Why are NK cells important for defense against *Aspergillus*?

Thomas Lehrnbecher

Pediatric Hematology and Oncology

Frankfurt/Main, Germany

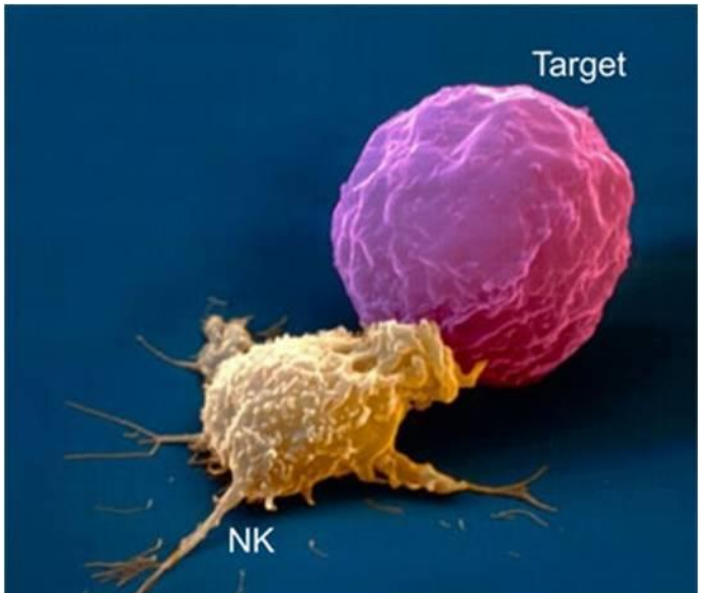
Conflict of Interest

Speakers bureau:	Astellas, Gilead Sciences, Merck/MSD, Pfizer
Advisory board:	Astellas, Basilea, Gilead Sciences, Merck/MSD
Research grant:	Gilead Sciences

NK cells

- 5-10% of leukocytes in the peripheral blood are Natural Killer cells
- NK cells are characterized by CD56 and by the absence of the T cell marker CD3
- Two main subpopulations: the cytotoxic $CD56^{dim}CD16^{bright}$ and the immune regulatory $CD56^{bright}CD16^{dim}$
- Name originates from ability to kill tumor cells *in vitro* and *in vivo* without previous stimulation (e.g., ALL, AML, neuroblastoma, multiple myeloma)
- Effects: Cytolytic effect by soluble factors such as perforin, granzyme

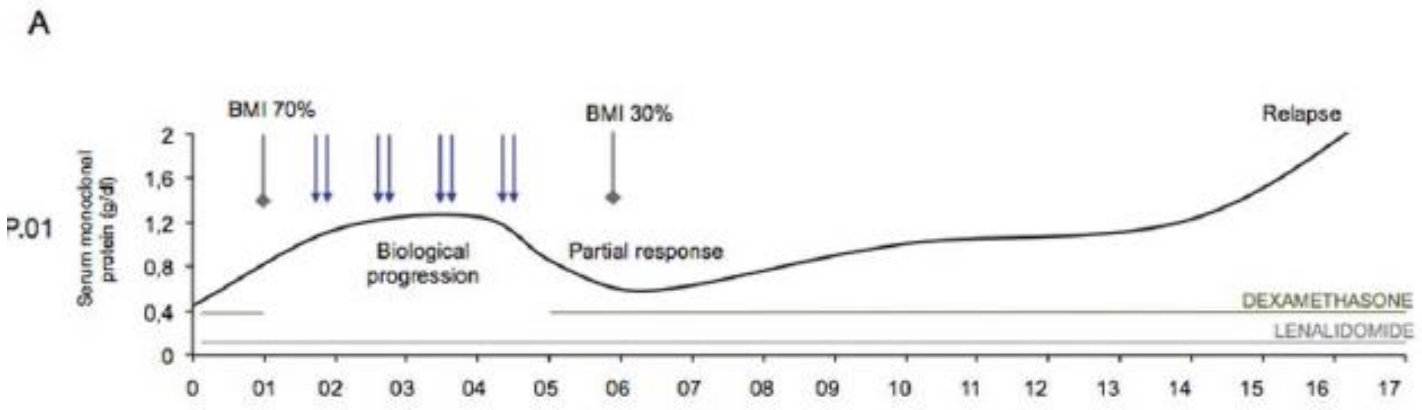
  Immunomodulation by secretion of chemokines and cytokines



