Resveratrol Inhibits Inflammation Induced by Heat-Killed *Listeria monocytogenes*

Dae-Weon Park,¹ Jin-Sik Kim,¹ Byung-Rho Chin,² and Suk-Hwan Baek¹

¹Department of Biochemistry & Molecular Biology, Aging-Associated Vascular Disease Research Center; and ²Department of Dentistry; College of Medicine, Yeungnam University, Daegu, Republic of Korea.

ABSTRACT Resveratrol is a polyphenolic compound in red wine that has antioxidant and cardioprotective effects in animal models. *Listeria monocytogenes* is a pathogen that mainly affects immunocompromised individuals and is initially detected at the cell surface or in phagosomes by toll-like receptor 2. Many antioxidants also exert anti-inflammatory activities; therefore, we evaluated the anti-inflammatory properties of resveratrol by studying the various inflammatory responses induced by heat-killed *L. monocytogenes* (HKLM). Resveratrol strongly blocked HKLM-induced NADPH oxidase-1 mRNA and reactive oxygen species production by macrophages. Resveratrol also suppressed monocyte chemotactic protein-1 expression, cyclooxygenase-2 expression, prostaglandin production, inducible nitric oxide (NO) synthase expression, and NO production induced by HKLM. We investigated the signaling pathway involved in the resveratrol effect. HKLM stimulated glycogen synthase kinase 3 β (GSK3 β) and extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation. The involvement of GSK3 β and ERK1/2 was tested using inhibitors. While the GSK3 β inhibitor LiCl potentiated the effect of HKLM, the MEK inhibitor U0126 blocked these responses. Additionally, pretreatment with resveratrol blocked phosphorylation of both kinases induced by HKLM. These results suggest that HKLM is strong inducer of inflammatory mediators, and that the inhibitory effect of resveratrol may be mediated by the GSK3 β and ERK1/2 pathways.

KEY WORDS: • COX-2 • HKLM • iNOS • MCP-1 • NADPH oxidase 1 • resveratrol

INTRODUCTION

RESVERATROL IS A POLYPHENOL present in grapes and various seeds. Resveratrol protects plants from fungal infection and exhibits antioxidative, anti-inflammatory, and chemopreventative properties in mammals.¹ Additionally, resveratrol has been proposed to have antiatherosclerotic properties.² A number of animal model studies support this suggestion.³ Mechanisms proposed to explain the anti-inflammatory properties of resveratrol include inhibition of monocyte chemotactic protein-1 (MCP-1) production^{4,5} and control of prostaglandins (PG) levels. However, the precise mechanisms responsible for the anti-inflammatory effects of resveratrol remain unclear.

Listeria monocytogenes is a pathogenic food-borne bacterium that causes diseases such as listeriosis. *L. monocytogenes* resides in the host depending on key virulence factors such as internalin or listeriolysin O.^{6,7} However, animals and humans defend against *L. monocytogenes* through innate receptors. Toll-like receptor 2 (TLR2) is the most well-known innate receptor for defense against *L.*

monocytogenes.⁸ Recent reports have shown that TLR4 cooperates in the recognition and regulation of a *Listeria* infection.⁹ TLR2-deficient mice are more susceptible to *L. monocytogenes* infection than that of wild-type mice; that is, bacterial loads are higher, and macrophage activation is reduced in TLR2 knockout mice.¹⁰ Previous reports have suggested that participation of MyD88, an intracellular adaptor protein in TLR2 signaling, is essential for defense against *L. monocytogenes*.¹¹ However, TLR2 signaling through MyD88 induces complicated signaling events, leading to increased expression of proinflammatory cytokines, chemokines, and other effector molecules.¹² Recent studies have reported that TLR is related various diseases. TLR2 has been associated with rheumatoid arthritis, type I diabetes, ischemia per reperfusion injury, and atherosclerosis.^{13–16} However, research on various inflammatory mediators induced by heat-killed *L. monocytogenes* (HKLM) is insufficient, and further study is necessary.

