The Journal of Microbiology (2012) Vol. 50, No. 3, pp. 401–408
DOI 10.1007/s12275-012-1621-8

Tularemia Progression Accompanied with Oxidative Stress and Antioxidant Alteration in Spleen and Liver of BALB/c Mice

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(Received December 22, 2011 / Accepted January 17, 2012)

Francisella tularensis **is the causative agent of tularemia. It is an intracellular pathogen with the ability to survive within phagosomes and induce pyroptotic cell death. In this study, we attempted to prove whether oxidative imbalance plays a significant role in tularemia pathogenesis. In our experimental model, we subcutaneously infected female BALB/c mice (dose 10^5 CFU of *F. tularensis* LVS). Liver, spleen, and blood were collected from mice at regular intervals from days 1–15 after infection. The bacterial burden was assessed by a cultivation test. The burden was unchanging from the 2nd to 6th day after infection. The bacterial burden corresponded to the plasmatic level of IFN-γ, IL-6, and liver malondialdehyde. After the phase of acute bacteraemia and the innate immunity reaction, the levels of reduced glutathione and total low molecular weight antioxidants decreased significantly and the activity of caspase-3 increased in the liver. The level of reduced glutathione decreased to 25% of the original level, and the total level of low molecular weight antioxidants was less than 50% of the initial amount. The demonstrated effects of tularemia-induced pathology had a more extensive impact on the liver than on the spleen.**

Keywords: *Francisella tularensis*, tularaemia, oxidative stress, inflammation, antioxidant, glutathione

Introduction

Tularemia is a zoonotic infectious disease caused by the Gram-negative bacterium *Francisella tularensis*. The disease is present in the northern hemisphere, where is it is transmitted by ticks and mosquitoes into reservoir hosts such as rabbits, hares, and muskrats (Nigrovic and Wingerter, 2008). In addition to wild mammals, arthropods can infect humans and livestock. The incidence of tularemia is reported to be approximately 1 case per 100,000 inhabitants in developed countries with the most frequent occurrence in regions with dry weather conditions and large forest areas suitable for ticks and rodents (Eisen *et al*., 2008). Recent outbreaks of tularemia have occurred in European countries including Norway (Larssen *et al*., 2011) and Bulgaria (Christova *et al*., 2004). Moreover, *F. tularensis* is considered a potential agent for biological warfare and is classified by the United States Centers for Disease Control and Prevention as a List A, Select Agent (Foley and Nieto, 2010).

After invading the host, *F. tularensis* is phagocytized by macrophages. The bacterium is able to survive the respiratory burst in phagosomes, become activated and even trigger proliferation (McCaffrey and Allen, 2006). In addition to its ability to survive in the phagosome, the pathogen is able to escape into the cytosol and induce pyroptotic death of the infected cells (Henry and Monack, 2007; Akimana *et al*., 2010). Caspase-1 activation, lysosome exocytosis and Ca^{2+} accumulation within cells are the most common attributes of pyroptotic processes (Orrenius *et al*., 2011). Unlike apoptosis, pyroptosis is a burden for the adjacent tissues as degradation products are released and these stimulate secretion of interleukin (IL)-1β (Bergsbaken *et al*., 2011).

Acute detrimental consequences in the course of tularemia can be recognized in the lymph nodes, lungs, brain, and bone marrow. The lymph nodes, spleen and liver, where necrotic lesions are most severe, are the most susceptible (Park *et al*., 2009). In our previous research, we reported significant alterations in biochemical responses and oxidative stress in a European brown hare infected with *F. tularensis* subsp. *holarctica* (Bandouchova *et al*., 2011). The assessed markers fluctuated in patterns that corresponded with symptomatic manifestation of tularemia. Unfortunately, only blood and plasma were sampled, and no measurement of oxidative stress in organs was carried out. Therefore, we could not come to a clear conclusion about the effects of tularemia. The production of endogenous, low molecular weight antioxidants seems to be necessary for the reduction of detrimental tularemia-induced processes (Pohanka *et al*., 2009). The work reported here was aimed at a comprehensive investigation of oxidative stress markers in the liver and spleen of tularemia-infected mice. In addition to estimating oxidative stress, the experiment was aimed at assessing the protective mechanisms represented by the production of low and high molecular weight antioxidants

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and their efficacy. To assess the protective mechanisms and to determine the level of oxidative stress, a battery of spectroscopic, electrochemical and immunological methods were used, and some correlations were made to uncover interactions between affected biochemical pathways. A broader understanding of the pathological processes could lead to a supplementary treatment of tularemia and amelioration of the pathological consequences of the disease.

