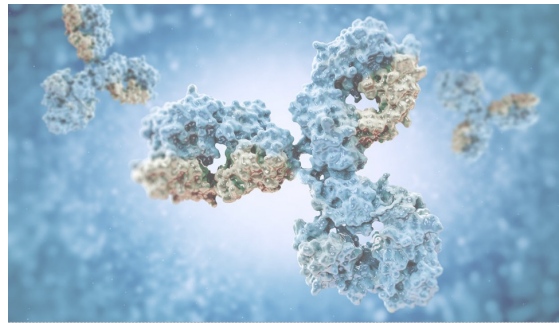


# An introduction to Fc-Dependent Immunomodulation induced by antiviral antibodies

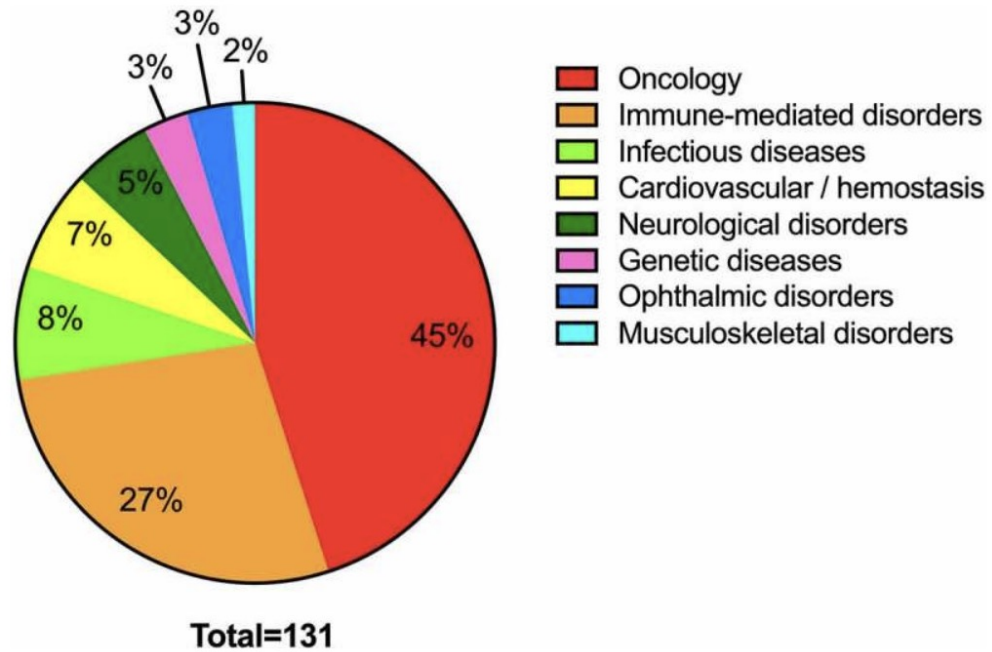
Mireia Pelegrin



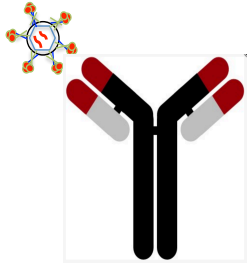
**IAS** “Vaccinal effect of monoclonal antibodies” Webminar  
14<sup>th</sup> November 2023



## Mab are the main class of biotherapeutics



# Antibodies-Immune actors interactions: High versatile system



+

**Multiple receptors**  
Fc-Receptors (FcR)

+

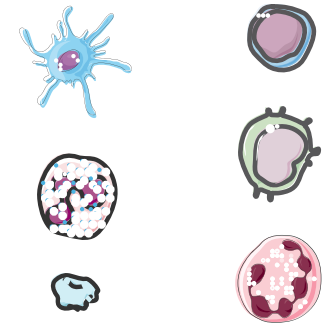
**Multiple immune cells**  
Expressing FcR

## Antibody properties

- Affinity
- Specificity
- Neutralization capacity
- Isotype
- Glycosilation
- ....

FcγRI	FcγRIIA	FcγRIIB	FcγRIIC <sup>o</sup>	FcγRIIA	FcγRIIB	FcRn
CD64	CD32A	CD32B	CD32C	CD16A	CD16B	-
ITAM T <sub>2</sub>		ITIM		ITAM T <sub>2</sub>	GPI	β <sub>2</sub> m

- activatory/inhibitory
- different affinities for IgGs isotypes
- differentially expressed on immune cells

