Cutting Edge

THE JOURNAL OF IMMUNOLOGY

Cutting Edge: Nitric Oxide Inhibits the NLRP3 Inflammasome

Eduardo Hernandez-Cuellar,*,† Kohsuke Tsuchiya,* Hideki Hara,* Rendong Fang,* Shunsuke Sakai,* Ikuo Kawamura,* Shizuo Akira,† and Masao Mitsuyama*

Although the NLRP3 inflammasome plays a pivotal role in host defense, its uncontrolled activation is associated with inflammatory disorders, suggesting that regulation of the inflammasome is important to prevent detrimental effects. Type I IFNs and long-term LPS stimulation were shown to negatively regulate NLRP3 activation. In this study, we found that endogenous NO is involved in the regulation of NLRP3 inflammasome activation by either IFN-β pretreatment or long-term LPS stimulation. Furthermore, *S*-nitroso-N-acetylpenicillamine (SNAP), an NO donor, markedly inhibited NLRP3 inflammasome activation, whereas the AIM2 and NLRC4 inflammasomes were only partially inhibited by SNAP. An increase in mitochondrial reactive oxygen species induced by ATP was only modestly affected by SNAP treatment. Interestingly, *S*-nitrosylation of NLRP3 was detected in macrophages treated with SNAP, and this modification may account for the NO-mediated mechanism controlling inflammasome activation. Taken together, these results revealed a novel role for NO in regulating the NLRP3 inflammasome. *The Journal of Immunology*, **2012, 189: 5113–5117.**

The inflammasome is a multiprotein complex that mediates the activation of caspase-1, which then processes pro–IL-1β and pro–IL-18 into mature IL-1β and IL-18. Among a number of sensor proteins reported to be involved in inflammasome activation, NLRP3, NLRP1b, NLRC4, and AIM2, have been established as the major sensors for the recognition of various pathogens or damage-associated molecular patterns.

NLRP3 activation is triggered by different types of stimuli (e.g., whole pathogens, uric acid crystals, nigericin, ATP) (1). The precise mechanism of NLRP3 inflammasome activation has not been determined; however, NLRP3 agonist-induced generation of mitochondrial reactive oxygen species (ROS) is likely to play a major role in NLRP3 activation (2–5). Despite the fact that NLRP3 inflammasome contributes to host defense against microbial pathogens, excessive activation due to mutations in the NLRP3 gene has been associated with a spectrum of autoinflammatory disorders collectively known as "cryopirin-associated periodic syndromes" (1). Additionally, NLRP3 has been implicated in obesity-induced inflammation and insulin resistance (6). Thus, appropriate regulation of inflammasome activation appears to be important to avoid detrimental effects.

It was demonstrated that activation of the inflammasome is regulated by several mechanisms. For instance, the protein level of NLRP3 is relatively low in resting macrophages, so that NLRP3 inflammasome formation is hardly induced until the expression level is increased by exogenous and endogenous factors, including TLR agonists and proinflammatory cytokines. This suggests that activation of the NLRP3 inflammasome is limited under normal conditions (7). In contrast, long-term priming with the TLR4 agonist LPS results in the suppression of NLRP3 inflammasome activity, reportedly owing to the induction of TRIM30 (8). It was also demonstrated that type I IFNs negatively regulate the activation of caspase-1 induced by NLRP3 agonists but not that induced by AIM2 or NLRC4 agonists, although the mechanism remains unclear (9). In a report published before the establishment of the inflammasome concept, it was suggested that NO directly inhibits caspase-1 activity, thereby preventing the release of mature IL-1β and IL-18 in response to LPS and IFN-γ (10). Because type I IFNs also induce the expression of IFN regulatory factor 1, a transcription factor required for the expression of inducible NO synthase (iNOS) (11), we addressed whether NO is involved in the inhibition of the NLRP3 inflammasome by type I IFNs. In the current study, we analyzed the role of NO in the regulation of NLRP3 inflammasome activation.

Materials and Methods

Mice

Female C57BL/6 (wild-type [WT]) mice were purchased from Japan SLC. IFN-α/β receptor 1 (IFNAR1) knockout (KO) mice were kindly provided

*Department of Microbiology, Kyoto University Graduate School of Medicine, Kyoto 606-8501, Japan; and †Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565-0871, Japan

Received for publication September 5, 2012. Accepted for publication October 4, 2012.

