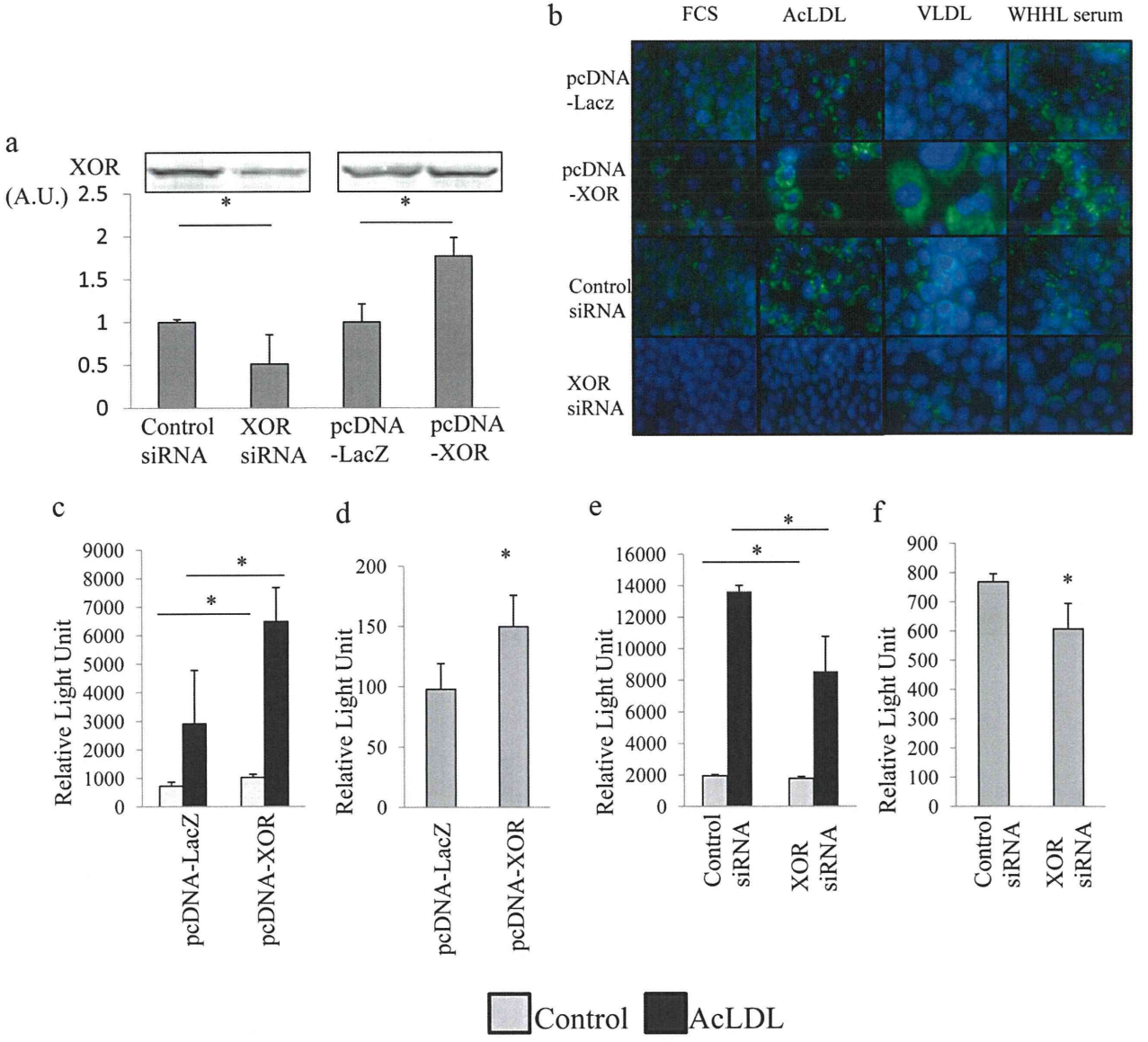


Figure 3. Effects of oxidoreductase (XOR) overexpression and knockdown on foam cell formation. **a**, Western blotting of XOR protein, representative bands and quantified band intensities, presented as means±SE, are shown for samples from J774.1 cell extracts. XOR knockdown mediated by siRNA and overexpression of XOR. Mean±SE of XOR band intensity is shown. **b**, The effects of XOR overexpression or siRNA mediated XOR gene knockdown on lipid accumulation induced by 10 ng/mL LPS, 50 μg/mL acetyl LDL (AcLDL), 50 μg/dL very low density lipoprotein (VLDL), and 1% Watanabe heritable hyperlipidemic rabbits (WHHL) rabbit serum. Cellular lipid is stained green by AdipoRed, the nucleus is stained blue by DAPI. **c** and **e**, Lipid accumulation in J774.1 cells incubated with 1% control rabbit serum or 1% WHHL rabbit serum. The effect of XOR overexpression (**c**) and siRNA-mediated XOR knockdown (**e**) are shown. **d** and **f**, Assays of DiI-AcLDL uptake. The effect of XOR overexpression (**d**) and siRNA-mediated XOR knockdown (**f**) are shown. The asterisk (*) indicates statistical significance at $P<0.05$.

Moreover, XOR overexpression with VLDL incubation induced cellular enlargement and multinucleation (Figure 3b).

Expressions of Lipoprotein Receptors and ABC Transporters Involved in Lipid Transport

XOR overexpression induced expressions of lipoprotein receptors, such as SR-B1, SR-B2, and VLDL. XOR siRNA knockdown diminished CD36, SR-A1, and LDL receptors. With XOR overexpression, ABCA1 and ABCG1 were diminished, whereas XOR knockdown induced expressions of ABC transporters such as ABCA1 and ABCG1 (Figure 4). In support of these data, allopurinol also suppressed lipoprotein receptors and induced ABCA1 and ABCG1 (Supplemental Figure III, available online at http://atvb.ahajournals.org).

