Division of Endocrinology[1], Department of Medicine, The Third Xiangya Hospital, Central South University; Division of Nephropathy[2], Department of Medicine, Xiangya Hospital; Division of Respiratory Medicine[3], Department of Medicine, Xiangya Hospital; State Key Laboratory of Medical Genetics of China[4], Changsha, Hunan, China

Losartan inhibits LPS + ATP-induced IL-1beta secretion from mouse primary macrophages by suppressing NALP3 inflammasome

Fang Wang[1], Ling Huang[2], Zhang-Zhe Peng[2], Yi-Ting Tang[2], Miao-Miao Lu[2], Yu Peng[2], Wen-Juan Mei[2], Lin Wu[2], Zhao-Hui Mo[1], Jie Meng[3], Li-Jian Tao[2,4]

Received November 13, 2013, accepted December 21, 2013

Prof. Li-jian Tao, MD, Ph.D, Division of Nephropathy, Department of Medicine, Xiangya Hospital, Central South University, Changsha, Hunan 410008, China
taolj@mail.csu.edu.cn

Pharmazie 69: 680–684 (2014) *doi: 10.1691/ph.2014.3926*

Objectives: IL-1beta is a potent proinflammatory, pro-fibrogenetic and pro-athrosclerosis cytokine which has been shown to play an important role in an expanding number of noninfectious, chronic inflammatory conditions including cardiovascular disease, renal fibrosis, rheumatoid arthritis and even type 2 diabetes. Losartan is an angiotensin II receptor antagonist widely used for the treatment of hypertension, diabetic nephropathy and congestive heart failure. In this study, we attempted to clarify whether losartan has an inhibitory effect on IL-1beta. To further elucidate the molecular mechanism underlying the anti-IL-1beta property of losartan, we studied the LPS + ATP-induced activation of NALP3 inflammasome which controls the muturation and secretion of IL-1beta. *Methods:* LPS and ATP were used to stimulate the release of IL-1beta from thioglycollate-elicited macrophages from BALB/c mice. The production of IL-1beta was evaluated by ELISA assay and NALP3,caspase-1, IL-1beta mRNA levels were determined by reverse transcription-polymerase chain reaction. *Results:* In cultured thioglycollate-elicited macrophages, we observed that LPS + ATP greatly enhanced IL-1beta secretion (6938.00 ± 83.45; $P < 0.05$) and the mRNA levels of NALP3, caspase-1 which are two main components of NALP3 inflammasome (60.88 ± 8.28; 1.31 ± 0.04, $P < 0.05$ for both). The macrophages co-cultured with losartan showed low production of IL-1beta (3907.50 ± 143.61; $P < 0.05$) and low production of NALP3, caspase-1mRNA (29.82 ± 6.92;1.12 ± 0.05, $P < 0.05$ for both). Losartan did not reduce IL-1beta mRNA($P > 0.05$). *Conclusions:* Our results show that the NALP3 inflammasome is up-regulated and activated in the mouse macrophage in response to LPS + ATP stimulation. Losartan is able to suppress the LPS + ATP-induced production of IL-1beta protein. In addition, this effect may be partially mediated by suppressing NALP3 inflammasome activation.

1. Introduction

Interleukin (IL)-1beta is a multifunctional cytokine that is generated mainly by monocytes/macrophages. It is a potent proinflammatory mediator which can promote the secretion of many other cytokines such as IL-6, IL-8 and TNF-α (Dinarello 2011b; Nee et al. 2004). Several autoinflammatory disorders such as Behcet's disease, Schnitzler syndrome have been suggested to be IL-1beta associated (Masters et al. 2009). *In-vitro* and *in-vivo* data show that IL-1beta plays an important role in a broader spectrum of disorders beyond the characterized autoinflammatory syndromes, including idiopathic pulmonary fibrosis (Mitroulis et al. 2010), chronic obstructive pulmonary disease (Mitroulis et al. 2010), renal fibrosis (Vesey et al. 2002), cardiovascular disease (Kher and Marsh 2004; Ross 1999; Dalekos et al. 1997), rheumatoid arthritis (Dinarello 2011b) and even type 2 diabetes (Masters et al. 2010).

