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論　文　内　容　の　要　旨
Synopsis of Thesis

氏　名 Name	馬　知秀
論文題名 Title	Selective and strain-specific NFAT4 activation by the *Toxoplasma gondii* polymorphic dense granule protein GRA6 （トキソプラズマ原虫の多型濃縮顆粒タンパク質GRA6による選択的・系統特異的NFAT4の活性化について）

論文内容の要旨

〔目　的(Purpose)〕

Toxoplasma gondii (*T. gondii*) infection results in co-option and subversion of host cellular signaling pathways. *T. gondii* GRA proteins, such as GRA15 and GRA16 were recently shown to directly influence gene expression in host cells. However, the role of the other GRAs still remains unknown. We have investigated the role of *T. gondii* GRAs in host immune responses.

〔方法ならびに成績(Methods/Results)〕

We constructed mammalian expression vectors for GRA proteins. Then, we assessed whether their overexpression, together with luciferase reporter plasmids harboring elements dependent on various transcription factors, activates the reporters. Overexpression of GRA6 dramatically activated the NFAT-dependent promoter in a calcineurin-dependent manner. We next analyzed the molecular mechanism by which GRA6 mediates activation of the NFAT. Immunoprecipitation study revealed an interaction between GRA6 and calcium modulating ligand (CAMLG) in 293T cells. GRA6-mediated activation of the NFAT-dependent promoter was selectively suppressed by down-regulation of NFAT4 gene expression but not by down-regulation of other NFAT members.

To assess the role of GRA6 in *in vivo* virulence in mice, we generated GRA6-deficient parasites by gene targeting. We found that local infection, in which parasites were injected into the right footpad, culminated in significantly slower systemic diffusion of GRA6-deficient parasites than that of WT parasites. In addition, mice locally infected with GRA6-deficient parasites survived for significantly longer than did those infected with WT parasites. Consistent with the in vivo imaging analysis, dpLNs from mice infected with WT parasites contained higher frequency of live cells infected with parasites than those from GRA6-deficient parasite-infected mice. Furthermore, we detected considerable proportion and numbers of $CD11b^{+}Ly6G^{+}$ cells in footpads of mice infected with WT parasites. The mRNA expression levels of *Cxcl2* and *Ccl2* in the footpads of mice infected with WT parasites were markedly higher than those in the footpads infected with GRA6-deficient parasites. To test whether NFAT4 is required for the GRA6-dependent full virulence directly, we generated NFAT4-deficient mice by the Cas9/CRISPR-mediated genome editing. Notably, NFAT4-deficient mice displayed prolonged survival, decreased recruitment of $CD11b^{+}Ly6G^{+}$ cells to the site of infection, and impaired expression of chemokines such as Cxcl2 and Ccl2.

The amino acid sequence of type II GRA6 contains amino acid substitutions and deletions compared with the sequences of type I and III GRA6. Compared with infection with type I parasites, infection with type II parasites resulted in markedly less nuclear translocation of NFAT4. We found that the C-terminal residue V227 in type I GRA6 (E221 in type IIGRA6) and the deletion of six other amino acids are the determinants of strain-dependent NFAT4 activation.

〔総　括(Conclusion)〕

Here, we demonstrate that GRA6-dependent NFAT4 activation is required for *T. gondii* manipulation of host immune responses to maximize the parasite virulence in a strain-dependent manner.

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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

トキソプラズマ原虫はエイズなどの免疫不全者で致死的な脳炎を引き起こす病原性寄生虫である。トキソプラズマ原虫は宿主の細胞の中でのみ増殖可能であるが、宿主細胞質内に放出するエフェクター分子群が細胞内シグナル伝達経路に影響を与え宿主の転写調節を行うことが近年明らかとなっており、その機能解析がトピックとなっている。

馬知秀氏はトキソプラズマ原虫の分泌装置であるデンスグラニュールから放出されるGRAタンパク質群の一つであるGRA6が宿主転写因子NFAT4を活性化することを突き止めた。さらに馬氏はGRA6欠損原虫を作成し病原性を免疫学観点から検討した結果、GRA6は局所感染におけるNFAT4依存的なケモカインの誘導と好中球の感染局所への動員を担うことを明らかにした。この研究成果は国内外から高く評価され、米国医学誌に発表されおよび日本のマスコミなどでも取り上げられた。

よって、馬知秀氏の本研究内容は、学位に値するものと考える。