Materials and Methods

Microorganism and experiment design

McLeod agar supplemented with bovine haemoglobin and Iso VitaleX (Becton-Dickinson, USA) was used for cultivation of *F. tularensis* LVS (collection code ATCC 29684). A temperature of 37°C and a moist atmosphere were used throughout the experiment. Cells were harvested after two days. Then they were suspended in saline solution and spun at 2,000×g for 10 min. The cell concentrations in saline solution were determined by a cultivation test using the above-mentioned agar and cultivation conditions.

In total, 96 female BALB/c mice (Velaz, Czech Republic) were used in the experiment. The mice weighed 24±2 g, and they were eight weeks old when the experiment began. Mice were kept in an air-conditioned room (22±2°C) with a humidity of 50±10% and a light period from 7 a.m. to 7 p.m. Full access to feed and water was provided for the whole experiment. Animals were infected with a dose of 10^5 CFU. One hundred microliter of a solution containing 10^6 CFU/ml was administered subcutaneously into the skin folds of the neck; controls (eight animals) were injected with saline only and sacrificed with CO_2 anaesthesia after 12 h. The infected animals were sacrificed on days 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, and 15 after infection. Eight animals were sacrificed at each interval. Blood was collected into heparinised tubes by puncturing the heart (BD Diagnostics, UK), and the plasma was taken after centrifugation at 3,000×g for 15 min. The livers and spleens of two animals from each interval were filtered through a strainer, and the bacterial burden was estimated by a cultivation test. The organs from the remaining six animals were homogenized using an Ultra-Turrax mill (Ika Werke, Germany). A total of 100 mg of freshly collected tissue was mixed with 1 ml of saline solution for 1 min.

Ethics statement

Animal use was supervised and permitted by the institutional ethical committee (Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic). The ethical committee approved the study (permission No. 6/10 in year 2011).

Ex vivo assay

Glutathione reductase (GR) and thiobarbituric acid reactive substances (TBARS) were assessed in compliance with previously optimized methods (Pohanka *et al.*, 2010, 2011). Plasmatic levels of IL-6 and IFN-γ were assessed using a "Murine IL-6 Eli-pair kit" and a "Mouse IFN-γ Eli-pair kit" (Abcam, USA) according to the manufacturer's instructions. A multichannel spectrophotometer Sunrise (Tecan, Austria) was used for measuring purposes. Tissue caspase-3 activity and total protein by the Bradford method were assayed with caspase-3 and total protein kits (Sigma-Aldrich, USA).

Metallothionein determination

The tissues were mixed with extraction buffer (100 mM potassium phosphate, pH 6.8) and subsequently homogenized using a Schuett homogen-plus, semiautomatic, homogenizer (Crea Laboratory Technologies Pty Ltd, Australia). The tissue homogenates were centrifuged at 10,000×g for 15 min at 4°C (Eppendorf 5402, USA). Then the samples were heat treated at 99°C in a thermomixer (Eppendorf Thermomixer Comfort, USA) for 15 min with occasional stirring, and then cooled to 4°C. The denatured homogenates were centrifuged at 4°C, 15,000×g for 30 min (Eppendorf 5402). Heat treatment effectively denatures and removes high molecular weight proteins from samples. The obtained supernatants were diluted 100× with extraction buffer (100 mM potassium phosphate, pH 6.8) prior to electrochemical measurements.

Electrochemical measurements were performed with a 747 VA Stand instrument connected to 746 VA Trace Analyzer and 695 Autosampler (Metrohm, Switzerland), using a standard cell with three electrodes and a cooled sample holder (4°C). A hanging mercury drop electrode with a drop area of 0.4 mm^2 was the working electrode. An Ag/AgCl/3M KCl electrode was the reference, and a glassy carbon electrode was the auxiliary electrode. GPES 4.9 supplied by EcoChemie was employed. The Brdicka supporting electrolyte containing 1 mM $Co(NH_3)_6Cl_3$ and 1 M ammonia buffer [$NH_3(aq)$ + NH_4Cl, pH = 9.6] was used and changed for each analysis. DPV parameters were as follows: initial potential of -0.7 V, end potential of -1.75 V, modulation time 0.057 sec, time interval 0.2 sec, step potential 2 mV, modulation amplitude -250 mV, E_{ads}=0 V. All experiments were carried out at a temperature of 4°C (Julabo F12 cooler, Germany).

