論文内容の要旨

論文題目；Functional analysis of an activating receptor LMIR4 and an inhibitory receptor LMIR3

和訳；活性型レセプターLMIR4と抑制型レセプターLMIR3の機能解析

指導教官；北村俊雄教授

東京大学医学系研究科

平成17年4月入学

医学博士課程

生殖・発達・加齢医学専攻

氏名；伊沢久未（学生証番号 41-57463）

The leukocyte mono-Ig-like receptor (LMIR) belongs to a new family of paired immunoreceptors. In this paper, I identified LMIR3 and LMIR4 from a BMMC cDNA library as new members of the LMIR family and analyzed an activating receptor LMIR4/CLM-5 and an inhibitory receptor LMIR3/CLM-1 (Figure 1.).

LMIR4 is expressed in myeloid cells, including granulocytes, macrophages, and mast cells, whereas LMIR3 is expressed in myeloid cells and B cells. Intraperitoneal administration of lipopolysaccharide strikingly up-regulated LMIR3 and down-regulated LMIR4, whereas that of granulocyte colony-stimulating factor up-regulated both LMIR3 and LMIR4 in granulocytes. Collectively, these results suggest that the innate immune system is at least in part regulated by the qualitative and quantitative balance of the paired receptors LMIR3 and LMIR4.

LMIR4 contained only a short cytoplasmic tail and has a negatively charged residue, glutamic acid in the transmembrane domain. The association of LMIR4 with FcRγ among immunoreceptor tyrosine-based activation motif (ITAM)- bearing molecules was indispensable for LMIR4-mediated functions of bone marrow-derived mast cells, but dispensable for its surface expression. Cross-linking of LMIR4 led to Lyn- and Syk-dependent activation of bone marrow-derived mast cells, resulting in cytokine production and degranulation, whereas that of LMIR3 did not. The triggering of LMIR4

and TLR4 synergistically caused robust cytokine production in accordance with enhanced activation of ERK, whereas the co-ligation of LMIR4 and LMIR3 dramatically abrogated cytokine production. Cross-linking of LMIR4 in bone marrow granulocytes also resulted in their activation, which was enhanced by lipopolysaccharide (Figure 2.).

LMIR3 posesses five cytoplasmic tyrosine residues (Y241, Y276, Y289, Y303, Y325) among which Y241 and Y289 fit the consensus sequence sequence of immunotyrosine-based inhibitory motif (ITIM) and Y325 fit the consensus sequence sequence of immunotyrosine-based switch motif (ITSM). Coligation of FcεRI and LMIR3 in BMMC significantly impaired the cytokine production induced by FcεRI crosslinking. In order to investigate the role of each tyrosine residue of LMIR3, we generated BMMC transduced with various LMIR3 mutants in which tyrosines were replaced with phenylalanines. As a result, the replacement of Y325 in addition to Y241 and Y289 with phenylalanine (Y241/289/325F) dampened the inhibitory function of LMIR3.

Interestingly, engagement of LMIR3 alone induced cytokine production in LMIR3 (Y241/289/325F) mutant- or LMIR3 (Y241/276/289/303/325F) mutant- transduced BMMC led to the significant cytokine production, indicating the existence of activating signal pathway independently of tyrosine phosphorylation of LMIR3. To explore whether LMIR3 associated with the adaptor protein, we performed immunoprecipitation experiments, demonstrating that ITAM-bearing FcRγ associated with LMIR3. The deficiency of FcRγ completely abolished the activating function of LMIR3 (Y241/276/289/303/325F). Collectively, LMIR3 transmits an inhibitory signal via Y241, Y289, and Y325, while does LMIR3 an activating signal by associating with FcRγ.

Furthermore, engagement of LMIR3 alone did not induce cytokine production in LMIR3 (WT), but in the presence of lipopolysaccharide, engagement of LMIR3 alone induced cytokine production, dependent on both cyoplasmic tyrosines and FcRγ. LMIR3, an inhibitory receptor, work as an activating receptor in the presence of lipopolysaccharide. (Figure 3.)

Although the identification of the ligands for LMIR3 and LMIR4 is indispensable for complete understanding of the functions, it has been unsuccessful despite an extensive trial using expression cloning or biochemical approaches. Fine-tuning of LMIR3, LMIR4, and their ligands might provide a new therapeutic strategy in the regulation of allergy and innate immunity. To fully understand the mechanism of LMIR3 and LMIR4 functions, further studies using knock-out mice are now under way.

Figure 1.