NK cells in antitumor immunotherapy

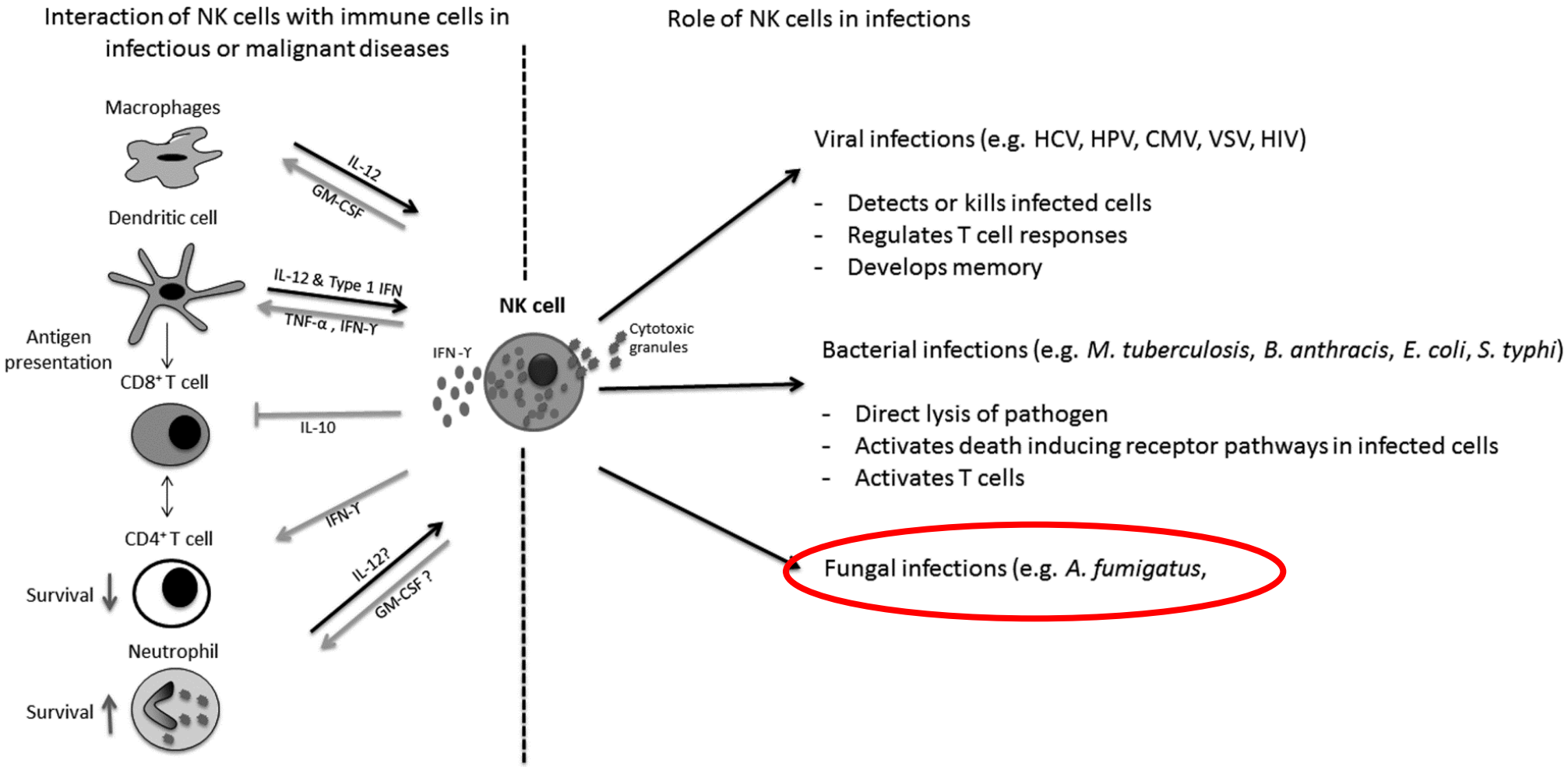
Based on the observation that NK cells kill malignant cells, multiple studies evaluate NK cells as antitumor immunotherapy

- Phase I study evaluating autologous activated and expanded NK cells in patients with multiple myeloma
- Five patients with relapsed or refractory MM who had received 2-7 prior lines of therapy
- Four cycles with two infusions of $7.5x10^6$/kg NK cells
- Safety excellent (no grade III/IV toxicities)
- Efficacy: 2 patients with partial response (50% reduction of bone marrow infiltration), 3 patients with stable disease



