 μg/mL) by significantly elevating the cytokine production.

Discussion

In this study we report several interesting findings associated with the role of LBP in TNF-α production by LPS in human peripheral blood mononuclear cell cultures. First, the actions of LPS, in terms of the ability to induce TNF-α production, depended on the reactivity of individual blood donors and LPS dose. We also showed that the actions of LPS were dependent on the presence of LBP, in low TNF-α producers. The requirement for LBP was, however, not absolute since in many cases removal of LBP resulted in a better stimulation of TNF-α by LPS. The addition of small doses of LBP to the serum-free medium reconstituted inducible TNF-α production to observed in control, unmanipulated cultures. However, high doses of LBP were, not effective. LF was regulatory with regard to LPS-induced TNF-α production in both

Table 1. Effects of LBP neutralization on LPS and BLF-induced production of TNF-α in human PBMC cultures: low dose of LPS (100 ng/mL)

Tabela 1. Wpływ neutralizacji LBP na wytwarzanie TNF-α w hodowlach ludzkich komórek jednojądrzastych indukowane LPS i BLF - zastosowanie małej dawki LPS (100 μg/ml)

Donor No. (Numer dawcy)	Experimental conditions (Warunki doświadczenia)	Autologous plasma TNF-a (pg/mL) (Dodatek autologicznego osocza)	Anti-LBP-treated plasma TNF-a (pg/mL) (Osocze traktowane przeciwciałem anty-LBP)
1	–	23	31
	LPS	281	39
	BLF	96	16
	LPS+BLF	121	204
2	–	38	48
	LPS	189	361
	BLF	48	70
	LPS+BLF	727	758
3	–	46	12
	LPS	352	709
	BLF	20	25
	LPS+BLF	706	787
4	–	48	31
	LPS	376	745
	BLF	73	87
	LPS+BLF	406	74
5	–	390	385
	LPS	427	1412
	BLF	432	112
	LPS+BLF	442	785
6	–	20	64
	LPS	734	839
	BLF	24	76
	LPS+BLF	723	760
7	–	38	45
	LPS	756	777
	BLF	37	104
	LPS+BLF	795	807
8	–	38	99
	LPS	841	1631
	BLF	32	109
	LPS+BLF	866	96
9	–	103	28
	LPS	883	806
	BLF	199	447
	LPS+BLF	415	890
10	–	29	33
	LPS	1511	803
	BLF	33	49
	LPS+BLF	1646	1638
11	–	1739	387
	LPS	1605	2988
	BLF	925	1306
	LPS+BLF	1616	1594
12	–	2272	2170
	LPS	2256	3793
	BLF	1824	1890
	LPS+BLF	1943	3576
13	–	2024	2240
	LPS	402	3496
	BLF	2002	1657
	LPS+BLF	2169	2076

PBMC (3×10^6/well/2 mL) were incubated for 24 h in culture medium containing autologous plasma or autologous plasma pretreated with neutralizing concentration of anti LBP antibody. LPS was used at concentration 100 ng/mL and BLF – 20 μg/mL. After the incubation the supernatants were assayed for TNF-α activity.

PBMC (3 x 10^6/hodowlę/2 ml) indukowano przez 24 godziny w medium hodowlanym zawierającym autologiczne osocze lub osocze uprzednio traktowane neutralizującym stężeniem przeciwciał anty-LBP. LPS użyto w stężeniu 100 ng/ml, a BLF – 20 μg /ml. Po inkubacji w supernatantach oznaczano aktywność TNF-α.

Table 2. Effects of LBP neutralization on LPS and BLF-induced production of TNF-α: high dose of LPS (5 μg/mL)

Tabela 2. Wpływ neutralizacji LBP na produkcję TNF-α indukowaną LPS i BLF – wysoka dawka LPS (5 μg/ml)

Donor No. (Numer dawcy)	Experimental conditions (Warunki doświadczenia)	Autologous plasma TNF-a (pg/mL) (Dodatek autologicznego osocza)	Anti-LBP-treated plasma TNF-a (pg/mL) (Osocze traktowane przeciwciałem anty-LBP)
1	–	390	385
	LPS	739	1529
	BLF	432	112
	LPS+BLF	403	438
2	–	60	62
	LPS	1464	198
	BLF	79	89
	LPS+BLF	2900	2944
3	–	1739	387
	LPS	1685	3173
	BLF	925	1306
	LPS+BLF	1792	1521
4	–	98	58
	LPS	3004	770
	BLF	348	109
	LPS+BLF	3137	689
5	–	2024	2240
	LPS	3528	3984
	BLF	2004	1657
	LPS+BLF	2984	3354
6	–	2272	2170
	LPS	3840	4254
	BLF	1824	1890
	LPS+BLF	3958	4070
7	–	718	691
	LPS	11 144	23 579
	BLF	110	411
	LPS+BLF	13 165	14 288

PBMC (3×10^6/well/2 mL) were incubated for 24 h in culture medium containing autologous plasma or autologous plasma pretreated with neutralizing concentration of anti LBP antibody. LPS was used at concentration 5 μg/mL and BLF – 20 μg/mL. After the incubation the supernatants were assayed for TNF-α activity.

