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Role of NLRP3 and CARD8 in the regulation of TNF-α induced IL-1β release in vascular smooth muscle cells

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Abstract. Interleukin (IL)-1β is known to be activated by the inflammasome. Inflammasome activities depend on a plethora of moieties including NLRP3 and CARD8, which have been reported to be associated with several inflammatory diseases. Aortic smooth muscle cells (AOSMCs) were transfected with siRNA targeting the *NLRP3* and *CARD8* genes, followed by tumor necrosis factor-α (TNF-α) treatment. We found that TNF-α induces *IL-1β, IL-1R*a and *NLRP3* genes but not CARD8. Silencing of the NLRP3 gene significantly decreased IL-1β expression and release, the *IL-1R*a expression showed a borderline non-significant increment, while *CARD8* knock-down did not affect the *IL-1β* and *IL-1R*a mRNA expression or IL-1β protein release. Our results suggest that mainly NLRP3 plays a role in the regulation of IL-1β expression and release in AOSMC and could be a potential future target for the treatment of atherosclerosis and other inflammatory diseases.

Introduction

Atherosclerosis is the major cause of cardiovascular disease (CVD), which is the leading cause of death in developed countries (1). During the last decade, increasing amount of evidence supports chronic systemic vascular inflammation as an essential requirement for the progression of atherosclerosis and that the innate and adaptive immune cells play an integral role (2). Activation of vascular endothelial and smooth muscle cells by several endogenous or exogenous moieties causes up regulation of inflammatory cytokines like interleukin (IL)-1β and IL-18, augment proliferation, accumulation of oxidized low density lipoprotein (oxLDL) and extracellular matrix (ECM) components by the activation of intracellular pattern-recognition receptors (PRRs) (3,4).

IL-1β, IL-1α and IL-1 receptor antagonist (Ra) are members of the IL-1 family protein encoded by genes located on chromosome 2 (5). IL-1Ra is known to be a natural competitor for similar binding sites with IL-1β, which is an important mediator of cellular processes such as differentiation, proliferation and apoptosis (5). IL-1β has been implicated for the recruitment of inflammatory cells to infection sites, while IL-18 is important for IFN-γ production and enhancement of natural killer cell activity (5,6). IL-1β and IL-18 are produced from their 31 and 24 kDa inactive forms to their 17 and 18 kDa bio-active forms, respectively, through a proteolytic cleavage by caspase-1 and this depends on a complex protein platform called the inflammasome (7-9).

Previous studies have identified the NLRP1, NLRP3, IPAF/NLRC4 and AIM2 inflammasomes to activate caspase-1 (10). These complex proteins consist of three major components: a receptor [nucleotide oligomerisation domain (NOD)-like receptor (NLR)], which acts as an intracellular sensor and induces complex formation; in most cases an adaptor protein [apoptosis speck-like protein containing (ASC) a caspase recruitment domain (CARD) and caspase-1] (10). It has been hypothesized that upon stimulation of the inflammasome receptors by exogenous and endogenous stimuli like pathogens, cellular stress, low K^+ concentrations, crystals or uric acid, the receptors undergo conformational changes forming inflammasome complexes by either recruiting an ASC via their pyrin-pyrin interaction before binding to caspase-1, or binding directly to pro-caspase-1 through CARD-CARD interaction (9,11-13). This leads to activation of caspase-1, which in turn converts inactive IL-1β to its active form (14). Unlike caspase-1 and NLRs that are expressed in resting cells, *IL-1β* mRNA is believed to emanate from the activation of the NF-κB pathway; or microbial induction via Toll-like receptor,

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Abbreviations: IL, interleukin; NLRP, NLR family, containing pyrin domain; CARD, caspase recruitment domain; AOSMCs, aortic smooth muscle cells; siRNA, short interfering RNA; TNF-α, tumor necrosis factor-α; Ra, receptor antagonist; CVD, cardiovascular disease; oxLDL, oxidized low density lipoprotein; ECM, extracellular matrix; PRR, pattern-recognition receptor; IFN-γ, interferon γ; NLR, NOD-like receptor or NOD and LRR containing; NLRC, NLR family, containing CARD domain; AIM2, absent in melanoma 2; NOD, nucleotide-binding oligomerization domain; ASC, apoptosis speck-like protein contaning CARD; NF-κB, nuclear factor-κ-light-chain-enhancer of activated B cells; IBD, inflammatory bowel disease; RA, rheumatoid arthritis; PBS, phosphate-buffered saline; BSA, bovine serum albumin

Key words: atherosclerosis, NLRP3, inflammasome, CARD8, vascular cells, inflammation

C-type lectin receptor or RIG-1-like receptor's ligand binding; or through MyD88 activation via IL-1 receptor binding (10).