Glucose and antioxidant assays

The tissues were mixed with an extraction buffer (100 mM potassium phosphate, pH 6.8) and subsequently homogenized using a polo automatic homogenizer (Shutt homogen plus, Germany). The tissue homogenates were centrifuged at 10,000×g for 15 min at 4°C (Eppendorf 5402). Supernatants were diluted 10× with potassium buffer solution (100 mM, pH 6.8) prior to spectrometric measurements. Antioxidant capacity (DPPH) and glucose concentration were determined. Spectrometric measurements were carried out using an automated chemical analyzer BS-200 (Mindray, China). Reagents and samples were placed on a cooled sample holder (4°C) and automatically pipetted directly into plastic cuvettes. Incubation proceeded at 37°C. The mixture was subsequently stirred. The washing steps with distilled water (18 mΩ) were done in the midst of the pipetting. The instrument was operated using software BS-200 (Mindray).

Glucose: A solution containing 200 µl, 0.1 M phosphate

buffer pH 7.5, 0.75 mM phenol, 0.25 mM 4-amino-antipyrin (4-AAP), glucose oxidase ≥ 15k U/L, peroxidase ≥ 1.5 U/L, was pipetted into plastic cuvettes. Then each sample (20 µl) was added. The absorbance was determined for ten minutes at λ=505. The concentration of glucose was calculated using absorbance of the mixture without sample and absorbance measured after ten minutes incubation at 37°C.

Antioxidant assays (DPPH and ABTS): A volume of 200 µl of reagent (gallic acid, Trolox®) was incubated with 20 µl of measured sample. Previously optimized protocol was used for assay of antioxidants (Sochor *et al*., 2010).

Determination of reduced and oxidized glutathione: Homogenates, prepared as described in the Glucose and DPPH assays were analyzed by high performance liquid chromatography with electrochemical detection (HPLC-ED). The chromatographic instrument consisted of two solvent delivery pumps operating in the range of 0.001–9.999 ml/min (Model 582 ESA Inc., USA), a reaction coil (1 m)/Metachem Polaris C18A reverse-phase column (150.0×2.1 mm, 5 µm particle size; Varian Inc., USA), and a CoulArray electrochemical detector (Model 5600A, ESA). The sample (5 µl) was injected using an autosampler (Model 540 Microtiter HPLC, ESA). Experimental conditions were as follows: gradient profile starting at 100:0 (80 mM TFA:methanol) kept constant for 9 min, then decreased to 85:15 over 1 min, then kept constant for 8 min, and finally increased linearly up to 97:3 from 18 to 19 min, a mobile phase flow rate of 0.8 ml/min, and a column temperature of 40°C, working electrode potential 900 mV.

Statistics

Origin 8 SR2 (OriginLab Corporation, USA) was used for significance testing using a Bonferroni test. Both P=0.05 and P=0.01 probability levels were calculated considering group size 6 specimens. This program was also used for correlation of the results.

Results and Discussion

The tularemia-infected mice (n=96) survived until sacrifice; no death due to tularemia occurred, as a sub-lethal infection dose was chosen. Disease symptoms were observed in the infected subjects. The first symptoms (lethargy, erect fur) of tularemia were recorded two days after infection. The signs disappeared after one week. Tularemia can influence several organs. The organs containing macrophages and sensitive to tularemia are spleen, liver, lung, kidney, intestine, central nervous system, and skeletal muscles (Ellis *et al*., 2002). Due to simplification of the experiment, we have chosen spleen and liver as representative organs implicated in basal metabolic homeostasis. Microbiological analysis of the bacterial burden in the spleen and liver corresponded well with the signs of disease: a noticeable bacterial burden appeared simultaneously with the first symptoms. As we predicted, the highest bacterial burden was measured in the spleen between the third and fourth day of the experiment (more than 120,000 CFU). After that, the bacterial burden decreased sharply, as the adaptive immune

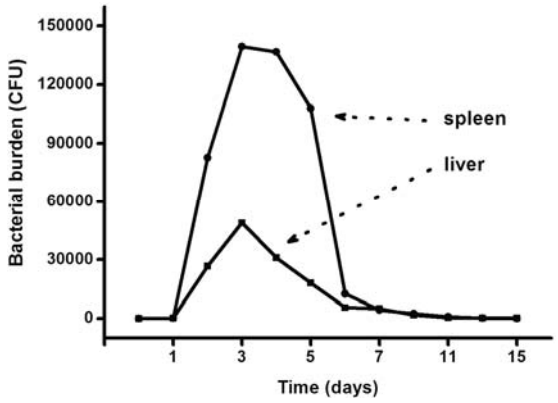

Fig. 1. Total bacterial burden (BB; count per one organ) in spleen and liver of tularemia-infected mice.