The glycogen synthase kinase 3 β (GSK3 β) and β -catenin pathways are crucial in the balance between pro- and anti-inflammatory cytokine production. This pathway plays an essential role in inflammation and immune cells.^{17,18} In particular, many groups have shown that GSK3 β , through TLR signaling, is necessary for inflammation. For example, GSK3 β regulates TLR-mediated cytokine production, and inactivation of GSK3 β by LPS has a negative effect on production of the proinflammatory cytokine interferon- β .¹⁹

Manuscript received 3 February 2012. Revision accepted 5 June 2012.

Address correspondence to: Suk-Hwan Baek, Ph.D., Department of Biochemistry & Molecular Biology, Aging-Associated Vascular Disease Research Center, College of Medicine, Yeungnam University, 210 Main Building, 170 Hyeonchungno (317-1 Dae-myung-5 Dong), Nam-gu, Daegu 705-802, Republic of Korea, E-mail: sbaek@med.yu.ac.kr

This study focused on the inhibitory mechanism of resveratrol inflammatory mediator production by the TLR2 agonist HKLM. Our results indicated that resveratrol in macrophages had a significant protective role in the HKLM-induced inflammatory response through multiple mechanisms, and that the extracellular signal-regulated kinase 1/2 (ERK1/2) and GSK3 β pathways are important for the anti-inflammatory effect.

MATERIALS AND METHODS

Reagents and cell culture

Cell culture reagents, including fetal bovine serum, were obtained from Life Technologies (Grand Island, NY, USA). Antibodies against phosphor-GSK3 β , GSK3 β , phosphor-ERK1/2, and ERK1/2 were obtained from Cell Signaling Technology (Beverly, MA, USA), and HKLM was obtained from InvivoGen (San Diego, CA, USA). The inducible nitric oxide synthase (iNOS) antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and the cyclooxygenase-2 (COX-2) antibody was obtained from Cayman Chemical (Ann Arbor, MI, USA). An MCP-1 immunoassay kit was obtained from R&D Systems (Minneapolis, MN, USA). Resveratrol, NADPH, lucigenin, and LiCl were obtained from Sigma-Aldrich (St. Louis, MO, USA). Carboxy-H₂-DCFDA was obtained from Life Technologies (Carlsbad, CA, USA), and various inhibitors were purchased from Merck KGaA (Darmstadt, Germany). The Raw264.7 macrophage cell line was obtained from the American Type Culture Collection (Manassas, VA, USA).

Measurement of reactive oxygen species generation

Production of reactive oxygen species (ROS), such as hydrogen peroxide, was determined by measuring changes in dichlorofluorescein diacetate (DCF-DA) fluorescence, an oxidation-sensitive fluorescence probe. Briefly, cells were seeded at 5×10^5 per well in six-well tissue culture plates. On the second day after seeding, cells were loaded with 1 mL fresh DMEM containing 50 μ M DCF-DA and incubated at 37°C for 40 min. The change in fluorescence intensity was monitored by flow cytometry using a FACSCalibur apparatus (Becton-Dickinson, Franklin Lakes, NJ, USA).

Measurement of NO generation

Synthesis of NO was determined by assaying culture supernatants for nitrite, the stable reaction product of NO. Briefly, 100 μ L of culture supernatant was reacted with 100 μ L of Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride, and 2.5% phosphoric acid) at room temperature for 10 min. The optical density of the assay sample was measured spectrophotometrically at 570 nm. A fresh culture medium served as the blank in all experiments. The nitrite concentration was calculated from a standard curve derived from the reaction of NaNO₂ under assay conditions.

Enzyme-linked immunosorbent assay

Supernatants from treated macrophages were collected and analyzed for PGE₂ or PGI₂ using sandwich enzyme-

linked immunosorbent assay (ELISA) kits (R&D Systems), according to the manufacturer's instructions.

Western blot analysis

Macrophages were cultured in six-well plates and treated with HKLM in the presence or absence of an inhibitor. Proteins were separated by 8% reducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotted onto nitrocellulose membranes in 20% methanol, 25 mM Tris, and 192 mM glycine. Membranes were then blocked with 5% nonfat dry milk and incubated with the primary antibody for 18 h. The membranes were then washed, incubated for 90 min with the secondary antibody conjugated to horseradish peroxidase, rewashed, and finally developed using an enhanced chemiluminescence system.