CARD8 is a member of the CARD family and has been implicated to be a co-regulator in both inflammatory and apoptotic signaling pathways, although their exact role in these processes remain elusive and thus needs to be further explored (15). The *CARD8* gene is located on chromosome 19q13 (16) and polymorphisms in this gene have been associated with several auto-inflammatory diseases, such as inflammatory bowel disease (IBD) and rheumatoid arthritis (RA) (17,18). Five isoforms of CARD8 have been identified but a contradictory functional role has been reported between the 48- and 54-kDa isoforms (19). The 48-kDa isoform has been implicated to interact directly with caspase-1 to induce apoptosis, while the 54-kDa isoform suppresses caspase mediated apoptosis (19,20). The isoforms have been reported to show a different expression pattern in different tissues and tumor cell lines (19).

Concurrently, studies have been carried out to explore the functional role of NLRP3 and CARD8 mainly in inflammatory (monocytes and macrophages) and cancer cells, but less is known about their functional role in vascular cells. Due to the expression of IL-1 family proteins in vascular cells and in atherosclerotic plaque (21-23) together with detrimental effects associated with excess IL-1β, it is therefore crucial to understand how vascular cells sense infection and metabolic stress and initiate vascular cell inflammation. The aim of this study is to examine the actual role of NLRP3 inflammasome and CARD8 protein in IL-1β expression and release from aortic smooth muscle cells (AOSMCs).

Materials and methods

Materials. Human AOSMC, OPTI-MEM reduce serum medium, Lipofectamine 2000, AOSMC Basal medium (M-231-500) and specific primers for Tucan 47F, Tucan 48F, Tucan 54F and Tucan Ex-9-R (19); and TaqDNA polymerase (18038/042) were purchased from Invitrogen (Stockholm, Sweden), Universal negative control and specific siRNA's for *NLRP3* and *CARD8* were obtained from Sigma-Aldrich (Stockholm, Sweden), E.Z.N.A. total-RNA kit I from Omega Biotech (Doraville, GA, USA), 2X TaqMan Universal PCR Master Mix and probes for gene expression analysis were from Applied Biosystems (Foster City, CA), tumor necrosis factor-α (TNF-α) from PeproTech (Stockholm, Sweden), IL-1β and IL-1Ra kit for ELISA was obtained from DuoSet® Development System (R&D Systems, UK). Primary antibodies against NLRP3 (MAB3724) and CARD8 (PAB0218) were obtained from Abnova Corp. (Taipei City, Taiwan). 5X Green Go reaction buffer (M719A) was purchased from Promega Biotech AB (Stockholm, Sweden).

Cell culture. Human AOSMC cells were grown in smooth muscle cell growth medium with medium change every second day. For experiments, passages 5-10 cells were used and $2.5x10^5$ cells/well were seeded in 6-well plates and treated with or without TNF-α for 1, 6, 24 or 48 h. Cell culture supernatants were used for measuring IL-1β and IL-1Ra expression.

Knockdown of NLRP3 and CARD8. Knockdown of NLRP3 and CARD8 were performed on $2x10^5$ cells/well seeded in 6-well plates. The transfection mixture was prepared by using 4 μl of Lipofectamine 2000 and 10 or 20 pmol of *NLRP3* (Hs0200313821) or *CARD8* (Hs0034180) siRNA, respectively, to 500 μl of Opti-MEM. The tubes were incubated for 20 min. The mixture was then added to cells and incubated for 8 h followed by addition of antibiotic free growth medium and incubated for 24 h. Media was then replaced and cells stimulated with 50 ng/ml TNF-α and incubated for further 24 h.

Quantitative real-time-polymerase chain reaction (qRT- PCR). In accordance with the manufacturer's instruction, total-RNA was extracted using the E.Z.N.A. total-RNA kit. Using random polyhexamers, RNaseOUT and Superscript II, 0.75 mg of RNA was reverse-transcribed into cDNA. For gene expression analysis, *IL-1β* (Hs001740097), *NLRP3* (Hs00366465), *IL-1RN* (Hs00277299), *CARD8* (Hs01088228) and *cyclophilin A* (Hs04194521) were used, cDNA was amplified in the 7900HT Fast Real-Time PCR system (Applied Biosystems) on fast optical 96-well plates according to the manufacturer's instructions. Each sample was analyzed in duplicate and values obtained were normalized with *cyclophilin A*.