response conferred protection against the pathogen. A similar trend was observed in the liver; however, the highest bacterial burden observed in the liver was nearly four times

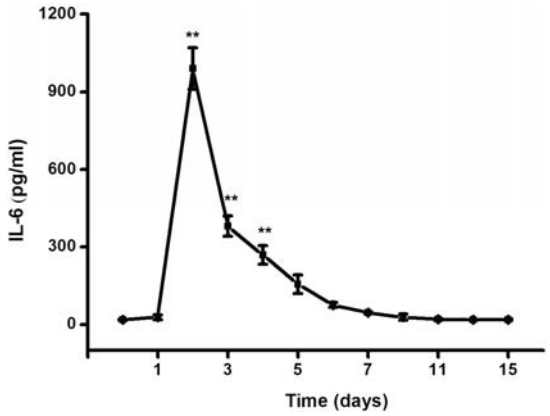

Fig. 2. Interleukin (IL)-6 level in plasma of tularemia-infected mice. Error bars indicate standard error of the mean for six specimens. Two asterisks represent the significance against the initial value at P<0.01.

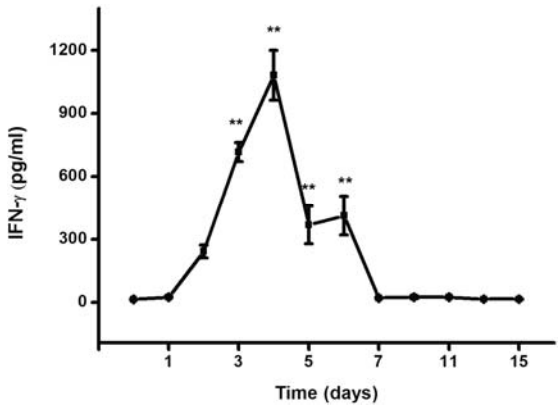

Fig. 3. Interferon (IFN)-γ level in plasma of mice suffering from tularemia. Error bars indicate standard error of the mean for six specimens. Two asterisks represent significance against the initial value at P<0.01.

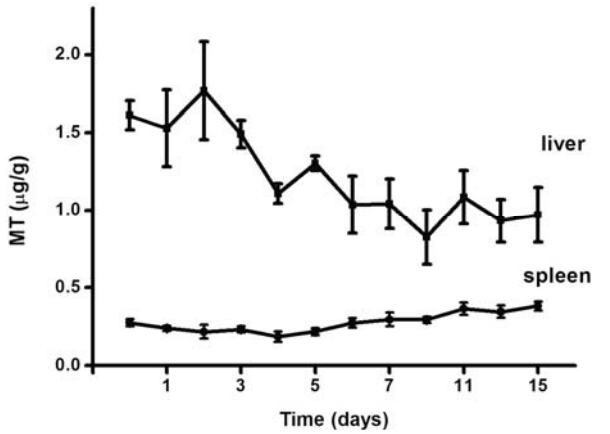

Fig. 4. Metallothionein (MT) level per g of protein in spleen and liver of tularemia-infected mice. Error bars indicate standard error of the mean for six specimens.

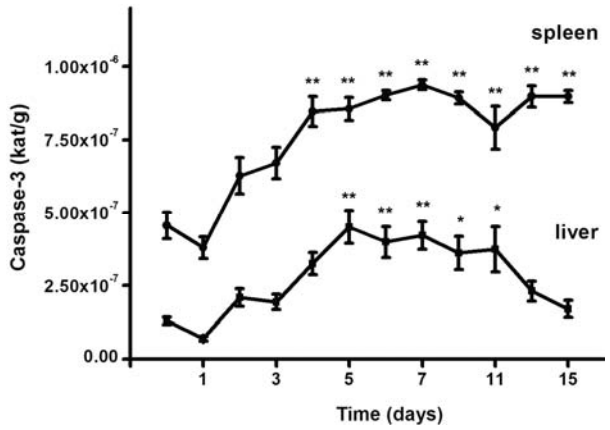

Fig. 5. Caspase-3 level per g of protein in the spleen and liver of tularemia-infected mice. Error bars indicate standard error of the mean for six specimens. Significance against the initial value is expressed by one ($0.01 \leq P < 0.05$) or two ($P < 0.01$) asterisks.