This work was supported by Grants-in-Aid for Scientific Research (B) and (C) and a Grant-in-Aid for Young Scientists (B) from the Japan Society for the Promotion of Science.

Address correspondence and reprint requests to Dr. Kohsuke Tsuchiya, Department of Microbiology, Kyoto University Graduate School of Medicine, Yoshida Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan. E-mail address: tsuchiya@mb.med.kyoto-u.ac.jp

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1202479

The online version of this article contains supplemental material.

Abbreviations used in this article: IFNAR1, IFN-α/β receptor 1; iNOS, inducible NO synthase; KO, knockout; L-NMMA, N^G-monomethyl-L-arginine; mtROS, mitochondrial reactive oxygen species; poly(dA:dT), poly(deoxyadenylic-thymidylic) acid; rIFN-β, recombinant mouse IFN-β; ROS, reactive oxygen species; SNAP, *S*-nitroso-*N*-acetylpenicillamine; WT, wild-type.

by Prof. Michel Aguet (Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland). KO mice for iNOS were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were maintained in specific pathogen-free conditions and used at 7–9 wk of age. All animal experimental procedures were approved by the Animal Ethics and Research Committee of Kyoto University Graduate School of Medicine.

Reagents

Ultra-pure LPS, Pam_3CSK_4, and *Salmonella* flagellin were purchased from InvivoGen (San Diego, CA); ATP was purchased from Amersham Biosciences (Piscataway, NJ); nigericin, poly(deoxyadenylic-thymidylic) acid [poly(dA:dT)], *S*-nitroso-*N*-acetylpenicillamine (SNAP), 8-bromoguanosine 3′,5′-cyclic monophosphate sodium salt, S-methyl methanethiosulfonate, and sodium L-ascorbate were purchased from Sigma-Aldrich (St. Louis, MO); N^G-monomethyl-L-arginine (L-NMMA) was purchased from Dojindo (Kumamoto, Japan); EZ-Link Biotin-HPDP was purchased from Pierce (Rockford, IL); recombinant mouse IFN-β, carrier-free (rIFN-β) was purchased from PBL Interferon Source (Piscataway, NJ); Griess reagent kit was purchased from Invitrogen (Grand Island, NY); anti-NLRP3 mAb (Cryo-2) and anti-ASC pAb (AL177) were purchased from Enzo Life Sciences (Exeter, U.K.); anti–caspase-1 p10 (M-20) pAb was purchased from Santa Cruz Biotechnology (Santa Cruz, CA); anti-mouse IL-1β biotinylated Ab was purchased from R&D Systems (Minneapolis, MN); anti-iNOS mAb was purchased from Transduction Laboratories (Lexington, KY); ELISA kit for mouse IL-1β was purchased from eBioscience (San Diego, CA); and recombinant mouse IL-18, anti-mouse IL-18 capture Ab, and anti-mouse 1L-18 biotin-labeled Ab were purchased from Medical & Biological Laboratories (Nagoya, Japan). The following primers were used: TRIM30 sense: 5′-GGCACAGTCTTTGCTCTGCAGTGTG-3′, antisense: 5′-GCAGTTGCCCTTCCCGTCTG-3′ and β-actin sense: 5′-TGGAATCCTGTGGCATCCATGAAAC-3′, antisense: 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′.

Cells

Peritoneal exudate cells collected 4 d after i.p. injection of thioglycollate medium were incubated for 3 h in RPMI 1640 medium supplemented with 10% FCS at 37°C in 5% CO_2. Nonadherent cells were washed out, and adherent macrophages were primed with LPS or Pam_3CSK_4 at a final concentration of 50 ng/ml in Opti-MEM I (Invitrogen). Then, the cells were treated with 5 μM nigericin for 60 min, 5 mM ATP for 30 min, 600 ng poly (dA:dT) using Lipofectamine LTX and Plus Reagent (Invitrogen) for 60 min, or 175 ng flagellin using GenomeONE-NEO Transfection Reagent HVJ Envelope vector Kit (Ishihara Sangyo, Osaka, Japan) for 60 min. rIFN-β and L-NMMA were used at final concentrations of 100 U/ml and 500 μM, respectively.