Enzyme-linked immunosorbent assay (ELISA). We employed the ELISA technique for the detection of IL-1β and IL-1Ra secreted in cell culture supernatant using the DuoSet® Development System kits. In accordance with the manufacturer's instruction with slight modification, 0.05% Tween-PBS was used as reagent diluent in place of 1% BSA. Samples were diluted in equal volumes with 0.05% Tween-PBS for IL-1β quantification, but not for IL-1Ra, and each sample was analyzed in duplicate.

Reverse transcription-polymerase chain reaction (RT-PCR). cDNA obtained from reverse transcriptase as mentioned above was amplified in a 50 μl PCR mixture. Tucan-54F/Ex9-R, Tucan-48F/Ex9-R and Tucan-47F/Ex9-R were used as forward and reverse primer pairs as previously described (19). The PCR mixture was amplified as follows: 10 min at 94˚C followed by 40 cycles at 45 sec at 94˚C, 30 sec at 58˚C, and 90 sec at 72˚C. The cycling procedure was followed by 10 min extension at 72˚C. The PCR mixture was viewed under UV light post electrophoresis on an ethidium bromide stained 1% agarose gel.

Western blot analysis. Protein lysates from transfected ASOMC were subjected to electrophoresis on 8 or 12% SDS-polyacrylamide gels and further transfered to PVDF membrane. Membranes were blocked with 5% non-fat milk in 0.01% Tween-PBS with shaking for 4 h and incubated overnight with primary antibodies (1:1,000) at 4˚C, followed by 2 h incubation with 1:1,000 dilution of secondary antibody for NLRP3 (anti-mouse IgG from GE Healthcare, Uppsala Sweden) and CARD8 (anti-rabbit IgG from AH Diagnostics, Stockholm, Sweden).

Statistical analysis. The independent two-tailed Student's t-test and one way ANOVA was used for data analysis and results are expressed as mean ± SD. The P-value was considered to be statistically significant at $P \leq 0.05$.