Leivas A et al *Oncoimmunol* 2016

NK cells and pathogens



Schmidt et al *Clin Vaccine Immunol* 2013

NK cells and *Aspergillus*: *in vitro* data

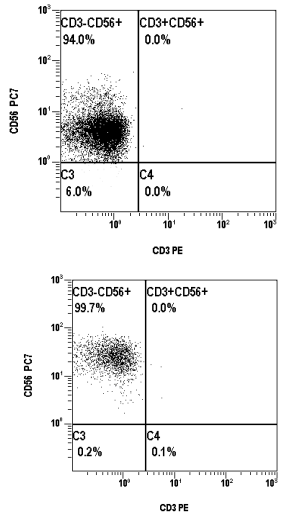
NK-cells and *Aspergillus fumigatus*

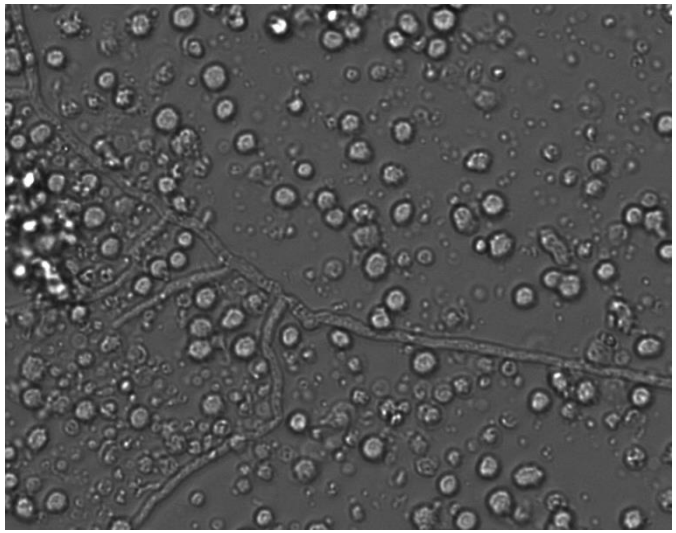
Purified human $CD56^{+}CD3^{-}$ NK cells

Aspergillus fumigatus (hyphae, conidia)

- unstimulated

- stimulated for
  7-10 days with IL-2
  (1000 U/ml)



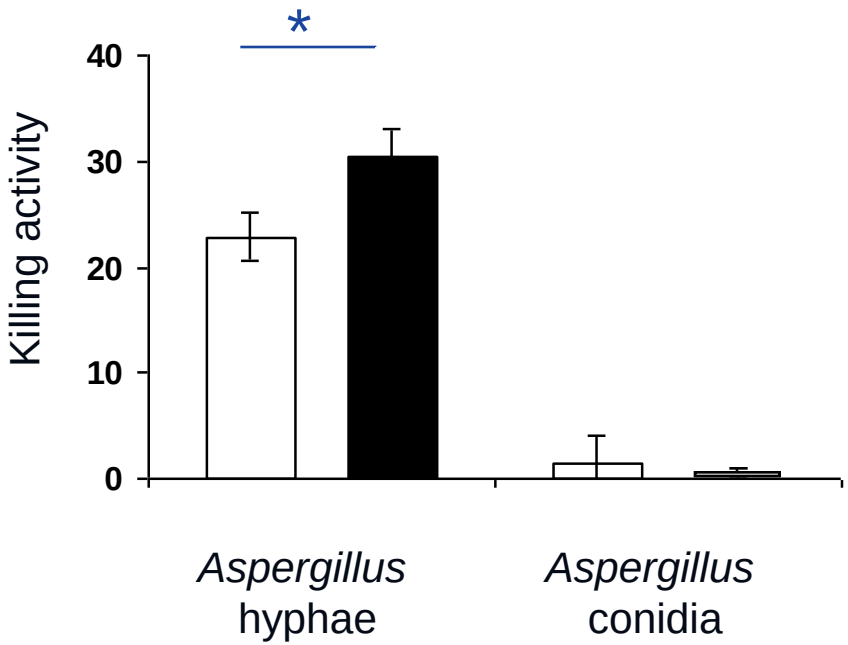


→ anti-*Aspergillus* activity [hyphal damage (microscopy, XTT)]
[anticonidial activity (CFU)]

→ Assessment of surface/activation markers on NK cells
soluble factors in supernatant
effect of perforin

Schmidt *et al*, *JID* 2011

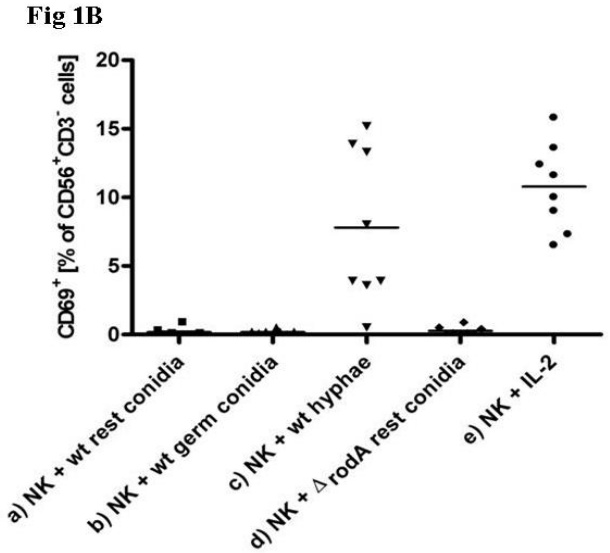
NK cells kill *A. fumigatus* hyphae, but not conidia



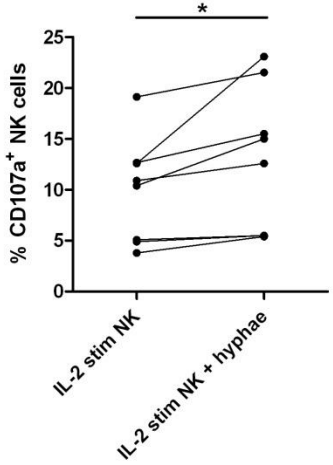
- Unstimulated and IL-2 stimulated human NK cells kill A. *fumigatus* hyphae, but do not affect resting *A. fumigatus* conidia
- Antifungal activity of IL-2 prestimulated NK cells significantly higher than of unstimulated NK cells

