Ph.D THESIS

PROTECTIVE ROLE OF CYPD-DEFICIENCY IN ENDOTOXEMIA INDUCED ACUTE LUNG INJURY

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1. INTRODUCTION

Sepsis or septic shock is a systemic inflammatory response syndrome associated with infection, caused by abnormal/overwhelming host defense mechanisms of the organism toward bacterial agents, such as lipopolysaccharide (LPS). The incidence of sepsis is still gradually rising despite tremendous development of medicine, at the same time mortality rates are improving. The pathogen spectrum is also changing overtime, however, Gram-negative bacteria play a prominent role. In the early phase of sepsis, the outer cell wall component of Gram negative bacteria, LPS activates the innate immune system and induces an uncontrolled inflammatory response. Proinflammatory cytokines (e.g. TNFα - tumor necrosis factor α and IL-1 - interleukin-1) produced during this stage induce the secretion of different toxic enzymes and oxidative agents leading to endothelial and tissue injury. The consequence is severe damage of distinct organs called multiple organ failure (MOF) or multiple organ dysfunction syndrome (MODS). Most often, the lungs are affected, resulting in acute lung injury (ALI) or a condition now known only as acute respiratory distress syndrome (ARDS). During sepsis induced indirect lung injury the alveolo-capillary unit, the integrity of endothelial and epithelial cells is damaged. These phenomena lead to hyaline membrane and edema formation, as well as infiltration of leukocytes causing decreased lung compliance, deteriorating gas exchange and hypoxia. LPS is recognized by the pattern recognition receptor (PRR) Toll like receptor 4 (TLR4). The ligand binding of TLR4 leads to the activation of the transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) through two distinct signaling pathways (Myd88-dependent or independent). Different members of TLR4 signal transduction activate IκB kinases (IKK). These phosphorylate IκB (inhibitor-κB) resulting in its ubiquitination and degradation. The process enables the release and nuclear translocation of NF-κB and initiation of target gene expression. In addition, many intracellular protein kinases, such as mitogen activated protein kinases (MAPK) (e.g. ERK (extracellular signal-regulated kinase), p38 or JNK (c-Jun NH2-terminal kinase)) and the serine-threonine kinase Akt are interfering with TLR4 signaling pathway contributing to the regulation of NF-κB. As a consequence of NF-κB activation, proinflammatory mediators, enzymes and reactive oxygen species are released from infiltrating neutrophils and resident cells. Leukocytes produce reactive oxygen (ROS) and nitrogen species (RNS), in order to eliminate pathogens. However, the excessive production of these reactive agents can cause tissue damage by lipid peroxidation, DNA-injury and alteration of protein amino acid sidechains. LPS-induced ROS can further enhance the activity of redox-sensitive inflammatory transcription factors (e.g. NF-κB) and

signaling kinases such as MAPKs and Akt. Reactive oxygen and nitrogen species are produced either intentionally or released as byproducts, in high amounts from the mitochondrial respiratory chain. Mitochondrial ROS production contributes to the development of many diseases/ is considered in the background of many diseases. Dissipation of mitochondrial membrane potential during the so called mitochondrial permeability transition (mPT) facilitates ROS formation. The mPT is the result of the opening of a non-specific, multicomponent channel, the mitochondrial permeability transition pore (mPTP). It enables passage of solutes up to 1.5 kDa from the matrix to the cytoplasm. Beside many others, oxidative stress, anorganic phosphate and mitochondrial Ca^{2+} overload promote pore opening. The exact molecular composition of the pore is still under debate. The classical model for mPTP included the voltage-dependent anion channel (VDAC) and the adenine nucleotide translocase (ANT), while lately it has been postulated that FoF1 ATP-synthase might be a mPTP component. However, cyclophilin D (CypD), a matrix peptidyl-prolyl *cis-trans*-isomerase, encoded by the nuclear *Ppif* gene, has remained an indispensable regulatory element of the pore. Mitochondria lacking CypD demonstrated very low Ca^{2+}-sensitivity, as pore opening required extreme high Ca^{2+} load similar to that seen during cyclosporine A (CsA) inhibition of wild type mitochondria. Although the exact physiological role of mPTP is still not elucidated, it contributes to the pathogenesis of different diseases and conditions accompanied by oxidative stress like ischemic/reperfusion injury, neurodegenerative disorders (Alzheimer's and Parkinson's disease) as well as muscular dystrophies. As CypD is an inevitable regulatory component of mPTP it is a well-established method to examine the relationship between certain diseases and the mPTP either by genetical or chemical ablation of CypD. There are only few studies regarding the role of mPTP in endotoxemia and consequential organ injury, and these have been conducted using CsA. Nevertheless, CsA inhibits not only CypD, but also cyclophilin A, B, C and calcineurin, therefore has a wide range of signaling effects - including inflammatory signaling - unrelated to CypD. Thus, immunomodulatory effects of CsA make it unfavorable for investigating the role of mPT under inflammatory conditions.

