Inflamm. res. 52 (2003) 305–310
1023-3830/03/070305-06
DOI 10.1007/s00011-003-1178-4

Inflammation Research

Bovine lactoferrin decreases histopathological changes in the liver and regulates cytokine production by splenocytes of obstructive jaundiced rats

M. Zimecki [1], J. Dawiskiba [2], B. Zawirska [3], Z. Krawczyk [2] and M. Kruzel [4]

[1] Institute of Immunology and Experimental Therapy of Polish Academy of Sciences, R. Weigla 12, 53-114 Wrocław, Poland, e-mail: zimecki@immuno.iitd.pan.wroc.pl

[2] First Clinic of General and Endocrine Surgery, Wrocław Medical University, Poniatowskiego Str. 2, 50-326 Wrocław, Poland

[3] Department of Pathology, Wrocław Medical University, Marcinkowskiego Str. 1, 50-368 Wrocław, Poland

[4] Department of Integrative Biology, Pharmacology and Physiology, University of Texas, Medical School at Houston Texas, USA

Received 15 August 2002; returned for revision 18 February 2003; accepted byA. Falus 5 March 2003

Abstract. *Objective and design:* The aim of this study was to evaluate effects of bovine lactoferrin (BLF) on histopathological changes in the liver of 14 day obstructive jaundiced (OJ) rats and production of tumor necrosis factor (TNF-α) and interleukin 6 (IL-6) by splenocytes from 7- and 14-day OJ rats.
Material and subjects: In the study 50 male rats of the Buffalo strain (170-270 g, mean 230 g) were used.
Treatment: Rats were given 10 mg BLF in 0.5 ml saline daily, using a stomach tube. BLF was applied 2 days before operation and for 13 days following operation.
Methods: The specimens of liver were prepared using standard techniques. Sections, 5 µm thick were stained with hematoxylin and eosin and reviewed histologically. Microscopic estimation using semiquantitive 4-grade scale was used for evaluation of liver changes. For cytokine measurement splenocyte cultures were stimulated with 5 µg/ml lipopolysaccharide (LPS). After overnight incubation the activities of TNFα and IL-6 were determined using bioassays. For statistical evaluation of data the nonparametric Mann-Whitney test was applied.
Results: In rats with 14-day OJ, treated with BLF, the pathological changes in the liver were markedly reduced, in particular foci of necrosis with disseminated lymphocytes, cellular necrobiosis, bile duct proliferation and dilation. Neither proliferation of fibrous and reticular connective tissue nor activation of Kupffer cells was revealed. In the 7-day OJ rats treatment with BLF caused significant inhibition of both spontaneous (mean 253, median 275, vs mean 160, median 148 pg/ml, $p = 0.002$) and LPS-induced TNF-α production (mean 4967, median 4102, vs mean 2291, median 2234 pg/ml, $p = 0.004$) in the splenocyte cultures. The spontaneous (mean 120, median 81, vs mean 43, median 26 pg/ml, $p = 0.005$) as well as LPS-induced IL-6 production (mean 422, median 378, vs mean 293, median 230 pg/ml, $p = 0.025$) were also lowered. On the other hand, in 14-day OJ, BLF upregulated cytokine production, in particular spontaneous (mean 148, median 158, vs mean 338, median 196, $p = 0.001$) and LPS-induced TNF-α (mean 1331, median 1507, vs mean 2239, median 1707 pg/ml, $p = 0.027$).
Conclusion: We conclude that oral administration of BLF is beneficial in alleviating deleterious effects of OJ.

