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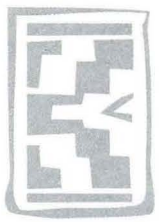

Changes in some factors of the innate immunity and serum zinc and iron concentrations in pigs following intravenous administration of *Escherichia coli* lipopolysaccharide

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ABSTRACT

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The changes in some factors of the innate immunity (phagocytosis, complement, lysozyme); haematological parameters—leukocytes, erythrocytes, differential white blood cell counts, haemoglobin, haematocrit and the serum concentrations of the microelements zinc and iron in six 2- to 3-months-old female piglets after the intravenous administration of lipopolysaccharide from *Escherichia coli* 0111:B4 were determined.

It was found out that 1 h after the administration of lipopolysaccharide at the dosage rate of 10 µg/kg body weight resulted in a decrease in the phagocytic parameters, i.e. the phagocytic number and the index of phagocytic activity, which was followed by an increase in their values between post treatment hours 2 and 4.

The leukocyte counts had decreased by hour 2 after the injection, but thereafter increased, and at post treatment hour 72, a leukocytosis was observed. The differential white blood cell counts were characterized by a shift to the left between hours 2 and 4 and a statistically significant increase in lymphocyte counts at hour 48 of the experiment.

The serum zinc concentrations were increased an hour after the lipopolysaccharide application; after which their average values were lower.

The haemolytic activities (CH_{50}) of the classical and the alternative pathways of complement activation decreased. The haemolytic activity (CH_{50}) for the classical pathway began to increase at hour 48 following the treatment.

Significant changes were not observed in lysozyme activity, serum iron concentrations or the related haematological parameters (erythrocytes and haemoglobin).

Keywords: Complement, *Escherichia coli*, lipopolysaccharide, phagocytosis, pigs, microelements

INTRODUCTION

Bacterial infection and septicaemia, in whose aetiology and development *Escherichia coli* one of several Gram-negative bacteria involved, are both a frequent cause of morbidity and mortality in domestic animals (Moore & Morris 1992; Olson, Kruse-Elliott & Dodam 1992; Olson, Dodam & Kruse-Elliott 1992; Cullor 1992). The principal factor responsible for the systemic effects of Gram-negative bacteria is lipopolysaccharide (LPS), an endotoxin. After gaining access to the circulation, the endotoxin exerts a variety of adverse effects including cardiovascular compromise, lactic acidosis and gastrointestinal disturbances. It can trigger a cascade of non-specific protective defence reactions to disease such as fever, inflammation, somnolence, lethargy, anorexia, vomiting, diarrhoea and decreased general activity. Its

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isolation in pure form has permitted the determination of new aspects of its pathogenicity (Green & Adams 1992; Abraham, Morrishardeman, Swenson, Knoppel, Ramanathah, Wright, Grieger & Minton 1998), which has motivated the use of contemporary means for the treatment of coliform infections (Sironi, Pozzi, Polentarutti, Benigni, Coletta, Guglielmotti, Milanese, Chezzi, Vecchi, Pinsa & Mantovani 1996; Malaisse, Nadi, Ladriere & Zhang 1997; Van Miert, Vanduin & Wensing 1997; MacKay, Clark, Logdberg & Lake 1999).

Initially, the reaction of an animal infected with *E.coli* to the LPS action includes a complex of early defence mechanisms involving phagocytosis, complement and lysozyme with the object of controlling the infection until an immune response can be mobilized.

Phagocytosis by white blood cells (monocytes and neutrophils) and tissue macrophages is one of the dominant defence mechanisms and there is no doubt that LPS suppresses it (Wonderling, Ghaffar & Mayer 1996). On the other hand, Barbour, Wong, Rabah, Kapur & Carter (1998) provided evidence that mature macrophages from different cell lines could exhibit viable responses to LPS and highlighted the inadvisability of making generalizations regarding the effects of LPS on macrophages. This paradox is rendered more complicated by the fact that LPS influences in different degrees the various factors responsible for its efficacy, such as the cytokines (Hostoffer, Krukoverts & Berger 1994; Tam, Ferrante, Goh, Robertson & Cripps 1995; Laichalk, Danforth & Standiford 1996), hormones (DeBowes & Anderson, 1991; Feola, Collins & Czupzynski 1999), the expression of Fc receptors and adhesion molecules (Graham & Brown, 1991; Grunwald, Fax, Jack, Workalemahu, Kallies, Stelter & Schutt 1996), and the specific (immunoglobulin) and non-specific (complement) opsonins.