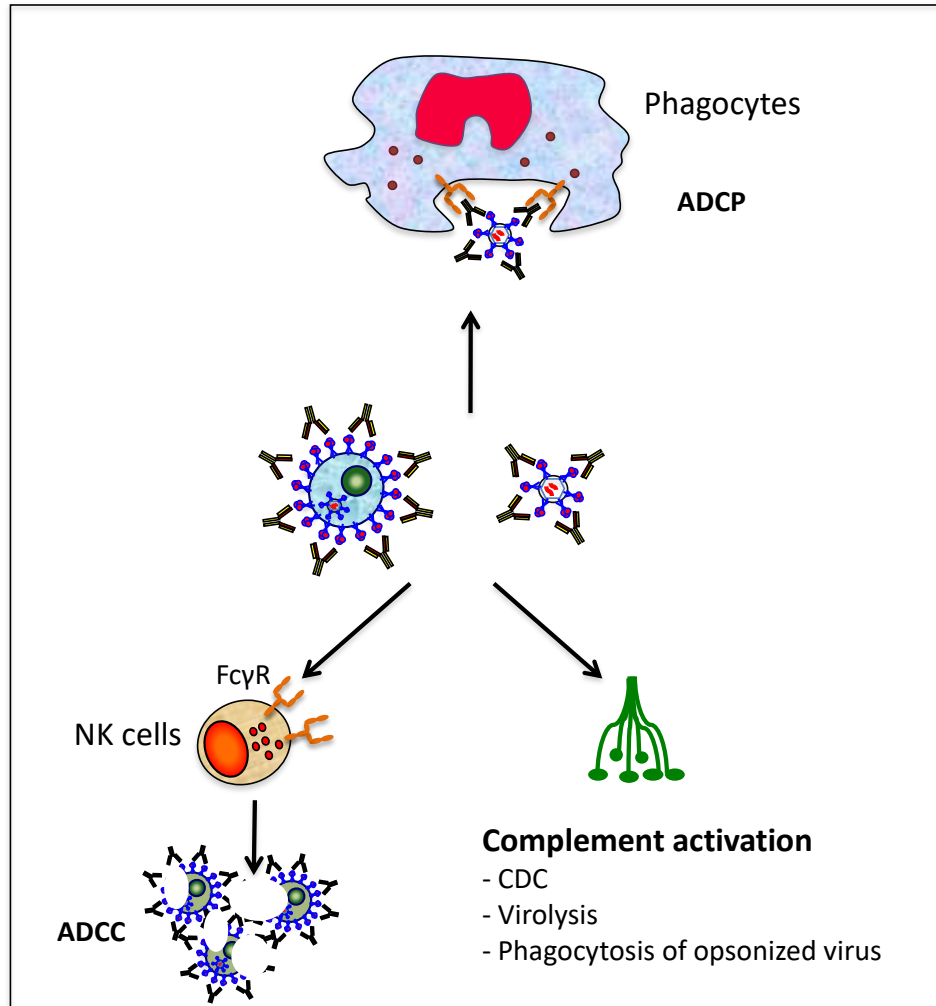


- Ag presentation
- ADCC
- Phagocytosis
- ....

## Myriad of immune outcomes

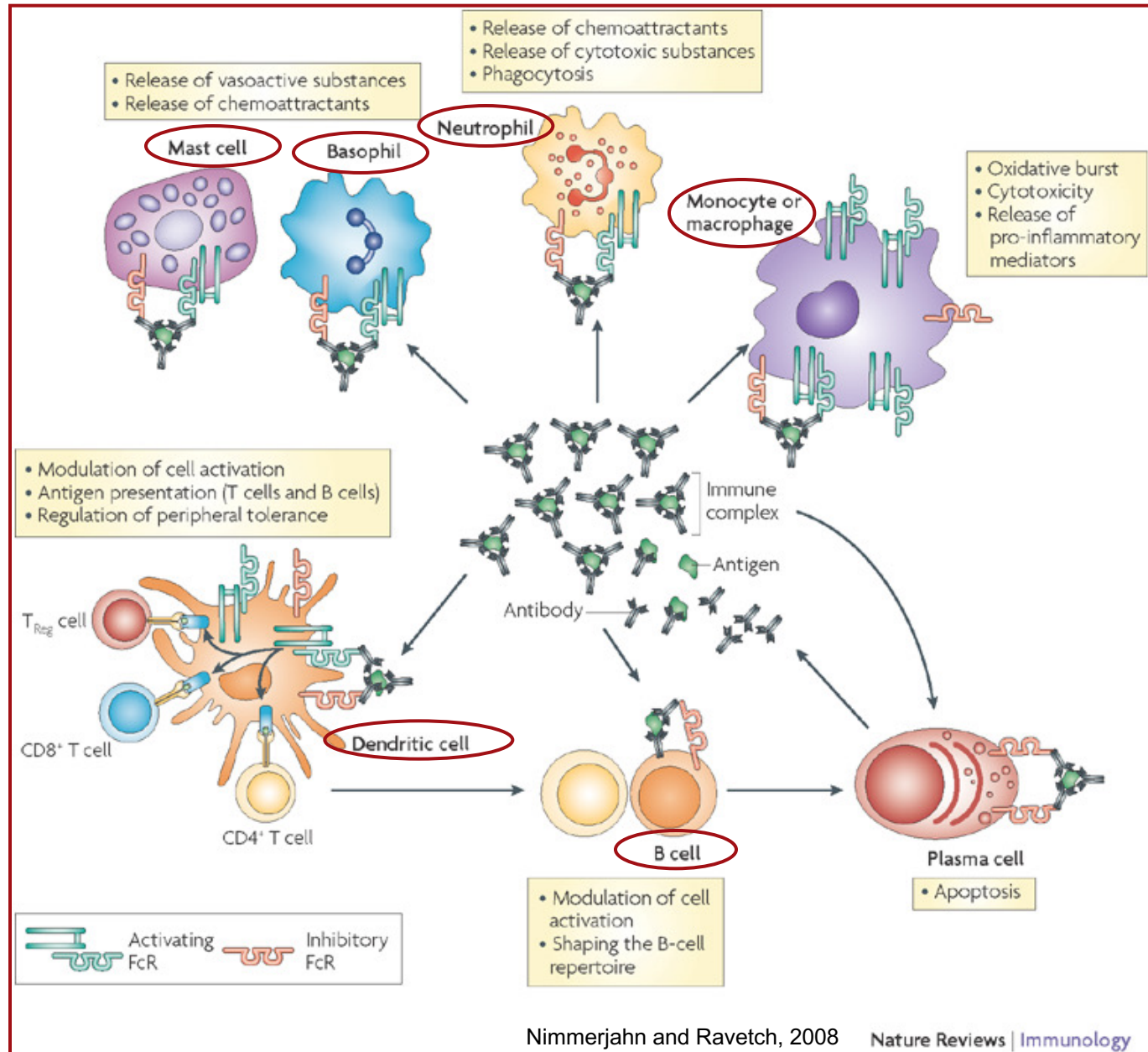
- Direct control of viral propagation
- Modulation of antiviral immune responses

