Innate and adaptive immune control of Mpox virus infection

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Background and preliminary data:
Mpox virus (MPXV) is a double stranded DNA-virus of the genus orthopoxvirus that was historically confined to Africa but has continued to spread globally since 2022. This led to the declaration of two public health emergencies of international concern, the latest starting on 14 August 2024 by the World Health Organization (WHO) (1). As of November 2024, there have been over 117,000 cases with 263 confirmed deaths worldwide (2). In endemic areas, pregnant women, children and immunosuppressed individuals (including PLHIV), are at risk for severe clinical disease. In non-endemic areas groups at high risk for MPXV infection are men who have sex with men and people living with HIV-1 (PLHIV). How HIV-1 infection (treated or untreated) affects an individual's risk for severe disease or viral spread of MPXV is at present not fully understood.

MPXV primarily infects human skin cells, particularly keratinocytes, but can also invade other tissues such as lymph nodes, liver, and spleen. Innate immune cells play key roles in the early immune response to MPXV infection and mice with low natural killer (NK) cell numbers are highly vulnerable to lethal MPXV infection (3,4) There are two primary clades of MPXV: the West African clade II, responsible for the current global outbreak, and the more virulent Central African clade I. The difference in the human NK cell response to these two different clades and how that influences virulence is currently unknown. This project aims to uncover how NK cells as innate effector cells react to and control MPXV infection, and whether the interaction between NK cells and infected target cells is dysfunctional in PLHIV.

Hypothesis:
The central hypothesis of this project is that NK cells kill MPXV-infected target cells via activating receptor-ligand interactions. Both immune-evasive MPVX proteins and NK-cell dysfunction can result in ineffective recognition of MPXV-infected targets by NK cells.

Aims and Work Program:

1. To characterise NK-cell receptor ligands differentially surface expressed on MPXV-infected epithelial cells, monocytes and hepatocytes.

2. To determine the NK-cell response against MPXV-infected targets in people living with HIV-1, healthy controls and individuals vaccinated with modified vaccinia virus Ankara (MVA vaccine against smallpox).

In Aim #1, we will analyse how MPXV infection modulates the surface expression of NK-cell receptor ligands on infected target cells using an established flow cytometry panel (5) and intracellular staining with an anti-nucleoprotein antibody against poxviruses. For MPXV infection, we will use epithelial cell lines (i.e. A549 cells), primary hepatocytes and autologous primary cells (i.e. monocytes/macrophages) as target cells and compare Clade Ia, Ib and Clade II MPXV strains.
Our collaborator recently showed that Sars-CoV2 infection induced shedding of MIC-A and B molecules from the cell surface of infected A549 cells (6), which was reversed by using the monoclonal antibody 7C6. This reduced NK-cell killing of infected cells by preventing engagement of the activating NKG2D receptor, which has also been implicated in NK-cell control of poxvirus disease (7,8). Here, we aim to investigate, whether MPXV employs similar

(potentially targetable) mechanisms to subvert NK-cell immunity. These studies will be done under S3 biosafety conditions, additionally training the ID Fellow candidate to perform experiments with viruses of biosafety level 3.

In Aim #2, we will investigate whether NK cells are able to prevent spread of MPXV infection in cell culture. Next to direct NK-cell killing of MPXV infected target cells, we will measure the production of antiviral cytokines such as Type II interferons in response to co-culture with MPXV-infected targets. We will compare the antiviral and cytotoxic activity of NK cells derived from peripheral blood from donors without HIV-1, with HIV-1 and of donors which have received one or two doses of the MVA vaccine against smallpox. This vaccine provides adaptive heterologous immunity to MPXV, and this work will investigate whether there is an additional effect of trained innate immunity next to adaptive immune control in vaccinated individuals. Additionally, the extent of persistent NK-cell dysfunction in treated PLHIV against MPXV will be investigated in vitro. Ethics approval has been obtained. Patient and vaccine samples have already been obtained, and recruitment is ongoing.

Project-related publications (* marks own references):

1. https://www.who.int/news/item/14-08-2024-who-director-general-declares-mpox-outbreak-a-public-health-emergency-of-international-concern (2024).

2. World Health Organization. Mpox: Multi-country External Situation Report no. 44, published 23 December 2024. WHO (2024).

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4. Earl, P. L., Americo, J. L. & Moss, B. Natural killer cells expanded in vivo or ex vivo with IL-15 overcomes the inherent susceptibility of CAST mice to lethal infection with orthopoxviruses. *PLoS Pathog.* 16, 1–25 (2020).

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8. Campbell, J. A., Trossman, D. S., Yokoyama, W. M. & Carayannopoulos, L. N. Zoonotic orthopoxviruses encode a high-affinity antagonist of NKG2D. *J. Exp. Med.* 204, 1311–1317 (2007).