The fact that the functional capacity of circulating neutrophils is affected by zinc (Jensen-Waern, Melin, Lindberg, Johannisson & Wallgren 1998) and that its direct interaction with monocytes results in release of interleukin -1 (IL-1), IL- 6, and tumour necrosis factor (TNF-a), gives sufficient reason to consider zinc as a microelement with an immense immunoregulatory capacity (Bodey, Siegeil & Kaiser 1998). Changes in trace element metabolism may therefore result in effective, though non-specific, defence mechanisms.

An integral component of such defence mechanisms is complement. Its haemolytic activity decreases significantly after LPS challenge (Uchiba, Okajima, Murakami, Okabe & Takatsuki 1997). According to some authors (Kushner & Mackiewicz 1986), the levels of native complement proteins decrease in patients with sepsis, but the concentrations of complement activation fragments, such as C_{3a}, C_{4a} and C_{5a} increase (McCabe, Treadwell & DeMaria 1983).

During endotoxin-induced inflammation, leukocytes appeared to be the source of lysozyme (Persson, Carlsson, Hambleton & Guidry 1992). An analysis of some factors with lysozyme-like activity in blood sera was performed by Zyczko & Zyczko (1998). Lysozyme expresses its activity primarily against Gram-positive bacteria (Bucharin, Suhih, Suleimanov & Frolov 1979), but other studies (Carroll 1979a,1979b) have shown that it is also involved in the lysis of coliform bacteria.

Bibliographical data provide substantial evidence that pathological overaction of the immunoregulatory and inflammatory defence systems mediates the key elements involved in the host response to LPS.

The aim of the present experiment therefore was to study the changes in innate immunity—phagocytosis, complement, lysozyme and serum zinc and iron concentrations, induced in pigs after the intravenous administration of *E.coli* LPS.

MATERIALS AND METHODS

Animals and experimental design

The animals used in the experiments were six 2- to 3-months-old female Landrace x Large White crossbred piglets, weighing 28 ± 3 kg. The experimental group was formed two weeks prior to the study and was housed under standard conditions at an environmental temperature of 25 °C and a humidity of 60 %.

The jugular vein of each piglet was canulated with a heparinized polyuretane cathether [(Vygoflon-T-Vicon) Germany].

Only female piglets were selected to exclude the potential influence of sex hormones on the parameters that were to be studied. Possible effects of circadial rhythm were eliminated by taking the required blood samples at 8:00 before the animals were fed.

The piglets were inoculated intravenously with LPS [(*Escherichia coli* 0111:B4-phenol extract purified) Sigma] in sterile 0.85 % saline solution, at the dosage rate of 10 mg/kg body weight.

Collection of blood

Blood samples were collected immediately before (0) and at hours 1, 2, 4, 24, 48 and 72 after administration of the LPS. Part of each sample, comprising 1 mℓ to be used for the phagocytosis assays were heparinized (30 IU), stored in ice and used within an hour of collection.

Serum production

The remainder of each blood sample was incubated immediately after collection for 1 h at 37 °C after

which it was centrifuged (1000 x *g*, 10 min, 20 °C) and the serum supernatant harvested.

The sera to be used for the complement, lysozyme, zinc and iron assays were stored at –20 °C and the analyses were performed within 3 days of the collection.

Determination of haematological parameters

Total leukocyte and erythrocyte counts were determined in a Bürker chamber [(Transmedimpex) Vienna, Austria]. Differential white blood cell (WBC) counts were performed on blood smears stained with May Grunwald and Giemsa solutions by counting 200 leukocytes. Haemoglobin (Hb) content was determined with the use of an acid-base analyzer [(ABL-3, Radiometer) Denmark] after correction to core body temperature. Haematocrit (Ht) values were determined by the microhaematocrit method (Betke & Savelsberg 1950).

Phagocytic assay

The phagocytic number (PHN) and the index of phagocytic activity (IPH) were determined according to the method of Samnaliev, Mladenov, Drashkova, Samnalieva, Padeshky & Radinov (1995), using fluorescein isothiocyanate (FITC), (Sigma) labeled *E. coli* bacteria and whole blood.