Different from other pro-inflammatory cytokines, the synthesis and processing of IL-1beta is controlled by two separate signals (Van et al. 2011): first, pattern recognition of microorganisms such as LPS induces transcription of IL-1beta mRNA and subsequently translation of pro-IL-1beta protein, but this inactive precursor will be degraded within the cell without a second stimulus; second, danger signals such as extracellular ATP (Mariathasan et al. 2006), gout-associated uric acid crystals (Martinon et al. 2006) and inhaled silica (Dostert et al. 2008) trigger the assembly of components of the NALP3 inflammasome. NALP3 recruits intracellular protein pro-caspase-1 through the adaptor protein ASC to form an complex termed the"NALP3 inflammasome", resulting in the activation of caspase-1, a protease that cleaves the inactive precursor pro-IL-1beta into bioactive form (Tschopp and Schroder 2010). As a result, the mature and active IL-1beta is released from the cell (Latz 2010). The processing of IL-1beta is tightly regulated by caspase-1 since caspase-1 inhibitor could cause a dramatically reduction of IL-1beta secreted from the cells (Thornberry et al. 1992; Cerretti et al. 1992). It has also been demonstrated that IL-1beta secretion was abrogated by caspase-1

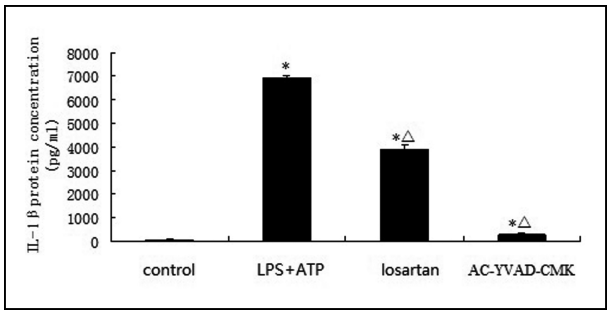

Fig. 1: Effect of losartan on the LPS + ATP induced secreation of IL-1beta from primary macrophages. Losartan group was pretreated with 10-5 mM losartan for 24 h, and then stimulated with 500ng/ml LPS for 3 hours and 5 mM ATP for another 1 hour. The caspase-1 inhibitor(AC-YVAD-CMK) was added 1 hour before the addition of LPS. Release of IL-1beta was determined by ELISA. All data represent the mean ± SD of three independent experiments. *$p < 0.05$ vs. the control group; $\Delta p < 0.05$ vs. the LPS + ATP group.

inhibitor AC-YVAD-CMK, indicating that IL-1beta maturation was indeed inflammasome-dependent (Rajamäki et al. 2010). As the importance of IL-1beta in noninfectious, chronic inflammatory conditions, some researchers argue that IL-1beta may become a standard target in the therapy of these diseases (Simon et al. 2007; Masters et al. 2009). Thus, the development of novel drugs which could inhibit IL-1beta will offer advantages for the treatment of an expanding number of chronic inflammatory conditions.

Inokuchi et al. (2005) demonstrated that losartan can suppress the level of IL-1beta in mice with experimental immune-mediated colitis. Decreased levels of IL-1beta were also noted in the liver of dehydrated Wistar rats treated with losartan after lipopolysaccharide (LPS) administration. Also Andrzejczak et al. (2007) observed that losartan significantly decreased LPS-stimulated IL-1beta levels in spontaneously hypertensive rats. However, the exact mechanism of losartan on IL-1beta is still unknown. In our study, we hypothesized that losartan could inhibit IL-1beta secretion by suppressing the NALP3 inflammasome.