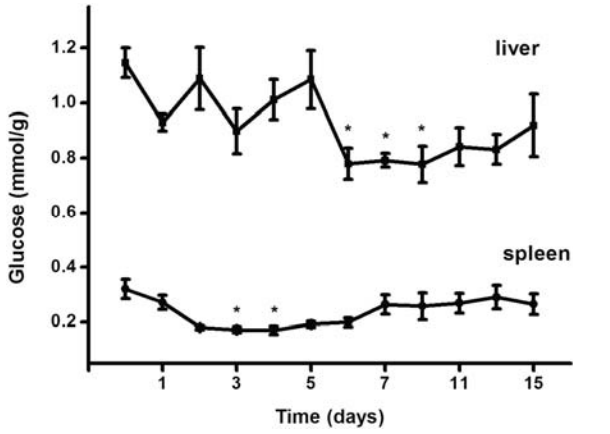

Fig. 6. Glucose level per g of protein in the spleen and liver of tularemia-infected mice. Error bars indicate standard error of the mean for six specimens. An asterisk represents significance against the initial value at $0.01 \leq P < 0.05$.

lower than the level in the spleen (Fig. 1).

Immune reaction

Inflammation, represented by the level of IL-6, intensified two days after the infection began (increased 100-fold) and reached a maximum on the ninth day. After that point, the level of IL-6 was similar to values measured in healthy animals (Fig. 2). Similarly, the level of interferon (IFN)-γ was elevated two days after infection and its increase culminated two days later than the level of IL-6 stopped increasing, reaching a maximum value higher that 100 pg/ml (Fig. 3). The IFN-γ level returned to the initial level within seven days. The enhanced immune response likely caused the sharp decrease in the bacterial burden; however, the enhanced immune response cannot be linked to significant alterations in oxidative stress or the antioxidant barrier, as discussed below.

Metallothionein, caspase-3, and glucose

The second group of assayed markers were metallothionein (MT), caspase-3 and glucose. The level of MT (Fig. 4) was not significantly altered in the examined organs (spleen and liver). However, the level of MT appeared to decrease in the liver, unlike the spleen, where its level remained steady or slightly increased. The level of MT decreased from 1.5 μg/g tissue (start of infection) to 1.0 μg/g tissue (the end of the experiment) in the liver. In the spleen, the differences were negligible. Caspase-3 is a marker of apoptosis, and its levels are shown in Fig. 5. In the spleen, caspase-3 activity was significantly ($P < 0.01$) elevated from day 4 through the end of the experiment. Caspase-3 activity in the liver was significantly elevated from days 5–11 after infection, ($P < 0.01$) for days 5–7, $0.01 \leq P < 0.05$ for days 9–11). Glucose was altered in the course of tularemia in both the spleen and the liver (Fig. 6). Significant decreases in glucose were observed in the liver from days 6–9 and in the spleen from days 3–4 ($0.01 \leq P < 0.05$).

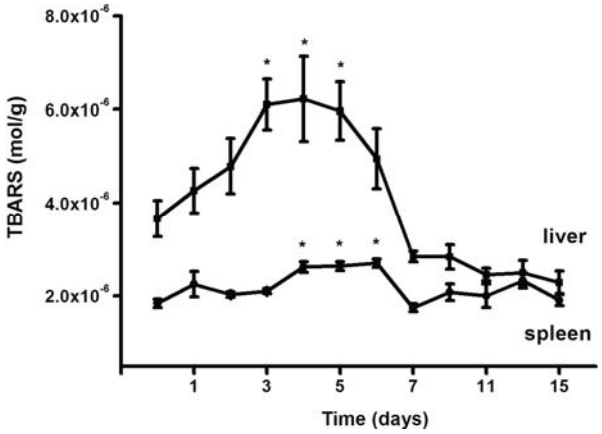

Fig. 7. Thiobarbituric acid reactive substances (TBARS) per g of protein in the spleen and liver of tularemia-infected mice. Error bars indicate standard error of the mean for six specimens. An asterisk represents significance against the initial value at $0.01 \leq P < 0.05$.