A summary of the method used is as follows:

- Mononuclear leukocytes were isolated using a modification of the procedure of Birmingham & Jeska (1980). Briefty, whole blood samples were centrifuged at 1000 x *g* for 10 min at 20 °C after which the plasma and buffy coat were discarded. The remaining packed red cells were twice hypotonically lysed and isotonicity subsequently restored with 5 mℓ of phosphate-buffered saline solution (PBS). The suspension was centrifuged (150 x *g*, 10 min, 10 °C). Mononuclear cells were then washed twice with PBS. A viability test was performed by using 0.5 % trypan blue [(Merck) München, Germany] exclusion technique and cell suspensions were adjusted to 3 x 10^6 cells/mℓ.
- Fluorescent labeling of bacteria. The bacterial strain *E. coli* 0111:B4 was prepared by culturing it until the logarithmic growth phase was reached. The culture was washed once in saline, resuspended in PBS and adjusted to a final concentration of 10^9 colony-forming units/ml. A volume of 1 mℓ of the bacterial suspension and 0.06 % FITC solution were mixed by rotation (100 rpm) for 60 min at room temperature, protected from light. After staining, FITC-labeled bacteria were washed twice in PBS and the concentration of live bacteria was adjusted to 3 x 10^7/mℓ.
- Phagocytosis assays using fluorescence microscopic evaluation were performed. Fifty microlitres of FITC-labeled bacterial suspension (3 x 10^7/mℓ) were preincubated (10 min) by 50 ml autologous serum, obtained at the same day. The incubation was necessitated because of the possible influence of serum elements on phagocytosis (Arnaout, Todd, Dana, Melamed, Schlossman & Colten 1983; Fallman, Andersson & Andersson 1993). Serum was added to opsonize bacteria. A control tube was prepared with PBS as a substitute for the serum. Phagocytosis was initiated by adding 100 µl of leukocyte suspension (3 x 10^6 cells/mℓ). During the incubation period (20 min) the assays were moved gently in a shaker at a temperature of 37 °C, and were then placed on ice to inhibit further phagocytosis. The cells were washed three times with ice cold PBS. The phagocytosis assays were stained with ethidium bromide [(EB - Sigma) 0.5 µg/mℓ working solution]. Quenching solution—trypan blue [(Merck), München, Germany]—was used to extinguish any extracellular fluorescence (Trowald-Widh & Thoren-Tolling 1990).

The IPH was expressed from 150 mononuclear cells as the percentage of cells able to phagocytize more than three bacteria. The PHN was calculated as the ratio of the number of phagocytized bacteria and the number of phagocytizing cells.

Determination of the alternative and classical pathways of complement

The alternative pathway of complement activation (APCA) was determined by the method of Sotirov (1986). A veronal-veronal Na buffer (85 g NaCl; 3.75 g sodium 5.5-diethylbarbiturate) (Loba Chemie, Austria); 5.75 g 5.5-diethylbarbituric acid (Reanal, Hungary); 0.01M EDTA (Sigma, USA); 0.008 M $MgCl_2$ (Polskie Odczynniki Chemiczne, Poland) were used. All these chemicals were diluted in 2 ℓ distilled water at pH = 7.5. The buffer was diluted to 1:5 before use. Then, 100 µℓ from each serum sample were mixed with 300 mℓ buffer. From these mixtures, seven dilutions were made using "U"-bottomed microplates (Flow Laboratories, UK). To each dilution, 100 µℓ of 1 % rabbit erythrocyte suspension was added and then incubated at 37 °C for 1 h. The optical density was measured by a "Sumal-PE2" ELISA reader (Carl Zeiss, Germany) at 540 nm.

The classical pathway of complement (CPCA) was determined by a modification of the method of Stelzner & Stein (1971). First, baseline serum dilutions were prepared from 170 µℓ buffer and 30 µℓ serum. The wells of "U"-bottomed microplates were filled with 50 µℓ buffer. In each well of the first row of wells, 50 µℓ diluted sera were placed using a 12 channel pipette (Labsystem, Finland). One hundred microlitres of baseline dilutions were used to obtain further dilutions from the second to the fifth row of wells. In each well, 100 µℓ buffer and an equal amount of 1 % sheep erythrocyte suspension were added by an

automated device AD96 belonging to the Sumal system (Carl Zeiss, GDR). The plates were then incubated at 37 °C for 1 h. Afterwards they were centrifugated at 2 000 x *g* for 3 min and 150 mℓ of the supernatant was put into flat-bottomed microplates. They were read at 540 nm using a Sumal PE spectrophotometer (Carl Zeiss, GDR) and printed. Afterwards, the activity of the classical pathway of complement was calculated by the method of Stelzner & Stein (1971).

