氏　　名	Almeida Mariana Silva
学位の種類	博士（医学）
学位記番号	博甲第　10359　号
学位授与年月	令和　4　年　3　月　25　日
学位授与の要件	学位規則第４条第１項該当
審査研究科	人間総合科学研究科
学位論文題目	A study on a ligand for an inhibitory immunoreceptor, Allergin-1 (抑制性免疫受容体のリガンドに関する研究)
主　　査	筑波大学教授　博士（医学）　柳沢　裕美
副　　査	筑波大学教授　博士（工学）　広川　貴次
副　　査	筑波大学准教授　博士（医学）　松本　　功
副　　査	筑波大学助教　博士（医学）　宮寺　浩子

論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, the author conducted a study in which she identified and characterized a putative ligand for an inhibitory immunoreceptor, Allergin-1.

The summary is as follows:

（目的　Purpose）

Type I allergic reactions, such as atopic dermatitis and asthma, are increasing worldwide and are estimated to occur in 20-30% of the population. Type I allergic reactions are mediated by allergen-triggered generation of immunoglobulin E (IgE) antibodies, which bind to the high affinity IgE receptor (FcεRI) on mast cells (MCs) and induce degranulation and release of inflammatory mediators. Despite the accumulating knowledge of the molecular mechanism of type I allergic reactions, there is still a need to develop new therapies for better treatment and management of allergic disorders. It has been reported that inhibitory immune receptors on MCs can ameliorate type I allergic reactions by suppressing the IgE-dependent FcεRI-mediated signaling. Therefore, to develop a new drug that effectively inhibits the initiation/progression of type I allergic reactions, the author focused on the inhibitory immunoreceptor, Allergin-1, expressed on MCs and proposed to identify and characterize a functional ligand for Allgerin-1.

（対象と方法　Materials and Methods）

The author first generated mouse soluble recombinant Allergin-1 (msAlg) with a C-terminal FLAG tag (3 x FLAG) and investigated its binding to Allergin-1 ligand expressed on bone marrow-derived dendritic cells (BMDCs) by FACS analysis. Allegin-1-mediated signaling was monitored by mouse Allergin-1/NFAT/Ba/F3 reporter cell line, which detects binding of a specific ligand to Allergin-1-FcεRI chimeric receptor, resulting in induction of NFAT signaling and GFP expression. Allergin-1 interacting molecules on BMDC were isolated by immunoprecipitation followed by liquid chromatography-mass spectrometry (LS-MS). Bioinformatics analysis and Western blots were

performed to identify 36-38 kDa Allergin-1 ligand expressed in extracellular vehicles (EVs) of BMDCs. After BMDC-derived EVs were analyzed by LS-MS, functional analysis was performed by incubating wild-type and Allergin-1-deficient (*Milr1*$^{-/-}$) IgE-sensitized BMMCs in the presence or absence of the prospective ligands and measured degranulation levels of BMMCs by β-hexosaminidase assay.

（結果　Results）Fluorescence-activated cell sorting (FACS) analysis showed that msAlg 3 x FLAG binds to BMDCs. Stimulation of Allergin-1 reporter cells with BMDCs and BMDC-derived culture supernatant induced GFP expression, suggesting that BMDCs express and secrete an Allergin-1 ligand. Immunoprecipitation of Allergin-1 and its interacting proteins from BMDCs detected six proteins, and database analysis demonstrated that they localize in exosomes. BMDC-derived EVs-loading beads induced GFP expression in Allergin-1 reporter cells, suggesting that an allergin-1 functional ligand is expressed on BMDC-derived EVs. Immunoblots of EVs with msAlg 3 x FLAG detected binding to approximately 36-38 kDa protein bands and MS-LS analysis revealed that these bands were Annexin A1-A5, in which only Annexin A5 induced GFP expression in Allergin-1 reporter cells. The expression of Annexin A5 on BMDC-derived EVs was detected by FACS and was increased upon LPS stimulation. The interaction between Annexin A5 and msAlg 3 x FLAG in the presence of phosphatidylserine (PS) was detected by ELISA. Further analysis showed that administration of Annexin A5 significantly inhibited IgE-dependent degranulation in WT BMMCs but not in *Milr1*$^{-/-}$ BMMC, suggesting that Annexin 5 is a functional ligand of Allergin-1.

（考察　Discussion）
The binding of Allergin-1 to Annexin A5 required the presence of plate-coated PS. Annexin A5 tridimensional structure suggests that Annexin A5 undergoes conformational changes and exhibits an “open form” in the presence of Ca^{2+} or PS in a Ca^{2+}-dependent manner. The author discussed that it is likely that structural change of Annexin A5 is required for the binding to Allegrin-1. Since the regulatory role of Annexin A5 and contribution of PS in type I allergic reactions remains unknown, the author stated that further studies are necessary to characterize Annexin A5 in Allergin-1-mediated suppression of type I allergic reactions.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）
The author focused on Allergin-1, an inhibitory receptor rather than activating receptor, to build a basis for the development of medications for anti-Type I allergic reactions. In addition, the author was able to characterize the Allergin-1-binding proteins and identify Annexin A5 as its putative ligand. Although it is needed to investigate the molecular detail of the binding between Allergin-1 and Annexin A5, this discovery may lead to future drug discovery for allergic diseasese.

（最終試験の結果　Assessment）
The final examination committee conducted a meeting as a final examination on January 7th, 2022. 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）
The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.