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博士の専攻分野の名称	博　士（医学）
学位記番号	第　２５８９４　号
学位授与年月日	平成25年３月25日
学位授与の要件	学位規則第４条第１項該当 医学系研究科予防環境医学専攻
学位論文名	Nitric Oxide Inhibits the NLRP3 Inflammasome （一酸化窒素はNLRP３インフラマソームの活性化を抑制する）
論文審査委員	（主査） 教　授　審良　静男 （副査） 教　授　竹田　　潔　　教　授　荒瀬　　尚

論文内容の要旨

〔目　的 (Purpose)〕

Caspase-1 is involved in the processing and maturation of the inflammatory cytokines IL-1β and IL-18. Activation of caspase-1 is accomplished by the formation of multiprotein complexes called inflammasomes. NLRP3 inflammasome is important for host defense against microbial pathogens. However, its excessive activation has been associated to several autoinflammatory diseases, highlighting the importance of negative regulation of this inflammasome. Exposure of macrophages to type I IFNs or long-term LPS stimulation has been shown to negatively regulate NLRP3 activation. As both type I IFN signaling and LPS stimulation lead to the expression of inducible nitric oxide synthase (iNOS) and the production of NO, we addressed whether NO inhibits the NLRP3 inflammasome.

〔方法ならびに成績 (Methods/Results)〕

Female C57BL/6 (WT), IFN-α/β receptor 1 (IFNAR1) knockout (KO), and iNOS KO mice were used in this study. To activate each inflammasome, peritoneal exudate macrophages from mice were primed with LPS or Pam_3CSK_4. Then, cells were stimulated with nigericin or ATP (NLRP3 inflammasome agonists), poly(dA:dT) (AIM2 inflammasome agonist), or flagellin (NLRC4 inflammasome agonist). Activation of caspase-1 was monitored by Western blotting with an anti-caspase-1 antibody. IL-1β and IL-18 were measured by using specific ELISA kits. Recombinant IFN-β and L-NMMA (a NOS inhibitor) were used at final concentrations of 100 U/ml and 500 μM, respectively. *S*-nitroso-N-acetylpenicillamine (SNAP), an NO donor, was used at 500 μM.

The inhibitory effect of IFN-β on the NLRP3 inflammasome activation was not observed when macrophages were treated with L-NMMA or in iNOS-deficient macrophages, suggesting that NO is responsible for this inhibitory effect. We also found that the inhibition of the NLRP3 inflammasome by long-term LPS stimulation depends on NO production. Furthermore, SNAP treatment markedly inhibited the NLRP3 inflammasome activation, while the AIM2 and NLRC4 inflammasomes were only partially inhibited by SNAP. The inhibitory effect of NO on the NLRP3 inflammasome was neither due to a change in the mitochondrial ROS production nor to the induction of the second messenger cGMP. Interestingly, treatment with SNAP resulted in the S-nitrosylation of NLRP3, which may account for the NO-dependent regulatory mechanism.

〔総　括 (Conclusion)〕

Nitric oxide was revealed as a negative regulator of the NLRP3 inflammasome via a mechanism that depends neither on the mitochondrial ROS production nor on the synthesis of new proteins. We suggest S-nitrosylation as an inhibitory modification that affects the NLRP3 activation.

論文審査の結果の要旨

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.

以上の結果は、学術的に非常に有意義な成果であり、学位の授与に値すると考えられる。