Key words: Obstructive jaundice (OJ) – Rats – Bovine lactoferrin (BLF) – TNF-α – IL-6 – Liver

Introduction

Clinical observations confirm increased risk of postsurgical complications in patients with obstructive jaundice (OJ) [1, 2]. OJ in humans and experimental animals is associated with characteristic functional [3–5], histopathological [6], and biochemical changes [7–9], predominantly found in the liver and spleen. In addition, the immune status of patients [1, 2] and experimental animals [10] is impaired. It has been shown that the pathological changes in OJ are initiated by translocating of bacteria from the gastrointestinal tract to mesenteric lymph nodes [11] and stimulation of reticuloendothelial system cells by endotoxins [12, 13]. Therapeutical approaches in OJ were mainly focused on administration of bile salts, taking advantage of their property to bind endotoxin [14]. Other compounds, such as allopurinol and lactulose [15, 16] were also used. Such strategies, applied preoperatively, prevented deterioration of renal function and postoperative renal failure or systemic endotoxemia [14, 15].

We have recently turned attention to lactoferrin as a potential therapeutic agent in OJ. Lactoferrin is a 80 kDa protein found in secretory fluids of mammals [17] and sec-

Correspondence to: M. Zimecki

ondary granules of neutrophils [18]. This protein exhibits a broad range of immunoregulatory [19] and protective activities [20].

In particular, lactoferrin and lactoferrin-derived peptides, are bacteriocidal [21] or bacteriostatic [22] and are thought to be responsible for maintenance of a proper gut microflora [23]. Furthermore, lactoferrin protects gut integrity upon infection [24] or in experimental endotoxemia [25] probably by inhibition of inducible nitric oxide [26] which is destructive for gut epithelium [27]. The premise on which a potential lactoferrin application in OJ is based, would be presence of that protein in the bile duct, and gut [28] and its ability to bind LPS [29].

For our studies we have chosen a 7- and 14-day OJ experimental model in rats. We have previously shown that the cytokine production in early (7-day) and fully developed (14-day) OJ is different (stimulation and inhibition of TNF-α and IL-6, respectively) [3, 4]. The inhibition of cytokine production (both spontaneous and LPS-induced) in the late OJ was also correlated with impairment of phagocytic RES function [4]. In this report we decided to evaluate effectiveness of oral treatment of OJ rats with bovine lactoferrin by determination of cytokine production in splenocyte cultures in 7- and 14-day OJ and microscopic examination of pathological changes in the liver in 14-day OJ.

Materials and methods

Animals

Male rats of the Buffalo strain (170–270 g, mean 230 g) were used. The rats were maintained in stable conditions, fed standard commercial diet with free access to food and water. All experiments were conducted under approved guidelines of the animal ethics committee at the Wrocław Medical School, Wrocław.

Surgical procedures

Procedures were performed under general anaesthesia after intramuscular administration of Bioketan® (Biowet-Poland), dose 60 mg/kg b.w. in clean, but not sterile conditions. The abdominal cavity was opened with a midline incision after disinfecting the skin. The common bile duct (CBD) was located and obstructive jaundice induced by a double ligation with 5/0 silk and transsection of the CBD in the supraduodental part between the lowermost tributary of the bile duct and the uppermost tributary of the pancreatic duct. The control group rats underwent opening of the abdominal cavity and dissection of the CBD without ligation.

At the end of the experiment, after opening of the abdominal cavity, in all rats blood for bilirubin measurement was taken by direct cardiac puncture.

In rats with obstructive jaundice the bilirubin level was high, the liver was markedly enlarged with characteristic discolouration and the CBD was significantly dilated. In both groups of rats specimens of liver were collected for pathological examination.

Treatment of rats with lactoferrin

Rats were given 10 mg of low endotoxin BLF in 0.5 ml saline (4.4 E.U./mg, < 25% iron saturated, Sigma, MO USA) using a stomach tube. BLF was applied daily 2 days before operation and for 13 days following procedure.

