

Novel cooperation between CX3CL1 and CCL26 inducing NK cell chemotaxis via CX3CR1: a possible mechanism for NK cell infiltration of the allergic nasal tissue

206

Amr E. El-Shazly¹, Hugo Castillo-Dolorier², Bettina Bisig², Philippe Lefebvre¹, Philippe Delvenne², Nathalie Jacobs²

¹Department of Otolaryngology and Head and Neck Surgery, Liege University Hospital-CHU, Liege, Belgium, ²GIGA-I3, Laboratory of Experimental Pathology-Virology, University of Liege, Liege, Belgium, n.jacobs@ulg.ac.be

Background Recent data indicated that natural killer (NK) cells and chemokines could play a pivotal role in nasal inflammation. CX3CR1, the only receptor for fractalkine/ CX3CL1, is abundantly expressed by NK cells, and was recently shown to also be a receptor for eotaxin-3/CCL26. However, no reports explored the NK cells-CX3CL1-CCL26 axis via CX3CR1 in allergy.

Objective Our goals were first to determine specifically NK cell recruitment pattern in nasal tissue of allergic chronic rhinosinusitis (ACRS) and non-allergic chronic rhinosinusitis (NACRS) patients in comparison with healthy controls, and secondly, to investigate the function of CX3CR1 in NK cell migration.

Methods Immunohistochemistry, microchemotaxis chambers, flow cytometry and confocal microscopy were used in this study. **Results** Herein, we showed that NK cells infiltrated the epithelial layers of nasal tissue only in ACRS patients and not in NACRS patients or controls. NK cells were also more numerous in the stroma of the nasal tissue from ACRS patients compared with NACRS patients or controls. This migration could be mediated by both CX3CL1 and CCL26, as these two chemokines induced NK cell migration. Moreover, both molecules also stimulated cytoskeleton changes and F-actin reorganisation in NK cells. Chemotaxis and cytoskeleton changes were sensitive to genistein, a tyrosine kinase inhibitor. By flow cytometry, we demonstrated that a single antigen nasal provocation challenge increased the expression of CX3CR1 on NK cells in allergic rhinitis (AR) patients. The function of this receptor was associated with a significant augmentation of NK cell chemotaxis against the optimal doses of CX3CL1 and CCL26.

Conclusions and Clinical Relevance Our results highlight a novel role for CX3CR1 in NK cell migration that may contribute to the NK cell trafficking to the allergic upper airway. This could be mediated largely by CX3CL1 and CCL26 stimulation of the tyrosine kinase pathway.

A Study of Peripheral Blood and Cord Blood Natural Killer Cell Activation

207

Rehab Alnabhan², Alejandro Madrigal¹, Aurore Saudemont¹

¹Anthony Nolan Research Institute, London, UK, ²UCL Cancer Institute, The Royal Free Campus, London, UK, rehab.alnabhan.10@ucl.ac.uk

Natural killer (NK) cells play substantial role in clearing infected cells and tumour cells, and in mediating graft versus leukaemia effect (GvL) in transplantation settings. In this study, we aimed to investigate the mechanisms of cord blood (CB) NK cell activation and compare it to peripheral blood (PB) NK cells to identify which conditions lead to fully activated functional NK cells for immunotherapy. Cytokines involved in stimulation and proliferation of NK cells were used including Interleukin (IL)-2, IL-12, IL-15, IL-18, the combination of IL-15 and IL-18 or the combination of IL-15 and IL-2. We studied both the phenotypic characteristics and functional capabilities of cytokine-activated NK cells. Our results indicated that CB NK cells are readily responsive to cytokine treatment presenting a comparative amount of most cytokines' receptors. However, CB NK cells express significantly less IL-2 receptors correlating with the lower levels of activation reached after IL-2 stimulation. CB NK cells acquire the phenotype of activated NK cells upon activation with cytokines showing up-regulation of activation markers, adhesion molecules, and secondary lymphoid tissues homing markers. The analysis of CB NK cell cultures' supernatants revealed high production of IFN-g and inflammatory cytokines (IL-6, IL-8) but only traces of TNF- α . Nonetheless, differential findings depending on which cytokine used was observed. CB NK cells gained the tendency to proliferate in the presence of cytokines diluting CFSE by day 2. Interestingly, better proliferation was observed in cultures containing a combination of IL-15 and IL-18. Cytokines' treated CB NK cells when co-cultured with K562 were able to kill this cell line in 51Cr release cytotoxicity assay. Our early finding of relative gene expression revealed significant differences in IFN-g, Granzyme-B, Perforin, and BCL-2 expression based on the cytokine condition used. Our results from transwell migration assay revealed that CB NK cells when treated with the combination of IL-15 and IL-2 or IL-15 and IL-18 were able to migrate toward CCL19/21 and CXCL10/11 chemokine signal showing similar pattern of migration to PB NK cells. Using cytokines, it was also feasible to induce memory NK cells from CB NK cells that exhibited sustained ability to produce IFN-g at day 21. All together, this study aims to identify which cytokine or combination of cytokines promotes fully functional CB NK cells that could be used for therapeutic purposes.