2. AIM OF THE STUDY

ROS play an important role in the development of LPS and endotoxemia induced acute lung injury. The role of mitochondrial ROS, especially their excessive production due to the opening of the pore has been implicated in many pathological conditions. Although the exact molecular composition of the pore is still controversial, the indispensable modulatory role of CypD is proven. According to these data our aim was to investigate the role of CypD-dependent mPT in endotoxemia induced ALI using CypD *knock-out* mice.

1. First we investigated its effect on the survival rate after lethal dose of intraperitoneal LPS.

2. To assess the severity of acute lung injury classical histological characteristics were studied.

3. To explore the balance of pro- and anti-inflammatory processes, cytokines functioning as effector molecules were determined by ELISA.

4. Endothelial dysfunction during ALI was confirmed using fibrin-specific staining and electron microscopic examination of ultrastructural damage.

5. The location and severity of ROS induced injury was demonstrated by immunohistochemistry using antibodies against stable ROS-endproducts.

6. The effect on phosphorylation of MAPKs and Akt, which are closely related to TLR4 signaling, was verified by Western blot.

7. Events of canonical NF-κB-activation, a transcription factor at the end of TLR4 signaling and of outstanding significance for acute lung injury, were performed by determining the phosphorylation of the components by Western blot. The actual effect on gene expression was confirmed by Real Time-PCR.

4. RESULTS

4.1 Mice lacking CypD survive lethal endotoxemia

Survival study was carried out with age-matched wild type (n=8) and CypD *knock-out* mice (n=8) using high dose of LPS. Survival was monitored for 7 days after the intraperitoneal LPS injection. Out of the 8 $CypD^{-/-}$ mice two (25%) died within the first 30 hours but after that no deaths occurred. However, all of the 8 wild type mice died within 60 hours. These results show that the loss of CypD massively reduces mortality.

4.2 CypD *knock-out* mice are protected against LPS-induced histopathological changes

We prepared 5 μm sections from paraformaldehyde (10%; pH=7,2) fixed lobe of the right lung. Hematoxylin-eosin staining was performed using standard protocol. For making histological examination quantitative, five slides in each group were scored in a double blinded manner by an independent expert using the scoring system recommended by the American Thoracic Society. Alveolar wall thickening, blood vessel congestion, thrombosis as well as perivascular edema and robust interstitial neutrophil infiltration were seen, which are suggestive of impaired lung tissue architecture and function. Histopathological characteristics were significantly more severe in LPS treated wild type mice compared to CypD *kock-out* animal. These findings mainly resulted from marked differences in interstitial neutrophil accumulation and alveolar thickening, which are the most important features of endotoxemia induced ALI according to the literature.

3.3 CypD$^{-/-}$ mice do not exhibit marked fibrin exudation in the lungs after LPS treatment

To prove the interstitial appearance of fibrin containing exudate due to endothelial damage Mallory's phosphotungstic acid-hematoxylin staining was used. Collagen fibers were packed into dense, contiguous layers in the perivascular region and around the bronchovascular bundle in control animals. On the contrary LPS-treated CypD $^{+/+}$ mice showed severe edema and fibrous changes around the vessels. Exudation with blue fibrin was detectable among the loose connective tissue and partially in the alveoli. Despite mild widening of the perivascular area no significant amounts of fibrin was detectable in *knock-out* animals.