Histological procedures

The specimens of liver were preserved in 5% buffered formaldehyde, then embedded in paraffin using standard techniques. Section, 5 µm thick, were cut, stained with hematoxylin and eosin (H&E) and subsequently reviewed histologically; the pathologist viewing and interpreting the slides was blinded to type of experiment and treatment. Microscopic estimation using semiquantitative 4-grade scale (normal, light, moderate and significant) was used to evaluate various signs of extrahepatic cholestasis in the liver tissue, such as focal liver necrosis with lymphocytic infiltration, hepatocytes necrobiosis, proliferation and dilation of small bile ducts, hepatocytes regeneration, hyperplasia of connective fibrous and reticular tissue and activation of Kupffer cells.

Preparation of spleen cell cultures and induction of cytokines in cell cultures

In brief, spleens were removed, a single cell suspension prepared by pressing the organs through a plastic screen, washed 2× with Hanks' solution and resuspended in the culture medium at concentration 10^7/ml. PEC (10^6/ml) and splenocytes (10^7/ml) were incubated overnight in 24-well plates without or with 5 µg LPS/ml from *E. coli* strain No 011:B4 lot 5711148 (Sigma). Supernatants were harvested and kept frozen until cytokine determination.

Determination of IL-6 activity

The assay was performed according to Van Snick et al. [30]. Briefly, 7TD1 indicator cells were washed 3 times with Hanks' medium and resuspended in Iscove's medium supplemented with 10% FCS, HEPES buffer, glutamine and antibiotics in density of 2×10^4 cells/ml. Then, the cells were distributed in 100 µl aliquots into 96-well flat-bottom plates containing 100 µl of serially diluted plasma or supernatant in triplicate. After 72 h of culture the proliferation of 7TD1 cells was determined using MTT colorimetric method [31]. The results of IL-6 activity are presented in picograms per ml -such concentration of IL-6 corresponds to the activity of IL-6 expressed in units/ml [30]. One unit of IL-6 activity was calculated as the inverse dilution of a plasma sample where a half-maximal proliferation of 7TD1 cells was registered. The sensitivity limit in this assay is 0.5 pg IL-6 when tested a recombinant IL-6. 7TD1 line responds by proliferation only to IL-6 [30].

Determination of TNF-α activity [32]

For determination of TNF-α activity a highly specific 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 samples were prepared on separate plates and transferred to microtitter plates containing WEHI 164.13 cells. The medium contained in addition 1 µg/ml actinomycin D to increase sensitivity of the assay. After an overnight incubation the survival of cells was determined using MTT colorimetric assay [31]. 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. The sensitivity limit of this assay is 2.5 pg TNF-α.

Statistics

The nonparametric Mann-Whitney's test was applied and the results were given as mean value and median. The differences were regarded as significant when $p < 0.05$.

Results

Protective effects of BLF in preventing pathologic changes in liver of rats with OJ

In rats with 7 day OJ, the changes in liver were estimated as only functional and no pathological morphology could be observed.

In order to present semiqantitative evaluation of the protective role of BLF in preventing pathologic liver changes in rats with 14 day OJ, a four degree scale was used (Table 1). Changes seen in particular liver tissue samples were marked as: none (–), light – 1st degree (+), moderate – 2nd (++) and significant – 3rd degree (+++).

In animals with 14 day OJ, foci of necrosis of different degree with lymphocytic infiltration, hepatocyte necrocytosis, dilation and proliferation of small bile ducts, lymphocytic infiltrates in periportal areas, fibrosis with proliferation of fibrous and reticular connective tissue and activation of Kupffer cells, were seen.

In rats with 14 day OJ, which received BLF, the following pathologic changes were observed. A limited number of animals (two) exhibited foci of low degree (+) necrosis with disseminated lymphocytes, while hepatocyte necrobiosis was found sporadically. In seven rats, bile duct proliferation and dilation of a low degree (+) with no lymphocytic infiltrates was seen. Neither proliferation of fibrous and reticular connective tissue nor activation of Kupffer cells was noticed. A moderate degree proliferation (+) of a limited number of hepatocytes, indicating liver regeneration in three rats, was found.