Schmid et al, *JID* 2011

A. fumigatus hyphae, but not conidia activate NK cells



- A. *fumigatus* hyphae (c), but not resting (a) and germinating *A. fumigatus* conidia (b) up-regulate CD69 expression on unstimulated human NK cells
  - Percentage of activated NK cells (bar: median)



- A. *fumigatus* hyphae, but not resting *A. fumigatus* conidia up-regulate CD107a expression on stimulated human NK cells

Schmid et al, *JID* 2011

Perforin plays an important role in hyphal killing

Fig 3A

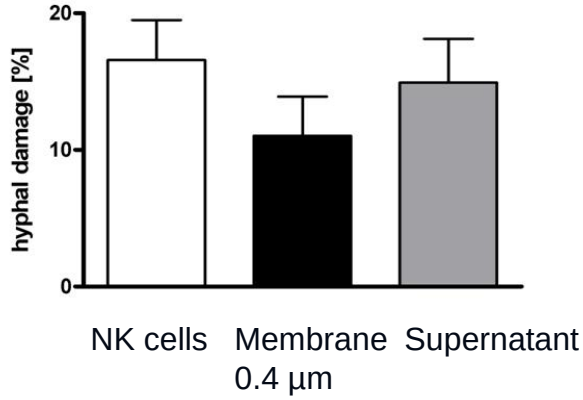
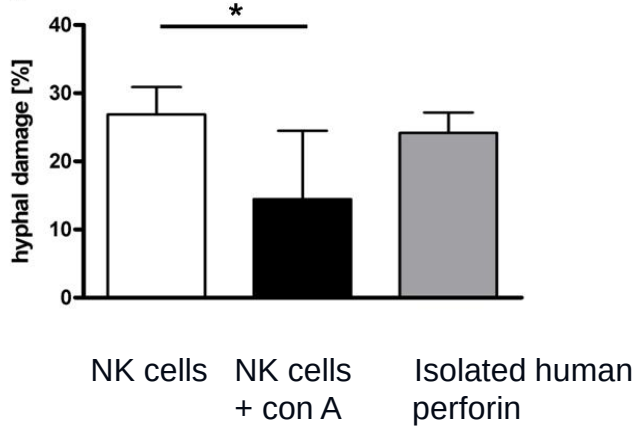


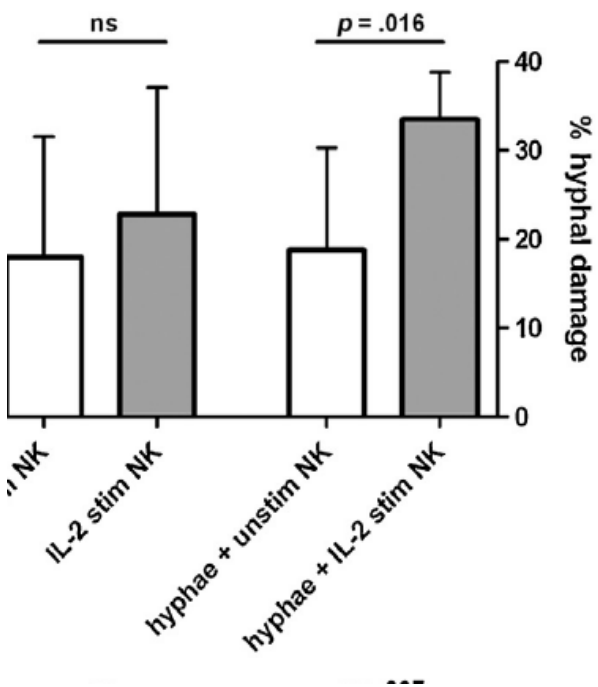
Fig 3B

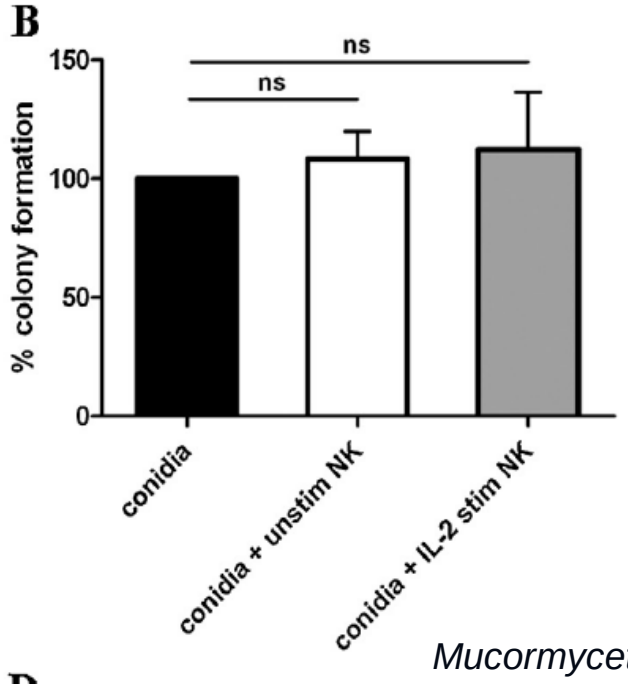


- Hyphal killing by NK cells induced by soluble factors
- Killing activity of IL-2 stimulated NK-cells significantly reduced by concanamycin A
- Killing of *Aspergillus* hyphae also induced by purified human perforin

