levels, indicating that DAP10 could associate with mLMIR5 under conditions where DAP10 is up-regulated. On the other hand, the finding that surface expression levels of exogenously transduced mLMIR5 did not alter between WT and DAP12-deficient BMMCs suggested that mLMIR5, if abundant in cells, did not necessarily require DAP12 for maintaining its surface expression levels. Thus, it is suggested that surface expression levels of mLMIR5 were determined by DAP12 and to a lesser extent DAP10.

We found that mLMIR5 was highly expressed in both mucosal mast cells, including BMMCs and connective tissue–type mast cells, including peritoneal mast cells. Unexpectedly, cross-linking of endogenous mLMIR5 induced a more pronounced activation in FLMCs than in BMMCs despite comparable surface expression levels of mLMIR5. This suggests that mLMIR5 plays an important role in specific types of mast cells, closely related to their differentiation and distribution in tissue. Notably, activation events caused by mLMIR5 cross-linking were almost completely dampened by the deficiency of DAP12 or its ITAM-associated kinase Syk, suggesting that mLMIR5 transmitted an activating signal through the phosphorylation of ITAM in a cytoplasmic region of DAP12.[17-19,27,43] It is noteworthy that Syk-dependent activation was demonstrated by cross-linking of endogenous mLMIR5. Interestingly, the deficiency of DAP10 in BMMCs also inhibited the functions of mast cells, albeit to a lesser degree. This is probably due to decreased expression levels of endogenous DAP12 in the absence of DAP10 (Figure 5F), although we could not completely rule out the involvement of DAP10 in the functions of mLMIR5. Thus, activating functions of mLMIR5 were predominantly regulated by DAP12 among adaptor proteins.

Analysis of differential regulation of immune receptors in mouse versus human cells sometimes sheds light on the evolutionary and physiologic significance of the paired receptor.[20-23] Indeed, several structural and functional differences were observed in mouse versus human LMIR5. Indeed, several structural differences were observed in mouse versus human LMIR5. For example, mLMIR5, but not hLMIR5, was an N-glycoprotein. In addition, mLMIR5 differed from hLMIR5 by several amino acid residues in the transmembrane domain sequence (Figure 1A). These differences in glycosylation and transmembrane structure may be related to differing surface expression levels or to capacities to associate with DAP10 in mLMIR5 versus hLMIR5 transduced into Ba/F3 cells (Figure 3A). It was noticed that hLMIR5 but not mLMIR5 possessed a putative phosphorylation motif (Y188) in the short cytoplasmic region. From the results using WT and *DAP12*$^{-/-}$ BMMCs, we concluded that hLMIR5 delivered an activation signal independently of the phosphorylation of Y188 in WT BMMCs, whereas it did so through phosphorylation in *DAP12*$^{-/-}$ BMMCs. Martinez-Barriocanal and Sayos[24] also reported that CD300b/hLMIR5 signal was dependent on Y188 phosphorylation in rat basophilic leukemia (RBL) cells that supposedly do not express DAP12. However, unlike our results, hLMIR5 signal in RBL cells was not completely blocked by Y188 mutation. How Y188 is phosphorylated by cross-linking of hLMIR5 in DAP12-deficient mast cells remains elusive. It is possible that Y188 in a cytoplasmic tail becomes readily accessible in the absence of DAP12 and thereby is phosphorylated by some tyrosine kinases. The finding that cross-linking of hLMIR5 induced cytokine production even in the absence of both DAP12 and DAP10 strongly suggested the existence of unknown adaptors. In addition, hLMIR5-mediated cytokine production was abrogated by the disruption of the charged residue (K158) in DAP12-deficient BMMCs, but not WT BMMCs, whereas the disruption of both K158 and Y188 dampened hLMIR5-mediated cytokine production in WT BMMCs as well as DAP12-deficient BMMCs. In contrast, Martinez-Barriocanal and Sayos[24] showed that the disruption of K158 abrogated cytokine production in DAP12-transduced RBL cells. This discrepancy might be explained by the cell-type difference between RBL cells and BMMCs. However, it is also possible that different experimental systems (RBL cells and DAP12 overexpression in RBL cells vs DAP12-knockout cells and the wild-type cells) are responsible for the discrepancy. One strange result is that Y188F mutant but not K158Q mutant of hLMIR5 induced cytokine production in DAP10/DAP12 double-knockout BMMCs but not in DAP12-knockout BMMCs. This difference could be explained by the presence of an additional adaptor that is up-regulated in the absence of both DAP10 and DAP12, but not DAP12 alone.

Recent advances[44-51] have demonstrated that DAP12 could mediate an activating or inhibitory signal depending on the associated receptor, and support a model that the avidity of the receptor for its ligand could modulate cellular response. Therefore, identification of the LMIR5 ligand, either endogenous protein or foreign bodies, and analysis of LMIR5 knock-out mice will be indispensable for elucidation of in vivo functions of LMIR5.

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Authorship

Contribution: Y.Y. did all the experiments and participated in writing the manuscript; J.K. oversaw all the experiments and actively participated in manuscript writing; I.K. assisted with the experiments including retroviral transfection; T.M. assisted with the experiments including retroviral transfection and flow cytometric analysis; T.O., Y.L., F.S., S.Y., H.K., and H.N. did the cloning and made the constructs of LMIRs; M.M.-Y. measured the released histamine; V.L.J.T provided Syk knock-out mice; T.T. provided several knock-out mice; and T.K. conceived of and directed the project, secured funding, and actively participated in manuscript writing.

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