## Direct target elimination

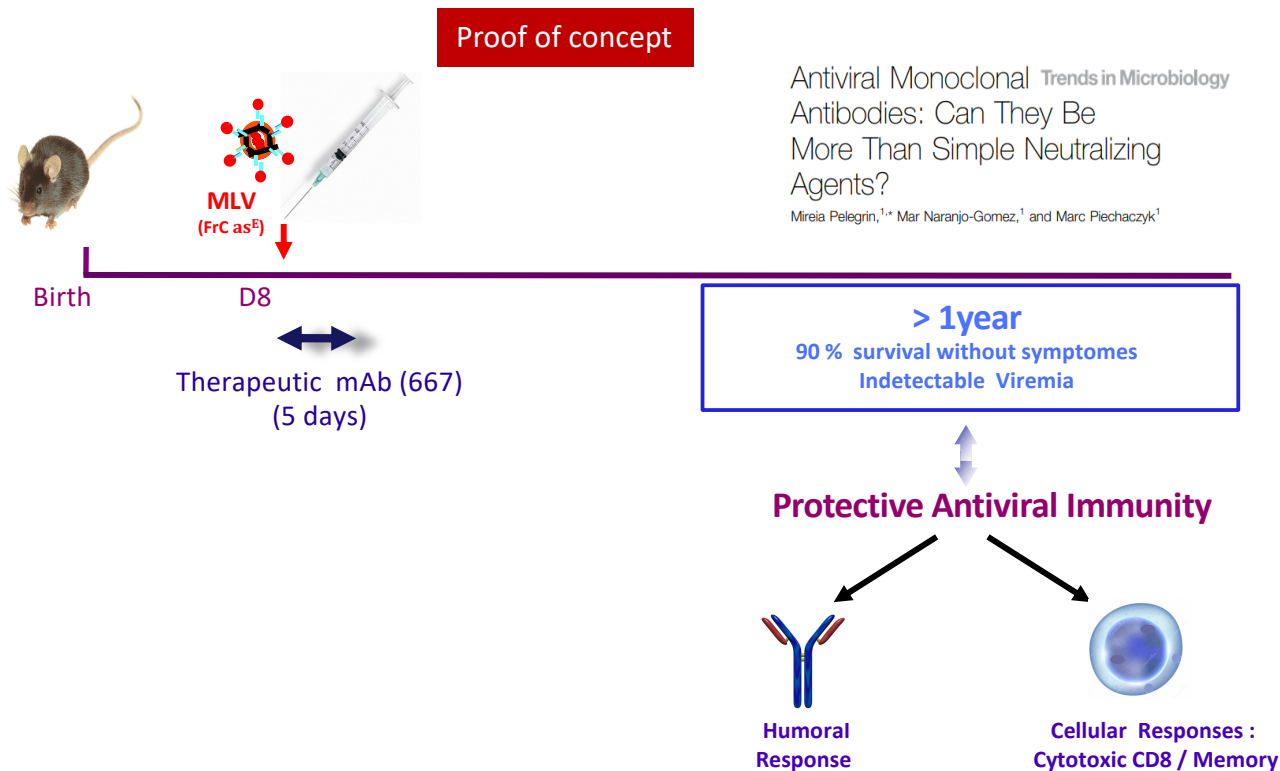




# Immunomodulatory functions of immune complexes (IC)



# Antiviral mAbs induce long-term protective immunity



Dreja et al, J. Virol, 2003  
Gros et al, J. Virol., 2005  
Gros et al, J. Virol, 2006  
Gros et al, J. Virol, 2008  
Michaud et al, Plos Pathogens, 2010  
Nasser et al, J Virol., 2010  
Nasser et al, Blood, 2013  
Pelegrin et al, Trends Microbiol, 2015

**Enhancement of humoral and cellular immune responses by mAbs have been reported in :**

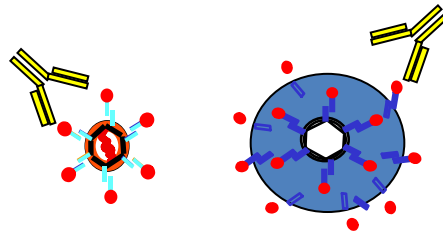
- breast cancer, lymphoma, melanoma
- viral infections (Influenza, HIV-1, Henipaviurs, ....)
- bacterial infections (*P. aeruginosa*)

Which are the mechanisms involved in the induction of long-term protective immunity by antiviral mAb?



*Lessons from a murine retrovirus infection model*

1/ Does Ab-treatment decrease the viral load and avoid overwhelming of the immune system?



