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学 位 論 文 名	Essential role of IPS-1 in innate immune responses against RNA viruses （RNA ウイルスに対する自然免疫反応における IPS-1 の役割）
論文審査委員	（主査） 教　授　審良　静男 （副査） 教　授　菊谷　　仁　　教　授　松浦　善治

論 文 内 容 の 要 旨

〔　目　　的　〕

Viruses are small, obligate intracellular parasites which cause infection by invading cells of the body and multiplying within them. After infection, viruses have a relatively short extracellular period and a longer intracellular period for replication. The host antiviral system has sensors known as pattern recognition receptors (PRRs) which detect viral components and induce type I interferon (IFN) and inflammatory cytokines, which are essential for virus elimination. Retinoic acid-inducible gene-I (RIG-I) and melanoma differentiation-associated gene 5 (Mda5) are cytoplasmic PRRs which recognize RNA of various RNA viruses. They contain a C-terminal RNA helicase domain which recognizes viral double stranded (ds) RNA and N-terminal caspase-recruiting domains (CARD) for downstream signaling. Upon recognition, RIG-I and Mda5 interact with a CARD-containing molecule known as Interferon-β promoter stimulator-1 (IPS-1) which we identified and activate transcription factors IRF3/7 and NFκB, which control expression of type I interferon (IFN) and inflammatory cytokines. However, the contributions of IPS-1 in antiviral immune responses in vivo remain unclear.

〔　方法ならびに成績　〕

We generated IPS-1 knockout (KO) mice by replacing the first two exons which encode the CARD with neomycin gene using the standard homologous recombination gene targeting method. IPS-1 KO mice were born at the expected Mendelian ratio and grew healthy.

First, we investigated cytokine production after infection with various single-stranded RNA viruses such as New castle disease virus (NDV), Vesicular Stomatitis virus (VSV) and Sendai virus (SeV), which are specifically recognized by RIG-I. The induction of type I IFN, IFN-inducible genes and IL-6 was completely abrogated in various cells from IPS-1 KO mice as investigated by ELISA and northern blot. In addition, the activation of

IRF3 and NFκB after NDV infection was also abrogated in IPS-1 KO cells. After in vivo infection with VSV, the virus titer was increased in the liver and brain of IPS-1 KO mice and IPS-1 KO mice died earlier than control mice.

Next, we investigated cytokine production after infection with another single-stranded RNA virus known as Encephalomyocarditis virus (EMCV) which is specifically recognized by Mda5. Type I IFN was completely abrogated in IPS-1 KO cells whereas IL-6 was significantly reduced in IPS-1 KO cells compared to the control cells. After in vivo infection with EMCV, the virus titer was increased in the heart of IPS-1 KO mice and IPS-1 KO mice were died earlier than control mice.

Finally, we investigated the involvement of IPS-1 in DNA virus recognition. Cells were infected with Modified Vaccinia virus Ankara (a DNA virus), and production of the type I IFN and other cytokines was measured. The production in IPS-1 KO cells was comparable to the wild type cells.

〔 総　　括 〕

These results show that the IPS-1 is the sole adaptor for RIG-I and Mda5, and an essential signal transducer for the activation of NF-κB and IRF3.

IPS-1 is not essential for DNA virus-mediated responses.

論文審査の結果の要旨

ウイルス感染を察知するセンサーとして RIG-I や Mda5 といった細胞質内 RNA ヘリカーゼが同定されている。これら分子は IPS-1 と呼ばれるアダプター分子を介して、I 型インターフェロンや炎症性サイトカインを誘導し、ウイルスを排除する。今回、IPS-1 欠損マウスを作製し、ウイルス感染に対する免疫応答の検討を行った。IPS-1 欠損マウスでは RIG-I により認識される RNA 型ウイルス（センダイウイルス、ニューカッスル病ウイルス、水疱性口内炎ウイルス）や Mda5 により認識される脳心筋炎ウイルス感染によるサイトカイン産生が顕著に減少していた。また、IPS-1 欠損マウスはウイルス感染により早期に死亡した。これらのことは、IPS-1 は RIG-I と Mda5 の共通のアダプター分子であることを示している。本論文は、ウイルス感染に応答した情報伝達経路に IPS-1 が生体内において必須の役割を果たしていることを明らかにしたものであり高く評価できる。したがって、博士（医学）の学位授与に値する。