3.4 Lack of CypD prevents the fine structural anatomy of lung tissue damaged by LPS

For more precise evaluation of endotoxemia induced barrier-dysfunction transmission electron microscopy was performed on lung tissue. Our results confirmed the injury of the endothelial layer and interendothelial junctions. Besides these changes pinocytotic vesicles and big vacuoles appeared in the cytoplasm of CypD$^{+/+}$ mouse lungs. Denudation of basal membrane as a consequence of cellular injury serves as potential surfaces to fine fibrin branches to attach. Cellular injury related denudation of basal membrane serves as potential surfaces to fine fibrin branches to attach. On the contrary fine structural changes were missing or only slight in LPS-treated CypD KO mice.

4.5 Loss of CypD protects lung epithelial cells against oxidative damage

To evaluate oxidative stress lung tissue sections were examined with HRP-conjugated polymer based immunohistochemistry using Dako *Autostainer Plus* automated slide processing system. The slides/sections were probed with antibodies against 4-hydroxy-2-noneal Michael adducts and nitrotyrosine. Nitrotyrosine is an in vivo marker of nitrosative stress originating from peroxynitrite-mediated nitration of tyrosine residues of target proteins due to excessive NO production. The lipid peroxidation product, 4-hydroxy-2-nonenal forms stable derivative through protein modification indicating oxidative stress. In wild type LPS-treated animals epithelial cells were intensively stained against both antibodies. Endothelium also showed prominent positivity in wild type, but not in *knock-out* LPS-treated animals. In wild type animals extensive 4HNE Michael adduct staining due to lipid peroxidation damage after LPS treatment was also visible regarding airways covered by bronchial mucinous cells, unlike in KO animals.

4.6 Absence of CypD impairs proinflammatory, but does not affect anti-inflammatory cytokine production

Determination of proinflammatory cytokines TNFα and IL-1β (B, and anti-inflammatory cytokine IL-10 24 h after LPS treatment (40 mg/kg) from total lung homogenates by ELISA. Clinical studies using bronchoalveolar lavage fluid proved the importance of these factors, in the development of ARDS. In our experiments, LPS treatment resulted in elevated TNFα and IL-1β levels, measured in lung homogenates, while the amount of these cytokines was markedly decreased in LPS-treated $CypD^{-/-}$ mice. In our study, there was no difference in the amount of anti-inflammatory IL-10 in total lung homogenates between wild type and *knock-out* animals 24h after LPS administration, as both increased significantly. Our result indicates similar anti-inflammatory reactions in both groups.

4.7 Deficiency of CypD affects the activation of MAPKs through MKP-1 and Akt in mouse lungs after LPS treatment

Activation of ERK, p38, SAPK/JNK, MKP-1 and Akt in lung total homogenates was determined 24 h after LPS treatment by immunoblotting utilizing phosphorylation specific and total primary antibodies. Total proteins (non-phosphorylated) and GAPDH were used as loading controls. In our experiments, phosphorylation levels of extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK) were significantly elevated 24 hours

after LPS treatment in wild type animals, while the activation of ERK and p38 was lower in the lungs of LPS-treated CypD$^{-/-}$ mice. No difference could be observed regarding JNK phosphorylation between *knock-out* and wild type animals after LPS challenge. MAP kinases are under the direct negative regulation through dephosphatase activity of MAPK-phosphatase-1 (MKP1). The level of MKP1 was up-regulated in CypD$^{-/-}$ mice compared to wild type animals after LPS treatment. Beside MAP kinases Akt contributes to the TLR4 signaling cascade leading to NF-κB activation and promoting inflammatory processes in the lung. In our experiment, LPS treatment significantly enhanced the phosphorylation of Akt in the lungs of wild type animals, while this effect was strongly reduced in CypD$^{-/-}$ animals.

4.8 CypD *knock-out* mice do not exhibit prominent NF-κB activation after LPS treatment

Phosphorylation of NF-κB p65 subunit and IκB in lung total homogenates was determined 24 h after LPS treatment by immunoblotting, utilizing phosphorylation specific primary antibodies. Total proteins (non-phosphorylated) and GAPDH were used as loading controls. Systemic LPS caused a significant activation of the analyzed NF-κB subunit in wild type mice compared to CypD$^{-/-}$ animals. Similarly, robust IκB phosphorylation was found in wild type animals after LPS treatment; however, CypD$^{-/-}$ mice showed decreased phosphorylation, which seems to confirm our data regarding NF-κB activation.