# Decreasing the viral load is not enough to induce protection

\* Reduce the viral load without therapy

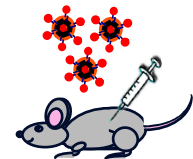


FrCas<sup>E</sup> low inoculum

No mAb



\* Keep the neutralizing effect without effector functions



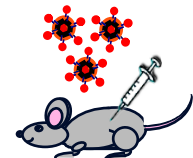
FrCas<sup>E</sup> high inoculum



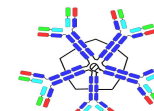
667 F(ab')<sub>2</sub>



\* Keep the neutralizing effect with different effector functions



FrCas<sup>E</sup> high inoculum



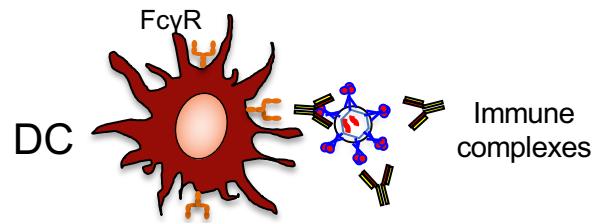
672 IgM



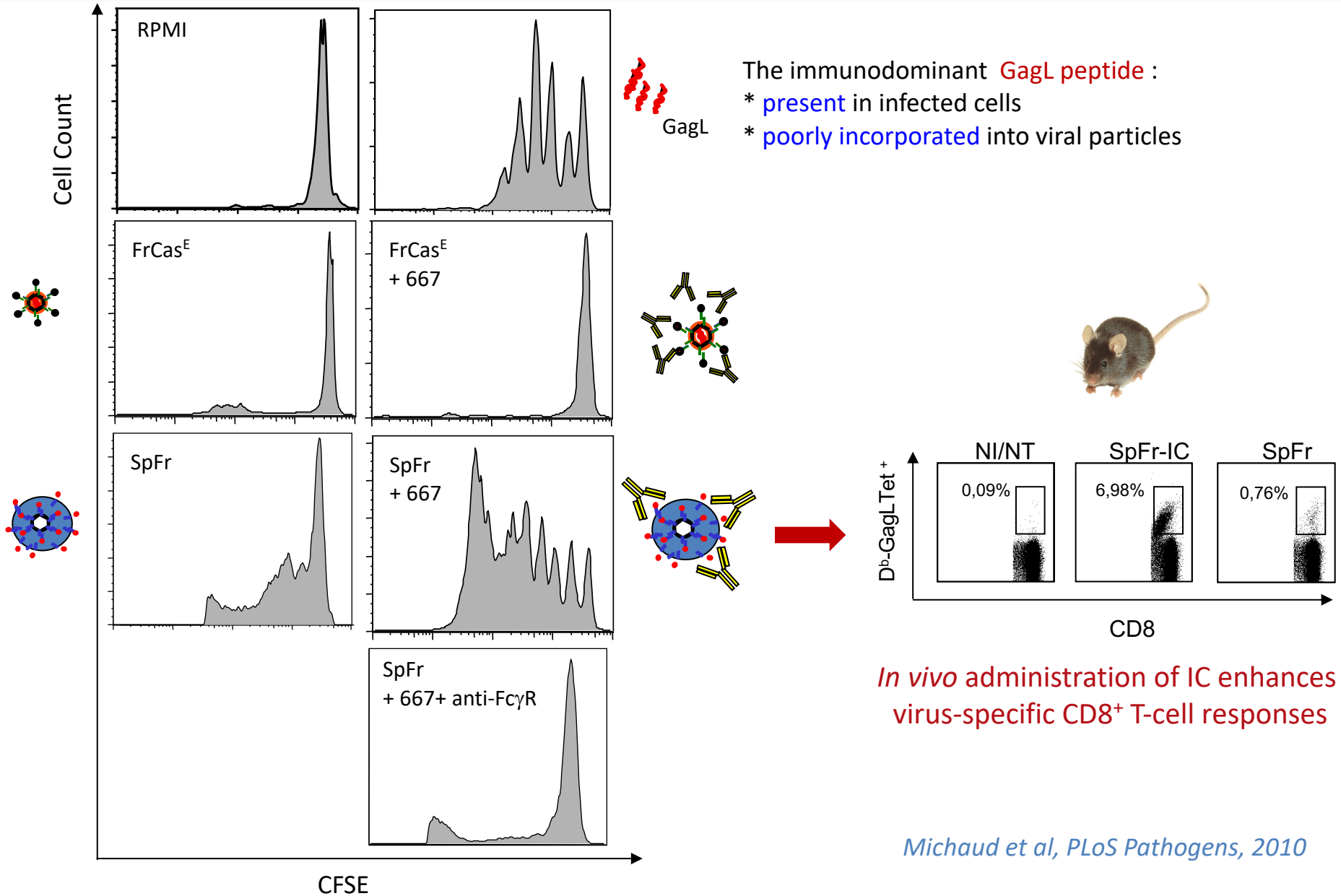
Fc-related functions are crucial for induction of protective immunity