Reverse transcription–polymerase chain reaction and real-time polymerase chain reaction

Total RNA was extracted from cells using a total RNA Miniprep kit (Axygen Biosciences, Union City, CA, USA). One microgram of total RNA was used as a template to make first-strand cDNA by oligo-dT priming using the Promega Reverse Transcriptase System (Promega, Madison, WI, USA). Polymerase chain reaction (PCR) was then performed using specific primers for target genes, as well as primers for β -actin as an internal standard. Real-time PCR was performed using a LightCycler 1.5 Thermal Cycler (Roche Diagnostics, Almere, The Netherlands) with SYBR-Green I as the fluorescent dye, according to the manufacturer's instructions. The cDNA obtained was then amplified using two synthetic primers specific for mouse Nox-1, MCP-1, and β -actin. The fluorescent synthetic gene-specific primer sets used for PCR were (1) Nox1 forward primer, 5'-AAG TGGCTGTACTGGTTGG-3', and reverse primer, 5'-GTGA GGAAGAGTCGGTAGTT-3', which amplified 238 bp of mouse Nox1 cDNA; (2) MCP-1 forward primer, 5'-AGAGA GCCAGACGGGAGGAA-3', and reverse primer, 5'-GTCAC ACTGGTCACTCCTAC-3', which amplified 520 bp of mouse MCP-1 cDNA; (3) β -actin forward primer, 5'-AGAGGGAAA TCGTGCCTGAC-3', and reverse primer, 5'-CAATAGTG ATGACCTGGCCGT-3', which amplified 137 bp of mouse β -actin cDNA.

RESULTS

Resveratrol inhibits HKLM-induced Nox-1 expression and ROS generation

ROS are well-known inducers of inflammation, and Nox-1 is an ROS-producing enzyme in macrophages.²⁰ Stimulation of HKLM, a well-known TLR2 agonist, resulted in a rapid increase in Nox-1 mRNA expression in Raw264.7 cells (Fig. 1A). The effects of resveratrol on Nox-1 mRNA expression were confirmed using a real-time PCR analysis. Similar to Figure 1A, HKLM showed a time-dependent increase in Nox-1 mRNA expression, but pretreatment with resveratrol effectively inhibited the Nox-1 mRNA expression caused by HKLM (Fig. 1B). While HKLM induced

FIG. 1. Resveratrol suppresses heat-killed *Listeria monocytogenes* (HKLM)-induced NADPH oxidase-1 (Nox-1) expression and reactive oxygen species generation. (A) Raw264.7 cells were incubated for 1–6 h with 10^8 cells/mL HKLM. Total mRNAs were isolated at the end of incubation. Nox-1 mRNA expression was determined by reverse transcription–polymerase chain reaction (RT-PCR) and was normalized to that of β -actin. (B) Cells were pretreated with $30 \mu\text{M}$ resveratrol for 1 h and stimulated with 10^8 cells/mL HKLM for the indicated times. Nox-1 mRNA expression was determined by real-time PCR or RT-PCR. The Nox-1 mRNA level was normalized to that of β -actin. (C) Cells were changed to a fresh serum-free DMEM containing dichlorofluorescein diacetate (DCF-DA) ($50 \mu\text{M}$) for 40 min. The loaded cells were then incubated with 10^8 cells/mL HKLM in the absence or presence of $30 \mu\text{M}$ resveratrol, and changes in fluorescence intensity were monitored by flow cytometry. Each data point represents the mean $\pm$ standard deviation of three independent experiments (* $P < .05$ and ** $P < .01$).

ROS production in macrophages, pretreatment with resveratrol partially inhibited the effects of HKLM (Fig. 1C). These results suggest that resveratrol inhibits ROS production by blocking HKLM-mediated Nox-1 expression, but the partial effects suggest the possibility of other types of ROS production mechanisms by HKLM.

Resveratrol suppresses HKLM-induced MCP-1 expression

MCP-1 is an essential chemokine for initiating inflammation.²¹ The effects of HKLM on MCP-1 expression were verified by reverse transcription–PCR. Treatment with HKLM time-dependently elevated MCP-1 mRNA expression (Fig. 2A). Pretreatment with resveratrol decreased the HKLM effects almost down to basal levels (Fig. 2B). Additionally, pretreatment with N-acetyl cysteine (NAC), an ROS scavenger, also inhibited HKLM-induced MCP-1 expression (data not shown). Therefore, we suggest that the inhibition of MCP-1 by resveratrol results from HKLM-induced ROS inhibition.