Effect of oral BLF treatment on cytokine production by splenocyte cultures in 7-day and 14-day OJ rats

We have previously demonstrated [3] that cytokine production by lymphoid cells, derived from OJ rats on day 7 and 14, significantly differed, i.e. in early OJ cytokine production was elevated and in late OJ was lowered compared with sham-operated controls. Tables 2 and 3 show how the treatment of rats with BLF affected TNF-α and IL-6 production, both spontaneous and LPS-induced. In the case of TNF-α the inhibition, by BLF, of inducible cytokine production was more profound comparing with IL-6.

On the other hand (Table 3) the effects of BLF on cytokine production in 14-day OJ were stimulatory. BLF significantly elevated both spontaneous and LPS-induced TNF-α production. In the case of IL-6 the enhancement of LPS-inducible cytokine production was small, but elevation of the spontaneous one, was significant.

We have also studied the effects of BLF treatment on serum cytokine levels. However, on day 7 no effects were observed and on day 14 a small, but significant decrease of TNF-α level was noted (data not shown).

Table 1. Type and intensity of changes in the liver of rats with 2-week OJ.

Experimental groups												
Type of changes in the liver	A		B		C		D		E		F	
	Control	BLF	Control	BLF	Control	BLF	Control	BLF	Control	BLF	Control	BLF
Mean value	2.29	0.17	1.29	0.25	2.29	0.58	–	0.25	1.00	–	0.43	–
Median	9.73	0.39	0.61	0.45	0.73	0.51	–	0.45	0.00	–	0.51	–
Statistics	$p < 0.001$		$p < 0.001$		$p < 0.001$		NS		$p < 0.001$		$p = 0.011$	
No changes (0)	0	10	0	9	0	5	14	9	0	12	8	12
Light (1)	2	2	11	3	2	7	0	3	14	0	6	0
Moderate (2)	6	0	2	0	6	0	0	0	0	0	0	0
Significant (3)	6	0	1	0	6	0	0	0	0	0	0	0
Total	32	2	18	3	32	7	0	3	14	0	6	0

Control group n = 14. BLF treated n = 12.
Legend:
A) foci of necrosis with disseminated lymphocytes. B) necrocytosis of single hepatocytes. C) proliferation of the bile ducts with their dilation. D) proliferation of single hepatocytes. E) proliferation of fibrous and reticular connective tissue. F) activation of Kupffer cells.

Table 2. TNF-α and IL-6 production by splenocytes of 7-day OJ rats.

	TNF-α (pg/ml)				IL-6 (pg/ml)			
	Control		BLF-treated		Control		BLF-treated	
	–[1]	LPS[2]	–[3]	LPS[4]	–[5]	LPS[6]	–[7]	LPS[8]
Mean value	253	4967	160	2291	120	422	43	293
Median	275	4102	148	2234	81	378	26	230

Statistical differences 1:3 p = 0.002, 2:4 p = 0.004, 5:7 p = 0.005, 6:8 p = 0.025. Number of rats: control group – 15; BLF group – 9.

Table 3. Production of TNF-α and IL-6 by splenocytes from 14-day OJ rats.

	TNF-α (pg/ml)				IL-6 (pg/ml)			
	Control		BLF-treated		Control		BLF-treated	
	–[1]	LPS[2]	–[3]	LPS[4]	–[5]	LPS[6]	–[7]	LPS[8]
Mean value	148	1331	338	2239	85	500	153	594
Median	158	1507	196	1707	101	553	172	584

Statistical differences 1:3 $p = 0.001$; 2:4 $p = 0.027$, 5:7 $p = 0.001$; 6:8 NS. Number of rats: control group n = 12; BLF group n = 14.