mAb isotype is crucial for optimal protection

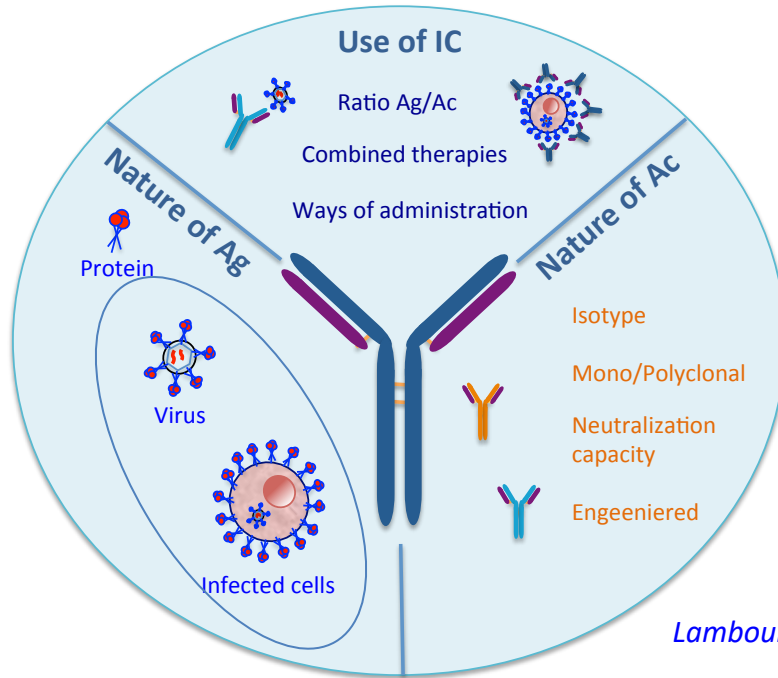
2/ Does antiviral mAb form immune complexes (IC) allowing better dendritic cell (DC) activation and antigen presentation?



# Activation of DC by IC (mAb/infected cells) enhances CD8<sup>+</sup> T-cell responses in a FcγR-dependent manner



# Nature of IC matters



Lambour et al, *Emerg Microbes Infect*, 2016

OPEN ACCESS Freely available online

PLoS PATHOGENS

## A Crucial Role for **Infected-Cell/Antibody** Immune Complexes in the Enhancement of Endogenous Antiviral Immunity by Short Passive Immunotherapy

Henri-Alexandre Michaud<sup>1,2,3</sup>, Tiphonie Gomard<sup>1,2,3</sup>, Laurent Gros<sup>1,2,3</sup>, Kevin Thiolon<sup>1,2,3</sup>, Roudaina Nasser<sup>1,2,3</sup>, Chantal Jacquet<sup>1,2,3</sup>, Javier Hernandez<sup>1,2,3</sup>, Marc Piechaczyk<sup>1,2,3\*\*</sup>, Mireia Pelegrin<sup>1,2,3\*\*</sup>

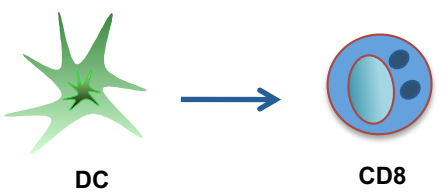
SCIENCE IMMUNOLOGY | RESEARCH ARTICLE

HIV

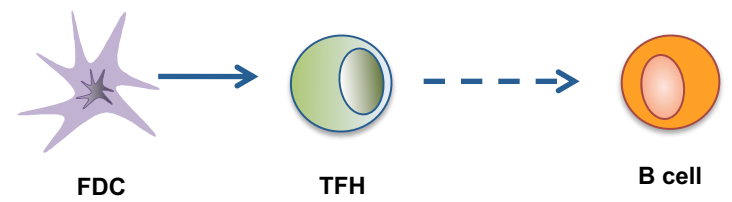
## Antigen-specific antibody **Fc glycosylation** enhances humoral immunity via the recruitment of complement

Giuseppe Lofano<sup>1\*</sup>, Matthew J. Gorman<sup>1\*</sup>, Ashraf S. Yousif<sup>1,2\*</sup>, Wen-Han Yu<sup>1,3\*</sup>, Julie M. Fox<sup>4</sup>, Anne-Sophie Dugast<sup>1</sup>, Margaret E. Ackerman<sup>5</sup>, Todd J. Suscovich<sup>1</sup>, Joshua Weiner<sup>5</sup>, Dan Barouch<sup>1,6</sup>, Hendrik Streeck<sup>7</sup>, Susan Little<sup>8</sup>, Davey Smith<sup>8,9</sup>, Douglas Richman<sup>8,9</sup>, Douglas Lauffenburger<sup>2</sup>, Bruce D. Walker<sup>1,10,11</sup>, Michael S. Diamond<sup>4</sup>, Galit Alter<sup>1\*</sup>

### FcγR-dependent mechanism



### Complement receptor-dependent mechanism





# IC-mediated enhancement of T-cell responses

REVIEW ARTICLE **OPEN**

From therapeutic antibodies to immune complex vaccines

Wang et al, *NPJ vaccines*, 2019

Review



EMBO  
Molecular Medicine

Immunoregulatory functions of immune complexes in vaccine and therapy

Wen et al, *EMBO Molecular Medicine*, 2016

REVIEW

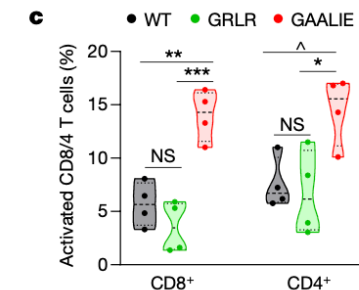
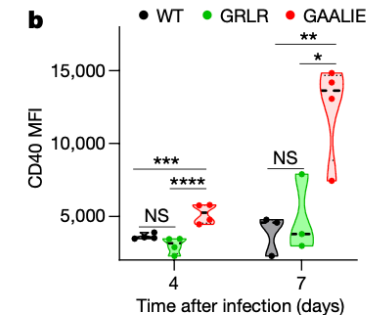
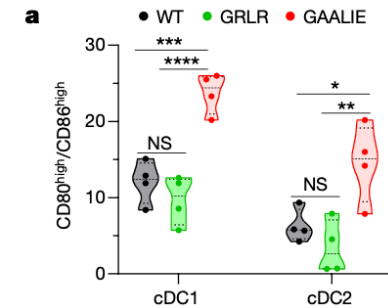
Converting monoclonal antibody-based immunotherapies from passive to active: bringing immune complexes into play

Lambour et al, *Emerg Microbes Infect*, 2016

- cancer
- viral diseases
  - MLV
  - SIV
  - HIV
  - HBV
  - Influenza
  - ....