Inflammatory Cytokine Secretions and Key Molecules in Atherosclerosis Development

Inflammatory cytokines such as IL-1β, IL-6, IL-12, and TNFα were dose-dependently inhibited by allopurinol when foam cell formation was triggered by WHHL serum (Supplemental Figure IVa–IVd), whereas secretions of LPS-induced cytokines, other than IL-6 (Supplemental Figure IVg), were unaffected by allopurinol (Supplemental Figure IVe, IVf, and IVh). Incubation with WHHL serum induced VCAM1,

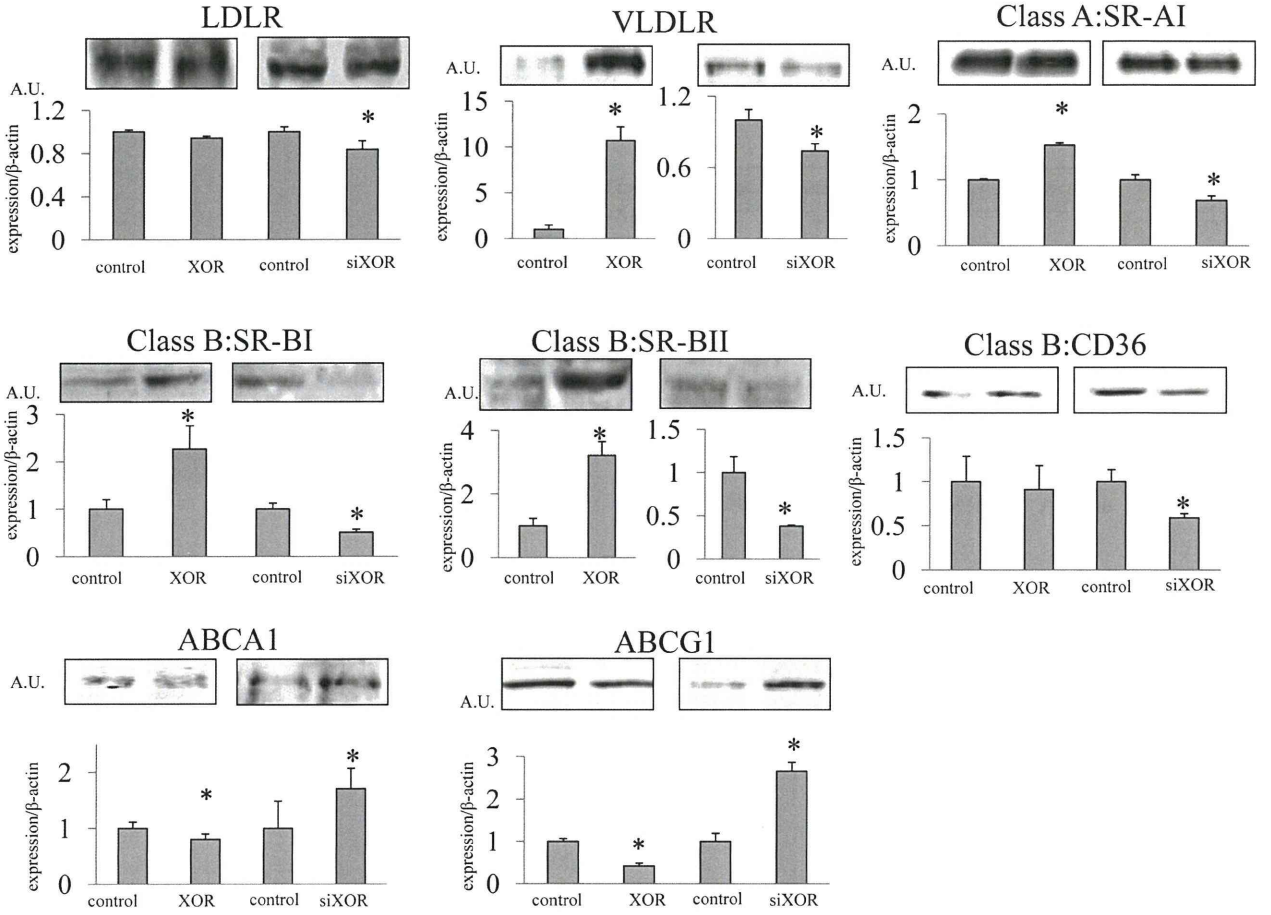


Figure 4. Quantification of lipoprotein receptors and ABC transporters involved in lipid transport with overexpression or suppression of oxidoreductase (XOR) in J774.1 cells. Western blotting was performed using specific antibodies for lipoprotein receptors and ABC transporters involved in lipid transporters. Representative bands and quantified band intensity are presented as means±SE. The asterisk (*) indicates statistical significance at $P<0.05$. LDLR indicates LDL receptor; VLDLR, very low-density lipoprotein receptor.

MCP-1, and MMP2 but neither ICAM1 nor MMP9 expression. Allopurinol suppressed these inductions and inhibited expression of MMP9. LPS induced MCP-1 and MMP2, and these expressions were slightly blunted by allopurinol administration. VCAM1 and MMP9 were suppressed by 10 ng/mL LPS, and adding allopurinol further suppressed both (Supplemental Figure V).