Resveratrol inhibits HKLM-induced COX-2 and iNOS expression and their metabolite production

COX-2 and its metabolites are well-known inflammatory mediators. COX-2 expression by macrophages in the presence of HKLM and the effects of resveratrol were analyzed by Western blot. HKLM treatment significantly induced

FIG. 2. Resveratrol inhibits HKLM-induced monocyte chemotactic protein-1 (MCP-1) expression. (A) Raw264.7 cells were incubated for 1–6 h with 10^8 cells/mL HKLM. Total mRNAs were isolated at the end of incubation. MCP-1 mRNA expression was determined by RT-PCR and was normalized to that of β -actin. (B) Cells were pretreated with $30 \mu\text{M}$ resveratrol for 1 h and stimulated with 10^8 cells/mL HKLM for the indicated times. MCP-1 mRNA expression was determined by real-time PCR or RT-PCR. MCP-1 mRNA was normalized to that of β -actin. Each data point represents the mean $\pm$ standard deviation of three independent experiments (* $P < .05$ and ** $P < .01$).

FIG. 3. Resveratrol suppresses cyclooxygenase-2 (COX-2) expression induced by HKLM. (A) Cells were treated with the indicated concentrations of resveratrol for 1 h before stimulation with 10^8 cells/mL HKLM. Total lysates were isolated at the end of incubation, and COX-2 proteins were analyzed by Western blot. (B) Cells were pretreated with the indicated concentrations of resveratrol for 1 h and stimulated with 10^8 cells/mL HKLM for 6 h. Prostaglandin E₂ (PGE₂) and PGI₂ levels in cell supernatants were analyzed with an enzyme immunoassay kit. Each data point represents the mean $\pm$ standard deviation of three independent experiments (** $P < .01$).

COX-2 expression in macrophages, whereas pretreatment with resveratrol dose-dependently decreased COX-2 expression (Fig. 3A). Changes in PGE₂ and PGI₂ production among COX-2 metabolites also demonstrated that resveratrol dose-dependently inhibits HKLM-induced COX-2 metabolite (PGE₂ and PGI₂) production (Fig. 3B). iNOS expression and NO production, also important inflammatory mediators, were analyzed by the Western blot and Griess reagent analyses. HKLM induced iNOS expression and NO production by macrophages, but pretreatment with resveratrol dose-dependently inhibited HKLM-induced iNOS expression (Fig. 4A) and NO production (Fig. 4B).

Both ERK1/2 and GSK3 β play a role in the resveratrol effect

Resveratrol inhibited production of various inflammatory mediators induced by HKLM. Studies of regulatory

FIG. 4. Resveratrol suppresses inducible nitric oxide synthase (iNOS) expression induced by HKLM. (A) Cells were treated with resveratrol at the indicated concentrations for 1 h before stimulation with 10^8 cells/mL HKLM. Total lysates were isolated at the end of incubation, and the iNOS protein was analyzed by Western blot. (B) Cells were pretreated with the indicated concentrations of resveratrol for 1 h and stimulated with 10^8 cells/mL HKLM for 24 h. NO amounts in cell supernatants were analyzed with the Griess reagent. Each data point represents the mean $\pm$ standard deviation of three independent experiments (* $P < .05$ and ** $P < .01$).

signaling proteins have shown a contribution by ERK1/2 and GSK3 β as well. HKLM induced phosphorylation of ERK1/2 and GSK3 β (Fig. 5A). However, pretreatment with resveratrol inhibited HKLM-induced ERK1/2 and GSK3 β phosphorylation (Fig. 5B). Specific inhibitors were used to confirm the direct participation of ERK1/2 and GSK3 β . Pretreatment with the ERK1/2 inhibitor U0126 inhibited HKLM-induced MCP-1, iNOS, and COX-2 protein expression (Fig. 5C). However, pretreatment with the GSK3 β inhibitor LiCl increased HKLM-induced MCP-1, iNOS, and COX-2 expression (Fig. 5D). These results show that ERK1/2 and GSK3 β are involved in HKLM-induced MCP-1, iNOS, and COX-2 expression.