Discussion

In this report we demonstrated that OJ rats, treated orally with bovine lactoferrin, showed significantly reduced histopathological changes in the liver on day 14 following bile duct ligation. In addition, treatment with BLF seems to be regulatory on production of cytokines by 7 and 14 day splenocyte cultures. Recently, we showed that production of TNF-α and IL-6 by splenocyte cultures was elevated in early (7 day) OJ as compared with sham-operated controls, but significantly suppressed in 14-day OJ [3, 4]. Those changes could be associated with activation of the immune system by translocating bacteria and endotoxin [11, 33]. The subsequent decrease in the immunological activity was consistant with other reports and could have different causes such as: decrease of the liver and spleen reticuloendothelial system activities [34, 35], possibly related to the injurious effect of the bile acids or toxic activity of conjugated bilirubin [15, 36, 37], direct action of endotoxin or their effects by the activation of coagulation factors [37, 38]. The lack of bile in the gut lumen, as a characteristic of OJ, is thought to be responsible not only for an increase in translocation of endotoxin but also for a reduction in liver reticuloendothelial system function leading to a diminished clearance of endotoxin by Kupffer cells [39, 40]. Although in our study we did not measure endotoxin concentration, we are strongly in favour of the hypothesis by Scott-Connor [41], who proposed that the immune dysfunction in OJ rats is a consequence of systemic endotoxemia and inappropriate elimination of enteric-derived antigens or to an increased production of cytokines.

In this study we demonstrated regulatory effects of BLF on splenocyte-derived TNF-α and IL-6. The production of spontaneous and LPS-induced TNFα that was initially high in 7-day culture was inhibited, and lower concentrations detected initially in 14-day cultures were elevated. As a result, the mean LPS-induced TNF-α production following BLF treatment was similar on respective days of cytokine determination (Table 2 and 3, i.e. 2.291 versus 2239 pg/ml. The spontaneous TNF-α production was also regulated. IL-6 production was markedly regulated on day 7 and to a much lesser degree on day 14 suggesting a need for sustained production of this cytokine in the late OJ. It is possible, that anti-inflammatory and protective activities of IL-6 are required to induce the acute phase proteins [42].

The regulatory effect of BLF on splenocyte-derived cytokines is relevant to significant decrease of the histopathological changes in the liver such as: foci of necrosis with lymphocyte infiltration, necrocytosis of hepatocytes, dilation and proliferation of small bile ducts, lymphocytic infiltrates in periportal areas, fibrosis with proliferation of fibrous and reticular connective tissue and activation of Kupffer cells. The reduction in the OJ-induced liver injury in rats treated with lactoferrin indicate that the protection of Kupffer cells may play an important role in active removal of endotoxin in OJ individuals. Also, we postulate that the damaging effects of OJ and the protective effects of lactoferrin may depend upon mediation of relative amounts of both proinflammatory TNF-α and antiinflammatory IL-6. Therefore, inhibition of deleterious effects of TNF-α by BLF, as demonstrated in the present study, would be relevant in the diminution of the histopathological changes in the liver of OJ rats [43].

Since lactoferrin is constitutively secreted into lumen of gastrointestinal tract it would be expected that when given orally would also protect against OJ-induced endotoxemia. In fact, Saito & Nakanuma [28] reported that in hepatolithiasis there was a significant increase in expression of lactoferrin and lysozyme in specific areas of intramural and extramural glands, suggesting that in the peribiliary glands in the stone-containing bile ducts produce significant amount of lactoferrin and lysozyme. The authors concluded that it could represent a local defense mechanism against bacterial infections. The role of lactoferrin in activating lysozyme-mediated bacteria lysis is well documented [44] and the protein is bacteriocidal itself [21]. In another report [45] BLF was shown to diminish endotoxin activity in plasma and bacterial contamination of the peritoneal cavity and mesenteric lymph nodes in rats given *E. coli* and antibiotic for increased LPS release. Lactoferrin also preserved gut mucosal integrity in experimental endotoxemia [25] and upon infection with Trichinella spiralis [24]. It is plausible that in some experimental models the protective property of lactoferrin may result from its ability to inactivate LPS [29]. That, in turn, may prevent induction of nitric oxide, a known, destructive agent for the gut epithelium [27]. BLF was also shown to protect against development of hepatitis elicited by sensitization of Kupffer cells by LPS in zymosan-primed mice [46]. Protective activity of BLF may be also associated with regulation of cytokine production by endotoxemic mice [47, 48]. It seems, therefore, that the mechanism of the protective action of BLF in prevention of harmful consequences of experimental OJ may be complex and based on its well-documented anti-inflammatory properties. In conclusion, we demonstrated that oral administration of BLF may significantly