PBMC (3×10^6/hodowlę/2 ml) indukowano przez 24 godziny w medium hodowlanym zawierającym autologiczne osocze lub osocze uprzednio traktowane neutralizującym stężeniem przeciwciał anty-LBP. LPS użyto w stężeniu 5 μg/ml, a BLF – 20 μg /ml. Po inkubacji w supernatantach oznaczano aktywność TNF-α.

untreated as well in anti-LBP treated cultures and relevant controls. More importantly, in low-responding blood donors, LF could substitute for the action of LBP in LBP-deprived culture medium. In addition, LF enhanced LPS-induced TNF-α production at high LBP concentration (Tab. 3).

Our results are in accordance with the recently postulated roles for LBP in mediating response of cells to LPS [16–19]. They also support the findings [35, 36] that LBP may in fact inhibit LPS-induced cytokine production. We demonstrated that removal of LBP, particularly in moderate and high responders, enhanced LPS-induced TNF-α production. On the other hand, in two low-responding individuals, inactivation of LBP significantly decreased the ability of LPS to induce TNF-α. Also, when cytokine induction was performed in serum-free medium, addition of small amounts of LBP was sufficient to restore high TNF-α production induced by LPS. Moreover, the removal of LBP had similar consequences when nanogram or microgram doses of LPS were used. This suggests that LPB plays a role in induction of anti cytokine response to both low (nanograms) and high (micrograms) LPS doses.

The described model of evaluating the role of LBP and LF in LPS-induced cytokine production is far from being perfect as most of the *in vitro* systems. However, assessment of regulatory actions of LBP and LF in such a system has apparent advantage over murine or cell line models since it takes into consideration the immune status of individual donors as reflected by variations in the susceptibility to the regulatory actions of the described regulatory proteins. It is well known, for example, that the ability to produce cytokines, in particular TNF-α [37] in is under genetic control.

Table 3. The role of LBP and LF in the induction of TNF-α by LPS

Tabela 3. Rola LBP i LF w indukcji TNF-α przez LPS

	Experimental conditions (Warunki doświadczenia)	Dose/mL (Dawka/ml)	TNF-α (pg/mL)
	Medium only containing 5% plasma	–	717
A	LPS	5 μg	11 144
	BLF	20 μg	110
	LPS+BLF	5/20 μg	13 165
B	Medium only	–	1498
	LPS	5 μg	208
	BLF	20 μg	68
	LPS+BLF	5/20 μg	711
C	LPS_5 + LBP	1.4 μg	119
		0.3 μg	122
		80 ng	7087
		20 ng	11 537
		5 ng	12 604
		1 ng	12 407
		250 pg	11 340
		80 pg	329
		20 pg	285
D	LPS_5 + LBP + BLF	1.4 μg	3593
		0.3 μg	6582
		80 ng	6722
		20 ng	11 087
		5 ng	13 024
		1 ng	6610
		250 pg	10 891
		80 pg	184
		20 pg	757

PBMC (3 x 10^6/well/2 mL) were incubated in autologous plasma-containing medium (A) or preincubated with medium without plasma with addition of LPS, BLF or BLF and LPS at indicated doses (B, C, D). The concentration of LPB ranged from 1.4 μg to 20 pg/mL. After 24 h incubation the supplements were harvested for determination of TNF-α activity.

PBMC (3 x 10^6/hodowlę/2 ml) były indukowane w obecności medium zawierającego autologiczne osocze (A) lub preinkubowane w medium bez osocza z dodatkiem LPS, BLF lub BLF i LPS podanych dawkach (B, C, D). Stężenie LBP było w granicach od 1,4 μg do 20 pg/ml. Po 24-godzinnej inkubacji supernatanty były zbierane do oznaczania aktywności TNF-α.