Results and Discussion

NO is involved in the inhibitory effect of IFN-β on caspase-1 activation via the NLRP3 inflammasome

IFN-β was shown to inhibit the expression of pro–IL-1β and caspase-1 activation via NLRP3 inflammasome, but the mechanism remains unknown (9). To analyze whether NO generated upon IFN stimulation is responsible for the inhibitory effect of type I IFNs on NLRP3 inflammasome activation, peritoneal macrophages were primed with Pam_3CSK_4, a TLR2 ligand, in the presence or absence of rIFN-β and/or L-NMMA, an NO synthase inhibitor, before being stimulated with nigericin. In agreement with the previous study, we observed clear decreases in nigericin-induced caspase-1 activation, the protein level of pro–IL-1β, and the secretion of mature IL-1β when priming was done in the presence of rIFN-β (Fig. 1A, 1B). The specificity of the inhibitory effect of IFN-β was confirmed using IFNAR1-deficient macrophages (Fig. 1A, 1B). As expected, both the expression of iNOS and NO production were strongly induced in WT macrophages primed with Pam_3CSK_4 and rIFN-β (Fig. 1A, 1C). Interestingly, the addition of L-NMMA, which inhibited the production of NO, canceled the inhibitory effect of IFN-β on caspase-1 activation (Fig. 1A, 1C). In contrast, L-NMMA only modestly restored the level of pro–IL-1β in rIFN-β–treated macrophages

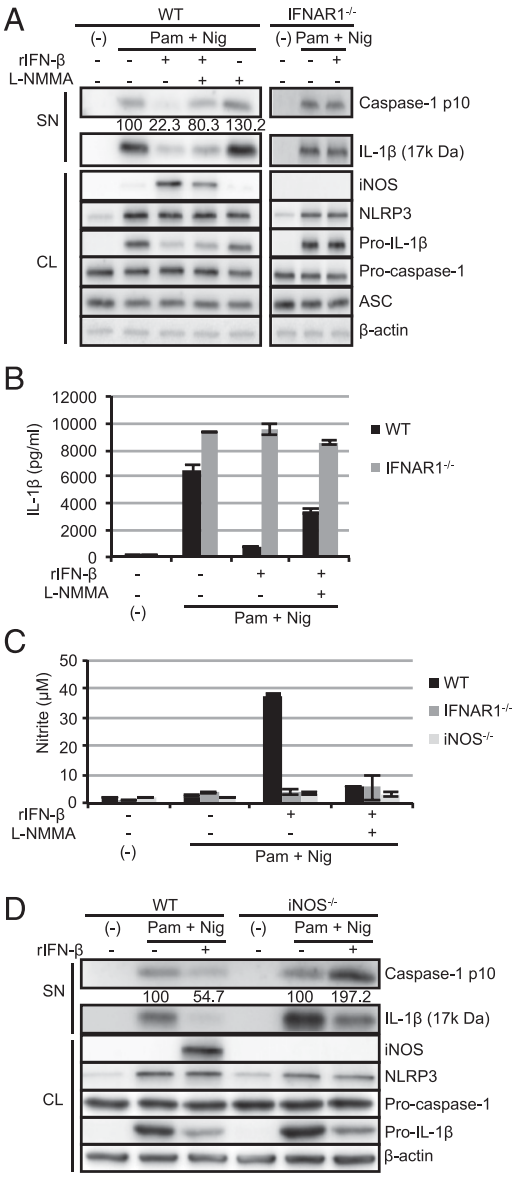

FIGURE 1. iNOS is required for inhibition of the NLRP3 inflammasome by IFN-β. Macrophages from WT mice (**A**–**D**), IFNAR1-deficient mice (A–C), or iNOS-deficient mice (C, D) were left unprimed or primed with Pam_3CSK_4 (Pam) in the presence or absence of rIFN-β and/or L-NMMA for 12 h. The cells were stimulated or not with nigericin (Nig) for 30 min. (A and D) Culture supernatants and cell lysates were collected and subjected to Western blotting with Abs specific to the indicated proteins. The numbers represent the relative intensities of the caspase-1 p10 fragment after normalization to control values (%). (B) The level of IL-1β in the culture supernatants was determined by ELISA. (C) The concentration of nitrite in the culture supernatants was measured using a Griess reagent kit. Experiments were repeated at least three times with consistent results. Data represent means and standard deviations of triplicate assays.

