論　文　内　容　の　要　旨
Synopsis of Thesis

氏　　名 Name	Thin Sandi Htun
論文題名 Title	Regnase-1 D141N mutation induces CD4+ T cell-mediated lung granuloma formation via upregulation of Pim2 (Regnase-1 D141N変異はPim2の上方制御を介してCD4+ T 細胞介在性肺肉芽腫を誘導する)

論文内容の要旨

〔目　的(Purpose)〕：Regnase-1, an RNase, plays a crucial role in downregulating immune responses by destabilizing inflammatory mRNAs. It can significantly contribute to various inflammatory diseases characterized by tissue damage and the infiltration of immune cells into organs in case of being disregulated. Our research aims to understand how RNase activity of Regnase-1 impacts the development of inflammatory diseases. To achieve this, we created mice with a single-point mutation (D141N mutation) at the catalytic center of RNase domain of Regnase-1, which leads to the loss of endonuclease activity. D141N mutation of has been widely used in the studies as a control to confirm Regnase-1 targets. However, the intricate immunological pathways affected by D141N mutation in vivo remain largely unknown. Our study delved into the phenotypic and mechanistic aspects of D141N mutation in vivo and in vitro.

〔方法ならびに成績(Methods/Results)〕：In Regnase-1 D141N mutant mice, systemic inflammation, immune cell infiltration into the lungs and progressive lung pathology were observed. Loss of endonuclease activity of Regnase-1 triggers immune reactions and organ inflammation. Moreover, endonuclease activity of Regnase-1 is indispensable to prevent aberrant activation of T and B lymphocytes. Notably, this mutation predominantly affected the lung tissue and $CD4^+$ T cells, leading to the upregulation of mTORC1 pathway and exacerbating chronic inflammation of the lungs. Although we observed certain resemblances in the immunological characteristics of Regnase-1 D141N and Regnase-1 KO mice, there were also discernible distinctions. Furthermore, we used D141N/Rag2 KO mouse model, as well as the mouse models involving the transfer of splenic CD4 cells and bone marrow cells to confirm the essential contribution of T cells in the manifestation of lung disease of D141N mutation in vivo. Transcriptomic assessment revealed the important genes including Pim2 kinase in D141N mutation. This mutation affects MAPK, NF-κB and Stat-3 pathways. Pharmacological inhibition of Pim2 ameliorated lung disease in D141N mice. We validated the importance of Pim2 kinase in CD4+ T cells in D141N mutation by ex vivo knocking down its gene and analyzing the activation and proliferation of CD4+ T cells.

〔総　括(Conclusion)〕Our study demonstrated that Regnase-1 D141N mutation results in elevated levels of Pim2 mRNA and protein, contributing to spontaneous activation, differentiation, proliferation and migration of lymphocytes and other leucocytes. Consequently, targeting Pim2 could be a promising therapeutic avenue for limiting abnormal immune responses caused by D141N mutation.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

Regnase-1は、IL-6, IL-12, いくつかのケモカインなどの炎症関連分子mRNAの3'非翻訳領域（3'UTR）のステム・ループ構造に結合し、これらのmRNAを分解するエンドリボヌクレアーゼで炎症反応にかかわる。本研究ではヌクレーゼ活性に必須のアミノ酸残基アスパラギン酸（D）をアスパラギン残基（N）に置換したノックインマウス（Reg1D141N）を作成し、Regnase-1のヌクレアーゼ活性を検討した。全身にRegnase-1を欠損したマウスと比較して炎症所見は減少し、主たる所見としては時間経過とともに肺に肉芽腫の形成が認められた。ノックインマウス由来の脾臓CD4＋細胞移入によって同様の所見が再現されることから、Regnase-1のヌクレアーゼ活性を欠損したCD4＋T細胞が肉芽腫形成に関与することがあきらかとなった。Regnase-1のヌクレアーゼ活性を欠損したCD4＋T細胞では細胞増殖が亢進し、Pim2キナーゼの遺伝子発現、蛋白量の増大が認められた。Pim2阻害剤投与によりこれらの肉芽腫形成が阻止された。さらにPim2キナーゼ遺伝子のノックダウンにおいてT細胞の増殖が抑制された。これらのデータは、Regnase-1のヌクレアーゼ活性がPim2キナーゼを制御することによりCD4T細胞の増殖を抑制している可能性を示唆する。これまで、あきらかでなかったRegnase-1のヌクレアーゼ活性の役割を解明したものとして博士（医学）に値するものと認める。