reduce OJ-induced injury to the liver and normalize inflammatory responses of jaundiced rats. These findings suggest that a preoperative treatment of jaundiced patients with lactoferrin would be beneficial in preventing postoperative complications. Such a notion is also consistent with the current views on prevention of postoperative complications [49].

References

[1] Jang WC, Puntis MCA. Immune dysfunction in patients with obstructive jaundice, mediators and implications for treatments. J. HPB Surgery 1997; 10: 129–42.

[2] Greig JD, Krukowski ZH Matheson NA. Surgical morbidity and mortality in one hundred and twenty-nine patients with obstructive jaundice. Br J Surg 1988; 75: 216–9.

[3] Dawiskiba J, Kornafel P, Kwiatkowskia D, Zimecki M. Alterations of tumor necrosis factor alpha and interleukin 6 production and activity of the reticuloendothelial system in experimental obstructive jaundice in rats. HPB 2002; 4: 11–20.

[4] Dawiskiba J, Kwiatkowska D, Zimecki M, Kornafel P, Tyran W, Czapińska E et al. The impairment of wound healing process is correlated with abnormalities of TNF-α production by peritoneal exudate cells in obstructive jaundiced rats. J HPB Surgery 2000; 11: 311–8.

[5] Clements WD, Halliday MI, McCaigue MD, Barclay RG, Rowlands BJ. Effects of extrahepatic obstructive jaundice on Kupffer cell clearance capacity. Arch Surg 1993; 128: 200–5.

[6] Cameron GR and Prasad LBM. Recovery from biliary obstruction after spontaneous restoration of the obstructed common bile duct. J Pathol Bact 1960; 80: 127–36.

[7] Tsai LY, Lee KT, Liu TZ. Evidence for accelerated generation of hydroxyl radicals in experimental obstructive jaundice of rats. Free Radic Biol Med 1998; 24: 732–7.

[8] Inan M, Sayek I, Tel BC, Sahin-Erdemli I. Role of endotoxin and nitric oxide in the pathogenesis of renal failure in obstructive jaundice. Br J Surg 1997; 84: 943–7.

[9] Quattropani C, Vogt B, Odermatt A, Dick B, Frey BM, Frey FJ. Reduced activity of 11beta-hydroxysteroid dehydrogenase in patients with cholestasis. J Clin Invest 2001; 108: 1299–305.

[10] Greve JW, Gouma DJ Soeters BP, Buurman WA. Suppression of cellular immunity in obstructive jaundice caused by endotoxins: a study with germ-free rats. Gastroenterology 1990; 98: 478–85.

[11] Deitch EA, Berg R., Specian R. Endotoxin promotes the translocation of bacteria from the gut. Arch Surg 1987; 122: 185–90.

[12] Dawiskiba J, Zimecki M, Kwiatkowska D, Kuźmiński A. The effect of endotoxin administration on cytokine production in obstructive jaundiced rats. Arch. Immunol. Ther Exp 2001; 49: 391–7.

[13] Saitoh N, Hiraoka T, Uchino R, Miyauchi Y. Endotoxemia and intestinal mucosal dysfunction after the relief of obstructive jaundice by internal and external drainage in rats. Eur Surg Res 1995; 27: 11–8.

[14] Hunt DR, Allison ME, Preusice CR, Blumgart LH. Endotoxemia, disturbance of coagulation and obstructive jaundice. Am J Surg 1982; 144: 325–9.