That phenomenon was evident as the ability of PBMC cultures to produce TNF-α, spontaneously or upon LPS addition was differential. High background TNF-α production observed in most cases, was probably due to specific culture conditions enabling contact of monocytes and T cells (such a fraction contains these cell types at 20:80 ratio). In our earlier studies we revealed phenomenon of IL-1 secretion in co-cultures of syngeneic macrophages and T cells [38]. Nevertheless, despite high spontaneous TNF-α production, LPS induced additional TNF-α in a dose-dependent manner (Tab. 1 and 2).

The ability of LF to induce cytokines *in vivo* [39] and *in vitro* [40] has been previously demonstrated and is dose-dependent. LF can interact with various cell types and, for example, it may induce in T cells signals used by mitogens [41]. Relevant to the present study was our investigation on the regulatory activity of LF in LPS-induced LFA-1 expression on PBMC [42]. We showed that the actions of LF were diverse depending on the constitutive expression of LFA-1 in individual blood donors and the effects of LPS. Interestingly, in that cases the regulatory actions of LF were blocked after additions of anti-TNF-α antibodies. An important finding of this study was the apparent lack of interference of the LBP function by lactoferrin which is in contrast with the results of Elass Rochard et al. [30]. The discrepancy in the results could be due to the different experimental models. In addition, LPS receptors other than CD14 may mediate LPS action.

In summary, the results indicate that LBP plays, in fact, an important regulatory role in LPS-induced cytokine production. It is, to our knowledge, the first report individually evaluating the role of LPB in LPS-induced cytokine production in PBMC from human blood donors. Nevertheless, LBP appears to represent only one of the elements of the immune system controlling reactivity to LPS. In addition, it seems that lactoferrin may also mediate the responsiveness of blood cells to LPS in the category of low-reactive individuals.

References

[1] Mayeux P. R.: Pathobiology of lipopolysaccharide. J. Toxicol. Environ. Health 1997, 51, 415–435.

[2] Fenton M. J., Golenbock D. T.: LPS-binding proteins and receptors. J. Leuk. Biol. 1998, 64, 25–32.

[3] Schroder N. W., Opitz B., Lamping N., Michelsen K. S., Zahringer U., Gobel U. B., Schumann R. R.: Involvement of lipopolysaccharide binding protein, CD14 and Toll-like receptors in the initiation of innate immune responses by *Treponema glycolipids*. J. Immunol. 2000, 165, 2683–2693.

[4] Frey E. A., Miller D. S., Jahr T. G., Sundan A., Bazil V., Espevik T., Finaly B. B., Wright S. D.: Soluble CD14 participates in the response of cells to lipopolysaccharide. J. Exp. Med. 1992, 176, 1665–1671.

[5] Le Roy D., Di Padova F., Adachi Y., Glauser M. P., Calandra T., Heumann D.: Critical role of lipopolysaccharide-binding protein and CD14 in immune responses against Gram-negative bacteria. J. Immunol. 2001, 167, 2759–2765.

[6] Haziot A., Ferrero E., Köntgen F., Hijiya N., Yamamoto S., Silver J., Steward C. L., Goyert S. M.: Resistance to endotoxin shock and reduced dissemination of Gram-negative bacteria in CD14-deficient mice. Immunity 1996, 4, 407–414.

[7] Ingalls R. R., Golenbock D. T.: CD11c/CD18 a transmembrane signaling receptor for lipopolysaccharide. J. Exp. Med. 1995, 181, 1473–1479.

[8] Thome Z. N., Amar S., Van Dyke T. E.: Moesin functions as a lipopolysaccharide receptor on human moncytes. Infect. Imm. 1999, 67, 3215–3220.

[9] Triantafilou K., Trantafilou M., Dedrick R. L.: Interactions of bacterial lipopolysaccharide and peptidoglycan with a 70 kDa and an 80 kDa protein on the cell surface of $CD14^+$ and $CD14^-$ cells. Hum. Immunol. 2001, 62, 50–63.

[10] Haziot A., Hijiya N., Gangloff S. C., Silver J., Goyert S. M.: Induction of a novel mechanism of accelerated bacterial clearance by lipopolysaccharide in CD14-deficient and toll-like receptor 4-deficient mice. J. Immunol. 2001, 166, 1075–1078.

[11] Tanke T., Van De Loo J.-W., Rhin H., Leventhal P. S., Proctor R.A., Bartics P.J.: Bacterial lipopolysaccharide-stimulated GTPase activity in RAW 264.7 macrophage membranes. Biochem. J. 1991, 277, 379–385.

[12] Bellezzo J. M., Britton R. S., Fox E. S.: LPS-mediated NF kappa activation in rat Kupfer cells can be induced independently of CD14. Am. J. Physiol. 1996, 270, G956–G961.