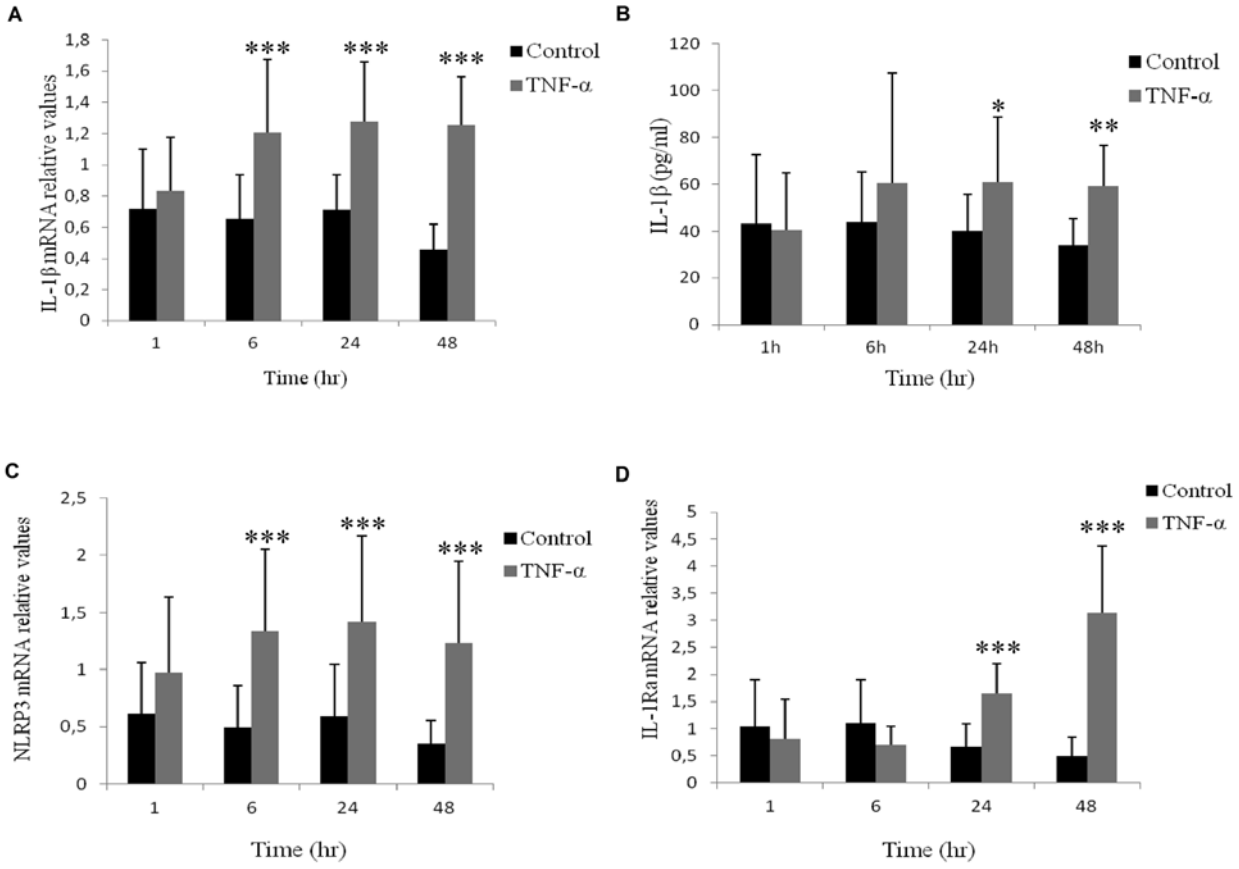

Figure 1. Effects of 50 ng/ml TNF-α at different times points (1-48 h), on the expression and release of IL-1β and its regulators in AOSMCs compared to the unstimulated controls. (A) qRT-PCR expression of *IL-1β* mRNA, (B) ELISA quantification of IL-1β protein secreted, (C) qRT-PCR expression pattern of *NLRP3* mRNA, (D) qRT-PCR expression pattern of *IL-1Ra* mRNA. (A, C and D) All values were normalized with *cyclophilin A*. $^{*}P \leq 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

Results

Effects of TNF-α on the expression and release of IL-1β and its regulators in AOSMCs. qRT-PCR revealed that stimulation of AOSMC with 50 ng/ml of TNF-α for different time points (1-48 h), induces a 2-3-fold increment of *IL-1β* mRNA expression, compared to their unstimulated controls (Fig. 1A). Furthermore, ELISA was used to quantify IL-1β protein release to the media from AOSMC stimulated with 50 ng/ml TNF-α and significant increases were observed after 24 and 48 h (Fig. 1B). We further analyzed the effects of TNF-α on IL-1β regulators: IL-1Ra, which is a natural competitor with IL-1β for the binding site to the IL-1 receptors; NLRP3, which is essential for NLRP3 inflammasome assembly; and CARD8 a co-inflammatory regulator. qRT-PCR revealed that stimulation of AOSMC with 50 ng/ml of TNF-α for different time points (1-48 h), induces a 1-3-fold increase in *NLRP3* mRNA expression at time points 6, 24 and 48 h and a 2.5-6-fold increment of *IL-1R*a mRNA expression at 24 and 48 h, compared to their respective unstimulated controls (Fig. 1C and D). *CARD8* levels were not affected by TNF-α (data not shown).

Effects of NLRP3 knockdown on IL-1β expression and release. Specific siRNAs were used to knockdown the NLRP3 gene to investigate the effect on the expression and release of IL-1β in AOSMC (Fig. 2A). The knockdown of NLRP3 was associated with a significant decrease in IL-1β expression and release (Fig. 2B and C), while a non-significant, but borderline increment of *IL-1R*a mRNA expression was revealed (P=0.07; Fig. 2D). We also found that there was no difference in IL-1Ra protein release 24 h after *NLRP3* knockdown, compared to the control (data not shown).

Effects of CARD8 knockdown on IL-1β expression and release. CARD8 has been reported to inhibit caspase-1 activity and also negatively regulate NF-κB activation in monocytes and other cell lines (15). We therefore hypothesized that knockdown of CARD8 may increase the expression and release of IL-1β. However, no significant change was evident in *IL-1β* mRNA (Fig. 3B) and protein release (Fig. 3C) but the *IL-1R*a mRNA expression showed a non-significant, but borderline increment (P=0.08) (Fig. 3D) in AOSMC after knockdown of *CARD8*.

Expression of CARD8 isoforms in AOSMCs. Considering the contradictory functional role reported between the CARD8 (T54 and T48) isoforms (19), we quest to determine which isoforms of CARD8 are expressed in AOSMC. We found that AOSMC express the T47, T48 and T54 isoforms and they were all knockdown by our siRNA. THP1 cells were used as positive control since it has previously been shown to express several CARD8 isoforms (19). Our results show that T54 is 250 bp, T48 is 200 bp while T47 is 300 bp (Fig. 4).