4.9 Marked differences between wild type and CypD *knock-out* animals regarding NF-κB-mediated gene expression

The expression of NF-κB-mediated inflammatory genes, CD14, IFN-γ, TNFα, IL-1α, Cxcl2 and iNOS was determined 24h after LPS treatment in lung tissue by Real Time-PCR. Actin was used as reference gene for ΔCt-determination. Results were normalized against ΔCt-values of controls. This way we wanted to proof whether differences in NF-κB regulation are only limited to the level of phosphorylation of key signaling enzymes, or it also affects the transcription of the related genes as well. Expression of all genes contributing to ARDS was elevated in wild type animals after endotoxemia. This LPS-induced overexpression was strongly reduced in every case in the *knock-out* mice.

4. DISCUSSION

In the present study, we demonstrated that deficiency of CypD, the regulatory component of mPTP, ameliorates pathological consequences of endotoxemia induced ALI. This effect could be observed both at tissue and molecular levels, and mortality rate was also massively reduced in KO animals. Mitochondrial dysfunction and ROS production related cellular damage has been implicated in the pathophysiology of LPS-induced inflammation and ALI. Many different inflammatory mediators can increase mitochondrial ROS formation. The opening of mPTP, a channel located in the inner-membrane, contributes to this phenomenon. Thus the role of mPTP opening has been implicated in many pathological conditions where ROS contribute to the organ damage. Examination is complicated as the exact structure of the multicomponent pore is still controversial. However, it is certain that CypD, a matrix-protein, is undoubtedly a modulatory constituent. Cyclophilins are ubiquitous proteins with peptidyl-prolyl *cis-trans* isomerase activity differing in subcellular localization and binding affinity to cyclosporine A (CsA). CypD is the only known mitochondrial member. Main target of CsA is calcineurin. This molecule participates in many signaling and inflammatory processes, like it effects cytokine expression through transcription factor NFATc3 in sepsis induced acute lung injury. CsA inhibits calcineurin thereby suppresses MKP-1 expression resulting in increased MAPK activation. Therefore, considering the importance of MAPKs in NF-κB activation, CsA is obviously unsuitable for studying the effect of mPT impairment on LPS-induced inflammatory response. To resolve this problem and to focus on the role of CypD and mPT on LPS-induced inflammation and lung injury, we used a $CypD^{-/-}$ model. Beside mitochondria, lung epithelial cells and macrophages, as well as sequestered neutrophils produce excessive amounts of ROS leading to cellular damage. It is characterized by endothelial barrier dysfunction, diffuse alveolar damage, accumulation of neutrophils, interstitial edema and thickening of the alveolar septa, hyaline membrane and formation of thrombi. Our histological results showed the same characteristics in the lungs of LPS-challenged wild type mice, but animals lacking CypD showed only mild tissue injury and histological scores supported these findings.

ROS induced barrier dysfunction and increased vascular permeability are important criteria of ALI animal model. Mallory's phosphotungstic acid-hematoxylin (PTAH) staining was performed which detects fibrin accumulation and fibrosis. The method is still in use to demonstrate the presence of fibrin rich alveolar and interstitial fluid caused by endotoxemia. In wild type animals the excessively widened and fibrous perivascular region contained large amounts of blue fibrin deposits, and partially blue coloration of fibrin was detectable in alveoli.

Endotoxemia induced damage of endothelial layer and interendothelial junctions was demonstrated at subcellular fine structural level using electron microscopy; however, a definitive protective effect was found in CypD-deficient mice. Our results suggest that the loss of CypD and thereby the impaired opening of the mPTP greatly diminishes oxidative stress after LPS treatment. To examine the extent of reactive oxygen and nitrogen species production immunohistochemistry was performed, Although, it is only a semi-quantitative method, it also gives information about the localization of the damage. As the quantification of reactive oxygen species is complicated due to their short half-lives, fluctuating amount and reactivity, stable byproducts have been used as indicators. Antibody against a protein-modification by 4 hydroxy-2 nonenal, a reactive aldehyde arising from lipid peroxidation of unsaturated fatty acids was used. It has been proven by animal as well as by human studies, that the amount of 4HNE increases during systemic inflammatory response syndrome, sepsis and ARDS. It has deleterious effects on the microvasculature and enhances permeability in the lung. However, it is not only a marker of oxidative stress, but is also affects cell signaling and causes mitochondrial dysfunction. Nitrotyrosine, the endproduct of reactive nitrogen species production, was used as indicator. It is the irreversible modification of tyrosin sidechains by peroxynitrite. LPS treatment markedly enhanced IHC staining in endothelial and lung epithelial cells of wild type animals, while Ppif knock out mice seemed to be protected and showed less intense staining. Our results suggest that loss of CypD greatly reduces ROS and RNs formation after LPS treatment, resulting in much milder histological and subcellular lesions as well as oxidative tissue damage in the lungs of mice.