DISCUSSION

We investigated the effects of resveratrol on inflammatory mediator production induced by HKLM, a TLR2 agonist. Resveratrol showed anti-inflammatory effects by inhibiting HKLM-induced Nox-1 expression, ROS production, MCP-1 expression, COX-2 expression, metabolite (PGE₂ and PGI₂) production, and NO production after iNOS expression. These results suggest that resveratrol prevents the severe inflammatory response by an *L. monocytogenes* infection by inhibiting various types of mediators.

TLR contributes to infection and septicemia at the early stage by fusing with bacterial ligands. TLR produces various

FIG. 5. Extracellular signal-regulated kinase 1/2 (ERK1/2) and glycogen synthase kinase 3 β (GSK3 β) are targets for the effects of resveratrol. **(A)** Raw264.7 cells were stimulated with 10^8 cells/mL HKLM for the indicated times. ERK1/2 and GSK3 β phosphorylation was determined by Western blot using phosphoprotein antibodies and was normalized to the total protein. **(B)** Cells were treated with 10^8 cells/mL HKLM in the absence or presence of 30 μ M resveratrol for the indicated times. ERK1/2 or GSK3 β phosphorylation was determined by Western blot using phosphoprotein antibodies and was normalized to the total protein. **(C)** Cells were treated with 10^8 cells/mL HKLM in the absence or presence of 1 μ M U0126. MCP-1 mRNA expression was determined by RT-PCR and was normalized to that of β -actin. iNOS and COX-2 proteins were analyzed by Western blot. **(D)** Cells were treated with 10^8 cells/mL HKLM in the absence or presence of 20 mM LiCl. MCP-1 mRNA expression was determined by RT-PCR and was normalized to that of β -actin. iNOS and COX-2 proteins were analyzed by Western blot.

inflammatory mediators such as cytokines, chemokines, PGEs, and NO.²² Among the many TLRs, TLR2 and TLR4 have been studied the most and are general TLRs that cause inflammation. There are many types of TLR2 ligands, including lipoproteins, and HKLM is known as a TLR2 ligand.²³

HKLM is Gram-positive coccobacillus heat-attenuated *L. monocytogenes*, which usually grows in clusters in a normal medium, and has a similar morphology to that of *Streptococcus*. While HKLM usually causes disease in

livestock, it infects humans as well, inducing intestinal diseases. HKLM is known as a TLR2 ligand, but research on its inflammatory mechanism is unclear. Our results confirmed that HKLM has a similar mechanism as that of other TLR2 ligands. HKLM induced MCP-1 expression, iNOS expression, NO production, as well as COX-2 and PG expression, resulting in inflammation. Additionally, HKLM significantly induced Nox-1 gene expression, which produces ROS. The HKLM mechanism is very similar to that of Pam3CSK4, a synthetic TLR2 ligand (data not shown). These results might provide a clue as to how *L. monocytogenes* induces inflammation.

ROS production induced by stimulating TLR2 has been reported previously,²⁴ but sources ROS within cells are diverse, leading to uncertainty of the ROS source. Recent reports have shown that *Mycobacterium* stimulates TLR2 receptors, and that TLR2 interacts with Nox-2 to produce ROS.²⁵ However, our results showed that Nox-1 expression is strongly induced by HKLM, suggesting that Nox-1 plays an essential role in ROS production caused by TLR2. While resveratrol dramatically inhibited Nox-1 expression, ROS production only decreased partially, suggesting the presence of other ROS sources.

Intracellular ROS production induces production of other types of mediators, including cytokines and chemokines, including the chemokine MCP-1. In particular, MCP-1 induces excess activation of macrophages and monocyte migration during initiation, eventually elevating the inflammatory response.^{26–28} HKLM-induced MCP-1 production was an anticipated result, but complete inhibition by resveratrol was a very exciting result. We speculate that inhibition of MCP-1 by resveratrol was probably induced by blocking ROS production. Many reports have shown that MCP-1 production is mediated by ROS.^{29–31} The effect of resveratrol on MCP-1 production was stronger than the most widely used antioxidant NAC. These results show that resveratrol is more effective than NAC in terms of general anti-inflammatory effects.

