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Characterization of the Signaling Pathway of CLEC12A: An Inhibitory Receptor involved in Gout.

Objectives: CLEC12A is a C-type lectin receptor that negatively regulates myeloid cell function. Monosodium urate crystals (MSU), the etiological agent of gout, cause a diminution in the expression of CLEC12A in neutrophils resulting in an enhanced activation of this leukocyte. Similarly, an exacerbated inflammatory reaction is observed in CLEC12A knock-out mice administered MSU. CLEC12A knock-out mice with collagen-induced arthritis also exhibit a more severe phenotype. These observations suggest that CLEC12A is a global negative regulator of inflammation. The molecular mechanisms underlying CLEC12A function remain, however, incompletely characterized.
The overall aim: To decipher the CLEC12A signalling pathway and determine the role(s) of its protein motifs in its function.
Methods: The CLEC12A signalling pathway was investigated in human neutrophils stimulated with MSU after inducing a decrease in its cell-surface expression with a specific antibody. Cells were then analysed by Western blot, immunoprecipitation, flow cytometry and inhibitors of signalling proteins. The role of the cytoskeleton in CLEC12A signalling was studied with similar techniques in addition to microscopy with compounds that disturb the integrity of various cytoskeletal components. Structure-function studies were performed in 293T cells transfected with CLEC12A variants containing mutations in residues predicted to be necessary for post-translational modifications, receptor oligomerization and/or phosphorylation.
Results: Early signalling events: The engagement of CLEC12A with a specific antibody on the surface of neutrophils induces its translocation to detergent resistant membranes (DRMS), phosphorylation and internalization. CLEC12A internalization does not appear to depend on a single cytoskeletal component. Moreover, we provide evidence for the Src-dependent phosphorylation of CLEC12A and the regulation of the PI3K and the p38 pathways in neutrophils stimulated with MSU by CLEC12A. Structure function studies: identified a cysteine residue that is crucial for the function of the receptor. When mutated, the glycosylation of CLEC12A is compromised as well as its dimerization and translocation into DRMs.
Conclusion: Together, our observations reveal that CLEC12A shares similarities with other inhibitory receptors including the Src-dependent phosphorylation of its signalling motif and its translocation to plasma membrane domains rich in signalling proteins. We also show that CLEC12A modulates more than one signalling pathway associated with MSU-induced inflammation which renders this receptor an attractive therapeutic target. These pathways are involved in the production of cytokines, reactive oxygen species and cell migration.