## Fc-optimized antibodies elicit CD8 immunity to viral respiratory infection

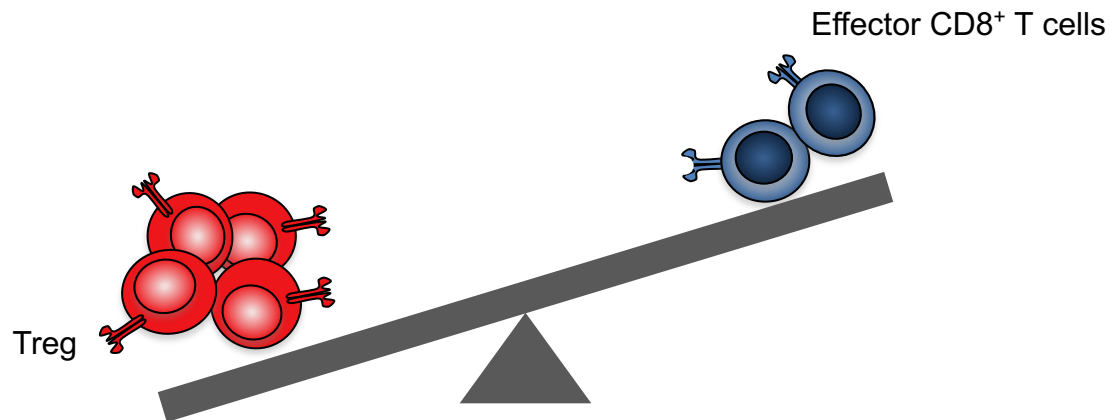
Bournazos et al, 2020



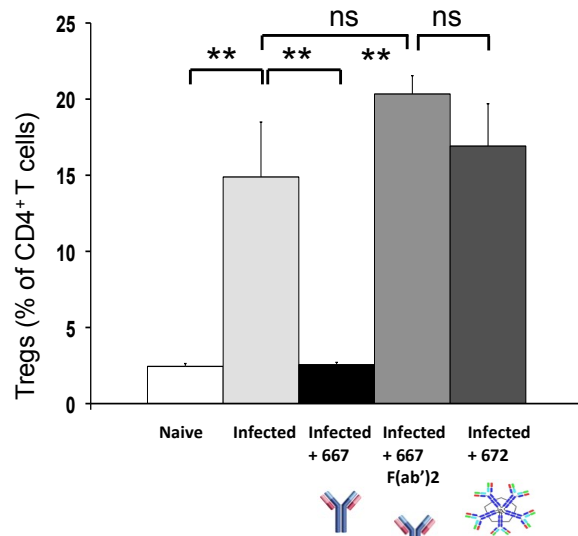
GAALIE Fc-mutated mAbs against SARS-CoV2, HBV, ... currently tested

### 3/ Are these vaccine-like effects induced by mAb therapy linked to an inhibition of the Treg response?

chronic viral infections are associated with Treg responses  
which dampen antiviral immune responses



## Early mAb therapy controls Treg development



- Tregs appear very early after infection (10 dpi)
- Tregs repress both **cellular** and **humoral** antiviral immune responses in infected mice
- 667 mAb controls Treg development

➔ neither 667- F(ab')<sub>2</sub> nor 672 (IgM) control Treg development

Treg control by 667 (IgG2a) is dependent on Fc-associated functions

➔ depletion of Tregs restores humoral and cellular antiviral responses

## Control of regulatory T cells is necessary for vaccine-like effects of antiviral immunotherapy by monoclonal antibodies