Recent interest in phenolic compounds derived from grapes has shown that they have a valuable function related to human health, and resveratrol is placed at the center of phenolic compound components. Grape components have cardiac reinforcing, antioxidant, anticancer, antiaging, and antibacterial effects,³² but the anti-inflammatory effect is the most important. The components of resveratrol have anti-inflammatory effects in rats, mice, and humans.³³ Bralley *et al.* reported that grape phenolic components inhibit inflammation, edema, and white blood infiltration in mice induced with 12-O-tetra decanoylphorbol 13-acetate.³⁴ Additionally, this component shows comparable effects to indomethacin, a widely used medication for degenerative joint diseases.³⁵ These observations demonstrate that resveratrol has clear anti-inflammatory effects. However, the anti-inflammatory mechanism of resveratrol is unknown, and hypotheses are insufficient. Therefore, we investigated whether the anti-inflammatory effects of resveratrol also act on the HKLM system.

The most well-known mechanisms in TLR2-induced reactions are pathways, including the transcription factors

nuclear factor- κ B and IRF-3 followed by IRAK and TRAF6, which are mediated by MyD88 and Mal.³⁶ However, recent studies suggest other delivery pathways than such factors. We hypothesized a function for GSK3 β . In fact, HKLM increased GSK3 β phosphorylation. GSK3 β phosphorylation weakens protein activation, and LiCl, a GSK3 β inhibitor, elevated HKLM-induced inflammatory gene expression. These results suggest that GSK3 β acts as a negative regulator of the HKLM-mediated inflammatory response. GSK3 β is inactivated by various pathways.

The Akt pathway is considered important among various pathways. Akt is a typical protein that directly phosphorylates and inactivates GSK3 β .¹⁸ We have previously investigated the importance of Akt as an inflammation mediator. Although the importance of Akt was studied for the TLR4-induced inflammatory response, the Akt pathway inhibitor LY294002 also inhibited HKLM-induced inflammation (data not shown). We demonstrated that inactivating GSK3 β elevated inflammation mediator production. Resveratrol restored the activation of GSK3 β weakened by HKLM. We believe that this result shows that resveratrol regulates inflammatory mediator production by inhibiting the Akt-GSK3 β pathway through HKLM. ERK1/2 is also a protein that weakens GSK3 β activation. The ERK1/2 inhibitor U0126 inhibited three types of inflammatory gene expression by HKLM. These results suggest the further possibility that ERK1/2 is the main pathway regulated by HKLM. However, further research is necessary.

Our results suggest that HKLM is a strong inducer of inflammatory mediators, and that the inhibitory effect of resveratrol may be mediated by the GSK3 β and ERK1/2 pathways.

ACKNOWLEDGMENT

This study was supported by a Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (2011-0013206 and 2011-0006181).