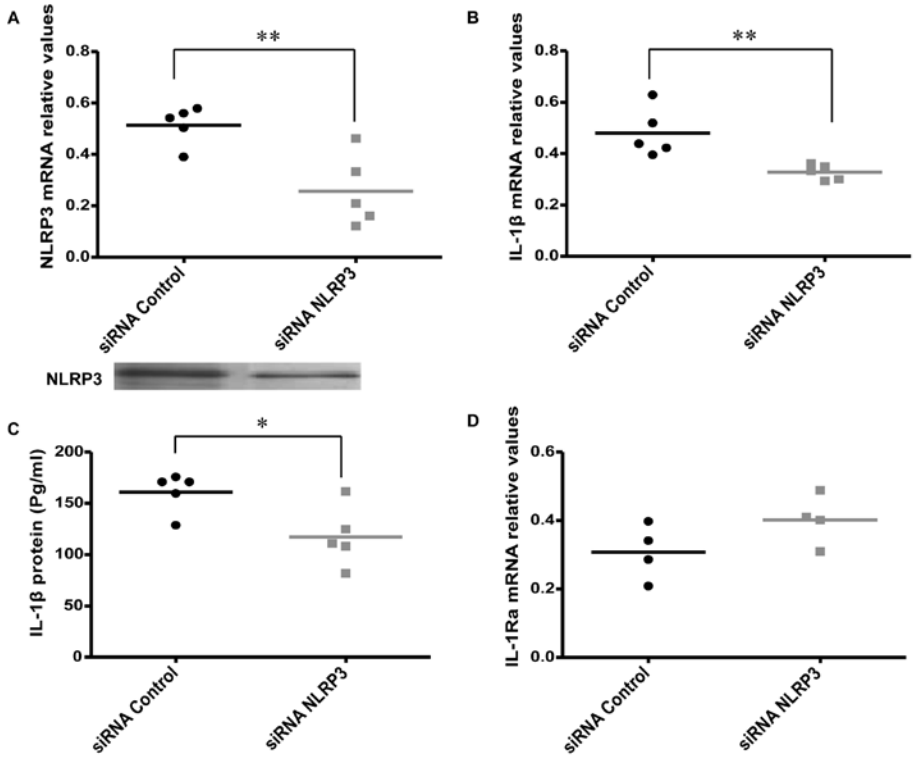

Figure 2. NLRP3 regulates IL-1β expression and secretion. AOSMC transfected with NLRP3 siRNA, followed by 24 h treatment with 50 ng/ml TNF-α, compared to the control without siRNA treatment. (A) Expression pattern of *NLRP3* mRNA and protein. (B) Expression pattern of *IL-1β* mRNA. (C) IL-1β protein secreted. (D) Expression pattern of *IL-1R*a mRNA. (A, B and D) In all experiments, values were normalized with *cyclophilin A*. n=5, *P≤0.05, **P<0.01 and ***P<0.001.

