Project: *Perforin-mediated killing of virus-infected macrophages*

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Background and Preliminary data:

Macrophages are directly infected by a large number of viruses, such as viral haemorrhagic fever viruses (VHF), herpesviruses and the human immunodeficiency virus. Clinically, acute infection can cause uncontrolled activation of macrophages, which can result in a severe sepsis-like condition that is called hemophagocytic lymphohistiocytosis with high fatality rate. Efficient killing of infected macrophages by the immune system is thus not only important to control viral replication but also to regulate the inflammatory macrophage response to infection. Virus-infected macrophages are more resistant to NK-cell killing compared to other cellular targets, as we and others (1) have observed in HIV-1 infection (Fig. 1). NK cells that cannot effectively kill their target cells are not released from the immunological synapse, resulting in prolonged proinflammatory cytokine production by NK cells (1), which in turn aggravates macrophage hyperinflammation.

NK cell killing can occur via two pathways, the upregulation of death-receptor signalling and by release of lytic granules that contain granzymes and perforin. Perforin is a human pore-forming protein, that inserts itself in the cell membrane so that granzymes can penetrate and activate intracellular pathways resulting in target cell death. The intracellular perforin content in NK cells is not fixed but modified by both priming of NK cells (Fig. 1C) and genetic *HLA class I* background (***1**). We have developed a standardized flow-cytometric killing assay set-up that enables the evaluation of interindividual differences in NK cell killing as well as testing of potential inhibitors of perforin using the perforin-sensitive K562 target cell line (Fig. 2).

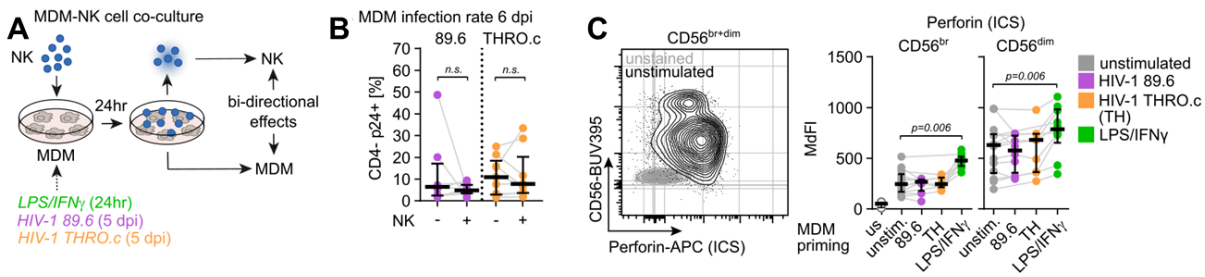

Figure 1: (A) Set-up of co-culture with monocyte-derived macrophages and natural killer cells derived from the same donor with/without HIV-1 infection and stimulation. **(B)** No significant changes in HIV-1 infection rates in co-culture after addition of NK cells for 24h, as measured by flow cytometry with HIV-1 p24 capsid staining. **(C)** Representative dot plot of intracellular perforin staining in NK cells (left); changes in median fluorescence intensity of perforin staining in NK cells after priming with proinflammatory or HIV-1 infected macrophages.

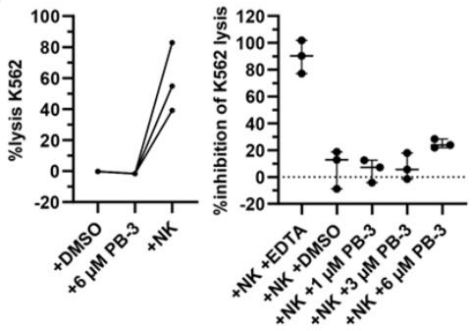

Figure 2: Flow-cytometry based killing assay of K562 targets by human NK cells after 3h of co-incubation at an E:T ratio of 1:5 in the presence or absence of indicated concentrations of the pneumolysin-inhibitor PB-3. DMSO was used as vehicle control, and EDTA as positive control for inhibition of NK cell killing. N = 3 biological donors

The central goal of this project is to elucidate the molecular and structural mechanisms underlying inefficient cytotoxic killing of macrophages either on the NK cell or the macrophage side, in order to rationally guide the development of interventions that will aid in controlling infected/inflammatory macrophages.

Project-related publications: (max. 5)

1.** Mensching L, Hoelzemer A, NK Cells, Monocytes and Macrophages in HIV-1 Control: Impact of Innate Immune Responses. ***Front Immunol.13:883728. (2022)

2.** Garcia-Beltran WF, Hoelzemer A, Martrus G, …, Altfeld M. Open conformers of HLA-F are high-affinity ligands of the activating NK-cell receptor KIR3DS1. ***Nat Immunol. 17(9):1067-74. (2016)

3:** Aziz, U.B.A., Saoud, A., Bermudez, M. …, Hoelzemer, A, …, Rademann, J, Targeted small molecule inhibitors blocking the cytolytic effects of pneumolysin and homologous toxins. ***Nat Commun 15, 4323 (2024).

4**: Marini, G., Poland, B., Leininger, C. …Topf, M., Saibil, H.R. Structural journey of an insecticidal protein against western corn rootworm. ***Nat Commun 14, 4171 (2023).

5.** Ivanova M.E., Lukoyanova, N., Malhotra, S., Topf, M. … Saibil, H.R. The pore conformation of lymphocyte perforin.Sci Adv*** 8, eabk3147 (2022).