Schmid et al, *JID* 2011

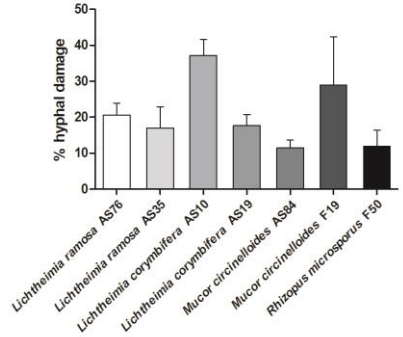
NK cells are able kill various mucormycetes and *Candida*





R. oryzae

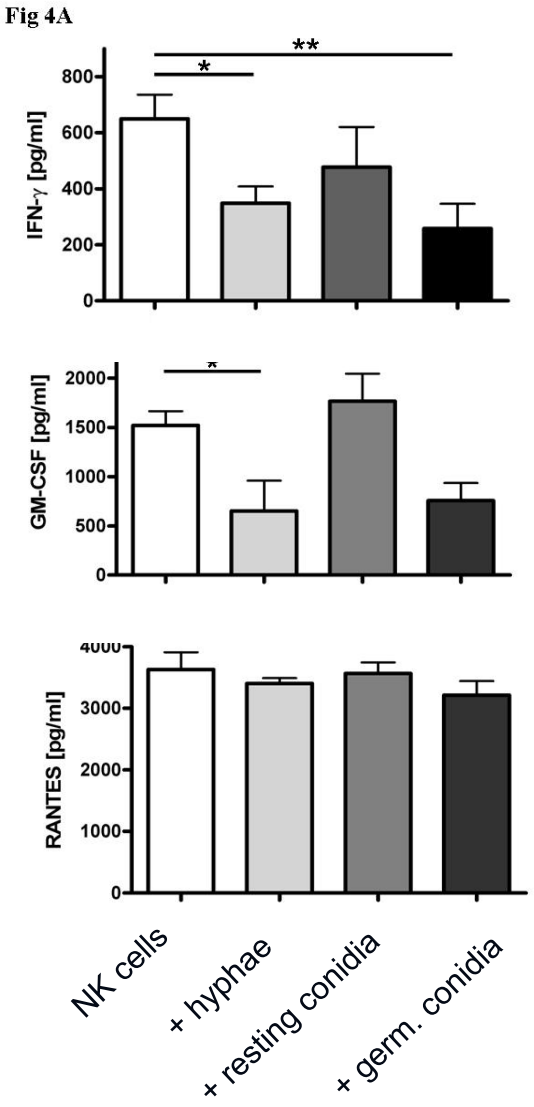
Mucormycetes (Lichtheimia, Mucor, Rhizopus)



Similar data for NK cells and C. albicans → NK cells with broad antifungal activity

Schmid et al, *Immunobiology 2013; Voigt et al J Infect Dis 2014; Schmidt et al, Mycoses 2016*

Immunosuppressive effect of *Aspergillus*

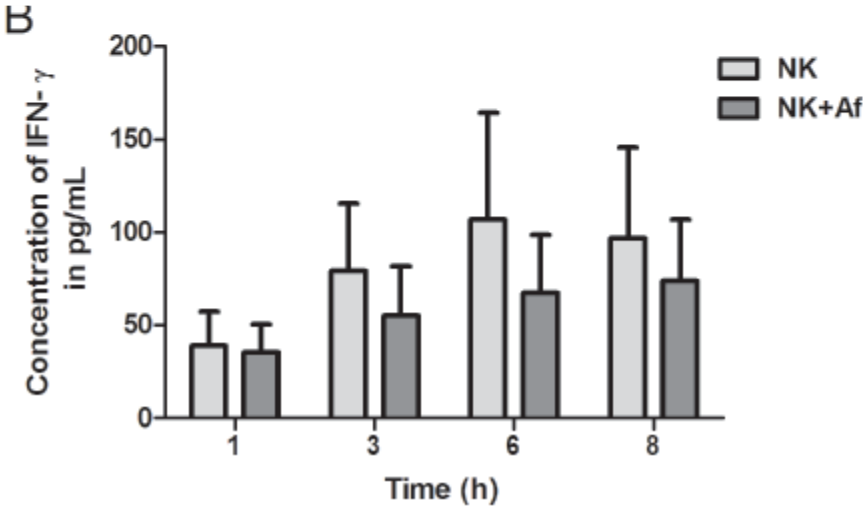


- Coincubation with *Aspergillus* hyphae and germinating conidia, but not with resting conidia leads to a
  - significant decrease of IFN-γ and GM-CSF in the supernatant
  - RANTES secretion not affected
  - Note: viability of NK cells unaffected

→ immunosuppressive effect of the fungus (mycotoxins?)