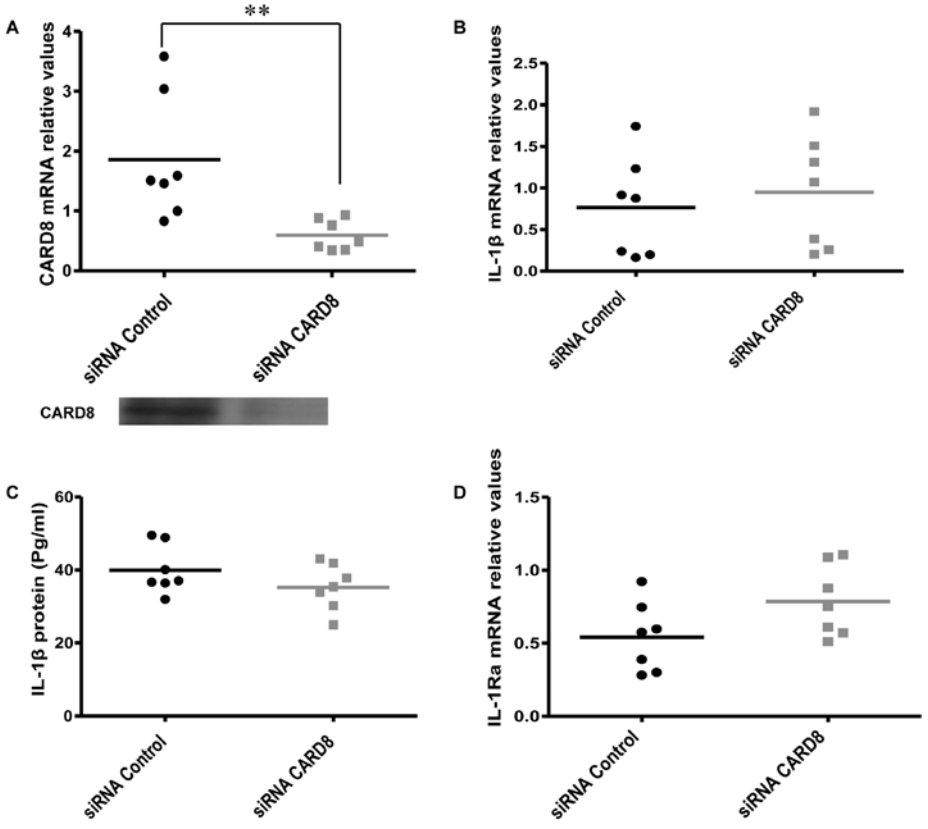

Figure 3. CARD8 regulates IL-1β expression and secretion. AOSMC were transfected with *CARD8* siRNA, followed by 24 h treatment with 50 ng/ml TNF-α, compared to the control without siRNA treatment. (A) Expression pattern *CARD8* mRNA and protein. (B) Expression pattern of *IL-1β* mRNA. (C) IL-1β protein secreted. (D) Expression pattern of *IL-1R*a mRNA. (A, B and D) In all experiments, values were normalized with *cyclophilin A*. n=7, *P≤0.05, **P<0.01 and ***P<0.001.

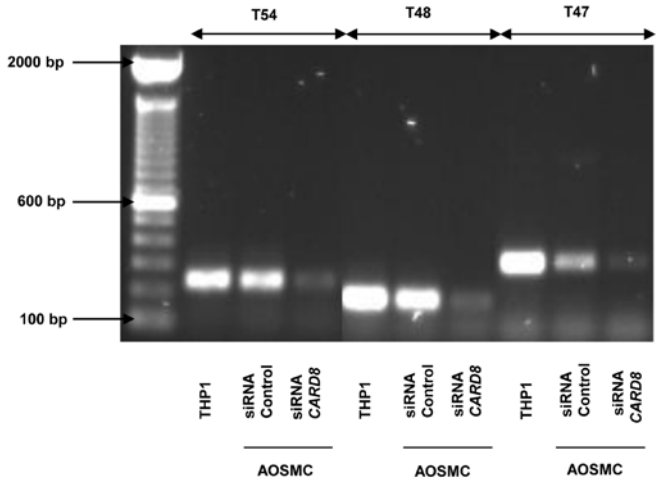

Figure 4. Comparative RT-PCR reveals that AOSMC express TUCAN 47, 48 and 54 isoforms (T47, T48 and T54, respectively). mRNA amplification of the three main isoforms was performed using the primer pair Tucan-54F/Tucan-Ex9-R for T54, Tucan-48F/Tucan-Ex9-R for T48 and Tucan-47F/Tucan-Ex9-R for T47. AOSMC were transfected with *CARD8* siRNA, followed by 24 h treatment with 50 ng/ml TNF-α, compared to the control without siRNA treatment. THP1 cells were used as control.

Discussion

Existing evidence suggests that vascular cells actively contribute to atherogenic inflammation as they express several inflammatory moieties, which are overexpressed in atherosclerotic plaques. It is now evident that NLRP3 forms an integral part of the inflammasome that catalyzes the maturation of inactive IL-1β and IL-18 (24). CARD8 on the other hand has been reported to be an inhibitor of caspase-1 and a negative regulator of NF-κB and conflicting evidence has been shown regarding CARD8 and some inflammatory diseases like IBD and RA (15,17,18,25).

Consistent with other studies, we also found that TNF-α affects *IL-1β* expression and release (21,26). Expression of *IL-1β* and *NLRP3* mRNA increased proportionally with time, while *IL-1R*a significantly increased at 24 and 48 h after treatment. This suggests that, during cellular stress or exposure to a foreign ligand, elevated levels of IL-1β are required to execute its protective role, but over time excess production of IL-1β could become detrimental. Thus, the increase of IL-1Ra after 24 h could be to compensate for IL-1β activity and to maintain cellular homeostasis. In addition, our data supports the hypothesis that pathogens, cellular stress or cytokines (TNF-α) stimulate NLR's to form inflammasome complexes which leads to the activation and secretion of IL-1β. To study the role of NLRP3 and CARD8 in *IL-1β* expression and release from AOSMC, cells were transfected with either siRNA against the *NLRP3* or *CARD8* genes. We observed that knockdown of *NLRP3* led to a decrease of *IL-1β* mRNA and protein secretion. Our results are similar to findings reported by Zhu *et al* (27), who showed that silencing of the *NLRP3* gene using siRNA, decreased the IL-1β release from Kupffer cells, hence antagonizing liver ischemic injury. The reason for the decreased *IL-1β* mRNA after *NLRP3* knockdown is unclear, but we suggest that, *IL-1β* mRNA may undergo self regulation, due to the decrease in IL-1β protein secreted from the cells. Our results are consistent with studies carried out by Yamasaki *et al* (28), and Kankkunen *et al* (29). On the other hand, from the observation that CARD8 is an inhibitor of caspase-1 and a negative regulator of NF-κB (15), we derived a hypothesis that knockdown of the *CARD8* gene may augment caspase-1 and NF-κB activities and thus increase the *IL-1β* expression and release. However, no significant change in *IL-1β* mRNA or protein release was evident.