2. Investigations and results

2.1. Losartan significantly inhibited LPS + ATP-induced IL-1beta protein secretion from peritoneal macrophages

The IL-1beta levels in the suspensions were examined using ELISA in different groups. Compared to an untreated control group, primary macrophages showed a significant increase in expression of IL-1beta after LPS + ATP stimulation ($P < 0.05$; Fig. 1), indicating that LPS and ATP were effective stimulators of IL-1beta secretion. The losartan group was incubated with lorsartan for 24 h before LPS and ATP were added. We saw that lorsartan prevented LPS + ATP-induced secretion of IL-1beta from murine macrophages ($P < 0.05$; Fig. 1). AC-YVAD-CMK is a specific inhibitor of caspase-1 upstream of IL-1beta. As a positive control, IL-1beta secretion from AC-YVAD-CMK group was significantly decreased ($P < 0.05$; Fig. 1). This results demonstrated that losartan has an effect of anti-IL-1beta.

2.2. Losartan reduced LPS + ATP-induced NALP3 and caspase-1 mRNA in peritoneal macrophages

To further determine whether losartan attenuates IL-1beta secretion through the way of impairing the NALP3 inflammasome, real-time PCR was performed. As shown in Fig. 2a, b, LPS plus ATP treated macrophages expressed significantly higher levels of NALP3mRNA and caspase-1mRNA ($P < 0.05$ for both), indicating that the NALP3 inflammasome was activated during the IL-1beta secretion process. However, in the losartan treated group, NALP3mRNA and caspase-1mRNA expression were significantly lower than in the model group ($P < 0.05$ for both;

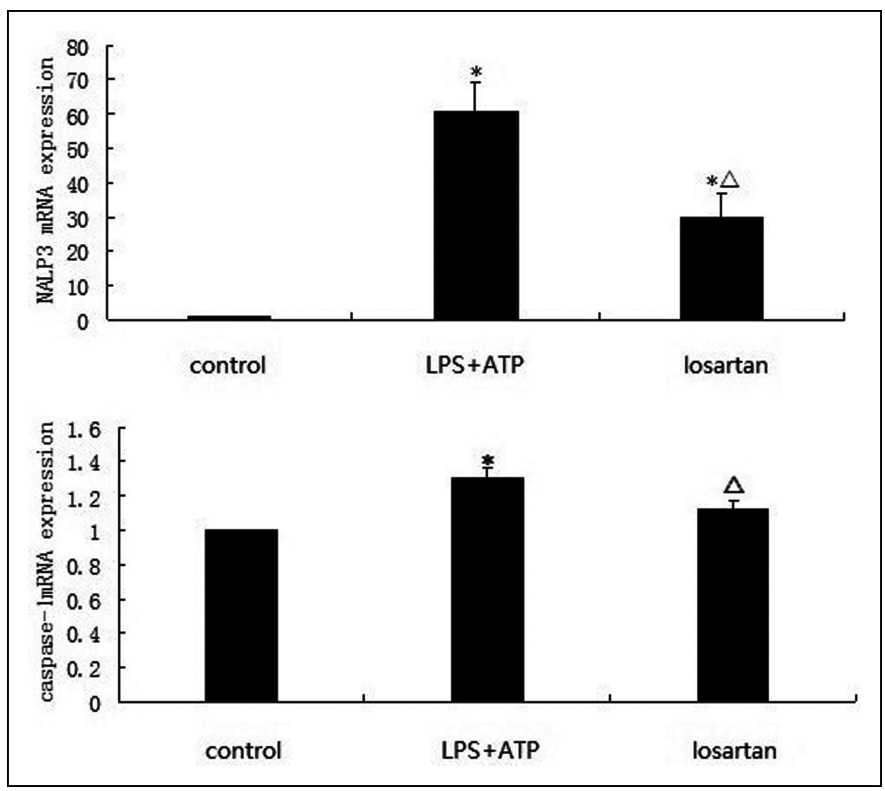

Fig. 2: Effect of losartan on the LPS + ATP induced expression of NALP3 mRNA and caspase-1 mRNA in primary macrophages. a Expression of NALP3 mRNA was detected by real-time RT-PCR in different groups.Losartan treated group expressed lower level of NALP3 mRNA than LPS + ATP treated group.b Lower level of caspase-1 mRNA was also expressed in losartan treated group than LPS + ATP treated group. All data represent the mean ± SD of three independent experiments. *$p < 0.05$ vs. the control group; $\Delta p < 0.05$ vs. the LPS + ATP group.