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Pervaiz S: Resveratrol: from grapevines to mammalian biology. *FASEB J* 2003;17:1975–1985.
2. Hou X, Xu S, Maitland-Toolan KA, Sato K, Jiang B, Ido Y, Lan F, Walsh K, Wierzbicki M, Verbeuren TJ, Cohen RA, Zang M: SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase. *J Biol Chem* 2008;283:20015–20026.
3. Zang M, Xu S, Maitland-Toolan KA, Zuccollo A, Hou X, Jiang B, Wierzbicki M, Verbeuren TJ, Cohen RA: Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice. *Diabetes* 2006;55:2180–2191.
4. Cullen JP, Morrow D, Jin Y, von Offenber Sweeney N, Sitzmann JV, Cahill PA, Redmond EM: Resveratrol inhibits expression and binding activity of the monocyte chemotactic protein-1 receptor, CCR2, on THP-1 monocytes. *Atherosclerosis* 2007;195:e125–e133.
5. Park DW, Baek K, Kim JR, Lee JJ, Ryu SH, Chin BR, Baek SH: Resveratrol inhibits foam cell formation via NADPH oxidase 1-mediated reactive oxygen species and monocyte chemotactic protein-1. *Exp Mol Med* 2009;41:171–179.
6. Cossart P, Sansonetti PJ: Bacterial invasion: the paradigms of enteroinvasive pathogens. *Science* 2004;304:242–248.
7. Vazquez-Boland JA, Kuhn M, Berche P, Chakraborty T, Dominguez-Bernal G, Goebel W, Gonzalez-Zorn B, Wehland J, Kreft J: Listeria pathogenesis and molecular virulence determinants. *Clin Microbiol Rev* 2001;14:584–640.
8. Janot L, Secher T, Torres D, Maillet I, Pfeilschifter J, Quesniaux VF, Landmann R, Ryffel B, Erard F: CD14 works with toll-like receptor 2 to contribute to recognition and control of Listeria monocytogenes infection. *J Infect Dis* 2008;198:115–124.
9. Corr SC, O'Neill LA: Listeria monocytogenes infection in the face of innate immunity. *Cell Microbiol* 2009;11:703–709.
10. Torres D, Barrier M, Bihl F, Quesniaux VJ, Maillet I, Akira S, Ryffel B, Erard F: Toll-like receptor 2 is required for optimal control of Listeria monocytogenes infection. *Infect Immun* 2004;72:2131–2139.
11. Seki E, Tsutsui H, Tsuji NM, Hayashi N, Adachi K, Nakano H, Futatsugi-Yumikura S, Takeuchi O, Hoshino K, Akira S, Fujimoto J, Nakanishi K: Critical roles of myeloid differentiation factor 88-dependent proinflammatory cytokine release in early phase clearance of Listeria monocytogenes in mice. *J Immunol* 2002;169:3863–3868.
12. Warren SE, Mao DP, Rodriguez AE, Miao EA, Aderem A: Multiple Nod-like receptors activate caspase 1 during Listeria monocytogenes infection. *J Immunol* 2008;180:7558–7564.
13. Eizirik DL, Colli ML, Ortis F: The role of inflammation in insulin and beta-cell loss in type 1 diabetes. *Nat Rev Endocrinol* 2009;5:219–226.
14. Huang QQ, Sobkoviak R, Jockheck-Clark AR, Shi B, Mandelin AM, 2nd, Tak PP, Haines GK, 3rd, Nicchitta CV, Pope RM: Heat shock protein 96 is elevated in rheumatoid arthritis and activates macrophages primarily via TLR2 signaling. *J Immunol* 2009;182:4965–4973.
15. Mersmann J, Koch A, Tran N, Zimmermann R, Granja TF, Larmann J, Herzog C, Theilmeier G, Bornstein SR, Kirschning CJ, Zacharowski K: Toll-like receptor 2 signaling triggers fatal arrhythmias upon myocardial ischemia-reperfusion. *Crit Care Med* 2010;38:1927–1932.
16. Monaco C, Gregan SM, Navin TJ, Foxwell BM, Davies AH, Feldmann M: Toll-like receptor-2 mediates inflammation and matrix degradation in human atherosclerosis. *Circulation* 2009;120:2462–2469.
17. Jope RS, Yuskaitis CJ, Beurel E: Glycogen synthase kinase-3 (GSK3): inflammation, diseases, and therapeutics. *Neurochem Res* 2007;32:577–595.
18. Kim JS, Yeo S, Shin DG, Bae YS, Lee JJ, Chin BR, Lee CH, Baek SH: Glycogen synthase kinase 3 β and beta-catenin pathway is involved in toll-like receptor 4-mediated NADPH oxidase 1 expression in macrophages. *FEBS J* 2010;277:2830–2837.
19. Wang H, Garcia CA, Rehani K, Cekic C, Alard P, Kinane DF, Mitchell T, Martin M: IFN- β production by TLR4-stimulated innate immune cells is negatively regulated by GSK3- β . *J Immunol* 2008;181:6797–6802.