Determination of lysozyme activity (LA)

Lysozyme levels were determined by the method of Lie (1985). Briefly, 20 mℓ of 2 % agarose (ICN, UK, Lot 2050) dissolved in phosphate buffer (0.07 M Na_2HPO_4 and NaH_2PO_4, pH = 6.2) was mixed with a 20 mℓ suspension of a 24 h culture of *Micrococcus lysodecticus* at 67 °C. This mixture was poured into a Petri dish (140 mm diameter). After solidifying at room temperature, 32 wells were made (5 mm diameter) in it. Fifty microlitres of undiluted sera were poured into each well. Eight standard dilutions (from 0.025–3.125 μℓ/mℓ) of lysozyme (Veterinary Research Institute, Veliko Tirnovo) each comprising 50 mℓ in volume, were added to each well. The samples were incubated for 20 h at 37 °C and lytic diameters were measured.

Determination of serum zinc and iron concentrations

Serum Fe and Zn levels were measured using an atomic absorption spectrophotometer AS-1N [(Carl Zeiss Jena) Germany].

Statistical analysis

Comparisons of average values were performed by the Student's *t*-test.

RESULTS

Effect of LPS on the factors of innate immunity

Phagocytic ability of blood leukocytes

The results of the measurements of the phagocytic ability of leukocytes using fluorescein-labeled *E. coli* bacteria are presented in Fig. 1.

As early as 1 h after the LPS challenge, the mean percentage of actively phagocytic cells (IPH) decreased gradually from 36 ± 1.04 % to 25 ± 0.67 % ($P < 0.001$). PHN decreased insignificantly from 1.15 ± 0.08 to 1.13 ± 0.07. Between hours 2 and 4 an interesting tendency in the behaviour of phagocytic parameters was observed. It resulted in significantly higher values of the phagocytic number (2.07 ± 0.06 and 2.00 ± 0.06, respectively) compared to baseline

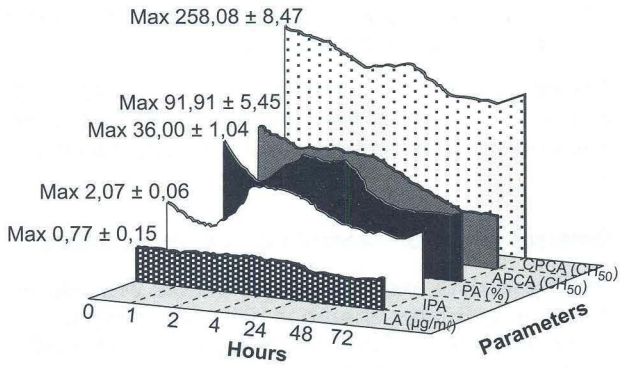

FIG. 1 Kinetics of changes in parameters of innate immunity after intravenous LPS inoculation

values ($P < 0.001$). On the contrary, IPH, although increasing, did not reach the initial level (34 ± 1.08 % at hour 2). Afterwards, the reduction was progressive and the most expressed at hour 48 when IPH reached its lowest value (24 ± 0.28 %). This decrease is statistically significant compared to the baseline level ($P < 0.001$) as well as to hours 2 and 4 ($P < 0.001$). A similar dynamic of changes was observed for PHN, whose value at hour 48 was 1.33 ± 0.03. This is insignificantly higher than the initial value and statistically significant compared to hours 2 and 4 ($P < 0.001$).

CPCA and APCA activities (CH_{50})

The intravenous administration of LPS in piglets induced parallel changes in the activity of both the classical and the alternative pathways of complement activity, manifested by a decrease in haemolytic activities (CH_{50}) as early as the first post treatment hour (Fig. 1). After slight deviations in the interval between hours 2–4, the CPCA and ACPA reached their lowest values at hour 48—206.47 ± 7.82 ($P < 0.01$ vs baseline) and 77.65 ± 6.38, respectively.

Lysozyme activity (LA)

The changes in blood serum LA after intravenous administration of LPS to the piglets were insignificant and were manifested by decrease at hour 48 (0.50 ± 0.06 μg/mℓ vs baseline value of 0.77 ± 0.15 μg/mℓ), as indicated in Fig. 1.

