氏　　名	王　亜秋　Yaqiu Wang
学位の種類	博士（　人間生物学　）
学位記番号	博甲第　９２６１　号
学位授与年月	令和元年８月３１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	Autonomous regulation of mast cell degranulation through an inhibitory receptor CD300a （抑制性受容体 CD300a を介した肥満細胞脱顆粒の自己調節）

	（職名）	（学位）	（氏名）
主　　査	筑波大学教授 （連携大学院）	医学博士	狩野　繁之
副　　査	筑波大学教授	博士（医学）	渋谷　彰
副　　査	筑波大学教授 （グローバル教育院）	博士（医学）	矢田　幸博
副　　査	筑波大学助教	博士（薬学）	船越　祐司

論文の要旨
Abstract of thesis

Background: Mast cells (MCs) degranulation is the central cellular event involved in the effector phase of allergic responses. Although various stimuli can induce MCs degranulation, antigen and antigen-specific IgE mediated MCs degranulation through FcεRI is the most common cause of MCs activation in allergic patients. Therefore, the identification of novel regulatory mechanism of FcεRI-mediated MCs degranulation will shed light on our understanding of allergic response and potentiate interventions.

Although phosphatidylserine (PS) confined to the inner leaflets of plasma membrane is exposed on the cell surface when cells undergo apoptosis, viable cells also externalize PS in certain cellular states. However, the pathophysiological significance of PS exposure on viable cells remains elusive. Interestingly, an ITIM-bearing inhibitory receptor CD300a is highly expressed by MCs. Intriguingly, the physiological ligands of CD300a is PS. Therefore, MCs PS exposure during degranulation and its CD300a expression provide a unique scenario to investigate the pathophysiological function of live cell exposed PS in the context of mast cell activation and allergic response.

Purpose: To investigate the role of exposed PS during MCs degranulation in the perspective of an inhibitory receptor

CD300a.

Results: Through imaging analyses of cultured bone marrow derived MCs (BMMCs) and human synovial mast cells (hMCs), the applicant found that PS was exposed on the plasma membrane of live MCs during degranulation within minutes after FcεRI stimulation. The PS exposure can be observed in all degranulating stimulations (e.g. ATP and ionomycin) but not in non-degranulating stimulation (e.g. LPS and IL-33). In addition, the exposed PS accumulated on the cell surface and polarized together with FcεRI as well as degranulation marker CD107a, suggesting that PS exposure on live MCs is a degranulation associated event.

Unlike PS exposed on apoptotic cells, the exposed PS on live MCs did not confer phagocytes engulfment by thioglycollate-elicited peritoneal macrophages, although the exposure lasted for hours on live MCs. Surprisingly, the applicant found that the externalized PS colocalized with CD300a, an inhibitory immunoreceptor that recognizes PS as its natural ligand. Fluorescence resonance energy transfer (FRET) experiment using a fluorescence PS probe and a non-blocking anti-CD300a antibody revealed the direct *cis*-binding between CD300a and PS during degranulation *in situ*. Functionally, the applicant observed that degranulation was greater after stimulation with an IgE–antigen complex in MCs deficient in CD300a than in wild-type MCs by comparing the degranulation of BMMCs from both wild type and *Cd300a*$^{-/-}$ mice. Pretreatment of MCs with a neutralizing anti-CD300a antibody efficiently upregulated the degranulation of wild-type MCs as a result of interference of *cis*-interaction between CD300a and PS as evidenced by decreased FRET efficiency. Moreover, western analysis indicated the phosphorylation of Syk, an important kinase downstream of FcεRI signaling, was strongly enhanced in *Cd300a*$^{-/-}$ than in wildtype BMMCs. These results indicate the presence of *cis*-interaction between CD300a and externalized PS on degranulating MCs as well as the suppressive functions of such interaction during MCs degranulation.

Consistently, CD300a-deficient mice or *in vivo* treatment with a neutralizing CD300a antibody showed slower recovery of body temperature compared with wild-type mice in a model of MCs-dependent passive systemic anaphylaxis (PSA). Importantly, in this PSA model, the ligand of CD300a, namely PS, was exposed and polarized on scattered tissue MCs themselves but not surrounding cells. These observations suggest that the *cis*-interaction between CD300a and PS on live MCs also happens *in vivo* and has potential to suppress the allergic response.

Discussion: In this study, the applicant found the function of live MCs exposed PS as a suppressive reagent of MCs degranulation through its *cis*-binding with the inhibitory receptor CD300a. By live imaging and imaging flow cytometry analysis of MCs degranulation, this study provides the detailed characterization of PS exposure of activated MCs. Distinct from apoptotic PS exposure takes hours to occur, degranulated MCs exposed their PS in minutes-scale. Interestingly, although it is clear that the PS exposure on MCs is a degranulation associated event, the exact organelle source of surface PS is intriguing, giving that PS^+ area contained both patches-like and dot-like PS^+ regions. This suggests that the externalized PS may come from different source (e.g. inner leaflet of plasma membrane, membrane of granules and granule itself).

Importantly, the externalized PS binds to its receptor CD300a and effectively suppressed the degranulation of the same cell, resulting in less degranulated MCs *in vitro* and better recovery of mice from systemic anaphylaxis *in vivo*. Given the rich inflammatory mediator and protease content released after MCs degranulation, MCs activation is a strong immunogenic and maybe disruptive process, therefore should be extensively controlled. However, MCs distribute in the tissue in a scattered manner and the ligands of its receptors may not be always available, therefore

such self-regulatory characteristic of MCs represents a novel strategy that MCs evolved to control their own activation.

In summary, the applicant suggests an autonomous regulation of MC degranulation through *cis*-interaction of PS with CD300a to down regulate allergic response, adding another layer of regulation in allergic responses.

審査の要旨
Abstract of assessment result

【批評 Review】

The applicant investigated the role of PS exposed on the plasma membrane of MCs during degranulation. He found that a PS receptor CD300a bound to the PS on MCs and autonomously regulated the degranulation by suppression of an activating signal mediated by FceRI. These results are extremely novel and important findings in cell biology as well as immunology and shed light on a new therapeutic approach to the treatment for allergic diseases.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 3 June, 2019. 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.