Discussion

This study is the first to show that XOR plays a key role in the transformation of macrophages into foam cells and the development of atherosclerotic plaque. It was clearly demonstrated that oral administration of allopurinol for 4 weeks significantly inhibited lipid accumulation and calcification in the aortas of ApoE KO mice. Interestingly, serum lipid levels were not significantly altered by allopurinol. TG was slightly, but not significantly, reduced. Rats with fructose-induced hyperuricemia given allopurinol had reduced serum TG levels[25] suggesting a weak TG-lowering effect in rodents. In contrast, allopurinol markedly reduced serum uric acid in ApoE KO mice to approximately one-third of normal.

While XOR activity is highest in the liver and intestines, a previous histological study revealed that XOR is also present in macrophages.[14] In good agreement with that report, we observed XOR to be abundant in macrophages clustered at the aortic root (Supplemental Figure IA and IB). Importantly, using XOR overexpression as well as XOR siRNA or allopurinol treatment, we demonstrated XOR activity to be critical for the transformation of J774.1 cells and human macrophages into foam cells. The allopurinol concentration was essentially within physiological range, ie, the trough serum oxypurinol concentration [>4.6 μg/mL (=30.2 μmol/L)] approximated that achieved by a 100 to 200 mg single allopurinol administration to hyperuricemic patients.[31] Thus, our in vitro results might be applicable to clinical practice. XO activation was induced by hypoxia, LPS, hypoxia-inducible factor 1, and inflammatory cytokines like IL-1β.[32] We also found secretion of inflammatory cytokines to be accompanied by foam cell formation, which was blocked by XOR inhibition.

Inflammatory cytokines were induced via XOR when foam cells formed, although not via, at least not entirely, the XOR pathway when stimulated by LPS. These observations suggest a vicious cycle of lipid accumulation and XOR activation to be involved in foam cell formation, which might contribute to the mechanism of plaque development. Phagocytosis was not affected by XOR inhibition (Supplemental Figure II). Thus, phagocytic activity does not involve XOR.

There are reports describing XOR as an endogenous regulator of cyclooxygenase (Cox)-2[33] in the inflammatory system, and XOR appears to be central to innate immune function.[34] In addition, XOR is critically involved in both differentiation and lipid metabolism of adipose tissue. Because XOR is a regulator of adipogenesis and peroxisome proliferator-activated receptor activity, XOR−/− mice demonstrate a 50% reduction in adipose mass versus their littermates.[35] PPARγ also plays roles in macrophage lipid efflux[36] in a manner opposite that of lipid retention. Cox-2 has central roles in innate immunity and inflammation and is regulated by a negative feedback loop mediated by PPARγ.[37] XOR is thought to be upstream from PPARγ in lipid retention[35] and also to induce Cox-2, which then promotes inflammation, also possibly constituting a feedback loop.

In macrophages, cellular mechanisms of lipid metabolism such as lipid influx and efflux are mediated by lipoprotein receptors such as scavenger receptors and some ABC transporters.[38,39] As to scavenger receptors, both class A, especially SR-A1, and class B, such as SR-B1 and -B2, receptors appear to be involved in this XOR system. Class A receptors include SR-A1, SR-A2, MARCO, and SRCL, receptors for oxidized LDL and AcLDL, whereas class B receptors include CD36, SR-B1, and mediators of MAPK or TLR signaling, leading to expressions of inflammatory cytokines and reactive oxygen species generation. Therefore, the SR-B receptor decrease with XOR inhibition observed herein might suppress cytokine expressions. Among proteins involved in lipid uptake, VLDL receptor was strongly induced by XOR overexpression. Although cholesterol efflux was not examined, according to our data, expressions of ABCA1 and ABCG1 were reduced by XOR overexpression. Inflammation reportedly inhibits expressions of these genes.[40,41]

In ApoE KO mice, atherosclerotic plaque was predominantly derived from ApoE deficiency in HDL leading to impaired efflux of cholesterol.[42] Influx of cholesterol in ApoE KO mice depends on high serum cholesterol levels, produced by serum ApoE deficiency,[43] dysfunction of the normal, rapid catabolism of chylomicron remnants entering the liver via ApoE,[44] and evident accumulation of VLDL containing cholesteryl esters.[45] Allopurinol did not change serum cholesterol levels, although XOR expression changed amounts of lipid transporting proteins in macrophages. Thus, the antiatherosclerotic effect of allopurinol is independent of lipoprotein metabolism in the liver and might be directed to macrophages.

A previous report revealed tungsten to prevent atherosclerosis development[46] but did not focus on lipid accumulation in plaque areas or foam cell formation by macrophages. In addition, tungsten inhibits XDH transcription and also proteolytic processing of XDH protein. Thus, proteolytic involvement of tungsten in more proteins than just XDH cannot be ruled out.

A major limitation in applying our results to clinical practice appears to be the differences in XOR distributions among humans and other species, in both physiological and pathophysiological states.[12] These differences are not yet well understood. However, macrophages express XOR and are known to play roles in such disorders as angina, congestive heart failure, and chronic kidney disease, and our results indicate allopurinol is directly and significantly involved in the transformation of macrophages into foam cells.

Although further investigation is needed, our results clearly suggest the importance of inhibiting XO activity for the prevention and treatment of atherosclerosis and may provide insights allowing the development of novel antiatherosclerotic drugs.

Acknowledgments

Akifumi Kushiyama, Hirofumi Okubo, and Tomoichiro Asano designed research; Akifumi Kushiyama, Hirofumi Okubo, Hideyuki Sakoda, Takako Kikuchi, Midori Fujishiro, Hirokazu Sato, Sakura Kushiyama, Misaki Iwashita Yusuke Nakatsu, Fusanori Nishimura, Toshiaki Fukushima, Yukihito Higashi, and Hiroki Kurihara performed research; S.K., Yukihito Higashi, and Hiroki Kurihara analyzed data; and Akifumi Kushiyama and Tomoichiro Asano wrote the paper.

