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A novel TLR-9 agonist (MGN1703) activates NK-cells and enhances NK-cell mediated viral killing of HIV-1 infected CD4+ T-cells ex vivo

Offersen R., Tolstrup M., Nissen S.K., Rasmussen T., Østergaard L., Denton P.W., Søgaard O.S.
Aarhus University Hospital, Dept. of Infectious Diseases, Aarhus, Denmark

Background: Toll-like receptor (TLR) agonists may have dual favorable effects in the context of 'kick and kill' HIV eradication approaches. First, as enhancers of anti-viral immunity via stimulation of immune effector cells and second as direct latency-reversing agents. To hasten the inclusion of a TLR agonist into an HIV cure strategy, we have performed extensive preclinical testing of a novel, specific and potent TLR-9 agonist, MGN1703. Classical TLR-9 agonists (e.g. CpG-ODN) exhibit toxicity and backbone-dependent activity associated with phosphorothioate modifications. In contrast, such chemical modifications are not required to maintain the structure of MGN1703, which greatly enhances the safety profile of this molecule.

Methods: PBMCs from HIV-patients were stimulated with MGN1703 or media. Unspliced HIV-1 RNA (usHIV-RNA) in subsequently enriched CD4+ T cells was quantified using RT-qPCR. NK-cell activation, intracellular IFN-gamma production and degranulation were assessed by flow cytometry. NK-mediated viral inhibition of HIV-1 (HBX2) infected, autologous CD4+ T cells was assessed using HIV-1 P24 ELISA and intracellular HIV-1 P24 staining of CD4+ T cells. Supernatant cytokines were quantitated by QuickPlex (MSD). Statistical analyses included one-sample and paired t-tests on log-transformed data.

Results: Regarding the ability of MGN1703 to improve antiviral immune responses, we found that MGN1703-stimulation led to: (i) increased CD69-expression on CD56dimCD16+ NK-cells (4.75-fold; p=0.0014); (ii) a higher proportion of CD107a+ NK-cells (1.50-fold; p=0.0016); and (iii) a higher proportion of CD107a+IFN-gamma+ NK-cells (2.04-fold; p=0.13). Furthermore, MGN1703-stimulated NK-cells suppressed supernatant HIV-1 p24 levels by 76% versus 51% for unstimulated NK-cells (culture day 5; p=0.03). PBMCs stimulated with MGN1703 exhibited significant increases in cytokine production from (e.g. IP-10 increased 6.16-fold; p=0.024). Regarding the potential of MGN1703 to activate transcription of latent HIV-1, we found that MGN1703 increased transcription of usHIV-RNA in CD4+ T cells by 1.51-fold over media alone (p=0.036).

Conclusions: MGN1703 stimulation activated and enhanced the degranulatory capacity of NK-cells. In addition, NK-cells stimulated with MGN1703 exhibited a significantly increased capacity to control HIV-1 replication in autologous CD4+ T cells. These findings combined with the observation that MGN1703 induced an increase in usHIV-RNA transcription in CD4+ T cells supports the incorporation of the TLR9-agonist MGN1703 into HIV eradication trials.