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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	Cathepsin D triggers dendritic cell necroptosis by modulating an IPS-1-dependent pathway to propagate adjuvant efficacy of poly IC （カテプシンDのIPS-1経路を介した樹状細胞の細胞死誘導とそれによるPoly ICのアジュバンド効果亢進について）
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論文内容の要旨

〔目　的 (Purpose)〕

Polyinosinic-polycytidylic acid (poly IC), a synthetic analogue of dsRNA, is recognized by RIG-I-like receptors (RLRs; RIG-I, MDA5) and Toll-like receptor (TLR) 3. However, the contribution of RLRs and TLR3 in recognition of poly IC is not well understood. Accumulating evidence has demonstrated that pathogen infection triggers various types of cell death, which also contribute to driving of innate and adaptive immune responses. In our study we investigated how poly IC facilitates adjuvant effects in dendritic cells (DCs).

〔方法ならびに成績 (Methods/results)〕

IPS-1 is required for poly IC-induced cytokine production in cDCs

We prepared GM-CSF-induced bone marrow DCs (GM-DCs) and examined their cytokine production after poly IC stimulation. Production of IFNβ and IL-6 following poly IC treatment was severely reduced in IPS-1-deficient GM-DCs. We also investigated the cytokine production in Flt3 ligand-induced bone marrow DCs (FL-DCs), and found that poly IC-induced IFNβ production was severely reduced in IPS-1-deficient $CD11b^{high}CD24^{low}$ cells ($CD8^-$ cDCs). The IFNβ mRNA level was also significantly reduced in IPS-1-deficient spleen-derived $CD8\alpha^-$ DCs compared with wild type cells after poly IC administration in vivo. Thus, an IPS-1-dependent pathway contributes to the poly IC-induced IFNβ production by $CD8\alpha^-$ DCs.

Cathepsin D is released to the cytoplasm following poly IC stimulation and interacts with IPS-1

We discovered that Cathepsin D could bind to IPS-1 by a yeast two-hybrid screen. We confirmed the interaction of IPS-1 with Cathepsin D in coimmunoprecipitation experiments using epitope-tagged proteins expressed after transient transfection into HEK293 cells. In unstimulated GM-DCs, Cathepsin D was localized in cytoplasmic vesicles with dot-like structures, which were merged with LAMP1-positive vesicles. Poly IC stimulation caused translocation of Cathepsin D to the cytoplasm.

Poly IC induces cell death in a Cathepsin D- and IPS-1-dependent manner

Poly IC stimulation increased necroptosis, and this type of cell death was impaired in IPS-1-deficient GM-DCs. Pep A, an inhibitor of Cathepsin D, treatment or Cathepsin D knockdown abrogated the poly IC-induced cell death. Pretreatment of GM-DCs with Necrostatin-1 (Nec-1), an inhibitor of RIP-1 kinase activity, reduced the ratio of dead cells. These suggest that the poly IC-induced necroptosis is mediated by IPS-1, Cathepsin D, and RIP-1.

HMGB1 enhances poly IC-induced IFNβ production

HMGB1 was released into the culture supernatant of GM-DCs stimulated with poly IC, and that this effect was abrogated by IPS-1-deficiency. Poly IC-induced production of HMGB1 was suppressed by treatment with Pep A or Nec-1. These findings suggest that poly IC-induced necroptosis is linked to the production of HMGB1. The poly IC-induced production of IFNβ by GM-DCs was augmented by costimulation with HMGB1. HMGB1 increased the enhancing effects of poly IC on IFNγ production by $CD4^+$ T cells. We also found that recombinant HMGB1 bound with biotinylated poly IC. Anti-HMGB1 blocking antibody, which inhibited the interaction between recombinant HMGB1 and poly IC, suppressed the poly IC-induced IFNβ production in GM-DCs, suggesting that HMGB1 is indeed involved in the acceleration of poly IC-induced IFNβ production.

〔総　括 (Conclusion)〕

Our data suggest that poly IC recognition is occurred through RLRs rather than TLR3 in $CD8^-$ DCs. Necroptosis of a minor population of DCs is a potential mechanism that increases the adjuvanticity of poly IC. This cell death is controlled by a signaling complex involving IPS-1 and RIP-1 along with Cathepsin D, which is leaked into the cytoplasm through lysosome rupture upon poly IC uptake. Furthermore, activation of this pathway results in release of HMGB1, which potentiates immune responses in concert with poly IC.

論文審査の結果の要旨

RIG-I-like receptors (RLRs)はウイルス由来のRNAやpolyinosinic-polycytidylic acid (poly IC)を認識し、抗ウイルス応答を惹起する。本研究では、poly ICによる免疫賦活化のメカニズムを解明した。poly ICはまず樹状細胞の貪食作用によって細胞内に取り込まれ、リソソームに局在したことを見出した。その後、リソソームの崩壊が誘導され、poly ICは細胞質に分散し、RLRsにより認識されることが示唆された。リソソームの崩壊に伴い、アスパラギン酸プロテアーゼのCathepsin Dがリソソームより細胞質に漏出し、RLRsのアダプター因子であるIPS-1と結合した。この複合体形成はRIP-1に依存的なProgrammed necrosis（necroptosis）を惹起した。死細胞から放出した内在性因子HMGB1は、poly ICと結合し、poly ICによるIFN-βの産生をさらに増強させた。これらのことから、Cathepsin Dを引き金としたIPS-1－RIP-1に依存的なnecroptosisがpoly ICによる免疫賦活を増強させることが明らかとなった。免疫賦活性を持つ自己成分の同定ならびに下流シグナルの解析をさらに深めることで、癌や感染症に対する新型アジュバントの開発に繋がることが期待できる。また、内在性因子の関与が疑われる自己免疫疾患や炎症性疾患の発症メカニズムの解明や治療法の開発にも繋がることが期待される。

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。