Contrary to NLRP3, the role of *CARD8* in *IL-1β* and *IL-1R*a expression and release in AOSMCs may therefore be limited. However, a contradictory functional role has been demonstrated in the CARD8 isoforms as they show different expression pattern in different cell types and tumor cell lines with some cells expressing both (19). We further sought to determine which isoforms of *CARD8* are expressed in AOSMC and found an expression of the 47, 48 and 54 kDa isoforms and that they were all knockdown by siRNA. However, due to lack of studies regarding CARD8, more studies are required to fully elucidate the functions of CARD8 and to demonstrate its role in inflammasome formation and activity.

In conclusion, our results show that TNF-α induces IL-1β, IL-1Ra and NLRP3 but not CARD8; knockdown of *NLRP3* gene significantly decreases 1L-1β expression and release and AOSMC express the 47, 48 and 54 kDa isoforms of *CARD8*, but the effect of *CARD8* on IL-1β and IL-1Ra seems to be limited. Our data therefore suggest that NLRP3 but not CARD8 play an important role in IL-1β expression and release in AOSMC and could be a therapeutic target or marker for atherosclerosis. Increased understanding of the mechanisms of initiation and progression of atherogenic vascular inflammation may lead to new approaches in the development of novel therapeutics for atherosclerosis and other inflammatory diseases.

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References

1. Libby P: Inflammation in atherosclerosis. Nature 420: 868-874, 2002.
2. Hansson GK and Hermansson A: The immune system in atherosclerosis. Nat Immunol 12: 204-212, 2011.
3. Westphal E, Herzberg M, Neumann I, Beibei L, Pilowski C, Li C, Werdan K and Loppnow H: Neutrophils process interleukin-1beta and interleukin-18 precursors in a caspase-1-like fashion - processing is inhibited by human vascular smooth muscle cells. Eur Cytokine Netw 17: 19-28, 2006.
4. Loppnow H, Werdan K and Buerke W: Vascular cells contribute to atherosclerosis by cytokine- and innate-immunity-related inflammatory mechanisms. Innate Immun 14: 63-87, 2008.
5. Gabay C, Lamacchia C and Palmer G: IL-1 pathways in inflammation and human diseases. Nat Rev 6: 232-241, 2010.
6. Sahoo M, Ceballos-Olvera I, del Barrio L and Re F: Role of the inflammasome, IL-1β, and IL-18 in bacterial infections. ScientificWorldJournal 11: 2037-2050, 2011.
7. Netea MG, Simon A, van de Veerdonk F, Kullberg BJ, van der Meer JW and Joosten LA: IL-1beta processing in host defense: beyond the inflammasomes. PLoS Pathog 6: e1000661, 2010.
8. Gracie JA, Robertson SE and McInnes IB: Interleukin-18. J Leukoc Biol 73: 213-224, 2003.
9. Schroder K and Tschopp J: The inflammasomes. Cell 140: 821-832, 2010.