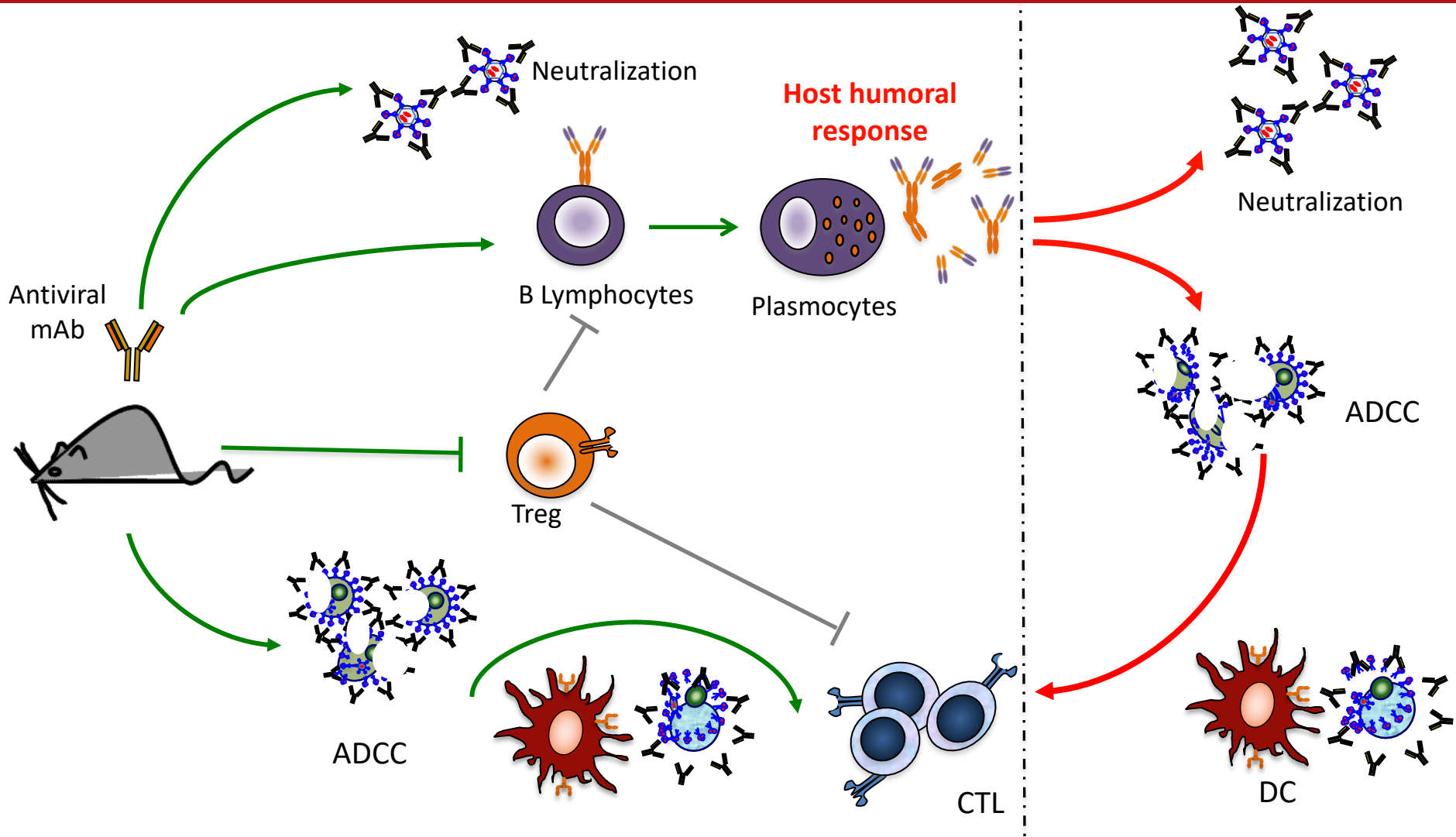
### Key Points

- Mab-based immunotherapy prevents Treg expansion and limits immunosuppressive activity.

*Nasser et al, J. Blood, 2013*

Control of immunosuppressive response might be necessary to achieve protective immunity by antiviral mAb.

# How antiviral mAb can induce long-term protective immunity



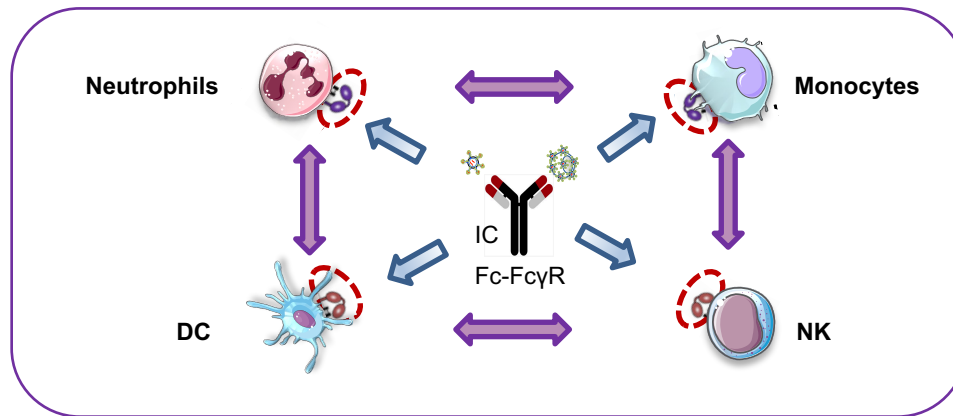
## Induction of the antiviral host response

- Control of viral propagation (neutralization, ADCC)
- Enhancement of antigen presentation
- Inhibition of Treg expansion