Schmid et al, *JID* 2011

Effect of *Aspergillus* on NK cell derived IFN-γ



Schneider et al , *Oncotarget* 2017

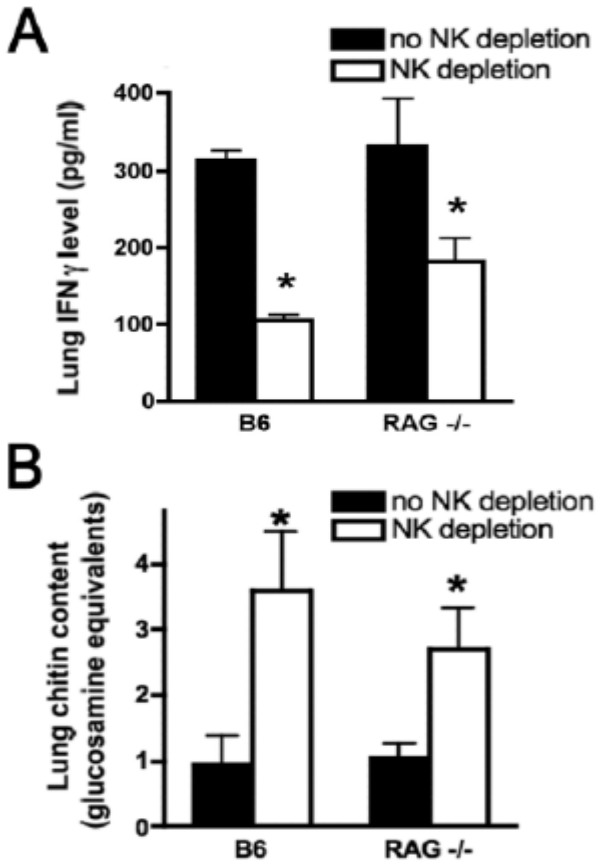
Evaluating NK cells *in vivo*: animal models

NK cells in the antifungal host defense: animal model

NK cells are main source of IFN-γ in neutropenic mice suffering from aspergillosis