20. Lee SH, Park DW, Park SC, Park YK, Hong SY, Kim JR, Lee CH, Baek SH: Calcium-independent phospholipase A2 β -Akt signaling is involved in lipopolysaccharide-induced NADPH oxidase 1 expression and foam cell formation. *J Immunol* 2009;183:7497–7504.
21. Lee JH, Woo JH, Woo SU, Kim KS, Park SM, Joe EH, Jou I: The 15-deoxy- Δ 12,14-prostaglandin J2 suppresses monocyte chemoattractant protein-1 expression in IFN- γ -stimulated astrocytes through induction of MAPK phosphatase-1. *J Immunol* 2008;181:8642–8649.
22. Simmons DP, Canaday DH, Liu Y, Li Q, Huang A, Boom WH, Harding CV: Mycobacterium tuberculosis and TLR2 agonists inhibit induction of type I IFN and class I MHC antigen cross processing by TLR9. *J Immunol* 2010;185:2405–2415.
23. Sato K, Ishikawa T, Okumura A, Yamauchi T, Sato S, Ayada M, Matsumoto E, Hotta N, Oohashi T, Fukuzawa Y, Kakumu S: Expression of Toll-like receptors in chronic hepatitis C virus infection. *J Gastroenterol Hepatol* 2007;22:1627–1632.
24. Blair P, Rex S, Vitseva O, Beaulieu L, Tanriverdi K, Chakrabarti S, Hayashi C, Genco CA, Iafrati M, Freedman JE: Stimulation of Toll-like receptor 2 in human platelets induces a thromboinflammatory response through activation of phosphoinositide 3-kinase. *Circ Res* 2009;104:346–354.
25. Yang CS, Shin DM, Kim KH, Lee ZW, Lee CH, Park SG, Bae YS, Jo EK: NADPH oxidase 2 interaction with TLR2 is required for efficient innate immune responses to mycobacteria via cathelicidin expression. *J Immunol* 2009;182:3696–3705.
26. Egashira K: Molecular mechanisms mediating inflammation in vascular disease: special reference to monocyte chemoattractant protein-1. *Hypertension* 2003;41:834–841.
27. Libby P: Inflammation in atherosclerosis. *Nature* 2002;420:868–874.
28. Niu J, Kolattukudy PE: Role of MCP-1 in cardiovascular disease: molecular mechanisms and clinical implications. *Clin Sci (Lond)* 2009;117:95–109.
29. Brand K, Page S, Rogler G, Bartsch A, Brandl R, Knuechel R, Page M, Kaltschmidt C, Baeuerle PA, Neumeier D: Activated transcription factor nuclear factor- κ B is present in the atherosclerotic lesion. *J Clin Invest* 1996;97:1715–1722.
30. Tumur Z, Shimizu H, Enomoto A, Miyazaki H, Niwa T: Indoxyl sulfate upregulates expression of ICAM-1 and MCP-1 by oxidative stress-induced NF- κ B activation. *Am J Nephrol* 2010;31:435–441.
31. Yao H, Yang Y, Kim KJ, Bethel-Brown C, Gong N, Funa K, Gendelman HE, Su TP, Wang JQ, Buch S: Molecular mechanisms involving sigma receptor-mediated induction of MCP-1: implication for increased monocyte transmigration. *Blood* 2010;115:4951–4962.
32. Henry-Vitrac C, Desmouliere A, Girard D, Merillon JM, Krisa S: Transport, deglycosylation, and metabolism of trans-piceid by small intestinal epithelial cells. *Eur J Nutr* 2006;45:376–382.
33. Das S, Das DK: Anti-inflammatory responses of resveratrol. *Inflamm Allergy Drug Targets* 2007;6:168–173.
34. Bralley EE, Greenspan P, Hargrove JL, Wicker L, Hartle DK: Topical anti-inflammatory activity of Polygonum cuspidatum extract in the TPA model of mouse ear inflammation. *J Inflamm (Lond)* 2008;5:1.
35. Buluc M, Demirel-Yilmaz E: Resveratrol decreases calcium sensitivity of vascular smooth muscle and enhances cytosolic calcium increase in endothelium. *Vascul Pharmacol* 2006;44:231–237.
36. Abe M, Matsuda M, Kobayashi H, Miyata Y, Nakayama Y, Komuro R, Fukuhara A, Shimomura I: Effects of statins on adipose tissue inflammation: their inhibitory effect on MyD88-independent IRF3/IFN- β pathway in macrophages. *Arterioscler Thromb Vasc Biol* 2008;28:871–877.