[15] Pain JA, Cahill CJ, Gilbert JM, Johnson CD, Trapnell JE, Bailey ME. Prevention of postoperative renal dysfunction in patients with obstructive jaundice: a multicentre study of bile salts and lactulose. Br J Surg 1991; 78: 467–9.

[16] Schimpl G, Pesendorfer P, Steinwender G, Feierl G, Ratschek M, Hollwarth ME. Allopurinol reduces bacterial translocation, intestinal mucosal lipid peroxidation, and neutrophil-derived myeloperoxidase activity in chronic portal hypertensive and common bile duct ligated growing rats. Pediatr Res 1996; 40: 422–8.

[17] Wang CS, Chan WY, Kloer HU. Comparative studies on the chemical and immunochemical properties of human milk, human pancreatic juice and bovine milk lactoferrin. Comp Biochem Physiol B 1984; 78: 575–80.

[18] Baggliolini M, de Duve C, Masson PL, Heremans JF. Association of lactoferrin with specific granules in rabbit heterophil leukocytes. J Exp Med 1970; 131: 559–70.

[19] Baveye S, Elass E, Mazurier J, Spik G, Legrand D. Lactoferrin: a multifunctional glycoprotein involved in the modulation of the inflammatory process. Clin Chem Lab Med 1999; 37: 281–6.

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

[21] Bellamy W, Takase M, Yamauchi K, Wakabayashi H, Kawase K, Tomita M. Identification of the bactericidal domain of lactoferrin. Biochim Biophys Acta 1992; 1121: 130–6.

[22] Teraguchi S, Shin K, Ozawa K, Nakamura S, Fukuwatari Y, Tsuyuki S, et al. Bacteriostatic effect of orally administered bovine lactoferrin on proliferation of Clostridium species in the gut of mice fed bovine milk. Appl Environ Microbiol 1995; 61: 501–6.

[23] Wharton BA, Balmer SE, Scott PH. Faecal flora in the newborn. Effect of lactoferrin and related nutrients. Adv Exp Med Biol 1994; 357: 91–8.

[24] Kruzel ML, Harari Y, Chen CY, Castro GA. The gut. A key metabolic organ protected by lactoferrin during experimental systemic inflammation in mice. Adv Exp Med Biol 1998; 443: 167–73.

[25] Kruzel ML., Harazi Y, Chen C-Y, Castro GA. Lactoferrin protects gut mucosal integrity during endotoxemia induced by lipopolysaccharide in mice. Inflammation 2000; 24: 33–44.

[26] Kruzel ML, Harari Y, Mailman D, Actor JK, Zimecki M. Differential effects of prophylactic, concurrent and therapeutic lactoferrin treatment on LPS-induced inflammatory responses in mice. Clin Exp Immunol 2002; 130: 25–31.

[27] Mercer DW, Smith GS, Cross JM, Russell DH, Chang L, Cacioppo J. Effects of lipopolysaccharide on intestinal injury; potential role of nitric oxide and lipid peroxidation. J Surg Res 1996; 63: 185–92.

[28] Saito K, Nakanuma Y.: Lactoferrin and lysozyme in the intrahepatic bile duct of normal livers and hepatolithiasis. An immunohistochemical study. J Hepatol 1992; 15: 147–53.

[29] Elass-Rochard E, Roseanu A, Legrand D, Trif M, Salmon V., Motas C et al. Lactoferrin lipopolysaccharide interaction: involvement of the 28–34 loop region of human lactoferrin in the high-affinity binding to Escherichia coli 055 B5 lipopolysaccharide Biochem J 1995; 312: 839–45.

[30] Van Snick J, Cayphas S, Vink A, Uyttenhove C, Coulie PG, Rubira MR, et al. Purification and NH_2-terminal aminoacid sequence of a T cell-derived lymphokine with growth factor activity for B cell hybridomas. Proc.Natl Acad Soc. USA 1986; 83: 9679–83.