primed with Pam_3CSK_4, resulting in a partial recovery of the secretion of mature IL-1β induced by nigericin (Fig. 1A, 1B). These data suggested that NO is the molecule contributing to the inhibitory effect of IFN-β on NLRP3 inflammasome activation. In iNOS-deficient macrophages, NO production in response to Pam_3CSK_4 and rIFN-β was not observed, and rIFN-β treatment did not reduce the nigericin-induced caspase-1 activation (Fig. 1C, 1D). Thus, we confirmed that NO plays a critical role in the inhibition of the NLRP3 inflammasome by IFN-β. Recently, a study showed that IFN-β induces the

expression of SOCS1, which mediates the inhibition of ROS production (12). Therefore, our findings suggest a novel mechanism of inhibition of inflammasome activation by type I IFNs in addition to the SOCS1-mediated one.

NO donor SNAP inhibits the NLRP3 inflammasome, whereas the AIM2 and NLRC4 inflammasomes are only partially affected

To further confirm the inhibitory effect of NO on the activation of NLRP3 inflammasome, macrophages primed with Pam_3CSK_4 were incubated in the presence of SNAP, an NO donor, before stimulation with inflammasome activators. Substantial levels of NO were generated in the cultures when various concentrations (125–500 μM) of SNAP were added (Fig. 2A). Under this experimental condition, caspase-1 activation and the secretion of mature forms of IL-1β and IL-18 induced by nigericin or ATP were significantly decreased by SNAP in a dose-dependent manner (Fig. 2B–E). This result substantiated the NO-dependent inhibition of the NLRP3 inflammasome. In contrast, SNAP treatment partially diminished these cellular responses induced by poly(dA:dT) or flagellin, agonists for AIM2 and NLRC4, respectively (Fig. 2B–E). Judging from these results, it appeared that the NLRP3 inflammasome is more susceptible to NO than are the AIM2 and NLRC4 inflammasomes and that the inhibitory effect of NO on the NLRP3 inflammasome is not simply due to direct inhibition of caspase-1 enzymatic activity by NO, as proposed in a previous report (10).

Endogenous NO induced by long-time LPS priming serves as a negative regulator of NLRP3 inflammasome activation

It was shown that, after long-term priming with LPS, macrophages become refractory to the activation of NLRP3. Actually, macrophages primed with LPS for 12 h secreted markedly lower levels of IL-18 after stimulation with nigericin or ATP compared with those primed for 4 h (Fig. 3A). A possible explanation for this inhibitory effect is that LPS induces the expression of TRIM30, which negatively regulates the NLRP3 inflammasome (8). However, we observed that long-term priming with Pam_3CSK_4 did not lead to suppression of NLRP3 inflammasome activation, even though TRIM30 expression was upregulated to some extent (Fig. 3B, 3C). LPS, but not Pam_3CSK_4, induced type I IFN production, iNOS expression, and NO generation in macrophages (Fig. 1C, Supplemental Fig. 1A, 1B) (11, 13). Therefore, we hypothesized that NO induced by long-term LPS priming may mediate the inhibitory effect. To test this possibility, iNOS-deficient macrophages were stimulated with nigericin or ATP after being primed with LPS for 4 or 12 h. We found that these NLRP3 agonists induced higher levels of IL-18 secretion from iNOS-deficient macrophages than from WT macrophages if the macrophages were primed for 12 h (Fig. 3D). Similar results were obtained in experiments using L-NMMA (Supplemental Fig. 1C, 1D). In contrast, there was no significant difference between iNOS-deficient macrophages and WT macrophages in the secretion of IL-18 in response to NLRP3 agonists when the cells were primed with LPS for 4 h, at which time iNOS was not expressed (Fig. 3D, Supplemental Fig. 1E). Accordingly, our results suggest that endogenous NO induced by long-time LPS priming inhibits NLRP3 inflammasome activation. Secretion of IL-18 induced by flagellin or poly(dA:dT) after long-term priming with LPS was even greater than was that after a 4-h LPS priming (Fig. 3E), supporting that NLRC4 and AIM2 inflammasomes are more resistant to NO than is the NLRP3 inflammasome.