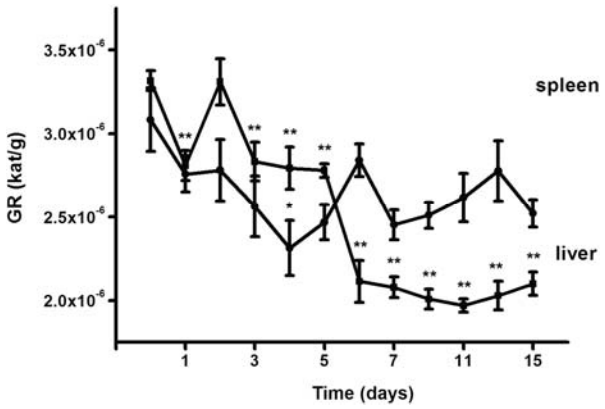

Fig. 8. Glutathione reductase (GR) activity per g of protein in the spleen and liver of tularemia-infected mice. Error bars indicate standard error of the mean for six specimens. Significance against the initial value is expressed by one (0.01≤P<0.05) or two (P<0.01) asterisks.

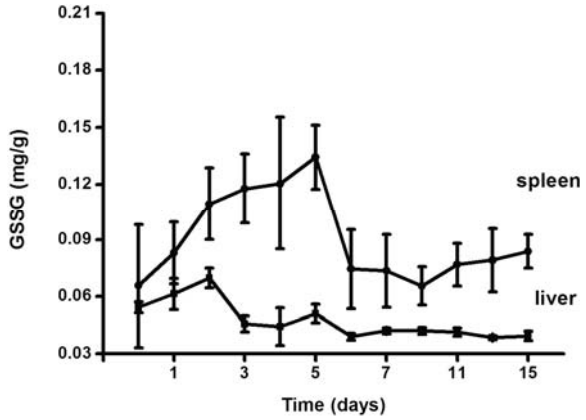

Fig. 10. Oxidized glutathione (GSSG) per g of protein in the spleen and liver of tularemia-infected mice. Error bars indicate standard error of the mean for six specimens.

Oxidative stress

Oxidative stress markers and antioxidant levels were investigated. Thiobarbituric acid reactive substances (TBARS; Fig. 7), glutathione reductase (GR; Fig. 8), reduced glutathione (GSH; Fig. 9), oxidized glutathione (GSSG; Fig. 10), the ratio of GSH to GSSG (Fig. 11), and the total level of antioxidants assayed by the 2,2-diphenyl-1-picrylhydrazyl test (DPPH; Fig. 12) are shown below. TBARS increased immediately after infection by *F. tularensis*. After reaching the maximum, which corresponds with the highest bacterial burden, TBARS decreased to the starting level. In the liver, the TBARS trend was similar; however, the values were several-fold lower. As confirmed by statistical evaluation, TBARS was significantly elevated in liver from days 3–5 and in the spleen from days 4–6 (0.01≤P<0.05). The level of GR remained stable in the spleen, with one exception on day 4 after infection. In contrast to the level in the spleen, the level of GR in the liver decreased from day 3 until the end of the experiment. The level of GSH and the GSH/GSSG ratio were altered in the liver only. The level of GSH decreased significantly (P<0.01) (0.2 to 0.05 μg/g) from day 7 until the end of the experiment. The GSH/GSSG ratio decreased from day 9 (P<0.01). The level of GSSG increased until day 5 of the experiment and then markedly decreased in the spleen and the liver. It must be emphasized that the GSH level in the liver dropped to 25% of the initial value. The GSH/GSSG ratio markedly increased in the beginning of the experiment, and the maximum was reached on day 5. The decrease of GSH might not only be due to oxidative stress but also to direct utilization of the GSH and other cysteine containing peptides, as a source of essential nutrients, by *F. tularensis* (Pohanka *et al.*, 2008; Alkhuder *et al.*, 2009). The GSH pathway in *F. tularensis* is unlike other intracellular pathogens such as *Mycobacterium tuberculosis*. Owing to the cysteine pathway, *M. tuberculosis* has specific utilization and catabolism of cysteine different from *F. tularensis* (Voss *et al.*, 2011); however, the both pathogens has GSH as a supplement. Patients infected with *M. tuberculosis* reportedly have a decreased GSH level under

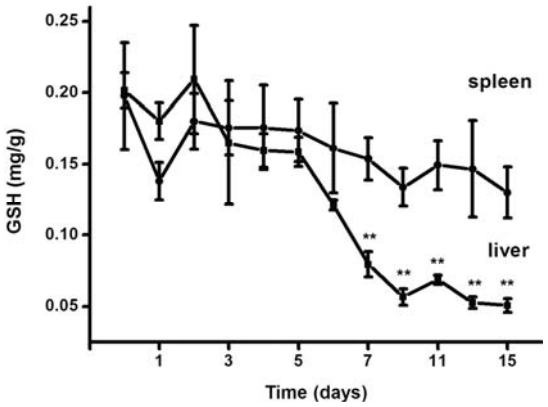

Fig. 9. Reduced glutathione (GSH) per g of protein in the spleen and liver of tularemia-infected mice. Error bars indicate standard error of the mean for six specimens. Two asterisks represent significance against the initial value at P<0.01.