10. Bauernfeind F, Ablasser A, Bartok E, Kim S, Schmid-Burgk J, Cavlar T and Hornung V: Inflammasomes: current understanding and open questions. Cell Mol Life Sci 68: 765-783, 2011.
11. Martinon F, Pétrilli V, Mayor A, Tardivel A and Tschopp J: Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 440: 237-241, 2006.
12. Pétrilli V, Papin S, Dostert C, Mayor A, Martinon F and Tschopp J: Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. Cell Death Diff 14: 1583-1589, 2007.
13. Martinon F, Agostini L, Meylan E and Tschopp J: Identification of bacterial muramyl dipeptide as activator of the NALP3/cryopyrin inflammasome. Curr Biol 14: 1929-1934, 2004.
14. Agostini L, Martinon F, Burns K, McDermott MF, Hawkins PN and Tschopp J: NALP3 forms an IL-1β-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. Immunity 20: 319-325, 2004.
15. Razmara M, Srinivasula SM, Wang L, Poyet JL, Geddes BJ, DiStefano PS, Bertin J and Alnemri ES: CARD-8 protein, a new CARD family member that regulates caspase-1 activation and apoptosis. J Biol Chem 277: 13952-13958, 2002.
16. Zhang H and Fu W: NDPP1 is a novel CARD domain containing protein which can inhibit apoptosis and suppress NF-kappaB activation. Int J Oncol 20: 1035-1040, 2002.
17. Fontalba A, Martinez-Taboada V, Gutierrez O, Pipaon C, Benito N, Balsa A, Blanco R and Fernandez-Luna JL: Deficiency of the NF-kappaB inhibitor caspase activating and recruitment domain 8 in patients with rheumatoid arthritis is associated with disease severity. J Immunol 179: 4867-4873, 2007.
18. McGovern DPB, Butler H, Ahmad T, Paolucci M, van Heel DA, Negoro K, Hysi P, Ragoussis J, Travis SPL, Cardon LR and Jewell DP: TUCAN (CARD8) genetic variants and inflammatory bowel disease. Gastroenterology 131: 1190-1196, 2006.
19. Bagnall RD, Roberts RG, Mirza MM, Torigoe T, Prescott NJ and Mathew CG: Novel isoforms of the CARD8 (TUCAN) gene evade a nonsense mutation. Eur J Hum Genet 16: 619-625, 2008.
20. Yamamoto M, Torigoe T, Kamiguchi K, Hirohashi Y, Nakanishi K, Nabeta C, Asanuma H, Tsurama T, Sato T, Hata F, *et al*: A novel isoform of TUCAN is overexpressed in human cancer tissues and suppresses both caspase-8- and caspase-9-mediated apoptosis. Cancer Res 65: 8706-8714, 2005.
21. Wågsäter D, Jatta K, Ocaya P, Dimberg J and Sirsjö A: Expression of IL-1beta, IL-1 receptor type I and IL-1 receptor antagonist in human aortic smooth muscle cells: effects of all-trans-retinoic acid. J Vasc Res 43: 377-382, 2006.
22. Olofsson PS, Sheikine Y, Jatta K, Ghaderi M, Samnegård A, Eriksson P and Sirsjö A: A functional interleukin-1 receptor antagonist polymorphism influences atherosclerosis development. The interleukin-1beta:interleukin-1 receptor antagonist balance in atherosclerosis. Circ J 73: 1531-1536, 2009.
23. Galea J, Armstrong J, Gadsdon P, Holden H, Francis SE and Holt CM: Interleukin-1 beta in coronary arteries of patients with ischemic heart disease. Atheroscler Thromb Vasc Biol 16: 1000-1006, 1996.
24. Arend WP, Palmer G and Gabay C: IL-1, IL-18, and IL-33 families of cytokines. Immunol Rev 223: 20-38, 2008.
25. Fisher SF, Mirza MM, Onnie CM, Soars D, Lewis CM, Prescott NJ, Mathew CG, Sanderson J, Forbes A, Todhunter C, *et al*: Combined evidence from three large british association studies rejects TUCAN/CARD8 as an IBD susceptibility gene. Gastroenterology 132: 2078-2080, 2007.
26. Libby P, Ordovas JM, Auger KR, Robbins AH, Birinyi LK and Dinarello CA: Endotoxin and tumor necrosis factor induce interleukin-1 gene expression in adult human vascular endothelial cells. Am J Pathol 124: 179-185, 1986.
27. Zhu P, Duan L, Chen J, Xiong A, Q Xu, Zhang H, Zheng F, Tan Z, Gong F and Fang M: Gene silencing of NALP3 protects against liver ischemia-reperfusion injury in mice. Hum Gene Ther 22: 853-864, 2011.
28. Yamasaki K, Muto J, Taylor KR, Cogen AL, Audish D, Bertin J, Grant EP, Coyle AJ, Misaghi A, Hoffman HM and Gallo RL: NLRP3/cryopyrin is necessary for interleukin-1beta (IL-1beta) release in response to hyaluronan, an endogenous trigger of inflammation in response to injury. J Biol Chem 284: 12762-12771, 2009.
29. Kankkunen P, Teirila L, Rintahaka J, Alenius H, Wolff H and Matikainen S: (1,3)-beta-glucans activate both dectin-1 and NLRP3 inflammasome in human macrophages. J Immunol 184: 6335-6342, 2010.