[31] Hansen MB, Nielsen SE, Berg K. Reexamination and further development of a precise and rapid dye method for measuring cell growth/kill. J Immunol Meth 1989; 119: 203–10.

[32] Espevik T, Nissen-Meyer J. A highly sensitive cell line WEHI 164 clone 13 for measuring cytotoxic factor/tumor necrosis factor from human monocytes. J Immunol Methods 1986; 95: 99–105.

[33] Reynolds JV, Murchan P, Redmond HP, Watson PWG, Leonard N, Hill A, et al. Failure of macrophage activation in experimental obstructive jaundice: association with bacterial translocation. Br J Surg 1995; 82: 534–8.

[34] Tanaka N, Ryden S, Bergqvist L, Christensen P, Bengmark S. Reticuloendothelial function in rats with obstructive jaundice. Br J Surg 1985; 72: 946–9.

[35] Holmberg JT, Bergqvist L, Hagerstrand J, Ihse J, Ryden S. Radiolabelled colloid uptake distribution and pulmonary content and localization of lysosomal enzymes in cholestatic rats. Scand J Gastroenterol 1986; 21: 291–9.

[36] Houdijk AP, Boermeester MA, Wesdorp RI, Hack CE, Van Leeuwen PA. Tumor necrosis factor unresponsiveness after surgery in bile duct-ligated rats. Am J Physiol 1996; 271: G980–G6.

[37] Drivas G, James O, Wardle N. Study of reticuloendothelial phagocytic capacity in patients with cholestasis. Br Med J 1976; 1: 1568–9.

[38] Saba M. Effect of surgical trauma on the clearance and localization of blood-borne particulate matter. Surgery 1972; 71: 675–85.

[39] Kimmings AN, van Deventer SJ, Obertop H, Rauws EA, Huibregtse K, Gouma DJ. Endotoxin, cytokines, and endotoxin binding proteins in obstructive jaundice and after preoperative biliary drainage. Gut 2000; 46(5): 725–31.
[40] Pain JA. Reticulo-endothelial function in obstructive jaundice. Br J Surg 1987; 74(12): 1091–4.
[41] Scott-Conner LE, Grogan JB. The pathophysiology of biliary obstruction and its effect on phagocytic and immune function. J Surg Res 1994; 57: 316–36.
[42] Bauman H, Gaudie J. The acute phase response. Immunol. Today 1994; 15: 74–80.
[43] Remick DG. Lung and gut injury by tumor necrosis factor. Res Immunol 1993; 144: 326–31.
[44] Ellison RT III, Giehl TJ. Killing of gram-negative bacteria by lactoferrin and lysozyme. J. Clin. Invest. 1991; 88: 1080–91.
[45] Nobermann L, Dohler JR, Perlick L. Treatment of enterogenic endotoxinemia with lactoferrin in rats. Langenbecks Arch Surg 2001; 386: 146–9.
[46] Yamaguchi M, Matsuura M, Kobayashi K, Sasaki H, Yajima T, Kuwata T. Lactoferrin protects against development of hepatitis caused by sensitization of Kupffer cells by lipopolysaccharide. Clin Diagn Lab Immunol 2001; 8: 1234–9.
[47] Machnicki M, Zimecki M, Zagulski T. Lactoferrin regulates the release of tumor necrosis factor alpha and interleukin 6 in vivo. Int J Exp Path 1993; 74: 433–9.
[48] Zimecki M, Kruzel M. Protective effect of lactoferrin against LPS-induced endotoxemia is mediated by IL-10. In: editor Faist E., Monduzzi Editore. 5th World Congress on Trauma, Shock, Inflammation and Sepsis. Munich, February 29-March 4, 2000: 221–5.
[49] Houdijk AP, Meijer C, Cuesta MA, Meyer S, Van Leeuwen PAM. Perioperative antiendotoxin strategies. Scand J Gastroent 1997; 32: 93–7.