Effects of LPS on haematological parameters

The analysis of the WBC counts before (hour 0) and after the LPS administration revealed prominent changes (Table 1). The leukocyte counts were significantly reduced at hour 2 of the study but afterwards they began to increase gradually and at hour 72, a leukocytosis was present. Initially the percentage of monocytes and eosinophils decreased significantly—the minimum in monocyte counts was at

TABLE 1 The effects of LPS (10 μg/kg body weight) on some haematological parameters in the pigs ($n = 6$)

(a) Significantly different from baseline values: a ($P < 0.05$); a_1 ($P < 0.01$); a_2 ($P < 0.001$)
(b) Significantly different from values at 2 h: b ($P < 0.05$); b_1 ($P < 0.01$); b_2 ($P < 0.001$)
(c) Significantly different from values at 4 h: c ($P < 0.05$); c_1 ($P < 0.01$); c_2 ($P < 0.001$)

Parameter	0 h	1 h	2 h	4 h	24 h	48 h	72 h
WBC (x $10^9/\ell$)	19.15 (0.47)	14.17 (2.09)	11.40 (1.14)	12.08 (1.23)	15.42 (1.50)	18.10 (2.20)	22.90 (2.09)$^{b1.c2}$
Lymphocytes (%)	49.17 (6.62)	56.50 (10.13)	27.50 (6.67)	21.66 (2.89)	51.33 (4.84)	56.83 (5.19)^{c}	47.00 (9.32)
Monocytes (%)	2.17 (1.25)	0.33 (0.23)	1.50 (0.55)	3.50 (1.16)	1.17 (0.43)	3.00 (1.05)	2.83 (0.82)
Neutrophils banded (%)	2.33 (0.36)	11.17 (3.34)	16.67 (1.74)^{a2}	20.33 (2.34)^{a2}	6.50 (1.67)$^{b.c1}$	4.83 (1.37)$^{b1.c2}$	6.17 (2.10)$^{b.c2}$
Neutrophils segmented (%)	43.17 (5.96)	28.16 (8.20)	53.33 (6.52)	53.83 (3.22)	33.00 (3.98)	33.17 (5.06)	40.0 (6.90)
Eosinophils (%)	3.17 (1.15)	3.17 (0.87)	0.83 (0.44)	0.67 (0.36)	7.33 (1.04)$^{b1.c1}$	2.17 (0.52)	2.83 (1.64)
Erythrocytes (x $10^{12}/\ell$)	6.33 (0.27)	6.37 (0.20)	6.44 (0.26)	6.31 (0.18)	5.78 (0.36)	5.68 (0.21)	5.92 (0.11)
Haemoglobin (g/ℓ)	124.30 (6.50)	121.70 (3.30)	126.30 (7.90)	119.30 (3.20)	108.30 (6.20)	106.70 (5.10)	108.00 (2.90)
Haematocrit (ℓ/ℓ)	0.35 (0.013)	0.33 (0.007)	0.33 (0.013)	0.33 (0.007)	0.31 (0.008)^{a}	0.29 (0.004)^{a1}	0.31 (0.004)^{a}

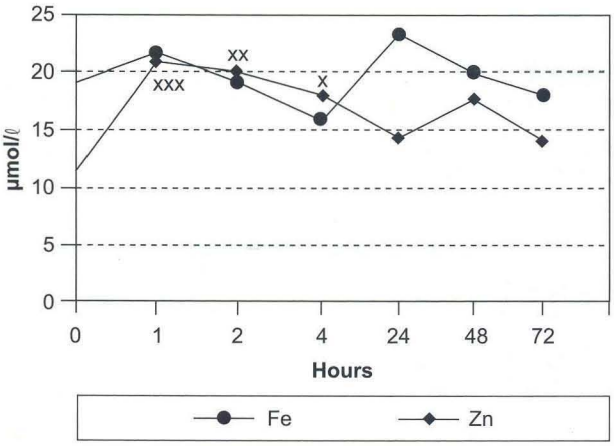

FIG. 2 Changes in serum Zn and Fe concentrations after intravenous LPS application (10 mg/kg body weight) in piglets. Statistically significant differences are indicated by:

xxx $P < 0.001$
xx $P < 0.01$
x $P < 0.05$

hour 1 and while those for eosinophils was at hour 4. The band neutrophils increased statistically significantly between hours 2 and 4 ($P < 0.001$), which provides evidence that a neutrophilia and a left shift had occurred. The lymphocytes reacted by decreasing in numbers between hours 2 and 4, and significantly increasing at hour 48 ($P < 0.05$).