Analysis of the mechanism by which NO regulates NLRP3 inflammasome activation

To elucidate the mechanism by which NO exerts its inhibitory effect, particularly on the NLRP3 inflammasome, we measured mitochondrial ROS (mtROS), which are known to promote

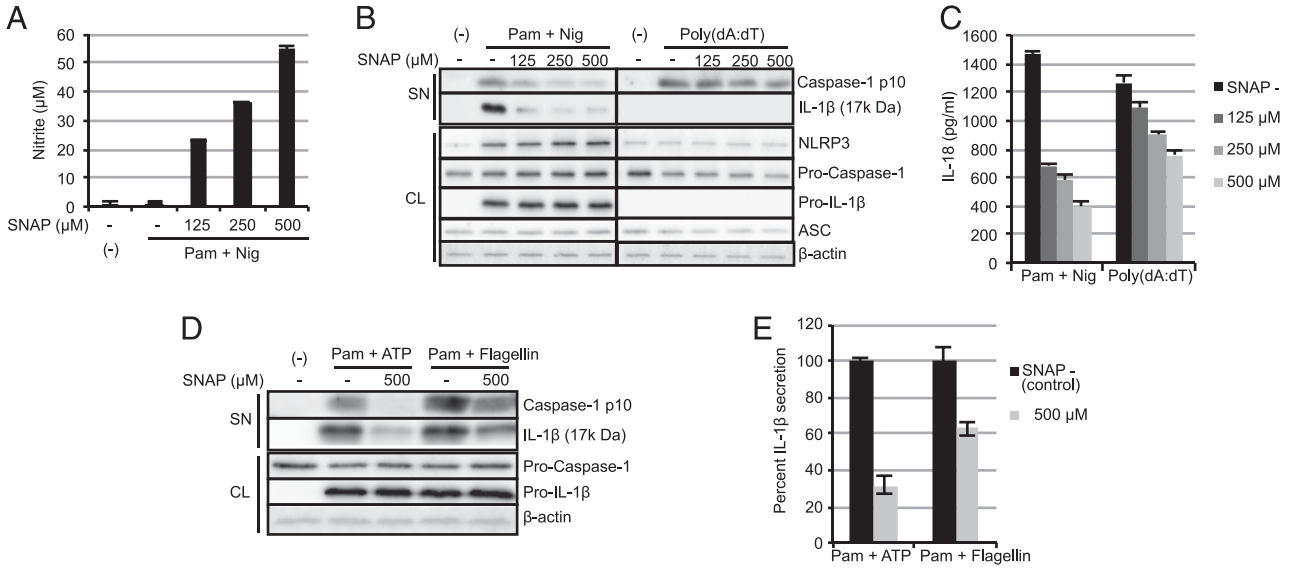

FIGURE 2. The NO donor SNAP inhibits the activation of the NLRP3 inflammasome. WT macrophages were primed with Pam_3CSK_4 (Pam) for 3 h and further treated or not with the indicated concentrations of SNAP for 1 h. The cells were then stimulated or not with nigericin (Nig) (**A**–**C**), ATP (**D**, **E**), or flagellin (D, E). (B and C) Unprimed cells were stimulated with poly(dA:dT) with or without SNAP pretreatment. (A) The concentration of nitrite in the culture supernatants was measured. (B and D) Culture supernatants and cell lysates were subjected to Western blotting with Abs specific to the indicated proteins. (C and E) The levels of IL-18 or IL-1β in the culture supernatants were determined by ELISA. (E) The percentage of IL-1β secretion was calculated as (IL-1β concentration in test sample)/(the average IL-1β concentration in control samples) × 100. Experiments were repeated at least two times with consistent results. Data represent means and standard deviations of triplicate assays.