Disclosures

None.

References

1. Ishizaka N, Ishizaka Y, Toda E, Nagai R, Yamakado M. Association between serum uric acid, metabolic syndrome, and carotid atherosclerosis in Japanese individuals. *Arterioscler Thromb Vasc Biol*. 2005;25:1038–1044.
2. Silbernagel G, Hoffmann MM, Grammer TB, Boehm BO, Marz W. Uric acid is predictive of cardiovascular mortality and sudden cardiac death in subjects referred for coronary angiography. *Nutrition, Metabolism, and Cardiovascular Diseases: NMCD*. 2011.
3. Rodrigues TC, Maahs DM, Johnson RJ, Jalal DI, Kinney GL, Rivard C, Rewers M, Snell-Bergeon JK. Serum uric acid predicts progression of subclinical coronary atherosclerosis in individuals without renal disease. *Diabetes Care*. 2010;33:2471–2473.
4. Facchini F, Chen YD, Hollenbeck CB, Reaven GM. Relationship between resistance to insulin-mediated glucose uptake, urinary uric acid clearance, and plasma uric acid concentration. *JAMA*. 1991;266:3008–3011.
5. Tanaka K, Hara S, Kushiyama A, Ubara Y, Yoshida Y, Mizuiri S, Aikawa A, Kawatzu S. Risk of macrovascular disease stratified by stage of chronic kidney disease in type 2 diabetic patients: Critical level of the estimated glomerular filtration rate and the significance of hyperuricemia. *Clinical and Experimental Nephrology*. 2011;15:391–397.
6. Ficociello LH, Rosolowsky ET, Niewczas MA, Maselli NJ, Weinberg JM, Aschengrau A, Eckfeldt JH, Stanton RC, Galecki AT, Doria A, Warram JH, Krolewski AS. High-normal serum uric acid increases risk of early progressive renal function loss in type 1 diabetes: Results of a 6-year follow-up. *Diabetes Care*. 2010;33:1337–1343.
7. Ekundayo OJ, Dell'Italia LJ, Sanders PW, Arnett D, Aban I, Love TE, Filippatos G, Anker SD, Lloyd-Jones DM, Bakris G, Mujib M, Ahmed A. Association between hyperuricemia and incident heart failure among older adults: A propensity-matched study. *Int J Cardiol*. 2010;142:279–287.
8. Spoon DB, Lerman A, Rule AD, Prasad A, Lennon RJ, Holmes DR, Rihal CS. The association of serum uric acid levels with outcomes following percutaneous coronary intervention. *J Interv Cardiol*. 2010;23:277–283.
9. Kowalczyk J, Francuz P, Swoboda R, Lenarczyk R, Sredniawa B, Golda A, Kurek T, Mazurek M, Podolecki T, Polonski L, Kalarus Z. Prognostic significance of hyperuricemia in patients with different types of renal dysfunction and acute myocardial infarction treated with percutaneous coronary intervention. *Nephron Clin Pract*. 2010;116:c114–c122.
10. Lazzeri C, Valente S, Chiostri M, Sori A, Bernardo P, Gensini GF. Uric acid in the acute phase of st elevation myocardial infarction submitted to primary pci: Its prognostic role and relation with inflammatory markers: A single center experience. *Int J Cardiol*. 2010;138:206–209.
11. Alvarez-Lario B, Macarron-Vicente J. Uric acid and evolution. *Rheumatology (Oxford)*. 2010;49:2010–2015.
12. Pritsos CA. Cellular distribution, metabolism and regulation of the xanthine oxidoreductase enzyme system. *Chemico-Biological Interactions*. 2000;129:195–208.