The intravenous administration the *E. coli* LPS did not provoke significant changes in erythrocyte counts or their haemoglobin content. The Ht was statistically decreased ($P < 0.05$) by post treatment hour 24—a tendency which remained until the end of the experiment (Table 1).

Serum Zn and Fe concentrations

In the first hours of the experiments (Fig. 2), serum Zn and Fe levels were increased at hour 1 (insignificantly for iron and statistically for Zn—$P < 0.001$) but thereafter there was a tendency for them to decrease up to hour 24 for zinc and hour 4 for iron. This was followed by an increase in iron levels (up to 23.28 ± 1.64 µmol/ℓ) and an increase in zinc levels (up to 17.77 ± 2.00 µmol/ℓ) in the latter hours (24–48) which, however, was transient and was followed again by a reduction in average values.

DISCUSSION

The difficulties in the therapy of diseases in which LPS is involved are a direct reflection of the complex participation of multiple tissue and organ systems (Johnson, Aarden, Choi, Groot & Creasey 1996; Petrowski, Sloane, Spath, Elsasser, Fisher & Gee 1996) as well as the complex cellular pathogenic mechanisms, which are triggered in the patient. Some of the responses to LPS are considered to be induced by cytokines (Kayama, Yoshida, Kodama, Matsui, Matheson & Luster 1997; Steege, Van de Ven, Forget, Brouckaert & Buurman 1998; Luttmann, Herzog, Matthys, Thierauch, Virohow & Kroegel 1999). The attempts to achieve a good therapeutic result by obstructing them are not always successful because of the complex interrelationships among endogenic mediators, antagonism of cellular and tissue effects, the mechanisms for regulation as well as the difficulties for the dosage of the means for correction (Andonova 1997).

Without the delineation of the principal non-specific defence systems and of the LPS-induced pathophysiological changes, specific treatment cannot be effective. The problem is further complicated by the possible influences of the extent of the LPS dose received by the patient (Nakajima, Momotani, Takahashi, Ishikawa, Ito, Kanesaki & Madarame 1995), the route of its acquisition and the animal species involved (Garcia, Hakt, Magnusson & Kindahl 1998).

Therefore, in our experiment we used an *E. coli* LPS at the dosage rate of 10 mg/kg body weight administered intravenously, therefore excluding the influence of local defence mechanisms (Heemken, Gandawidjaja & Hau 1997). This rate is lower than that of 20 mg/kg body weight used by Myers, Farrell, Evock-Clover, McDonald & Steele (1997) in their study on swine metabolism and inflammatory cytokine production, and is higher than that used by Garcia *et al.* (1998). It is difficult to establish a true threshold dosage rate at which endotoxins will cause clinical effects, but our aim was to induce the clinical signs of infection (fever, anorexia and decreased general activity) on the one hand, and to provoke defence and compensatory systemic mechanisms on the other.

Recognition of endotoxin by the cell is a prerequisite for biological activity. LPS affects a large number of cell types including mononuclear phagocytes, neutrophils, vascular endothelial cells and platelets as primary targets (Opdenakker, Fibbe & Van Damme 1998; Tsuchiya, Kyotani, Scott, Nishizono, Ashida, Mochizuki, Kitao, Yamada & Kobayashi 1999). Currently, however, phagocytes are considered the most critical cells in the host response to endotoxin and in the recruitment of components of the immune and inflammatory responses.

Phagocytes possess lectin-like receptors and can phagocytose Gram-negative bacteria by a CD-14 dependent mechanism (Grunwald *et al.* 1996).

Our studies upon the manifestations of one of the earliest mechanisms of defence—the phagocytosis in pigs after intravenous administration of the *E. coli* LPS—showed that as early as the first post treatment hour, the percentage of actively phagocytic cells had decreased but changes in PHN were insignificant

during this period. This is further supported by data reported by Wonderling *et al.* (1996) whose experimental model was, however, different from ours.