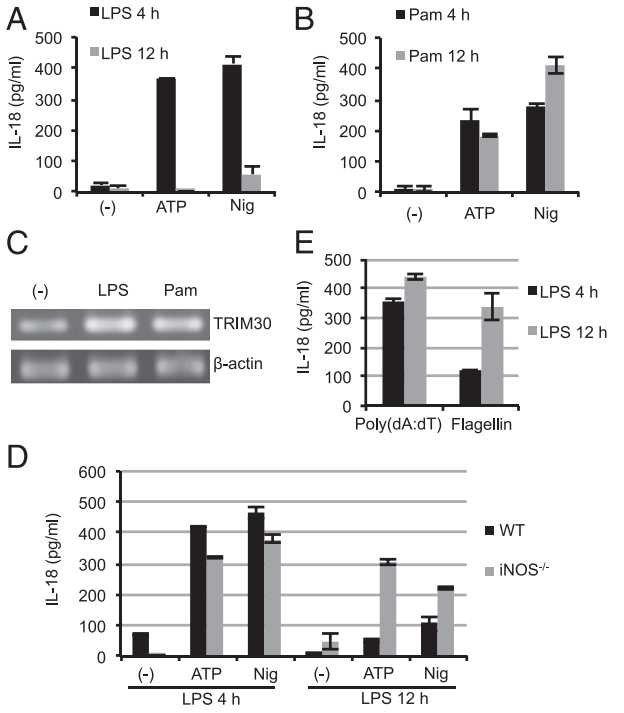

FIGURE 3. Long-term LPS priming suppresses NLRP3 inflammasome activation via induction of NO generation. Macrophages from WT mice or iNOS-deficient mice (**D**) were primed with LPS (**A**, D, **E**) or Pam_3CSK_4 (**B**) for 4 or 12 h. The cells were then stimulated or not with nigericin or ATP (A, B, D) or poly(dA:dT) or flagellin (E). The level of IL-18 in the culture supernatants was determined by ELISA. (**C**) Macrophages were stimulated with LPS or Pam_3CSK_4 for 12 h, and the expression of TRIM30 mRNA was analyzed by RT-PCR. Experiments were repeated at least two times with consistent results. Data represent means and standard deviations of triplicate assays.

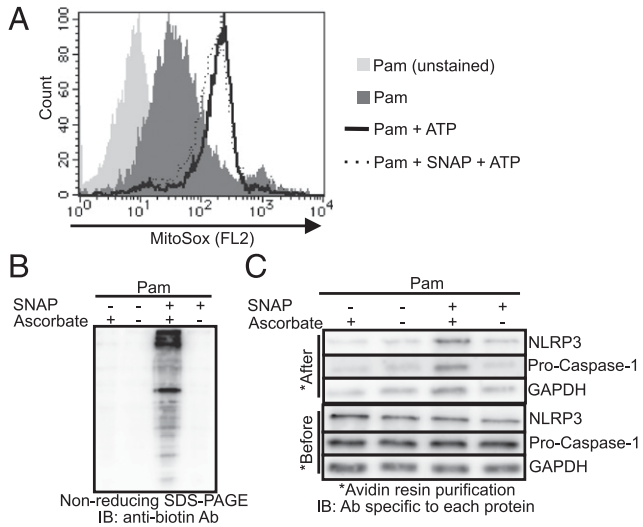

FIGURE 4. Analysis of the mechanism by which NO regulates the NLRP3 inflammasome. (**A**–**C**) WT macrophages were primed with Pam_3CSK_4 for 3 h and treated or not with SNAP (0.5 mM) for 1 h. (A) The cells were stimulated with ATP for 15 min, stained with MitoSOX (2.5 μM) for 15 min, and analyzed on a flow cytometer. (B and C) The cells were lysed, and free thiols were blocked with *S*-methyl methanethiosulfonate (20 mM). The lysates were reduced or not with sodium ascorbate (10 mM) and labeled with 200 μM of biotin-HPDP. (B) The labeled lysates were subjected to nonreducing SDS-PAGE and Western blotting with an anti-biotin Ab. (C) NLRP3, caspase-1, and GAPDH (positive control) were detected by Western blotting in the labeled lysates (*lower panels*) and in biotinylated protein fractions enriched from the same samples (*upper panels*). Experiments were repeated at least three times with consistent results.

