CLEC16A shows increased expression in multiple sclerosis and functions as a direct regulator of the HLA class II antigen presentation pathway

Van Luijn [1,*], M.M., Kreft [2,*], K.L., Jongsma [3], M.L.M., Mes [1], S.W., Wierenga-Wolf [1], A.F., Van Meurs [1], M., Melief [1], M.J., Van der Kant [3], R., Janssen [3], L., Janssen [3], H., Priatel [4], J.J., Tan,[4], R., Neefjes [3], J., Laman [1] , J.D., and Hintzen [2] , R.Q.

Departments of [1] Immunology, [2] Neurology, MS Centre ErasMS, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands; [3] Division of Cell Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands; [4] Department of Pathology and Laboratory Medicine, University of British Columbia, Child and Family Research Institute, Immunity in Health and Disease, British Columbia's Children's Hospital, Vancouver, BC, Canada.

Research question and background

C-type lectin CLEC16A is genetically associated with the risk to develop several autoimmune diseases, including multiple sclerosis (MS). However, the underlying mechanisms are poorly understood. In Drosophila, the orthologue of CLEC16A, Ema, controls endosome maturation. Since the formation of late endosomal compartments termed MIIC is an important step in the HLA class II (HLA-II) antigen presentation pathway, we hypothesize that CLEC16A has a key function in this process. To address this, CLEC16A expression was explored in primary MS material and modulated in both model and primary human antigen-presenting cells (APC). In post-mortem white, but not grey matter of MS cases, CLEC16A mRNA expression levels were significantly elevated compared to non-demented controls (NDC; $p=0.03$, $n=11$-14). *In situ* analyses showed high CLEC16A expression mainly in perivascular leukocyte infiltrates of MS white matter lesions, in contrast to both MS normal-appearing and NDC white matter. In peripheral blood mononuclear cells (PBMC), a two-fold increase in mRNA expression was found for MS patients compared to healthy controls ($p=0.003$, $n=46$-69).

Methods and tissues used

When comparing PBMC subsets, CLEC16A was predominantly expressed in monocytes and monocyte-derived dendritic cells (DC). In these DC, CLEC16A strongly correlated with CIITA mRNA levels ($p=0.005$, $r=0.87$) and was co-expressed with HLA-II, as shown by real-time qPCR and confocal microscopy, respectively. Treatment of these DC with vitamin D led to a reduced expression and a more perinuclear localization of CLEC16A and HLA-II. Silencing of CLEC16A in the model APC line MelJuSo caused a perinuclear-to-peripheral cytoplasmic scattering of HLA-II$^+$ late endosomes. Electron microscopy analysis of MelJuSo cells silenced for CLEC16A with shRNA lentivirus showed impaired maturation of multivesicular late endosomes, consisting of elevated numbers of HLA-II molecules ($p<0.0001$). In parallel to this, cell surface expression of HLA-II was significantly reduced after CLEC16A knockdown ($p<0.0001$). These results were confirmed with DC. Additionally, co-immunoprecipitation and RNAi experiments showed a direct association of CLEC16A with Rab7-interacting lysosomal protein (RILP) and the homotypic fusion and protein sorting (HOPS) complex, two members of the dynein motor complex regulating late endosome formation and transport.

Results and conclusion

In conclusion, we reveal that CLEC16A is upregulated in MS and serves as a key regulator of HLA-II expression by controlling late endosome biogenesis in human APC. We propose that by coupling the function of minor risk alleles such as CLEC16A to the strongest genetic factor in MS, HLA-II, important insights can be gained into selectively altered processes of antigen presentation as potential explanation for pathogenic T-cell activation in MS.