[13] Schumann R. R., Kirsching C. J., Unbehaun A.: The lipopolysaccharide-binding protein is a secretory class 1 acute-phase protein whose gene is transcriptionally activated by APRF/STAT-3 and other cytokine-inducible nuclear proteins. Mol. Cell. Biol. 1996, 16, 3490–3503.

[14] Froon A. H., Dentener M. A., Greve J. W., Ramsay G., Buurman W. A.: Lipopolysaccharide-toxicity-regulating protein in bacteremia. J. Infect. Dis. 1995, 171, 1250–1257.

[15] Tobias P. S., Soldan K., Ulevitch R. J.: Isolation of a lipopolysaccharide-binding acute phase reactant from rabbit serum. J. Exp. Med. 1986, 164, 777–793.

[16] Hailman E., Lichenstein H. S., Wurfel M. N., Miller D. S., Johnson D. A., Kelley M., Busse L. A., Zukowski M. M., Wright S. D.: Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. J. Exp. Med. 1994, 179, 269–277.

[17] Wright S. D., Ramos R. A., Tobias P. S., Ulevitch R. J., Mathison J. C.: C14, a receptor for complexes of lipopolysaccharide (LPS) and LPS-binding protein. Science 1990, 249, 1431–1433.

[18] Mathison J. C., Tobias P. S., Wolfson E., Ulevitch R. J.: Plasma lipopolysaccharide (LPS)-binding protein. A key component in macrophage recognition of Gram-negative LPS. J. Immunol. 1992, 149, 200–206.

[19] Klein R. D., Su G. L., Schmidt C., Aminlari A., Steinstraesser L., Alarcon W. H., Zhang H. Y., Wang S. C.: Lipopolysaccharide-binding protein accelerates and augments *Escherichia coli* phagocytosis by alveolar macrophages. J. Surg. Res. 2000, 94, 159–166.

[20] Lonnerdal B., Iyer S.: Lactoferrin: molecular structure and biological function. Annu. Rev. Nutr. 1995, 15, 93–110.

[21] Venge P., Nilsson L., Nyström S.-O., Åberg T.: Serum and plasma measurements of neutrophil granule proteins during cardiopulmonary bypass: A model to estimate human turnover of lactoferrin and myeloperoxidase. Eur. J. Haematol. 1987, 39, 339–345.

[22] Rich I. N. C.: The macrophage as a production site for hematopoietic regulator molecule: sensing and responding to normal and pathophysiological signals. Anticancer Res. 1998, 8, 1015–1040.

[23] Walkabayashi H., Mellamy W., Takase M., Tomita M.: Inactivation of *Listeria monocytogenes* by lactoferrin, a potent antimicrobial peptide derived from cow's milk. J. Food Protect. 1992, 55, 238–240.

[24] Zagulski T., Lipiński P., Zagulska A., Broniek S., Jarząbek Z.: Lactoferrin can protect mice against a lethal dose of *Escherichia coli* in experimental infection *in vivo*. Br. J. Exp. Pathol. 1989, 70, 697–704.

[25] Machnicki M., Zimecki M., Zagulski T.: Lactoferrin regulates the release of tumor necrosis factor alpha and interleukin 6 *in vivo*. Int. J. Exp. Pathol. 1993, 74, 433–439.

[26] Kruzel M. L., Harari Y., Chen C. Y., Castro G. A.: Lactoferrin protects gut mucosal integrity during endotoxemia induced by lipopolysaccharide in mice. Inflammation 2000, 24, 33–44.

[27] Cohen M. S., Mao J., Rasmussen G. T., Serody J. S., Britigan B.: Interaction of lactoferrin and lipopolysaccharide (LPS). Effects on the antioxidant property of lactoferrin and the ability of LPS to prime human neutrophils for enhanced superoxide formation. J. Infect. Dis. 1992, 166, 1375–1378.

[28] Appelmelk B. J., An Y.-Q., Geerts M., Thijs B. G., de Boer H. A., McLaren D. M., de Graaff J., Nuijens J. H.: Lactoferrin is a lipid A-binding protein. Inf. Immunity 1994, 62, 2628–2632.

[29] Brandenburg K., Jurgens G., Muller M. Fukuoka S., Koch M. H.: Biophysical characterization of lipopolysaccharide and lipid A inactivation by lactoferrin. Biol. Chem. 2001, 382, 1215–1225.