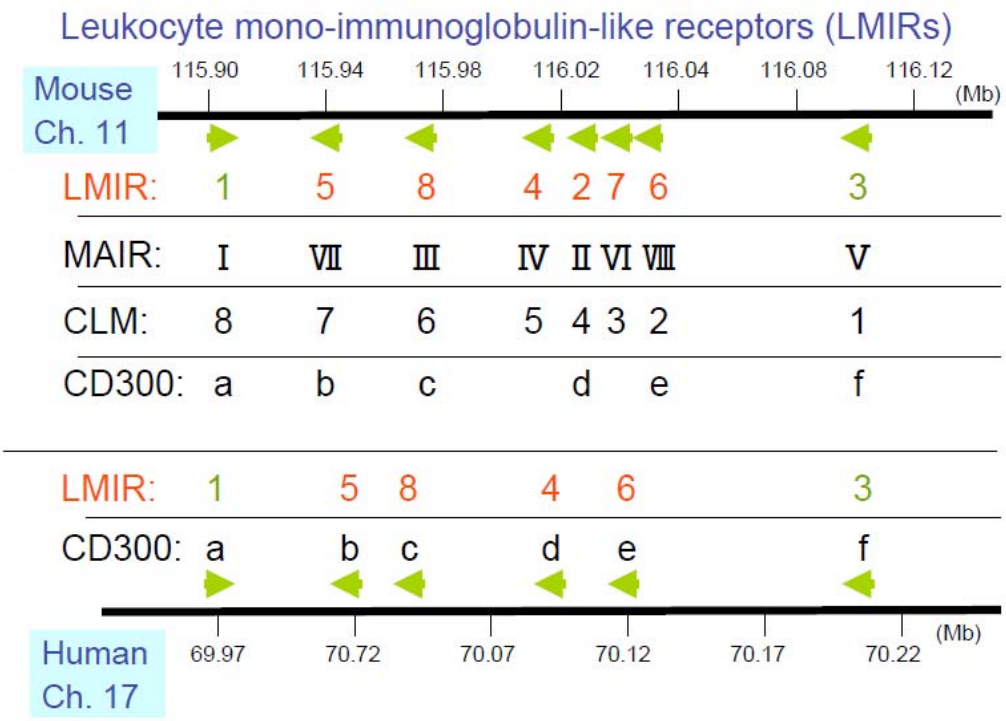
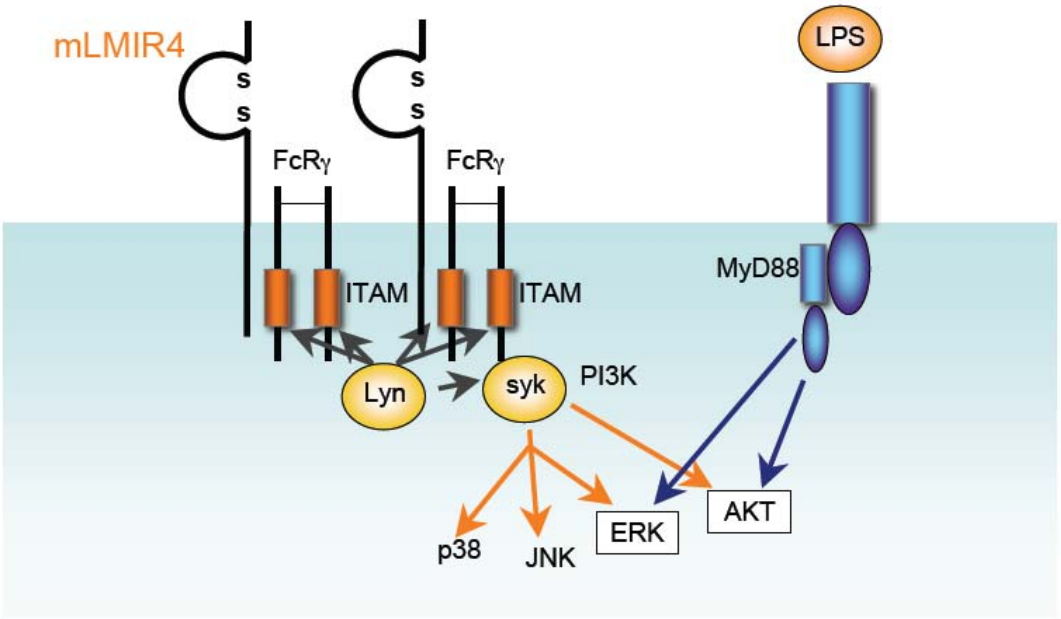
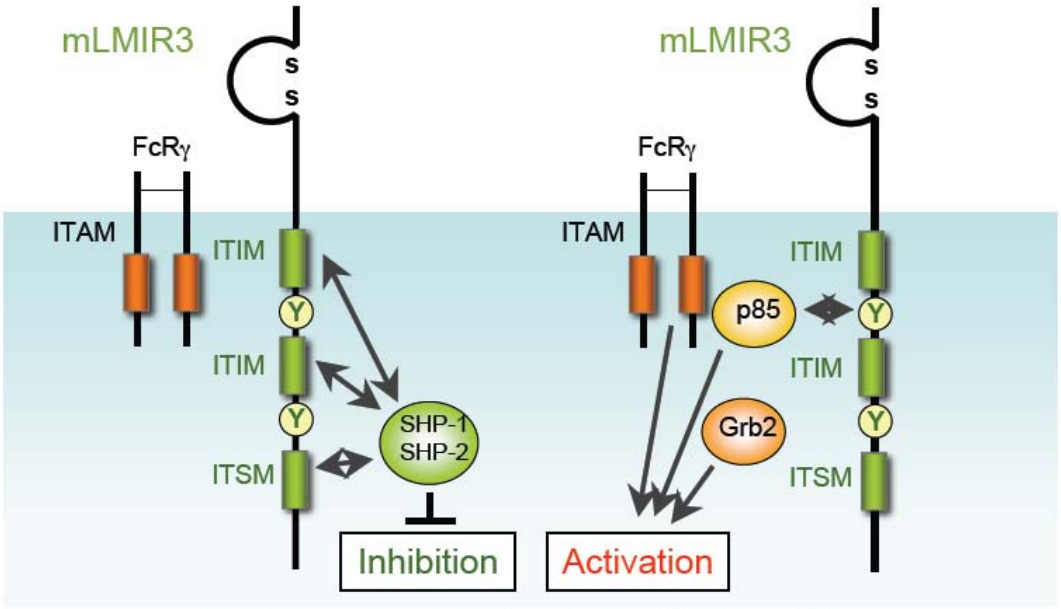


Figure 2.



LMIR4 transmits an activating signal by associating with FcRγ,
and crosslinking of LMIR4 synergize with LPS.

Figure 3.



LMIR3 transmits an inhibitory signal via Y1, Y3, and Y5,
while does LMIR3 an activating signal by associating with FcRγ.