Our evaluation of phagocytosis was based on the use of fluorescein-labeled *E. coli* bacteria and whole blood. The main advantages of this method were that only a small blood sample is necessary and no cell separation was needed. Although several flow cytometric methods of quantifying phagocytosis have been described for human polymorphonuclear cells, only a few reports deal with the quantitative assessment of phagocytosis by swine phagocytes (Busque, Higgins, Senechal, Marchand & Quessy 1998; Magnusson & Greko, 1998). Furthermore, these authors utilized FITC-labeled beads, bacteria, yeast and zymosan particles.

At the background of the reduction in leukocyte counts, outlined at the second hour of the study (Table 1), the phagocytic parameters IPH and PHB increased in the period between post treatment hours 2 and 4. The differential WBC count was characterized by a shift to the left which was probably due to the activation of defence mechanisms, in which neutrophils play a dominant role because of their high phagocytic activity.

These processes are also related to the endotoxin-stimulated release of soluble mediators, such as IL-1, TNF-a, prostaglandins, interferons and platelet-activating factor (PAF) (Klosterhalfer, Horstmann-Jundemann & Vogel 1992) but they were not determined in our studies. Some aspects of leukocyte trafficking are relatively well dissected at the molecular level: the identification of selectins, integrins, chemokines and proteases (Opdenakker *et al.* 1998).

The serum zinc concentrations showed statistically significant changes during the experimental period (Fig. 2). As early as the first post treatment hour, the levels of this microelement were increased. This can be interpreted as a sign for its involvement in the non-specific defence system of the animal.

It is stated (Jensen-Waern, Johannisson, Ederoth & Trowald-Wigh 1994) that the functional capacity of circulating neutrophils is affected by zinc—its deficiency impair the oxygen burst (Cook-Mills & Fraker 1993). Moreover, zinc is supposed to induce the release of some cytokines such as IL-1, IL-6 from monocytes (Bodey *et al.* 1998). Zinc is a component of several enzyme systems (Riordan 1976). Kolb & Kolb-Bachofen (1998) reported that nitric oxide, which level increased after LPS administration, disrupts zinc-finger configurations by releasing zinc from thiol groups. This leads to the reversible inactivation of zinc finger-containing transcription factors and intranuclear zinc release. Immediately after this increase, serum zinc concentrations decreased. Similar data are reported by Garcia *et al.* (1998).

Despite the significant number of publications on the involvement of iron in antibacterial activity (Reiter & Perrandin 1998; Zagulski, Jarzabek, Zagulska & Zimecki 1998), our results showed no significant changes in its serum concentrations after the LPS administration (Fig. 2). It could be supposed that this was due to a dose-dependent effect of LPS upon plasma iron concentrations. Lohuis, Verheijden, Burvenich & Van Miert (1988) consider that cytokines could be a possible regulator of iron levels, while Stangl & Kirchgessner (1998) emphasize the possibility of interrelationship of iron levels and thyroid hormones in blood. The related haematological parameters, haemoglobin and erythrocytes, were not changed (Table 1).

The changes in lysozyme activity were insignificant although lysozyme activity was decreased at hour 48 but this is not statistically significant (Fig. 1). On the basis of the studies of Sotirov (1990) who determined contradictions in lysozyme activity in pigs due to age and sex, we used only female piglets aged 2–3 months. The studies of Goranov (1978) on the muramidase (lysozyme) activity of blood cells of some domestic and laboratory animals showed that cells from the peripheral blood of cattle, sheep, pigs and buffaloes do not manifest a muramidase activity.

An interesting fact was the involvement of complement as a factor in the early host defences to LPS. The decreased activity of both classical and alternative pathways, especially at hour 48 after the LPS administration (Fig. 1) could be interpreted as an evidence for the depletion of adaptive systemic reactions.

The parallelism of changes in the activities of classical and alternative pathways showed that endotoxin fixed complement via both routes. The increase in the haemolytic activity of CPCA after that period was probably related to its participation in the triggered immune reactions related to the realization of the adaptive immune response.

In conclusion, the present data indicate that the intravenous application of LPS of *E. coli* 0111:B4 at a dosage rate of 10 mg/kg body weight to female piglets caused statistically significant changes in the phagocytic parameters (IPH, PHN), white blood cell picture, serum zinc concentrations, haemolytic activities of classic and alternative complement pathways, which all indicate a depletion of the early adaptive mechanisms of defence. There were no significant changes in erythrocyte counts and levels of haemoglobin, iron and lysozyme activity.

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