[30] Elas-Rochard E., Legrand D., Salmon V., Roseanu A., Trif M., Tobias P. S., Mazurier J., Spik G.: Lactoferrin inhibits the endotoxin interaction with CD14 by competition with the lipopolysaccharide-binding protein. Infec. Immunity 1998, 66, 486–491.
[31] Zimecki M., Właszczyk A., Cheneau P., Brunel A. S., Mazurier J., Spik G., Kübler A.: Immunoregulatory effects of bovine lactoferrin taken orally by healthy individuals. Arch. Immunol. Ther. Exp. 1998, 46, 231–240.
[32] Zimecki M., Właszczyk A., Wojciechowski R., Dawiskiba J., Kruzel M.: Lactoferrin regulates the immune responses in post-surgical patients. Arch. Immunol. Ther. Exp. 2001, 49, 325–333.
[33] Espevik T., Nissen-Mayer J.: A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. J. Immunol. Methods 1986, 25, 99–105.
[34] Hansen M. B., Nielsen S.E., Berg K.: Reexamination and further development of a precise and rapid dye method for measuring cell growth/kill. J. Immunol. Methods 1989, 119, 203–210.
[35] Gegner J.A., Ulevitch R. J., Tobias P. S.: Lipopolysaccharide (LPS) signal transduction and clearance: dual roles for LPS binding protein and membrane CD14. J. Biol. Chem. 1995, 270, 5320–5325.
[36] Zweigner J., Gramm H. J., Singer O. C., Wegscheider K., Schumann R. R.: High concentration of lipopolysaccharide-binding protein in serum of patients with severe sepsis or septic shock inhibit the lipopolysaccharide response in human monocytes. Blood 2001, 98, 3800–3808.
[37] Louis E., Franchimout D., Piron A., Gevaert Y., Schaaf-Lafontaine N., Roland S., Machieu P., Malaise M., de Groote D., Louis R., Belaiche J.: Tumor necrosis factor (TNF) gene polymorphism influences TNF-α production in lipopolysaccharide (LPS)-stimulated whole blood culture in healthy humans. Clin. Exp. Immunol. 1998, 113, 401–409.
[38] Zimecki M., Wieczorek Z., Kapp J., Pierce C. W.: Structures on T cell and macrophages involved in interleukin 1 (IL-1) secretion by macrophages upon contact with syngeneic thymocytes. Arch. Immunol. Ther. Exp.1989, 37, 587–592.
[39] Zimecki M., Kruzel M.: Protective effect of lactoferrin against LPS-induced endotoxemia is mediated by IL-10. In: Monduzzi Editore. 5th World Congress on Trauma, Shock, Inflammation and Sepsis; 2000 February 29 – March 4, Munich, Germany, p. 221–225.
[40] Actor J. K., Hwang S.-A., Olsen M., Zimecki M., Hunter R. L., Kruzel M.: Lactoferrin immunomodulation of DTH response in mice. Int. Immunopharm. 2002, 2, 475–486.
[41] Dhennin-Duthille I., Masson M., Damiens E., Fillebeen C., Spik G., Mazurier J.: Lactoferrin upregulates the expression of CD4 antigen through the stimulation of the mitogen-activated protein kinase in the human lymphoblastic T Jurkat cell line. J. Cell. Biochem. 2000, 79, 583–593.
[42] Zimecki M., Międzybrodzki R., Mazurier J., Spik G.: Regulatory effects of lactoferrin and lipopolysaccharide on LFA-1 expression on human peripheral blood mononuclear cells. Arch. Immunol. Ther. Exp. 1999, 47, 257–264.

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Received: 8.11.2002
Revised: 27.11.2002
Accepted: 27.11.2002

Praca wpłynęła do Redakcji: 8.11.2002 r.
Po recenzji: 27.11.2002 r.
Zaakceptowano do druku: 27.11.2002 r.

Komunikat

Uprzejmie zawiadamiamy, że

XXXV Zjazd Towarzystwa Internistów Polskich odbędzie się w Katowicach w dniach 9–12 września 2004 r.

Zjazd będzie miał charakter naukowo-szkoleniowy, będzie łączył prezentacje osiągnięć naukowo-badawczych z ich zastosowaniem praktyczno-klinicznym, a także przedstawieniem wykładów podsumowujących aktualne poglądy na najważniejsze zagadnienia specjalności wchodzących w skład nauki o chorobach wewnętrznych.

Zapraszamy do Katowic lekarzy internistów, lekarzy rodzinnych i wszystkich interesujących się problemami medycyny wewnętrznej i wierzymy, że dzięki Państwa obecności Zjazd stanie się świętem polskiej interny i przyniesie wiele korzyści zawodowych i miłych wspomnień.

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