NK cell stimulation induced by Dendritic cell-derived cytokines during *Candida* infection

Alessandra Marolda¹, Kerstin Hünig^{1,2}, Oliver Kurzai^{1,2}

¹ Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena; ² University of Würzburg, Würzburg

Introduction

Invasive fungal infections are emerging as a significant health risk for humans. In this regard, *Candida albicans* and *Candida glabrata* are two most prevalent pathogens in the genus *Candida* and account for the majority of candidiasis cases worldwide. Although these two species share some of the same virulence factors, they are phylogenetically quite distinct. Consequently, there is also evidence that the interplay of *C. albicans* and *C. glabrata* with the human immune system differs considerably. In order to get further insights into immune cell interactions in response to *Candida* infection, we investigated Natural Killer cell (NK cell) activation during whole-blood infection compared with those of isolated cells in more detail and detected markedly differences in the stimulation of NK cells dependent on the milieu and *Candida* ssp.

Results

Differences in the stimulation of NK cells dependent on the milieu and *Candida* ssp.

1 *C. glabrata* induces higher NK cell activation than *C. albicans* in blood

In contrast to primary NK cells, which are directly stimulated by fungal cells and showed an increased surface exposure of degranulation marker CD107a and decreased CD16 expression upon fungal confrontation, the cytotoxic cells *in vivo* seem to be activated indirectly by signals from other immune cells, resulting in different effectors mechanisms indicated by the up-regulation of activation marker CD69 and TNF-related apoptosis-inducing ligand (TRAIL).

2 *C. albicans* stimulates primary NK cells stronger than *C. glabrata*

Dendritic cell - NK cell crosstalk (Indirect)

1 DC activation by *C. albicans* and *C. glabrata*

DC activation phenotype during the infection with either *C. albicans* or *C. glabrata* showed increased levels of surface activation marker CD83 and release of cytokines, with significant different levels between the two fungal species for IL-1 β and IL-12p70.

Transwell experimental layout

NK cell activation induced by soluble factors released from activated DCs was analysed after 40h.

2 NK cell activation by soluble factors released from stimulated DC

NK cell - DC co-incubation in the presence of fungi using the transwell system (40 h). Dependent on the *Candida* species, NK cells showed different levels of CD69, TRAIL and FAS up-regulation on the surface, reflecting the activation phenotype of NK cells in infected blood. The high IFN- γ release by NK cells also shows a strong activation of these cells.

3 Effect of IL-12p70 neutralizing antibody on NK cells activation

Blocking of IL-12p70 to verify its regulatory function on NK cells resulted in a reduced activation marker up-regulation and inhibited release of IFN- γ by these cells.

Dendritic cell - NK cell crosstalk (Direct)

Direct NK cell - DC co-incubation in the presence of fungi (40 h). Dependent on the contact with DC and fungal cells, NK cells showed an up-regulation of the following markers: CD69, TRAIL, CD107a and Fas and a down-regulation of CD16 reflecting a mixed activation phenotype compared to primary and blood NK cells.

Conclusion

These results demonstrate distinct patterns of NK cell activation dependent on the differential induced cytokine secretion by DC and in human blood after contact with the two *Candida* species, *C. albicans* and *C. glabrata*. Among all measured cytokines, IL-12p70 represents the most interesting difference in the DC supernatants after confrontation with both species with a significantly higher release during *C. glabrata* infection. However, additional cytokines are required to fully stimulate the NK cells and will be in the focus of future studies.