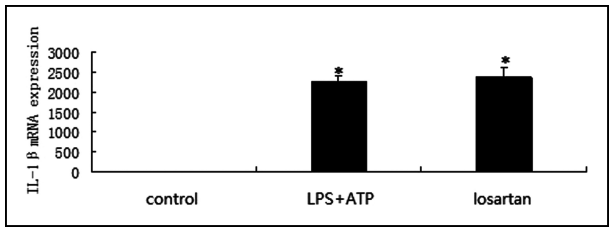

Fig. 3: Effect of losartan on the LPS + ATP induced expression of IL-1beta mRNA in primary macrophages. IL-1beta mRNA was significantly upregulated in LPS + ATP treated macrophages,but no significant difference was detected between losartan treated group and the LPS + ATP treated group.All data represent the mean ± SD of three independent experiments. $*p < 0.05$ vs. the control group.

Fig. 2a, b). These results demonstrate that losartan can reduce LPS + ATP-induced NALP3 and caspase-1 mRNA expression in peritoneal macrophages.

2.3. Losartan has no effect on LPS + ATP-induced IL-1beta mRNA in peritoneal macrophages

In parallel, higher levels of IL-1beta mRNA were seen in the LPS + ATP treated macrophages compared with the controls ($P < 0.05$; Fig. 3). However, no significant difference in IL-1beta mRNA was detected between the losartan treated group and the model group ($P > 0.05$; Fig. 3), revealing that losartan may have no effect on LPS + ATP-induced IL-1beta mRNA expression in peritoneal macrophages. It gives us a clue that losartan inhibits IL-1beta in the post-translation level which is controlled by the NALP3 inflammasome but not in the transcriptional level.

3. Discussion

IL-1beta is a potent pro-inflammatory cytokine. It is one of the most critical inflammatory mediators in inflammation reaction and one of the main regulators in immune response (Zhao et al. 2013). Dinarello (2011a) argues that IL-1beta acts as an inflammation "gatekeeper". Because of the importance of IL-1beta in the inflammatory response, some researchers believe IL-1beta may serve as a target in the treatment of inflammatory diseases (Dinarello et al. 2011b). IL-1beta is also involved in the pathogenesis of cardiovascular diseases including hypertension, atherosclerosis and other diseases like renal fibrosis (Kher and Marsh 2004; Ross 1999). Dalekos et al. (1997) reported that the expression level of IL-1beta was elevated in hypertensive patients, and its concentration was positively associated with the average blood pressure. In coronary artery disease, the expression level of IL-1beta was correlated with the severity of the disease (Masters et al. 2009). Many researchers had demonstrated that losartan could suppress IL-1beta in different disease models (Silveira et al. 2013). However, there is little data showing the detailed mechanism.

A large number of studies suggest that IL-1beta secretion was controlled on two levels (Dinarello et al. 2011a): (1) The transcriptional level: inflammatory stimuli such as LPS activate IL-1beta gene expression and increase IL-1beta mRNA through inflammatory signaling pathways; (2) post-translation level: IL-1beta mRNA translates to pro-IL-1beta, but the precursor form has no biological activity. The NALP3 inflammasome multiprotein complex converts the precursor pro-IL-1beta to the mature form. So what is the exact mechanism of losartan inhibiting IL-1beta? How does losartan function on IL-1beta, on the transcriptional or on the post-translation level? Further studies are required to elucidate this question.

