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Human Natural Killer Cells and Aspergillus Fumigatus Differentially Influence their Gene Expression Profile

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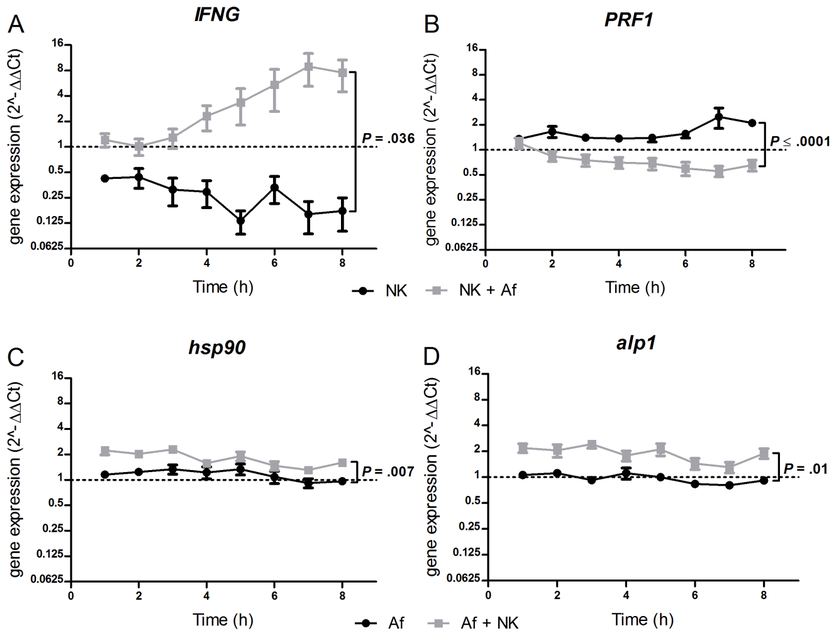
Background: Natural Killer (NK) cells are active against *Aspergillus fumigatus,* which, in turn produces proteins to impair host defense and to facilitate tissue invasion. However, little is known on the interaction of NK cells and *A. fumigatus.*

Methods: We investigated the mutual influence of gene expression profiles of human NK cells and *A. fumigatus*. Freshly isolated and interleukin-2 prestimulated human NK cells from healthy volunteers were coincubated with *A. fumigatus* for 8 hours. RNA samples were collected immediately prior to co-incubation and thereafter every hour during co-incubation for up to 8 hours to assess gene expression of selected genes of both NK cells and the fungus by quantitative real-time PCR (iQ5 platform; BioRad, Munich, Germany). Data were analyzed using the $2^{-\Delta\Delta Ct}$-method.



Gene expression of IFN-γ (*IFNG*; A) and perforin (*PRF1*; B) in IL-2 prestimulated human NK cells coincubated with *A. fumigatus* hyphae or incubated alone, and gene expression of heat shock protein 90 (*hsp90*, C) and alkaline protease 1 (*alp1*, D) in *A. fumigatus* coincubated with IL-2 prestimulated human NK cells or incubated alone, respectively.
Coincubation starts at hour 0, and first assessment of the change of transcript levels were performed at hour 1. The Y axis represent the relative fold-change of the mRNA expression of the gene of interest relative to the housekeeping gene (*GAPDH* and *TUBB,* respectively) and to time point hour 0, respectively, determined by the $2^{-\Delta\Delta Ct}$–method

Results: In the presence of *A. fumigatus,* a decrease of the gene expression of the cytotoxic molecules perforin, granzyme B, and granulysin was observed in both unstimulated and prestimulated NK cells. In contrast*, A. fumigatus* increased mRNA levels of the pro-inflammatory molecules IFN-γ, GM-CSF, TNF-α and MIP-1α in both unstimulated and prestimulated NK cells (Figure and Table).

The transcript levels of the fungal stress-related heat shock protein90 (*hsp90*), the fungal ferric chelate reductase (*freB*) and alkaline protease 1 (*alp 1*) were significantly higher when *A. fumigatus* was coincubated with IL-2 prestimulated NK cells as when incubated alone (Figure and Table). In contrast, neither unstimulated or prestimulated NK cells had a significant impact on the transcript levels of the other fungal genes tested (Table).

human NK cells		unstimulated NK cells ± *A. fumigatus*			IL-2 prestimulated NK cells ± *A. fumigatus*		
	gene (molecule)	$2^{-\Delta\Delta Ct}$; NK-Af/NK+Af[1]	*P*	x-fold change[2]	$2^{-\Delta\Delta Ct}$; NK-Af/NK+Af[1]	*P*	x-fold change[2]
	cytotoxic molecules						
	PRF1 (perforin)	2.5 ± 0.3/0.7 ± 0.2	0.0005	**-3.6**	2.1 ± 0.1/0.7 ± 0.1	< 0.0001	**-3**
	GZMB (granzyme B)	1.7 ± 0.3/0.4 ± 0.1	0.006	**-4.3**	0.8 ± 0.1/0.3 ± 0.1	0.01	**-2.6**
	GNLY (granulysin)	1.8 ± 0.4/0.6 ± 0.1	0.009	**-3**	0.9 ± 0.1/0.4 ± 0.1	0.013	**-2.2**
	pro-inflammatory molecules						
	IFNG (interferon-gamma)	0.2 ± 0.1/2.6 ± 0.9	0.031	**+13**	0.2 ± 0.1/7.6 ± 3.1	0.036	**+38**
	GMCSF (GM-CSF)	1.3 ± 0.5/7.3 ± 2.6	0.031	**+5.6**	0.7 ± 0.1/3.2 ± 0.7	0.015	**+4.6**
	TNFA (tumor necrosis factor α)	1.0 ± 0.2/4.3 ± 2.3	ns	**+4.3**	0.4 ± 0.1/0.6 ± 0.1	ns	**+1.5**
	MIP1A (macrophage inflammatory protein 1α)	1.1 ± 0.5/1.9 ± 0.8	ns	**+1.7**	0.6 ± 0.1/2.2 ± 0.5	0.0161	**+3.7**
	MIP1B (macrophage inflammatory protein 1β)	3.0 ± 0.8/2.4 ± 0.9	ns	**-1.3**	0.7 ± 0.1/2.6 ± 0.7	0.044	**+3.7**
	anti-inflammatory molecules						
	TGFB (transforming growth factor β)	1.0 ± 0.2/0.4 ± 0.1	0.044	**-2.5**	2.1 ± 0.4/1.1 ± 0.1	ns	**-1.9**