ROS contribute to the inflammatory phenotype, with the increased production of proinflammatory cytokines in lung cells. Elevated concentrations of proinflammatory chemokines and cytokines, including IL-8, IL-1β, and TNFα, in the lungs are clinically proven critical regulators of ALI-development. Detection of proinflammatory cytokines is also an extremely important criterion for ALI in animal models. Compared to wild type animals, in CypD-deficient mice, the level of TNFα and IL-1β produced by resident cells was decreased, indicating that the lack of CypD could severely interfere with cytokine generation, possibly due to reduced mitochondrial ROS production. This strong correlation between mitochondrial ROS and proinflammatory cytokine production was also reported by other research groups. Blockade of mitochondrial ROS generation efficiently reduced inflammatory cytokine secretion after treatment in cells from patients with TNF receptor-associated periodic syndrome and from healthy individuals. In microglia cells, the LPS-induced proinflammatory cytokine production could also be prevented by chemical inhibition of mitochondrial ROS formation. As a

counterbalance, IL-10 is a key anti-inflammatory cytokine in the down-regulation of inflammatory response. One of its key functions is regulation of the pathogen-mediated activation of macrophages and dendritic cells, consequentially inhibiting the expression of chemokines, inflammatory enzymes, and potent proinflammatory cytokines, thereby inhibiting the neutrophil granulocyte migration into the lung. The results in the literature show that, in contrast to proinflammatory cytokines, the change in IL-10 levels by LPS is highly dependent on the location of the stimulus and the time of measurement. Elevated levels of IL-10 after LPS exposure did not differ in the two LPS-treated groups, indicating that the ameliorated injury and inflammatory processes in lungs of CypD-deficient animals are not a consequence of anti-inflammatory mechanisms but of attenuated ROS production.

ROS are important chemical mediators that regulate signal transduction pathways, including members of the MAP kinases. MAPK activity increases during LPS-induced signaling as some components of the pathway also act as MAPKKKs. In line with previous studies, we found the increased phosphorylation of MAPKs in the lungs after intraperitoneal LPS treatment. Phosphorylation of redox-sensitive p38 and ERK was markedly decreased in CypD-deficient mice; however, JNK activation was unaltered in our experiments. This phenomenon can be explained by the different ROS sensitivity of MAPKs. ROS can activate all three MAPKs. This regulation is conducted either by direct modification of protein sidechains, or by different upstream regulators independently of each other. Our results are supported by previous findings that H_2O_2 stimulates JNK but not p38 and ERK via a pathway that is dependent on Src kinase The exact mechanisms for ROS-mediated p38 and ERK activation is not fully elucidated, but redox-sensitive IKK might also play a role. Based on our results the depletion of CypD exerts its effect on ROS-induced MAPK activation in p38- and ERK-dependent and JNK-independent ways. Besides the regulation of upstream mediators of MAPKs, direct control mechanisms could act also through MKP-1(MAPK phosphatase 1) activity. MKP-1, located in the nucleus, exerts inhibition on all three MAPKs, but the extent of its effect varies among different cell types. Many studies support its negative regulatory role in inflammatory processes. Expression is enhanced by JNK and IL10. Up-regulation of MKP-1 occurred in lungs upon LPS exposure, which was much stronger in CypD knock-out mice. However, there was no difference in IL10 concentration or JNK phosphorylation state between the two groups. Results suggest that not the decreased expression, rather higher degradation rates due to oxidative stress are responsible for reduced MKP1 amounts in wild type animals. Akt is another kinase participating in TLR4 pathway beside MAPKs. Previous studies similarly to ours have shown that LPS exposure

increases phosphorylation of the kinase in lungs. Akt regulates p38 as well, thereby positively influencing NF-κB activation. Indeed, the phosphorylation pattern of p38 followed that of Akt in our experiments which was also found to be less phosphorylated in the lungs of LPS treated CyD-deficient mice. This phenomenon can also be explained by upstream regulation of Akt via mitochondrial ROS. Indirect redox regulation of Akt can occur on the level of PI3K, which is activated by H2O2. On the other hand, ROS inactivate the inhibitory phosphatase, PTEN.