In our study, we showed that LPS + ATP strongly induce the secretion of IL-1beta protein and the expression of IL-1beta mRNA in mouse macrophages, which demonstrates that the mode of macrophage activation is successful which is in agreement with previous studies (Ferrari et al. 1997; Mariathasan et al. 2006). Moreover, the mRNA expression of the two main components in NALP3 inflammasome------NALP3 and caspase-1 in model groups is significantly higher than those in normal controls, which means that the NALP3 inflammasome is activated by LPS + ATP stimulation. When treated with lorsartan, the IL-1beta protein and NALP3mRNA, caspase-1mRNA expression are significantly lower than in LPS + ATP group. Unexpectedly, we found no significant difference of IL-1beta mRNA between the losartan treated group and the LPS + ATP group.

Taken together, the present findings demonstrate that losartan is able to suppress the LPS + ATP-induced production of IL-1beta protein but not IL-1beta mRNA in mouse primary macrophages. Thus, the mechanism of losartan inhibiting IL-1beta is mediated via suppressing the NALP3 inflammasome which controls the post-translational processing of IL-1beta. This study also provides new insight into a new target of an old drug.

The NALP3 inflammasome is a protein complex composed of NALP3 (also known as cryopyrin or CIAS), caspase-1 and an adaptor protein ASC. It stimulates caspase-1 activation to promote the processing and secretion of IL-1beta. Recent work indicates that the NALP3 inflammasome is a general detector of either exogenous or endogenous danger signals as an extraordinary number of NALP3 inflammasome activators have been identified (Schroder and Tschopp 2010). Exogenous danger signals like asbestos or silica have been reported to be activators of the NALP3 inflammasome which contributes to the pathogenesis of pulmonary fibrotic disorders, asbestosis and silicosis (Dostert et al. 2008). Except from the above diseases, NALP3 inflammasome is suggested to be a sensor of cellular stress and metabolic danger signals (De et al. 2011). For example, MSU (monosodium urate) crystals have been reported as capable of activating the NALP3 inflammasome in gout (Martinon et al. 2006). Saturated fatty acids and islet-derived amyloid polypeptides are demonstrated to activate the NALP3 inflammasome in type2 diabetes (Wen et al. 2011; Masters et al. 2010). The cholesterol crystals, which play a key role in the development of atherosclerosis, are also shown to activate the NALP3 inflammasome (Rajamäki et al. 2010). So why can the NALP3 inflammasome be activated by a wide range of danger signals? Interestingly, reactive oxygen species (ROS) are generated by all known NALP3 inflammasome activators, which indicates that those structurally distinct molecules trigger NALP3 inflammasome activation which is dependent on the generation of ROS (Zhou et al. 2010; Martinon et al. 2009). Tschopp and Schroder (2010) also suggested that NALP3 inflammasome activation is the convergence of multiple signaling pathways on ROS production. Additionally, inhibitors or scavengers of ROS can block NALP3 inflammasome activation and prevent IL-1beta secretion in response to various agonists (Dostert et al. 2008; Carta et al. 2011).

Angiotensin II is known to trigger ROS generation through AT1 receptor activation (Nakashima et al. 2006). Thus, there is growing evidence to suggest that angiotensin II receptor blockers (ARBs) suppress ROS production through the blockade of the AT1 receptor (Ogawa et al. 2006; Satoh et al. 2008). Among numerous ARBs, losartan is one of the most classical ARBs. However, besides blocking AT1 receptors, losartan also has AT1 receptor-independent actions which are not shared by other ARBs (Sadoshima 2002). For example EXP3179, one of the metabolites of losartan, was shown as a ROS scavenger which does not interfere with the AT1 receptor (Sadoshima 2002).

Taken the above together, the properties of losartan can explain its potential function on the NALP3 inflammasome and IL-1beta due to ROS suppression. Therefore, this study supports primary

Table: Real-time polymerase chain reaction primers

Target gene	Forward primer (5'→3')	Reverse primer (5'→3')
NLRP3	TTC ACT GCT ATC AAG CCC TCC	AAG CCT TTG CTC GAG ACC CT
Caspase-1	GTG GAG AGA AAC AAG GAG TGG T	CGC AGA TGC CCA CCA CT
IL-1beta	GGCTGGACTGTTTCTAATGC	ATG GTT TCT TGT GAC CCT GA
β-actin	CTTCCGCCTTAATACTTCATT	GGA GAC CAA AGC CTT CAT AC

evidence that suggests losartan has an anti-inflammasome quality and perhaps losartan can inhibit NALP3-related diseases, which adds a new dimension to the therapeutic potential of losartan.