A. fumigatus		*A. fumigatus* ± unstimulated NK cells			*A. fumigatus* ± IL-2 prestimulated NK cells		
	gene (molecule)	$2^{-\Delta\Delta Ct}$; Af-NK/Af+NK[1]	*P*	x-fold change[2]	$2^{-\Delta\Delta Ct}$; Af-NK/Af+NK[1]	*P*	x-fold change[2]
	heat shock response						
	hsp70 (heat shock protein 70)	0.9 ± 0.3/1.0 ± 0.3	ns	**+1.1**	0.9 ± 0.1/1.0 ± 0.1	ns	**+1.1**
	hsp90 (heat shock protein 90)	0.7 ± 0.1/1.0 ± 0.1	ns	**+1.4**	1.0 ± 0.1/1.6 ± 0.1	0.007	**+1.6**
	high affinity iron assimilation						
	freB (ferric chelate reductase)	1.4 ± 0.3/1.0 ± 0.1	ns	**-1.4**	0.9 ± 0.0/1.4 ± 0.1	0.0005	**+1.6**
	proteases/ peptidases						
	alp1 (alkaline protease 1)	1.4 ± 0.5/0.9 ± 0.1	ns	**-1.6**	0.9 ± 0.0/1.9 ± 0.3	0.01	**+2.1**
	dppIV (dipeptidyl peptidase IV)	1.2 ± 0.4/0.8 ± 0.1	ns	**-1.5**	0.6 ± 0.1/0.9 ± 0.1	ns	**+1.5**
	dppV (dipeptidyl peptidase V)	2.4 ± 0.4/1.8 ± 0.2	ns	**-1.3**	1.3 ± 0.2/1.3 ± 0.2	ns	**±1.0**
	ROS detoxification						
	sod1 (superoxide dismutase 1)	0.6 ± 0.2/0.7 ± 0.2	ns	**+1.2**	0.9 ± 0.2/0.9 ± 0.2	ns	**±1.0**
	sod2 (superoxide dismutase 2)	0.7 ± 0.1/0.7 ± 0.1	ns	**±1.0**	0.9 ± 0.2/1.3 ± 0.3	ns	**+1.4**
	sod3 (superoxide dismutase 3)	0.8 ± 0.1/0.8 ± 0.1	ns	**±1.0**	1.5 ± 0.0/1.6 ± 0.2	ns	**+1.1**
	cat1 (catalase 1)	0.6 ± 0.1/0.5 ± 0.0	ns	**-1.2**	0.6 ± 0.1/0.6 ± 0.1	ns	**±1.0**
	cat2 (catalase 2)	1.0 ± 0.1/0.9 ± 0.0	ns	**-1.1**	1.3 ± 0.0/1.7 ± 0.4	ns	**+1.3**
	cycA (cytochrome C)	1.0 ± 0.2/0.9 ± 0.2	ns	**-1.1**	0.8 ± 0.1/0.8 ± 0.2	ns	**±1.0**
	mycotoxins						
	mgl (mitogillin)	1.5 ± 0.3/1.0 ± 0.1	ns	**-1.5**	0.7 ± 0.1/1.1 ± 0.2	ns	**+1.6**
	gliT (gliotoxin)	1.2 ± 0.1/1.4 ± 0.3	ns	**+1.2**	1.2 ± 0.1/1.4 ± 0.4	ns	**+1.2**

Regulation of selected genes in human NK cells and *A. fumigatus*
[1] Relative change of the mRNA expression of the gene of interest after 8 hours of (co-) incubation relative to the housekeeping gene and to time point at hour 0, respectively, determined by the $2^{-\Delta\Delta Ct}$-method. The first number represents the result in NK cells (NK-Af) or in *A. fumigatus* (Af-NK) incubated alone, the second number the result in NK cells incubated with the fungus (NK+Af) or in *A. fumigatus* incubated with NK cells (Af+NK), respectively.
[2] x-fold change is defined by the comparison of means as described above [1]; negative values indicate down-regulation, positive values up-regulation, and the value of 1 unchanged mRNA levels.

Discussion: The increase of mRNA levels of the pro-inflammatory molecules is in line with previous data. The decrease of transcript levels of cytotoxic molecules in the presence *of A. fumigatus* is surprising, as these are important molecules in the NK cell mediated killing of fungi. This observation may be explained by an increased translation of the preexisting mRNA, as demonstrated in a murine model.

Human NK cells did not exhibit major impact on fungal gene expression except for minor up-regulation of mRNA levels of some stress related molecules and proteases.

Based on the present results, future studies have to focus on the products of the investigated genes in order to better characterize both direct and indirect NK cell-mediated anti-*Aspergillus* activity.

References
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