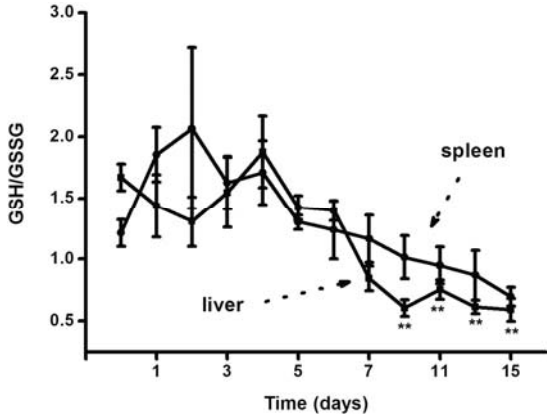

Fig. 11. Ratio of reduced to oxidized glutathione (GSH/GSSG) in spleen and liver of tularemia-infected mice. Error bars indicate standard error of the mean for six specimens. Two asterisks represent significance against the initial value at probability level P<0.01.

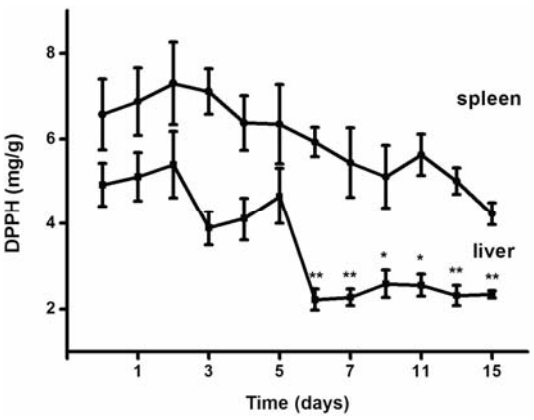

Fig. 12. Antioxidants assayed by 2,2-diphenyl-1-picrylhydrazyl test (DPPH) per g of protein in spleen and liver of tularemia infected mice. Error bars indicate standard error of the mean for six specimens. Significance against the initial value is expressed by one ($0.01 \leq P < 0.05$) or two ($P < 0.01$) asterisks.

stress conditions, as the bacterium makes demands on cysteine-rich compounds (Guerra *et al.*, 2011).

The sum of the low molecular weight antioxidants decreased as the DPPH value diminished. The decrease was significant in the liver from day 6 after infection, and the level of low molecular weight antioxidants in the liver decreased by more than 50% of the initial value.

The cytokine levels we observed correspond to our predictions and to past studies, such as research by Shen *et al.* (2010). Early onset of inflammatory markers can be predicted in tularemia-infected hosts, as has been reported by several researchers (Martinon *et al.*, 2010). In our experiment, the levels of both IL-6 and IFN-γ increased and decreased in keeping with the bacterial burden. The decrease of glucose levels and the increase in caspase-3 activity began when the bacterial burden reached the maximum. The induction of caspase-3 activity and the resulting apoptotic processes, by *F. tularensis* have been demonstrated in previous studies (e.g. Wickstrum *et al.*, 2009). The time of caspase-3 expression also corresponds with that team's findings. We infer that apoptotic processes are launched by the presence of the pathogen; however, these processes continue even after the decline of *F. tularensis* in tissues. These processes may also relate to the oxidative stress discussed in this report. The recognized decrease in the glucose level corresponds with our previous experiment in which hypoglycaemia was shown in European hares infected with *F. tularensis* subsp. *holarctica* (Bandouchova *et al.*, 2011). Glucose metabolism is typically accelerated and gluconeogenesis follows tularemia infection (Wannemacher *et al.*, 1980). In our previous paper, we discuss how differences in basic metabolism may impact susceptibility to tularemia infection (Bandouchova *et al.*, 2009).