13. Moriwaki Y, Yamamoto T, Suda M, Nasako Y, Takahashi S, Agbedana OE, Hada T, Higashino K. Purification and immunohistochemical tissue localization of human xanthine oxidase. *Biochimica et Biophysica Acta.* 1993;1164:327–330.
14. de Jong JW, Schoemaker RG, de Jonge R, Bernocchi P, Keijzer E, Harrison R, Sharma HS, Ceconi C. Enhanced expression and activity of xanthine oxidoreductase in the failing heart. *J Mol Cell Cardiol.* 2000; 32:2083–2089.
15. Massey V, Komai H, Palmer G, Elion GB. On the mechanism of inactivation of xanthine oxidase by allopurinol and other pyrazolo[3,4-d]pyrimidines. *J Biol Chem.* 1970;245:2837–2844.
16. Noman A, Ang DS, Ogston S, Lang CC, Struthers AD. Effect of high-dose allopurinol on exercise in patients with chronic stable angina: A randomised, placebo controlled crossover trial. *Lancet.* 375:2161–2167.
17. Johnson WD, Kayser KL, Brenowitz JB, Saedi SF. A randomized controlled trial of allopurinol in coronary bypass surgery. *Am Heart J.* 1991;121:20–24.
18. Cingolani HE, Plastino JA, Escudero EM, Mangal B, Brown J, Perez NG. The effect of xanthine oxidase inhibition upon ejection fraction in heart failure patients: La plata study. *J Card Fail.* 2006;12:491–498.
19. Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest.* 1993;91: 2546–2551.
20. Ishigaki Y, Katagiri H, Gao J, Yamada T, Imai J, Uno K, Hasegawa Y, Kaneko K, Ogihara T, Ishihara H, Sato Y, Takikawa K, Nishimichi N, Matsuda H, Sawamura T, Oka Y. Impact of plasma oxidized low-density lipoprotein removal on atherosclerosis. *Circulation.* 2008;118:75–83.
21. Ito T, Yamada S, Shiomi M. Progression of coronary atherosclerosis relates to the onset of myocardial infarction in an animal model of spontaneous myocardial infarction (whhlmi rabbits). *Exp Anim.* 2004;53: 339–346.
22. Shiomi M, Ito T, Yamada S, Kawashima S, Fan J. Development of an animal model for spontaneous myocardial infarction (whhlmi rabbit). *Arterioscler Thromb Vasc Biol.* 2003;23:1239–1244.
23. Buja LM, Kita T, Goldstein JL, Watanabe Y, Brown MS. Cellular pathology of progressive atherosclerosis in the whhl rabbit. An animal model of familial hypercholesterolemia. *Arteriosclerosis.* 1983;3:87–101.
24. Seneviratne AN, Sivagurunathan B, Monaco C. Toll-like receptors and macrophage activation in atherosclerosis. *Clinica Chimica Acta; International Journal of Clinical Chemistry.* 2011.
25. Nakagawa T, Hu H, Zharikov S, Tuttle KR, Short RA, Glushakova O, Ouyang X, Feig DI, Block ER, Herrera-Acosta J, Patel JM, Johnson RJ. A causal role for uric acid in fructose-induced metabolic syndrome. *Am J Physiol Renal Physiol.* 2006;290:F625–F631.
26. Catalano RA, Lillie RD. Elimination of precipitates in oil red o fat stain by adding dextrin. *Stain Technol.* 1975;50:297–299.
27. Fraulob JC, Ogg-Diamantino R, Fernandes-Santos C, Aguila MB, Mandarim-de-Lacerda CA. A mouse model of metabolic syndrome: Insulin resistance, fatty liver and non-alcoholic fatty pancreas disease (nafpd) in c57bl/6 mice fed a high fat diet. *Journal of Clinical Biochemistry and Nutrition.* 2010;46:212–223.
28. Cui X, Kushiyama A, Yoneda M, Nakatsu Y, Guo Y, Zhang J, Ono H, Kanna M, Sakoda H, Kikuchi T, Fujishiro M, Shiomi M, Kamata H, Kurihara H, Kikuchi M, Kawazu S, Nishimura F, Asano T. Macrophage foam cell formation is augmented in serum from patients with diabetic angiopathy. *Diabetes Res Clin Pract.* 2010;87:57–63.
29. Kushiyama A, Shojima N, Ogihara T, Inukai K, Sakoda H, Fujishiro M, Fukushima Y, Anai M, Ono H, Horike N, Viana AY, Uchijima Y, Nishiyama K, Shimosawa T, Fujita T, Katagiri H, Oka Y, Kurihara H, Asano T. Resistin-like molecule beta activates mapks, suppresses insulin signaling in hepatocytes, and induces diabetes, hyperlipidemia, and fatty liver in transgenic mice on a high fat diet. *J Biol Chem.* 2005;280: 42016–42025.
30. Pfaffl MW. A new mathematical model for relative quantification in real-time rt-pcr. *Nucleic Acids Research.* 2001;29:e45.
31. Takada M, Okada H, Kotake T, Kawato N, Saito M, Nakai M, Gunji T, Shibakawa M. Appropriate dosing regimen of allopurinol in japanese patients. *J Clin Pharm Ther.* 2005;30:407–412.
32. Nicholas SA, Bubnov VV, Yasinska IM, Sumbayev VV. Involvement of xanthine oxidase and hypoxia-inducible factor 1 in toll-like receptor 7/8-mediated activation of caspase 1 and interleukin-1beta. *Cell Mol Life Sci.*
33. Ohtsubo T, Rovira II, Starost MF, Liu C, Finkel T. Xanthine oxidoreductase is an endogenous regulator of cyclooxygenase-2. *Circ Res.* 2004;95:1118–1124.
34. Vorbach C, Harrison R, Capecchi MR. Xanthine oxidoreductase is central to the evolution and function of the innate immune system. *Trends Immunol.* 2003;24:512–517.
35. Cheung KJ, Tzameli I, Pissios P, Rovira I, Gavrilova O, Ohtsubo T, Chen Z, Finkel T, Flier JS, Friedman JM. Xanthine oxidoreductase is a regulator of adipogenesis and ppargamma activity. *Cell Metab.* 2007;5: 115–128.
36. Liu W, He P, Cheng B, Mei CL, Wang YF, Wan JJ. Chlamydia pneumoniae disturbs cholesterol homeostasis in human thp-1 macrophages via jnk-ppargamma dependent signal transduction pathways. *Microbes and Infection/Institut Pasteur.* 2010;12:1226–1235.
37. Inoue H, Tanabe T, Umesono K. Feedback control of cyclooxygenase-2 expression through ppargamma. *The Journal of Biological Chemistry.* 2000;275:28028–28032.
38. Hansson GK, Robertson AK, Soderberg-Naucler C. Inflammation and atherosclerosis. *Annu Rev Pathol.* 2006;1:297–329.
39. Zhao B, Song J, Chow WN, St Clair RW, Rudel LL, Ghosh S. Macrophage-specific transgenic expression of cholesteryl ester hydrolase significantly reduces atherosclerosis and lesion necrosis in ldlr mice. *J Clin Invest.* 2007;117:2983–2992.
40. Panousis CG, Zuckerman SH. Interferon-gamma induces downregulation of tangier disease gene (atp-binding-cassette transporter 1) in macrophage-derived foam cells. *Arterioscler Thromb Vasc Biol.* 2000;20: 1565–1571.
41. Khovidhunkit W, Moser AH, Shigenaga JK, Grunfeld C, Feingold KR. Endotoxin down-regulates abcg5 and abcg8 in mouse liver and abca1 and abcg1 in j774 murine macrophages: Differential role of lxr. *J Lipid Res.* 2003;44:1728–1736.
42. Hayek T, Oiknine J, Brook JG, Aviram M. Role of hdl apolipoprotein e in cellular cholesterol efflux: Studies in apo E knockout transgenic mice. *Biochem Biophys Res Commun.* 1994;205:1072–1078.
43. Kashyap VS, Santamarina-Fojo S, Brown DR, Parrott CL, Applebaum-Bowden D, Meyn S, Talley G, Paigen B, Maeda N, Brewer HB, Jr. Apolipoprotein E deficiency in mice: Gene replacement and prevention of atherosclerosis using adenovirus vectors. *J Clin Invest.* 1995;96: 1612–1620.
44. Mortimer BC, Martins I, Zeng BJ, Redgrave TG. Use of gene-manipulated models to study the physiology of lipid transport. *Clin Exp Pharmacol Physiol.* 1997;24:281–285.
45. Woollett LA, Osono Y, Herz J, Dietschy JM. Apolipoprotein E competitively inhibits receptor-dependent low density lipoprotein uptake by the liver but has no effect on cholesterol absorption or synthesis in the mouse. *Proc Natl Acad Sci U S A.* 1995;92:12500–12504.
46. Schroder K, Vecchione C, Jung O, Schreiber JG, Shiri-Sverdlov R, van Gorp PJ, Busse R, Brandes RP. Xanthine oxidase inhibitor tungsten prevents the development of atherosclerosis in apoe knockout mice fed a western-type diet. *Free Radical Biology & Medicine.* 2006;41: 1353–1360.