4. Experimental

4.1. Isolation and culture of primary macrophages from mice

Six to eight-week-old male BALB/c mice were used in the experiments. Macrophages were harvested by injecting 2 ml of 3% thioglycollate solution into the peritoneal cavity of BALB/c mice. Three days later macrophages were collected by peritoneal lavage and were centrifuged for 10 min at 4 °C.Then cells were adjusted to 2×10^6 cells/ml and the cell suspensions were plated onto 96-well flat-bottomed plates (1 ml/well) in RPMI 1640 medium supplemented with 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 ug/ml streptomycin. Cells were incubated for 3 h for adherence in a humidified atmosphere with 5% CO_2 at 37 °C. The nonadherent cells were removed by washing the wells with PBS. The adherent cells were almost macrophages.

4.2. Cell treatments

Thioglycollate-elicited macrophages from male BALB/c mice were co-cultured for 24 h with losartan (10^{-5}mM) or with medium. Losartan-primed 24 h macrophages were stimulated with 500 ng/ml LPS from Escherichia coli serotype O111:B4 (Sigma) for 3 h before ATP (5 mM) was added for another 1 h. After stimulation culture supernatants were collected and then assayed for subsequent IL-1beta ELISA assay. Antibody pair for the IL-1beta ELISA was from R&D Systems. The caspase-1 inhibitor(AC-YVAD-CMK) was added 1 h before the addition of LPS. And the cells were harvested for RNA extraction.

4.3. Enzyme-linked immunosorbent assay for IL-1beta

The supernatants from cultured primary macrophages were collected. The protein levels of IL-1beta in the supernatants were detected using ELISA kits (R&D Systems) according to the manufacturer's instructions.

4.4. RNA Isolation, cDNA synthesis, and real-time RT-PCR assays for NALP3, caspase-1 and IL-1beta

Total RNA from mouse peritoneal macrophages was isolated by Trizol (Invitrogen) according to the manufacturer's instructions. 1–2 μg RNA were converted into single-stranded cDNA using superscript reverse transcriptase II (Fermentas). The resulting cDNA was amplified by the PCR supermix kit (TaKaRa). Sequences of primers are shown in the Table. Amplification reactions were performed for one cycle of 94 °C for 2 min, 30 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min, followed by one cycle of 72 °C for 7 min. All reactions were performed in the ABI Prism 7900 Sequence Detection System (Applied Biosystems). PCR products were resolved on 1% agarose gels and visualized under UV lamps.

4.5. Statistical analysis

Data are presented as the mean ± SE. Comparisons between two groups were made by unpaired Student t test. Differences among multiple groups were determined with the one-way analysis of variance (ANOVA) using Tukey's method for post hoc analysis. $P < 0.05$ was considered to be statistically significant.

Acknowledgements: This research was supported by the National Natural Science Foundation (grant no. 81001467), the National Natural Science Foundation (grant no. 81273575), the National Natural Science Foundation (grant no. 81200048) and the Key Project of the Natural Science Foundation (no. 11-JJ2051) of Hunan Province to Dr. Lijian Tao. Thanks for the technical support by National Key Laboratory of Medical Genetics of China.

References

Andrzejczak D, Górska D, Czarnecka E (2007) Influence of enalapril, quinapril and losartan on lipopolysaccharide (LPS)-induced serum concentrations of TNF-a, IL-1b, IL-6 in spontaneously hypertensive rats (SHR). Pharmacol Rep 59: 437–446.

Carta S, Tassi S, Pettinati I, Delfino L, Dinarello CA, Rubartelli A (2011) The rate of interleukin-1beta secretion in different myeloid cells varies with the extent of redox response to Toll-like receptor triggering. J Biol Chem 286: 27069–27080.