→ depletion of NK cells results in diminished IFN-γ levels in the lungs

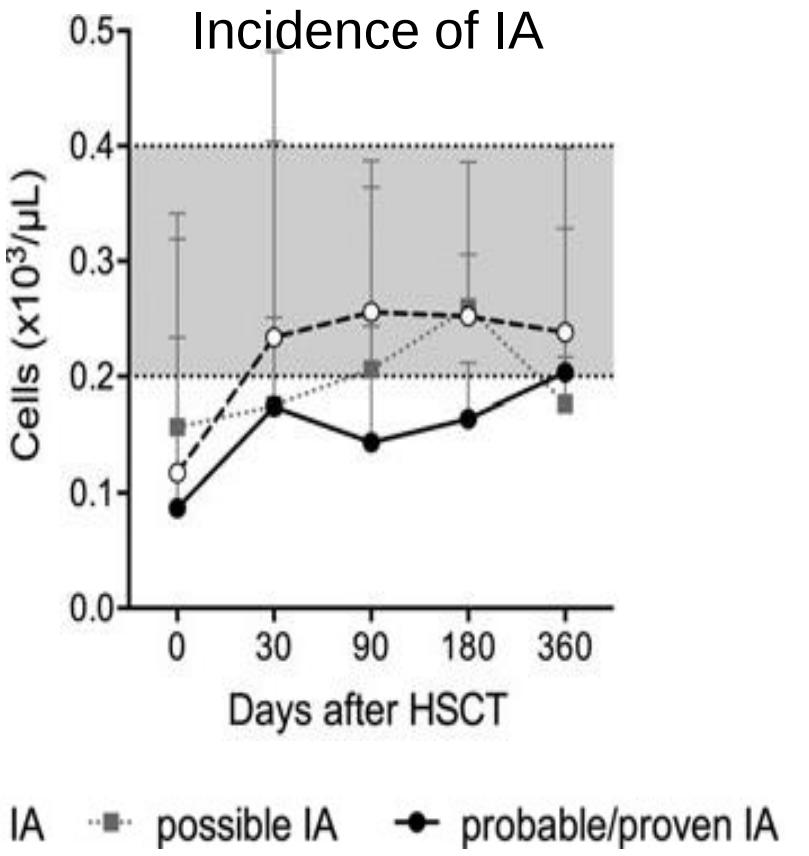
→ followed by increased fungal load

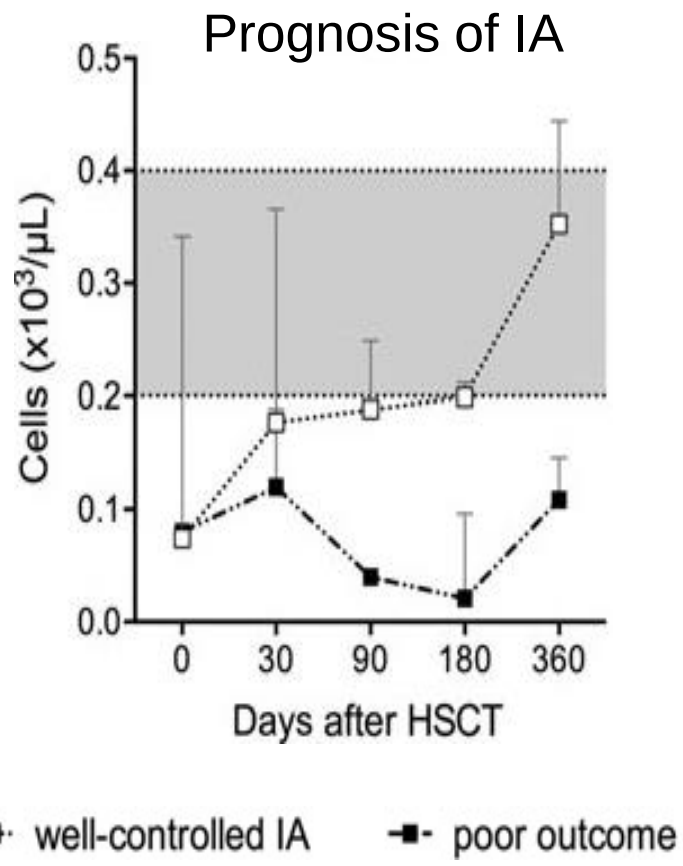


Park SJ, *J Immunol* 2009

Clinical Data

NK cells influence risk and outcome of IA after HSCT



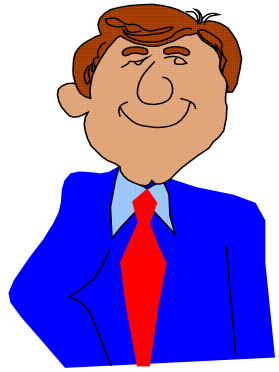


→ Rationale for immunotherapy with NK cells („adoptive immunotherapy“)

Stuehler et al, *J Infect Dis*. 2015

Adoptive immunotherapy

Principle of adoptive immunotherapy after SCT



„Third party“ „Donor“

Collection of stem cells

Transplantation

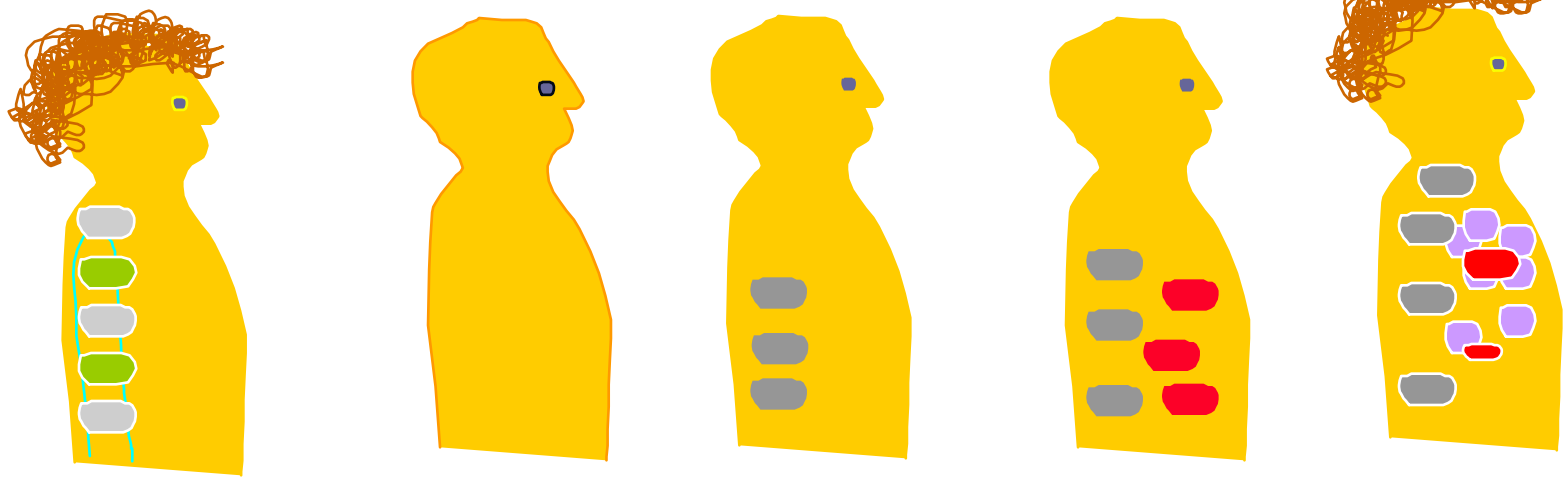
Immunotherapy

Infection

High dose chemotherapy

Effector cells

„from the shelf“



Normal cell Leukemic cell Fungus

Adoptive Immunotherapy in aspergillosis

Granulocyte transfusions: conflicting results regarding efficacy

Safety concerns in patients with pulmonary aspergilllosis

Transfusion of anti-*Aspergillus* T cells

„proof-of-principle“ study in 10 patients after haploidentical SCT

GMP conform generation possible – donor needed

epitopes?