Supplemental Materials and Methods

Reagents

Primary Ab for western blotting were purchased from Santa Cruz: anti-VCAM1 (#sc-8304), anti-ICAM1 (#sc-1511), anti-MCP-1 (#sc-1784), anti-MMP2 (#sc-10736) and anti-MMP9 (#sc-6840).

Assay of phagocytic activity

The indicated concentration of allopurinol was added to the medium 2h prior to additional incubation with 10ng/ml LPS or 1% WHHL rabbit serum for 24h. The phagocytic activity of J774.1 cells, treated as described above, was assayed using a phagocytosis assay kit (IgG FITC, Cayman Chemicals) according to the manufacturer's instructions. In brief, latex beads coated with fluorescently-labeled rabbit-IgG served as a probe for quantification of the phagocytic process *in vitro*. The engulfed fluorescent-beads were detectable using a Leica DMIRB microscope, with excitation and emission at 485 and 535 nm.

Supplemental Figure Legends

Supplemental Figure I

XOR expression. a. XOR expression in the aortic root. Macrophages and XOR

expression were visualized as green and red, respectively. b. Magnified view within the gray square in supplemental figure Ia.

Supplemental Figure II

The phagocytic capability of J774.1 cells was accelerated by stimulation with 1% WHHL serum or 10ng/ml LPS, but neither effect was diminished by allopurinol administration

Supplemental Figure III

Quantification of lipoprotein receptors and ABC transporters involved in lipid transport in J774.1 cells incubated with 30μM allopurinol. Western blotting was performed using specific antibodies for lipoprotein receptors and ABCA1 and ABCG1. Representative bands and quantified band intensities are presented as means±SE. * indicates statistical significance at $p<0.05$. Lipoprotein receptors were suppressed, while ABCA1 and G1 were up-regulated, by allopurinol.

Supplemental Figure IV

Allopurinol effects on secretions of inflammatory cytokines from J774.1 cells induced by 1% WHHL rabbit serum and 10ng/ml LPS. 5a-d, incubation with 1% WHHL rabbit serum, 5e-h, incubation with 10ng/ml LPS. IL indicates interleukin, TNF, tumor necrosis factor. Inflammatory cytokines such as IL-1β, IL-6, IL-12 and TNFα, were dose-dependently inhibited by allopurinol when foam cell formation was triggered by

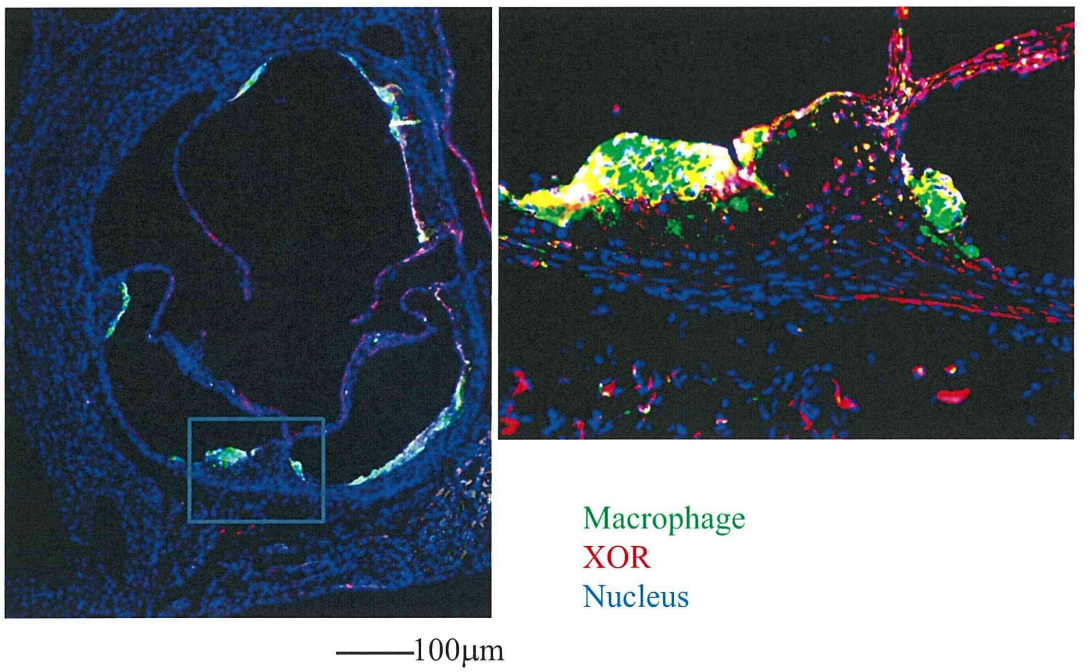
WHHL serum (IVa-d), while secretions of LPS-induced cytokines, other than IL-6 (IVg), were unaffected by allopurinol (IVe, IVf, and IVh).