CypD-dependent decreased oxidative stress influences canonical activation of NF-κB through redox-sensitive kinases. In accordance with these findings, NF-κB and IκB phosphorylation increased dramatically after LPS treatment in the lungs of wild type but not CypD-deficient animals. Moreover, we proved the functional inhibition of NF-κB activity in the absence of CypD, analyzing NF-κB-related inflammatory genes at the mRNA level. In CypD-deficient mice, the expression of important participants of TLR4 signaling (CD14, iNOS) and mediators of ALI, like chemokines and cytokines (Cxcl2, IFNγ, TNFα, IL-1α), showed a significant decrease compared to wild type animals. Our findings can be explained by reduced kinase-activity of IKK responsible for phosphorylation of IκBα on Ser32 arising from decreased oxidative stress in case of CypD deficiency. Decreased dephosphorylation may contribute to this phenomenon as IKK are inactivated by redox-sensitive phosphatases.

Our results support that the moderate oxidative environment due to CypD deficiency led to decreased activity of redox-sensitive signaling participants, p38, ERK, and Akt. These, together with the oxidative stress-responsive components of NF-κB signal transduction, resulted in decreased p65 phosphorylation and gene expression. As a consequence, the amount of proinflammatory cytokines in KO animals was lower and the lesions characteristic of acute lung damage were much milder, which also helped the animals to survive.

5. SUMMARY

We are the first group who demonstrated that the loss of the essential pore-component CypD can intensely ameliorate LPS-induced lung injury in mice. Our results can be summarized as follows:

1. Loss of CypD enhances survival in mice after intraperitoneal LPS challenge.
2. CypD knock out mice displayed significantly milder histological characteristics of ALI supported by objective scoring system.

3. Level of proinflammatory cytokines was lower in lungs of $CypD^{-/-}$ mice while the concentration of anti-inflammatory IL10 did not differ after systemic LPS treatment. We consider it as a consequence of lower ROS and thereby decreased cytokine expression.
4. Ameliorated endothelial dysfunction was demonstrated in lungs of *Ppif* deficient mice after endotoxemia using fibrin-specific staining and electron microscopy.
5. ROS and RNS induced injury was attenuated in KO animals after i.p. LPS as shown by immunohistochemistry using antibodies against lipid peroxidation endproduct, 4-hydroxy-2-nonenal Michael adduct and nitrosative stress marker, nitrotyrosine.
6. Deficiency of CypD attenuates LPS induced activity of certain kinases participating in TLR4 signaling like ERK, p38 and Akt. However, phosphorylation of JNK is not altered. Higher levels of MKP1, responsible for inactivation of MAPKs, might contribute to this phenomenon.
7. TLR4 related canonical NF-κB-activation was diminished after LPS in CypD *knock-out* mice. Both phosphorylation status of IκB and p65 subunit of NF-κB were decreased. Functional inhibition of NF-κB was also evident on gene expression profile of inflammatory mediators.

6. List of publications, posters, lectures

Publication related to the thesis

Fonai F, Priber JK, Jakus PB, Kalman N, Antus C, Pollak E, Karsai G, Tretter L, Sumegi B, Veres B. (2015) ***Lack of cyclophilin D protects against the development of acute lung injury in endotoxemia.*** Biochim Biophys Acta. 1852(12):2563-73.

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Turai R, Schandl MF, Dergez T, Vass RA, Kvárik T, Horányi E, Balika D, Mammel B, Gyarmati J, Fónai F, Vida G, Funke S, Gaál V, Reglődi D, Ertl T. (2019) ***Early and late complications of hyperglycemic extremely low birth-weight infants.*** Orv Hetil. 160(32):1270-1278.
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Posters and lectures

Posters based on the thesis

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FEBS3+ Meeting, 2015. szeptember 16-19., Portorož, Szlovénia

A ciklofillin D hiányának hatása a lipopoliszacharid indukálta tüdőkárosodásra egérben

Fónai F, Pribér JK, Kálmán N, Jakus PB, Antus C, Radnai B, Sümegi B, Veres B.

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