Cerretti DP, Kozlosky CJ, Mosley B, Nelson N, Van Ness K, Greenstreet TA, March CJ, Kronheim SR, Druck T, Cannizzaro LA (1992) Molecular cloning of the interleukin-1 beta converting enzyme. Science 256: 97–100.

Dalekos GN, Elisaf M, Bairaktari E, Tsolas O, Siamopoulos KC (1997) Increased serum levels of interleukin-1beta in the systemic circulation of patients with essential hypertension: additional risk factor for atherogenesis in hypertensive patients? J Lab Clin Med 129: 300–308.

Dinarello CA (2011a) A clinical perspective of IL-1beta as the gatekeeper of inflammation. Eur J Immunol 41: 1203–1217.

Dinarello CA (2011b) Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood 117: 3720–3732.

Dostert C, Petrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J (2008) Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. Science 320: 674–677.

Ferrari D, Chiozzi P, Falzoni S,Dal Susino M, Melchiorri L, Baricordi OR, Di Virgilio F (1997) Extracellular ATP triggers IL-1 beta release by activating the purinergic P2Z receptor of human macrophages. J Immunol 159: 1451–1458.

Inokuchi Y, Morohashi T, Kawana I,Nagashima Y, Kihara M, Umemura S (2005) Amelioration of 2,4,6-trinitrobenzene sulphonic acid induced colitis in angiotensinogen gene knockout mice. Gut 54: 349–356.

Kher N, Marsh JD (2004) Pathobiology of atherosclerosis-a brief review. Semin Thromb Hemost 30: 665–672.

Latz E (2010) The inflammasomes: mechanisms of activation and function. Curr Opin Immunol 22: 28–33.

Mariathasan S, Weiss DS, Newton K, McBride J, O'Rourke K, Roose-Girma M, Lee WP, Weinrauch Y, Monack DM, Dixit VM (2006) Cryopyrin activates the inflammasome in response to toxins and ATP. Nature 440: 228–232.

Mariathasan S, Weiss DS, Newton K, McBride J, O'Rourke K, Roose-Girma M, Lee WP, Weinrauch Y, Monack DM, Dixit VM (2006) Cryopyrin activates the inflammasome in response to toxins and ATP. Nature 440: 228–232.

Martinon F, Mayor A, Tschopp J (2009) The inflammasomes: guardians of the body. Annu Rev Immunol 27: 229–265.

Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J (2006) Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 440: 237–241.

Masters LS, Simon A, Aksentijevich I, Kastner DL (2009) Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease. Annu Rev Immunol 27: 621–668.

Masters SL, Dunne A, Subramanian SL, Hull RL, Tannahill GM, Sharp FA, Becker C, Franchi L, Yoshihara E, Chen Z, Mullooly N, Mielke LA, Harris J, Coll RC, Mills KH, Mok KH, Newsholme P, Nuñez G, Yodoi J, Kahn SE, Lavelle EC, O'Neill LA (2010) Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1beta in type 2 diabetes. Nat Immunol 11: 897–904.

Masters SL,Dunne A,Subramanian SL,Hull RL, Tannahill GM, Sharp FA, Becker C, Franchi L, Yoshihara E, Chen Z, Mullooly N, Mielke LA, Harris J, Coll RC, Mills KH, Mok KH, Newsholme P, Nuñez G, Yodoi

J, Kahn SE, Lavelle EC, O'Neill LA (2010) Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1 beta in type 2 diabetes. Nat Immunol 11: 897–904.

Mitroulis I, Skendros P, Ritis K (2010) Targeting IL-1beta in disease; the expanding role of NLRP3 inflammasome. Eur J Intern Med 21: 157–163.

Miyoshi M, Nagata K, Imoto T,Goto O, Ishida A, Watanabe T (2003) ANG II is involved in the LPS-induced production of proinflammatory cytokines in dehydrated rats. Am J Physiol Regul Integr Comp Physiol 284: R1092-R1097.