Concerns regarding safety, such as graft-versus-host disease

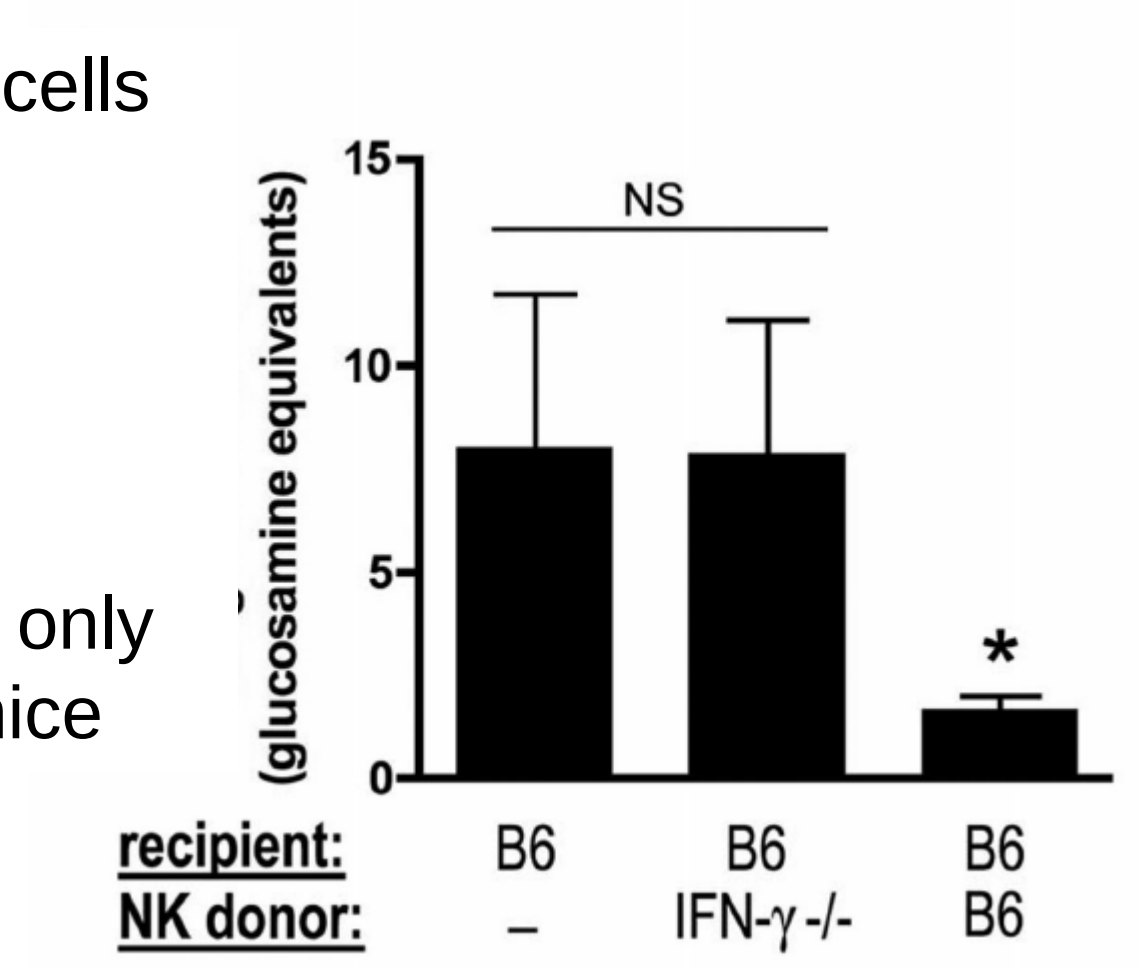
→ Natural Killer cells ?

Perruccio et al, *Blood* 2005

Transfer of activated NK cells to neutropenic mice

- wild-type mice
- IFN-γ deficient mice

Better clearance of *A. fumigatus* from the lungs only by transfer of wild type mice



Park SJ, *J Immunol* 2009

Summary – NK cells

- Both unstimulated and IL-2 stimulated NK cells exhibit killing activity against a variety of fungi (e.g. *A. fumigatus*, mucormycetes)
- Direct killing activity mediated – at least in part – by perforin
- Immunosuppressive effect of *A. fumigatus* by decreasing IFN-γ release
- Animal models support the important role of NK cells in the antifungal host response – more details by re-population studies needed
- NK cells as adoptive immunotherapy in patients with IFD?
  - autologous / allogeneic NK cells?
  - NK cell lines? Genetically modified NK cells?

Thank you for your attention!