The oxidative stress marker TBARS responds to lipid peroxidation and oxidative degradation of cell membranes (Catala, 2011). We found few oxidative insults in the spleen; oxidative stress primarily affected the liver, in which the TBARS level doubled. The TBARS level reached the maximum in livers at the same time that the bacterial burden was maximal. We infer that lipid peroxidation was triggered indirectly by alteration of the metabolism because the spleen appeared to remain intact. Malondialdehyde, a marker assayed by the TBARS method, is synthesized due to normal oxidative metabolism. A disturbance in liver function induced by hypoxia is well known to lead to oxidative stress and malondialdehyde over-production (Jusman *et al.*, 2010). Physical activity is another source of malondialdehyde genesis (Misra *et al.*, 2009). Live *F. tularensis* or lipopolysaccharide antigens can initiate the fatty acid metabolism pathway when they come in contact with peroxisome proliferator-activated receptors (Mohapatra *et al.*, 2010). Here we report that the elevation of TBARS can be caused just by fatty acid metabolism. The above-mentioned glucose level decrease can be caused solely by the activation of competing metabolic pathways.

Low molecular weight antioxidants were found to be depleted in livers. We must emphasize that this depletion was significant not only from a diagnostic point of view. We recognized a serious depletion in the level of antioxidants, which could result in serious adverse effects. The depletion of antioxidants was confirmed independently by assays for GSH, DPPH, and GSH/GSSG. Surprisingly, the depletion began after the resolution of lipid peroxidation, and it continued until the end of experiment. Similar to the level of low molecular weight antioxidants, the level of enzymatic antioxidant GR was also diminished. This may be related to the decrease of GSH, a natural substrate of GR. Activation of caspase-3 could result from the decrease of antioxidants or from mitochondrial degradation. Because the binding of metal ions to proteins can confer the ability to scavenge reactive oxygen species (Eckschlager *et al.*, 2009), changes in MT content may alter all of the above-mentioned factors. One may suggest that liver function could also be altered, which could cause an increase in this protein in the spleen. We infer that changes in the levels of low molecular weight antioxidants are caused by the above-mentioned changes in the host's metabolism rather than the direct effect of the pathogen, because the bacterial burden disappeared by the time there were changes in antioxidant levels. In our previous research, we investigated antioxidants in the plasma of tularemia-infected mice and common voles. They had shown different abilities in recovering from oxidative stress (Mohapatra *et al.*, 2010). More sensitive species have a lower ability to maintain the level of low molecular weight antioxidants. Their susceptibility to tularemia could decrease with the application of exogenous antioxidants. The supplementary administration of antioxidant-rich food or food supplements would be convenient for recovery from the disease. Surprisingly, the role of antioxidants in tularemia pathogenesis has not yet been extensively researched.

We conclude that tularemia is related to oxidative stress. The link between oxidative stress and tularemia has been discussed in several reports (Pohanka *et al.*, 2009; Bandouchova *et al.*, 2011); however, it has not been sufficiently investigated until now. In the experiment reported here, the oxidative stress is proved to be a complication of tularemia, and we deduce that this resulting stress can prolong recovery from the disease. Based on our experimental findings, we infer that providing low molecular weight antioxidant supplements to infected individuals would be beneficial in the

healing process. These findings are in agreement with the recent recommendation of many scientists to ameliorate sepsis by antioxidant therapy (Huet *et al*., 2011; Sebai *et al*., 2011). Our primary aim was to assess the oxidative stress that occurs in tularemia-targeted organs. We proved that oxidative insults occurred in the targeted organs. On the other hand, we chose bacterial doses below that which would result in the median mortality (Pohanka *et al*., 2009; Bandouchova *et al*., 2011). More serious pathogenesis and oxidative stress would be expected if the dose was increased. Considering the organs, we find the liver more vulnerable to tularemia caused oxidative stress than the spleen. It may be linked with systematic inflammation in course of the disease. The finding is not surprising as livers are vulnerable by oxidative stress due to multiple pathologies related to systematic inflammation (Kitada *et al*., 2001; Ypsilantis *et al*., 2010).

Conclusions

Oxidative homeostasis is significantly disturbed in the course of tularemia and the disturbance remains even after elimination of the pathogen from the host. The differential susceptibility of organs was also clearly demonstrated. Correcting the oxidative stress and depletion of antioxidants can be considered as an opportunity for therapy of tularemia induced pathologies. The therapy would be useful not only during the acute phase of tularemia but also in the convalescence after the acute disease.

Acknowledgements

The Ministry of Education, Youth and Sports of the Czech Republic is kindly acknowledged for project LH111023 and the Grant Agency of the Academy of Sciences of the Czech Republic is acknowledged for project: ANOSEMED GA AV KAN208130801. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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