Supplemental Figure V

Quantification of key cytokines and molecules which can induce cell migration or contribute to the development of unstable plaques. Monocyte chemoattractant protein (MCP)-1, VCAM-1, ICAM-1 and metaloprotenase (MMP)-2, 9 in J774.1 cells were investigated. J774.1 cells were incubated with 1% WHHL serum or 10ng/ml LPS, with 2 hr prior incubation with 30μg/ml allopurinol. Western blotting was performed using specific antibodies for these proteins. Quantified band intensities are presented as means±SE. * indicates statistical significance at $p<0.05$. Incubation with 1% WHHL serum induced VCAM1, MCP-1 and MMP2, but not ICAM1 or MMP9, expression under these conditions. Allopurinol suppressed the inductions and inhibited MMP9. LPS induced MCP-1 and MMP2, and these inductions were slightly blunted by allopurinol. VCAM1 and MMP9 were suppressed by 10ng/ml LPS, and addition of allopurinol further suppressed both.

Supplement material

Supplemental Figure I



Supplement material

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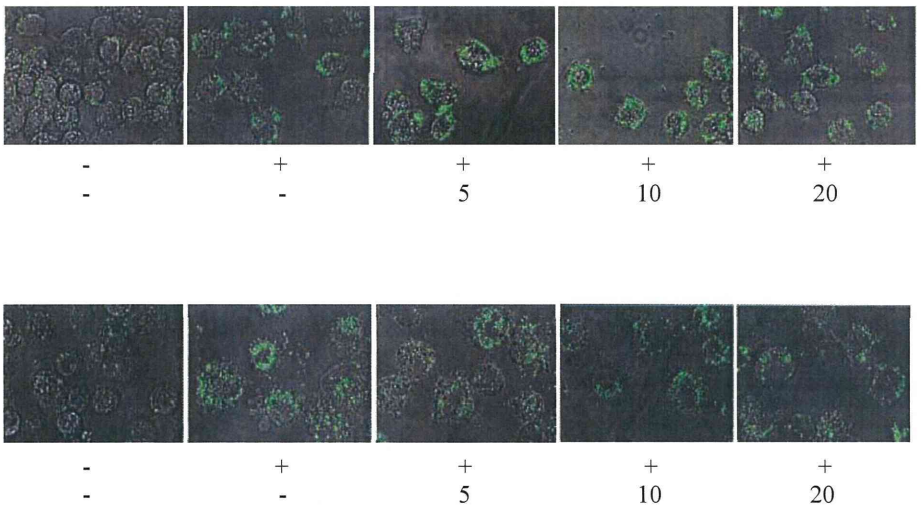
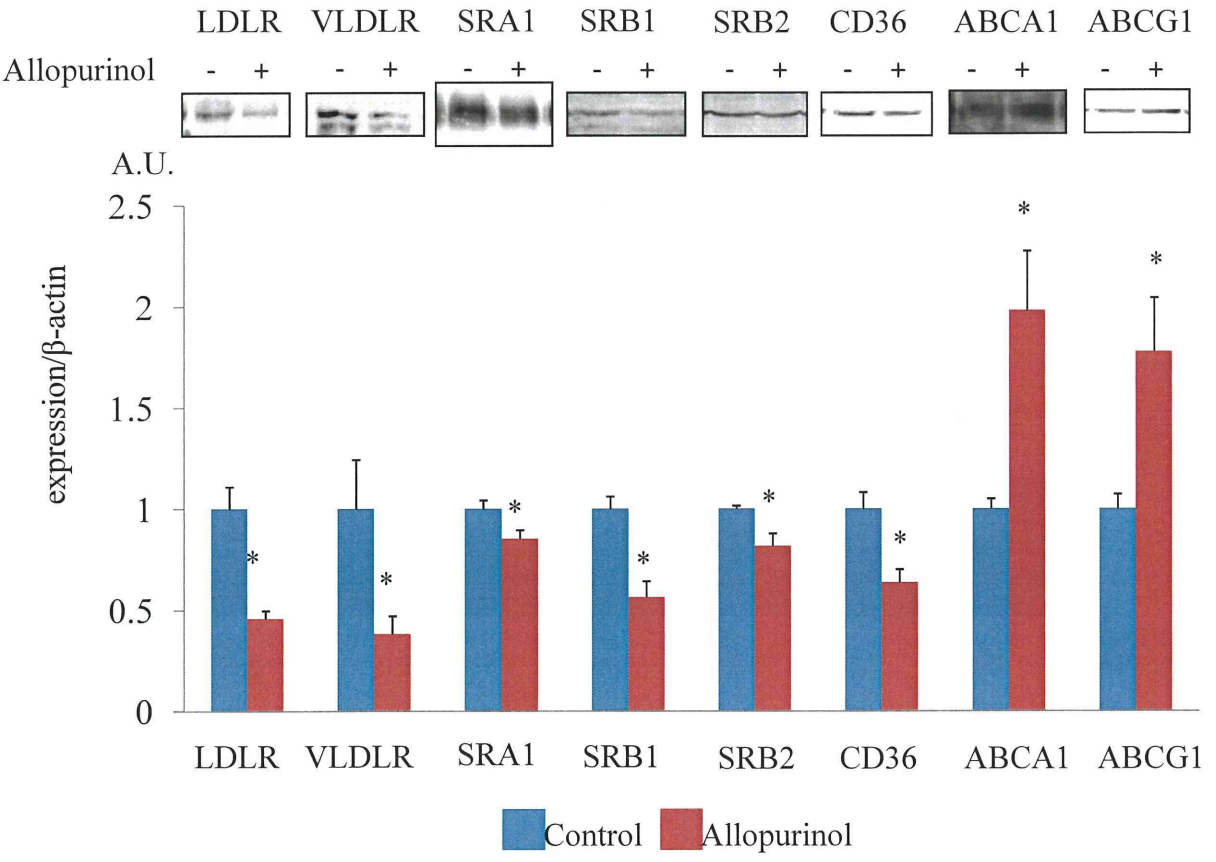
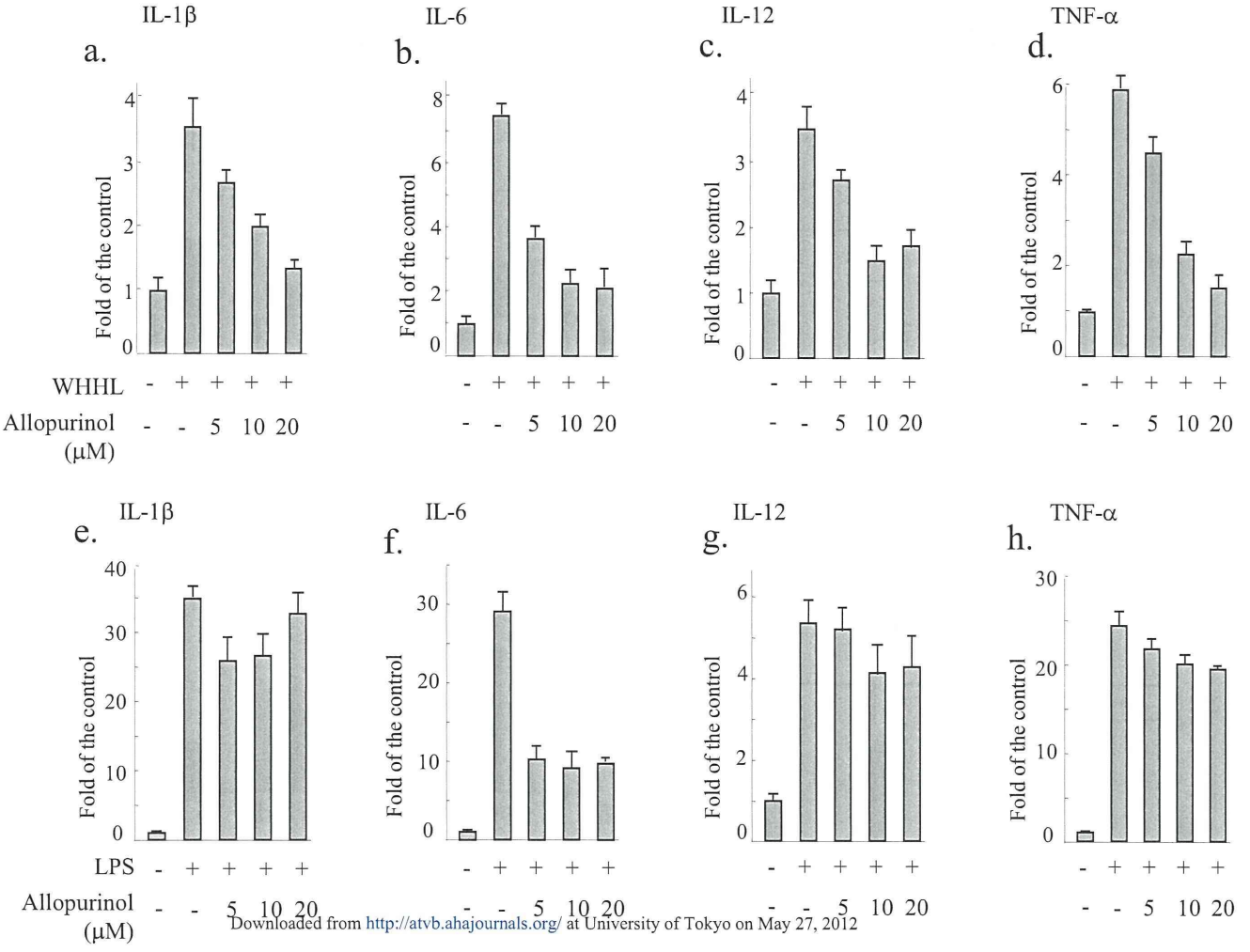


Supplement material

Supplemental Figure III.



Supplemental Figure IV.



Supplemental Figure V.