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Involvement of TLR-9 pathway on NK cells in Systemic Lupus Erythematosus

Objective(s): Natural killer (NK) cells participate in systemic lupus erythematosus (SLE) pathogenesis by promoting dendritic cell (DC) activation and/or interferon(IFN)γ over-production. NK c ells activation pathways remain unknown. One mechanism leading to pDC activation in SLE is the binding of auto-antigens (nucleic acids) with their cognate intracellular toll-like receptors (TLR), which includes TLR-9. Using synthetic CpG-ODN-A-2216 as TLR-9 ligand, the aim of this study was to determine if this pathway is similarly involved in NK cell activation and if hydroxychloroquine (HCQ) has an effect on this pathway.

Method(s): From 2013-2018, 41 HCQ-free and 8 HCQ-treated SLE patients were compared to 29 controls. SLEDAI ≥ 6 defined active patients. Fresh CD3-CD56+ NK cells were stained with anti-CD69-ECD and TLR-9-PE after permeabilization. Polyfunctionality assays detected both degranulation (anti-CD107a-FITC) and intracellular IFNγ (anti-INFγ-AF700) after a 5-hour-co-culture of either PBMCs or purified NK cells with K562 target cells to a 1:1 ratio. CpG-ODN-A-2216 was added overnight to 106 cells/ml cultures at 12.5 µM/ml. When added, soluble HCQ was used at 0.1, 0.5, 1, or 5 µg/ml.

Result(s): In HCQ-free SLE patients, % of TLR9+-NK cells was increased in active patients as compared to inactive patients: 31 ± 17% vs. 13 ± 15%. TLR-9 expression correlated with the % of CD69+-activated NK cells (r2=0.68; p=0.004). Overnight stimulation of PBMCs by CpG-ODN-A-2216 led to activation of NK cells (CD69 increasing from 31 ± 18% to 70 ± 18%, p<0.001) and resulted in degranulation and slight production of IFNγ with similar effects in patients and controls: 50 ± 14% vs. 49 ± 16% and 7 ± 6% vs. 8 ± 6%, p<0.001, respectively. CpG-ODN-A-2216 had a direct effect in purified-NK cell activation: % CD69 increasing from 25 ± 15% to 39 ± 19%, p=0.008. However, it was not sufficient to induce both their degranulation and IFNγ production in patients vs. controls. Adding HCQ prior to the PBMCs stimulation inhibits CpG-ODN-A-2216, with a dose effect in vitro. Interestingly, the significant effect started at the in vivo-target dose of 1 µg/ml: % CD69 decreasing from 70 ± 24% to 38 ± 31%, p=0.036. Furthermore, CpG-ODN-A-2216 stimulation of both PBMCs (and purified NK cells) from patients treated with HCQ (concentration > 0.8 µg/ml) did not show any effect: CD69 remaining stable at 24 ± 20% vs. 34 ± 24%, p=0.5.

Conclusion(s): Although CpG-ODN-A-2216 had only a partial direct effect on NK cell polyfunctionality, these results indicate that TLR-9 pathway is involved in NK cell activation in SLE. Further investigations are needed to determine if the “physiological” auto-antigens act like the CpG-ODN-A-2216.