Molecular and functional characterization of novel immunoreceptors CD300H and Allergin-1 involved in innate immune responses

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year	2018
その他のタイトル	自然免疫応答を制御する新規免疫受容体CD300Hと Allergin-1の機能解明
学位授与大学	筑波大学 (University of Tsukuba)
学位授与年度	2017
報告番号	12102甲第8748号
URL	http://hdl.handle.net/2241/00152849

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学位の種類	博士（　人間生物学　）
学位記番号	博甲第　８７４８　号
学位授与年月	平成　３０年　３月　２３日
学位授与の要件	学位規則　第４条第１項該当（昭和２８年４月１日文部省令第９号）
審査組織	グローバル教育院
学位論文題目	Molecular and functional characterization of novel immunoreceptors CD300H and Allergin-1 involved in innate immune responses (自然免疫応答を制御する新規免疫受容体 CD300H と Allergin-1 の機能解明)

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論文の要旨
Abstract of thesis

Innate immune system is the initial response of host defense. It prevents, control, or eliminates invading pathogens and important for the induction of adaptive immunity. The function of innate immune cells is regulated by the positive and negative signals received from the activating and inhibitory cell surface immunoreceptors. However, molecular mechanisms regulating the innate immune responses, especially by immunoreceptors, remain still elusive. Here the applicant has studied the molecular mechanisms for regulation of innate immune cells by analyzing the roles of immunoreceptors. The applicant characterized a newly identified activating immunoreceptor CD300H and tried to identify an orphan ligand for an inhibitory receptor, Allergin-1. Recruitment of blood leukocytes to sites of infection is essential for host defense against infection. Circulating monocytes and neutrophils are especially important effectors in the initiation of inflammatory responses to microbes. The CD300 family molecules are type 1 immunoreceptors belonging to the immunoglobulin superfamily and are encoded by seven genes on human chromosome 17 and nine genes on mouse chromosome 11. They are expressed on myeloid lineage cells, including monocytes-macrophages, granulocytes, dendritic cells, and mast cells, suggesting that they play an important role in innate immunity. The applicant identified a previously unannotated gene encoding an immunoglobulin-like receptor, designated CD300H. CD300H has a short cytoplasmic tail and associates with an ITAM-containing adaptor, DAP12 and DAP10. CD300H is

expressed on CD16+ monocytes and myeloid dendritic cells. Ligation of CD300H on CD16+ monocytes with anti-CD300H monoclonal antibody induced production of inflammatory cytokines and neutrophil chemoattractants. The applicant also found that the CD300H expression is variable among healthy individuals; they can be classified into two groups with “positive” and “negative” expression. Genomic sequence analysis revealed the existence of a single-nucleotide mutation (rs905709 (G→A)) at splice donor site in intron 1 in either single or both allele(s). The international HapMap project database demonstrated that the homozygous for the A allele of SNP rs905709 (negative expression) is observed at high frequency in Han Chinese in Beijing, Japanese in Tokyo and European, but extremely rare in Sub-Saharan African population. CD300H-reporter assays demonstrated that the ligands for CD300H exist in the supernatants of a macrophage cell line, stimulated by lipopolysaccharide, suggesting that they exist at inflammatory condition.

Anaphylaxis is a life-threatening allergic reaction that is accute in onset after exposure to an allergen. Mast cells play critical roles in allergic reaction by producing proteases, vasodilating substances, cytokines, and lipid mediators. Allergin-1 is an inhibitory receptor that contains an immunoreceptor tyrosine-based inhibitory motif (ITIM)-like domain in its cytoplasmic domain. It is preferentially expressed on mast cell and regulates IgE-mediated mast cell-dependent anaphylaxis in mice. However, a ligand for Allergin-1 is still unknown. To identify an Allergin-1 ligand, the applicant generated extracellular domain of Allergin-1 tagged with 3xflag as a bait. The applicant identified glucose-regulated protein (GRP) 78 as a binding partner of Allergin-1. However, recombinant GRP78 protein did not efficiently bind to Allergin-1 by enzyme-linked immuno-sorbent assay. Cell surface biotinylation assay of HEK293T cells demonstrated that Allergin-1 interacts with ~95 KDa and ~34 KDa proteins on the cell surface.

審査の要旨
Abstract of assessment result

【批評 Review】

The applicant has identified the eighth member of the CD300 immunoreceptor superfamily, CD300H, and characterized its roles on the innate immunity. The applicant also found a single nucleotide polymorphism of CD300H that abolishes its expression in Asian population. These are the first reports on the CD300H molecule and have further strengthen our understanding on the intricate regulation of the innate immunity. The applicant further tried to identify the ligand for CD300H and that for inhibitory immunoreceptor, Allergin-1. The identification and characterization of the ligands for activating and inhibitory cell surface immunoreceptors are important issues remained in this research filed. Although the ligands for both immunoreceptors are not yet identified, the research materials constructed in this study and the obtained results have provided invaluable clues for their clarification in near future.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 17 01, 2018. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded a Doctor of Philosophy in Human Biology.