Nakashima H, Suzuki H, Ohtsu H,Chao JY, Utsunomiya H, Frank GD, Eguchi S (2006) Angiotensin II regulates vascular and endothelial dysfunction: recent topics of Angiotensin II type-1 receptor signaling in the vasculature. Curr Vasc Pharmacol 4: 67–78.

Nee LE, McMorrow T,Campbell E,Slattery C, Ryan MP (2004) TNF-alpha and IL-1beta-mediated regulation of MMP-9 and TIMP-1 in renal proximal tubular cells. Kidney Int 66: 1376–1386.

Ogawa S, Mori T, Nako K,Kato T, Takeuchi K, Ito S (2006) Angiotensin II type 1 receptor blockers reduce urinary oxidative stress markers in hypertensive diabetic nephropathy. Hypertension 47: 699–705.

Rajamäki K, Lappalainen J, Oörni K,Välimäki E, Matikainen S, Kovanen PT, Eklund KK (2010) Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation. PLoS One 5: e11765.

Ross R (1999) Atherosclerosis-an inflammatory disease. N Engl J Med 340: 115–126.

Sadoshima J (2002) Novel AT1 receptor-independent functions of losartan. Circ Res 90: 754–756.

Satoh M, Fujimoto S, Arakawa S,Yada T, Namikoshi T, Haruna Y, Horike H, Sasaki T, Kashihara N (2008) Angiotensin II type 1 receptor blocker ameliorates uncoupled endothelial nitric oxide synthase in rats with experimental diabetic nephropathy. Nephrol Dial Transplant 23: 3806–3813.

Schroder K, Tschopp J (2010) The inflammasomes. Cell 140: 821–832.

Silveira KD, Coelho FM, Vieira AT, Barroso LC, Queiroz-Junior CM, Costa VV, Sousa LF, Oliveira ML, Bader M, Silva TA, Santos RA, Silva AC, Teixeira MM (2013) Mechanisms of the anti-inflammatory actions of the angiotensin type 1 receptor antagonist losartan in experimental models of arthritis. Peptides 46: 53–63.

Simon A, van der Meer JW (2007) Pathogenesis of familial periodic fever syndromes or hereditary autoinflammatory syndromes. Am J Physiol Regul Integr Comp Physiol 292: R86–98.

Thornberry NA, Bull HG, Calaycay JR, Chapman KT, Howard AD, Kostura MJ, Miller DK, Molineaux SM, Weidner JR, Aunins J (1992) A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. Nature 356: 768–774.

Tschopp J, Schroder K (2010) NLRP3 inflammasome activation:The convergence of multiple signaling pathways on ROS production? Nat Rev Immunol 10: 210–215.

van de Veerdonk FL, Netea MG, Dinarello CA, Joosten LA (2011) Inflammasome activation and IL-1beta and IL-18 processing during infection. Trends Immunol 32: 110–116.

Vesey DA, Cheung C, Cuttle L, Endre Z, Gobe G, Johnson DW (2002) Interleukin-1beta stimulates human renal fibroblast proliferation and matrix protein production by means of a transforming growth factor-beta-dependent mechanism. J Lab Clin Med 140: 342–350.

Vesey DA, Cheung CW, Cuttle L, Endre ZA, Gobé G, Johnson DW (2002) Interleukin-1beta induces human proximal tubule cell injury, alpha-smooth muscle actin expression and fibronectin production. Kidney Int 62: 31–40.

Wen H, GrisD, Lei Y, Jha S, Zhang L, Huang MT, Brickey WJ, Ting JP (2011) Fatty acid-induced NLRP3–ASC inflammasome activation interferes with insulin signaling. Nat Immunol 12: 408–415.

Zhao R, Zhou H, Su SB (2013) A critical role for interleukin-1β in the progression of autoimmune diseases. Int Immunopharmacol 17: 658–669.

Zhou R, Tardivel A, Thorens B,Choi I, Tschopp J (2010) Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol 11: 136–140.