Abstract

Rheumatoid arthritis (RA) is a worldwide problem representing one of the most prevalent autoimmune diseases in the world. Despite the commonness of the disease, its pathogenesis has not been fully described. Immune cells ranging from antigen-presenting cells to T, B and NK cells playing various roles participate in the rheumatic process. In this work we concentrated on NK cells expressing a repertoire of activating and inhibitory receptors which influence their function in health and disease. We focused on the analysis of NK cell function and described its possible modulation by rheumatic autoantigens and multivalent glycodendrimers bearing 4 (GN4C) or 8 (GN8P) N-acetyl glucosamine moieties. The effect on NK cells and the glycosylation pathways was further studied *in vitro*. Finally, an *in vivo* study was performed on an animal model of RA – collagen-induced arthritis (CIA) to assess the effect of the compounds on clinical development of the disease and selected immune parameters.

Comparison of NK cell cytotoxicity in patients suffering from RA, other inflammatory diseases and healthy donors showed its impairment particularly in RA patients. Peripheral blood NK cells reacted to GN8P glycoconjugate by inhibition of their effector function in CD161 high-expressing samples. The MGAT5 glycosyltransferase mRNA expression was increased in RA patients' synoviocytes but not in peripheral blood, suggesting the involvement of aberrant glycosylation in the autoimmune process. We found out that NK cells react to RA-specific autoantigen (MCV) by increased expression of CD161 in healthy donor samples but not in those from RA patients where the levels had already been elevated due to the ongoing disease. Moreover, NK cells are capable of PAD4 citrullination enzyme expression, showing their possible role in the citrullination processes leading to production of RA-specific autoantigens.

GN4C was used for *in vitro* study of the expression of glycosyltransferases in NK cells. The glycomimetic was found to interfere with the glycosylation processes downmodulating the expression of both MGAT3 and MGAT5 which was proven in both fresh NK cells of healthy donors and NK-92 cell line. In both models we also observed an increase of cytotoxicity and the stimulation of Th1 cytokines – TNF-α, IFN-γ and particularly IL-2 promoting NK cell cytotoxic activity.

In the mouse model of RA we confirmed the gradual impairment of NK cell activity similar to human RA, corresponding with NK cell relative distribution. GN4C proved to be more potent in terms of amelioration of the clinical symptoms, reduction of inflammatory synovial infiltration as well as suppression of activated APCs, necessary for autoreactive T cell activation. Evaluation of the humoral immune response to the treatment showed inhibition of IFN-γ and TNF-α rise and stimulation of IL-4 corresponding with a reduction of CII-specific IgG2a.

This work brings a comprehensive overview of NK cell involvement in RA and the role of glycans in different *in vitro* and *in vivo* conditions. NK cells are able to react to specific RA autoantigens and participate in the citrullination processes leading to the progression of RA. The use of multivalent glycodendrimers *in vivo* is, however, capable of inhibition of the autoimmune processes bringing significant alleviation of CIA clinical symptoms in mice, suggesting potential new prospects for more effective therapeutic interventions in the early stages of RA.