NLRP3 activation (3–5). SNAP treatment only modestly affected the increase in mtROS induced by ATP (Fig. 4A), and it was difficult to conclude whether the inhibition of NLRP3 by NO depends solely on the decrease in mtROS generation. NO induces the upregulation of antioxidant genes, including thioredoxin reductase-1, which may disturb NLRP3 inflammasome activation (2) (Supplemental Fig. 2A). However, inhibition of NLRP3 inflammasome by just a 1-h treatment with SNAP was also observed in the presence of cycloheximide (Supplemental Fig. 2B, 2C). Thus, the de novo synthesis of antioxidant proteins did not appear to account for the inhibitory effect of NO on NLRP3 inflammasome, at least at an early time after exposure to SNAP. NO is known to increase the synthesis of the second messenger cyclic GMP, but IL-1β secretion was not affected by 8-bromoguanosine 3′,5′-cyclic monophosphate sodium salt, an analog of cyclic GMP (Supplemental Fig. 2D). *S*-nitrosylation is a covalent addition of an NO group onto protein cysteine thiols to form *S*-nitrosoproteins, and it has been increasingly recognized as a post-translational modification regulating the protein functions (14). We used the biotin-switch technique, as previously described, to determine whether the NLRP3 protein can be a target of *S*-nitrosylation (15). Biotinylated proteins were detected in cell lysates from macrophages treated with SNAP for 1 h, only when the lysates were reduced with ascorbate before labeling of protein cysteine thiols with biotin (Fig. 4B), indicating that *S*-nitrosoproteins in the lysates were successfully biotinylated. Interestingly, caspase-1 and NLRP3 were detected in *S*-nitrosylated proteins enriched from the labeled lysates using an avidin resin (Fig. 4C). We also found that the C-terminal region of NLRP3 is more susceptible to *S*-nitrosylation than is the N-terminal region (Supplemental Fig. 2E). From these results, we propose that a direct modification of the NLRP3 protein by *S*-nitrosylation is the mechanism responsible for the early inhibition of NLRP3 inflammasome activation by NO. A direct *S*-nitrosylation of caspase-1 itself may explain the partial inhibition of the AIM2 and NLRC4 inflammasomes by NO. Further analysis is necessary to confirm the relevance of *S*-nitrosylation as a regulatory mechanism of the NLRP3 inflammasome. Another possibility to be addressed is that NO interferes with other proteins involved in NLRP3 inflammasome activation or with mitochondrial DNA release, thereby inhibiting the NLRP3 inflammasome (4, 5).

In this study, we found that NO regulates the activation of NLRP3 inflammasome. Because both NO and NLRP3 are known to play roles in a wide range of physiological responses, NO-mediated inhibition of the NLRP3 pathway might have significance not only in the beneficial host defense against microbial pathogens but also in the pathophysiology of NLRP3-associated detrimental diseases. For example, NLRP3 was implicated in the pathogenesis of ischemia–reperfusion injury (16), and the involvement of NO was demonstrated in preconditioning-induced tissue protection from ischemia–reperfusion injury (17). Therefore, it is worth considering whether inhibition of NLRP3 activation by NO is at least one of the mechanisms of tissue protection by ischemic preconditioning. Further studies are necessary to fully describe the role and mechanism of the inhibitory effect of NO on NLRP3, and a better understanding of the mechanism may shed new light on the activation process of the NLRP3 inflammasome.

Acknowledgments

We thank Prof. Michel Aguet for permission to use the IFNRA1 KO mice and Prof. Jürg Tschopp, University of Lausanne, Epalinges, Switzerland, for permission to use the NLRP3 KO mice. We thank Prof. Shigekazu Nagata, Kyoto University, for providing the IFNRA1 KO mice and Prof. Hiroko Tsutsui, Hyogo College of Medicine, Hyogo, Japan, for providing the NLRP3$^{-/-}$ mice.

Disclosures

The authors have no financial conflicts of interest.

References

1. Rathinam, V. A., S. K. Vanaja, and K. A. Fitzgerald. 2012. Regulation of inflammasome signaling. *Nat. Immunol.* 13: 333–2.
2. Zhou, R., A. Tardivel, B. Thorens, I. Choi, and J. Tschopp. 2010. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nat. Immunol.* 11: 136–140.
3. Zhou, R., A. S. Yazdi, P. Menu, and J. Tschopp. 2011. A role for mitochondria in NLRP3 inflammasome activation. *Nature* 469: 221–225.
4. Nakahira, K., J. A. Haspel, V. A. Rathinam, S. J. Lee, T. Dolinay, H. C. Lam, J. A. Englert, M. Rabinovitch, M. Cernadas, H. P. Kim, et al. 2011. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat. Immunol.* 12: 222–230.
5. Shimada, K., T. R. Crother, J. Karlin, J. Dagvadorj, N. Chiba, S. Chen, V. K. Ramanujan, A. J. Wolf, L. Vergnes, D. M. Ojcius, et al. 2012. Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity* 36: 401–414.
6. Vandanmagsar, B., Y. H. Youm, A. Ravussin, J. E. Galgani, K. Stadler, R. L. Mynatt, E. Ravussin, J. M. Stephens, and V. D. Dixit. 2011. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat. Med.* 17: 179–188.
7. Bauernfeind, F. G., G. Horvath, A. Stutz, E. S. Alnemri, K. MacDonald, D. Speert, T. Fernandes-Alnemri, J. Wu, B. G. Monks, K. A. Fitzgerald, et al. 2009. Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *J. Immunol.* 183: 787–791.
8. Hu, Y., K. Mao, Y. Zeng, S. Chen, Z. Tao, C. Yang, S. Sun, X. Wu, G. Meng, and B. Sun. 2010. Tripartite-motif protein 30 negatively regulates NLRP3 inflammasome activation by modulating reactive oxygen species production. *J. Immunol.* 185: 7699–7705.
9. Guarda, G., M. Braun, F. Staehli, A. Tardivel, C. Mattmann, I. Förster, M. Farlik, T. Decker, R. A. Du Pasquier, P. Romero, and J. Tschopp. 2011. Type I interferon inhibits interleukin-1 production and inflammasome activation. *Immunity* 34: 213–223.
10. Kim, Y. M., R. V. Talanian, J. Li, and T. R. Billiar. 1998. Nitric oxide prevents IL-1β and IFN-γ-inducing factor (IL-18) release from macrophages by inhibiting caspase-1 (IL-1β-converting enzyme). *J. Immunol.* 161: 4122–4128.
11. Jacobs, A. T., and L. J. Ignarro. 2001. Lipopolysaccharide-induced expression of interferon-β mediates the timing of inducible nitric-oxide synthase induction in RAW 264.7 macrophages. *J. Biol. Chem.* 276: 47950–47957.
12. Inoue, M., K. L. Williams, T. Oliver, P. Vandenabeele, J. V. Rajan, E. A. Miao, and M. L. Shinohara. 2012. Interferon-β therapy against EAE is effective only when development of the disease depends on the NLRP3 inflammasome. *Sci. Signal.* 5: ra38.
13. Takeuchi, O., and S. Akira. 2010. Pattern recognition receptors and inflammation. *Cell* 140: 805–820.
14. Sengupta, R., and A. Holmgren. 2012. The role of thioredoxin in the regulation of cellular processes by *S*-nitrosylation. *Biochim. Biophys. Acta* 1820: 689–700.
15. Jaffrey, S. R., and S. H. Snyder. 2001. The biotin switch method for the detection of S-nitrosylated proteins. *Sci. STKE* 2001: pl1.
16. Iyer, S. S., W. P. Pulskens, J. J. Sadler, L. M. Butter, G. J. Teske, T. K. Ulland, S. C. Eisenbarth, S. Florquin, R. A. Flavell, J. C. Leemans, and F. S. Sutterwala. 2009. Necrotic cells trigger a sterile inflammatory response through the Nlrp3 inflammasome. *Proc. Natl. Acad. Sci. USA* 106: 20388–20393.
17. Ogawa, T., A. K. Nussler, E. Tuzuner, P. Neuhaus, M. Kaminishi, Y. Mimura, and H. G. Beger. 2001. Contribution of nitric oxide to the protective effects of ischemic preconditioning in ischemia-reperfused rat kidneys. *J. Lab. Clin. Med.* 138: 50–58.