## Maintenance of the antiviral response

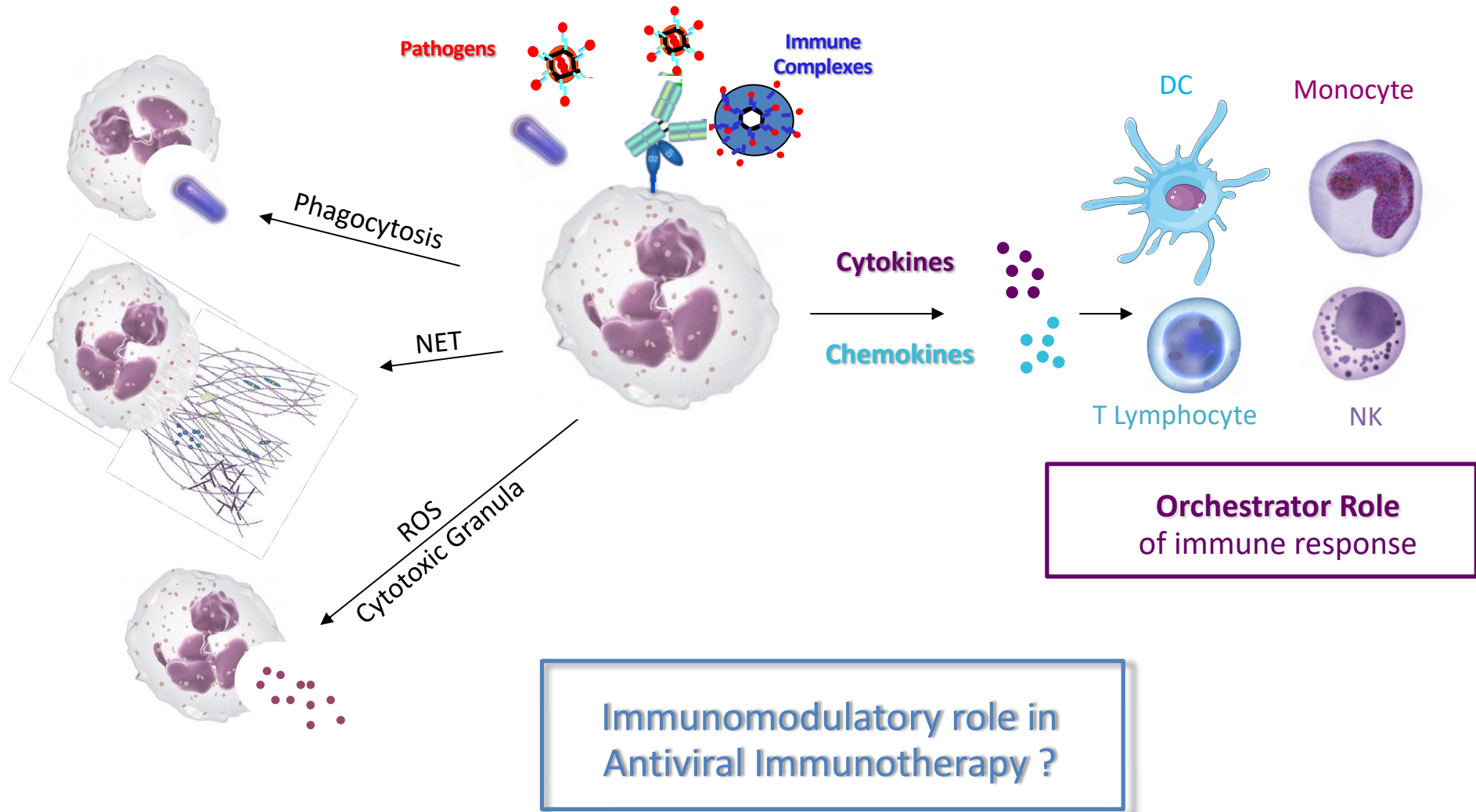
Long-term immune control

Are FcγRs-expressing cells other than DC involved in the induction of protective immunity?

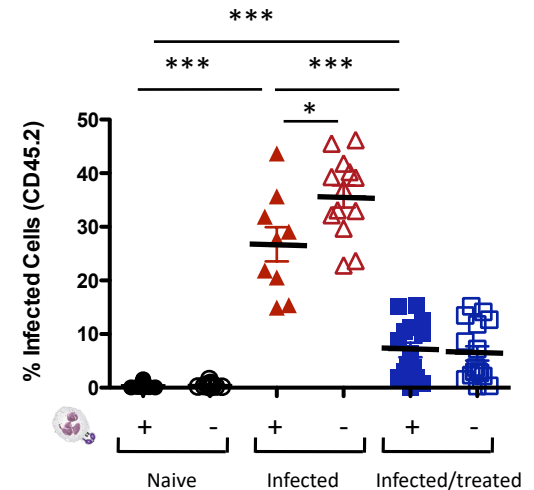
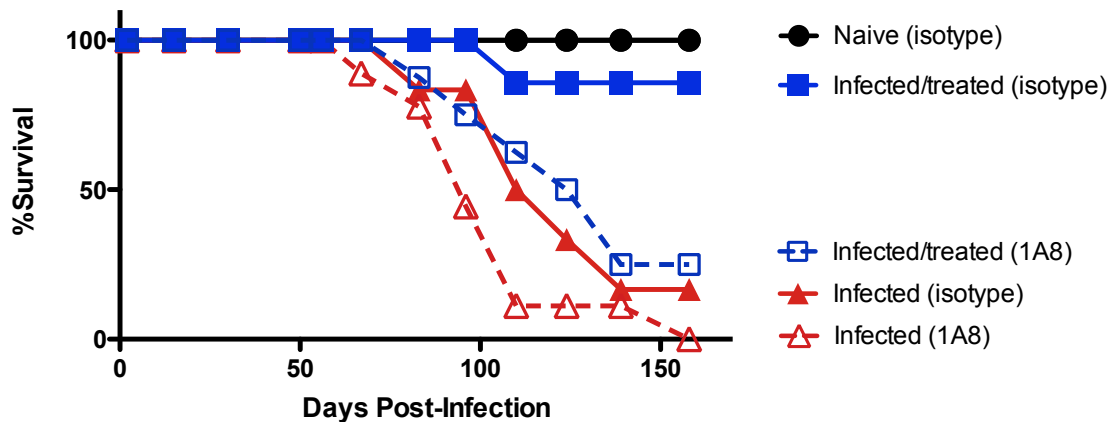


Do different FcγR-expressing cells cooperate in modulating antiviral immune responses during antibody therapy?

# Neutrophils: killers and immunomodulators



# Neutrophil depletion abrogates the protection by the therapeutic mAb despite having no impact in the control of viral propagation



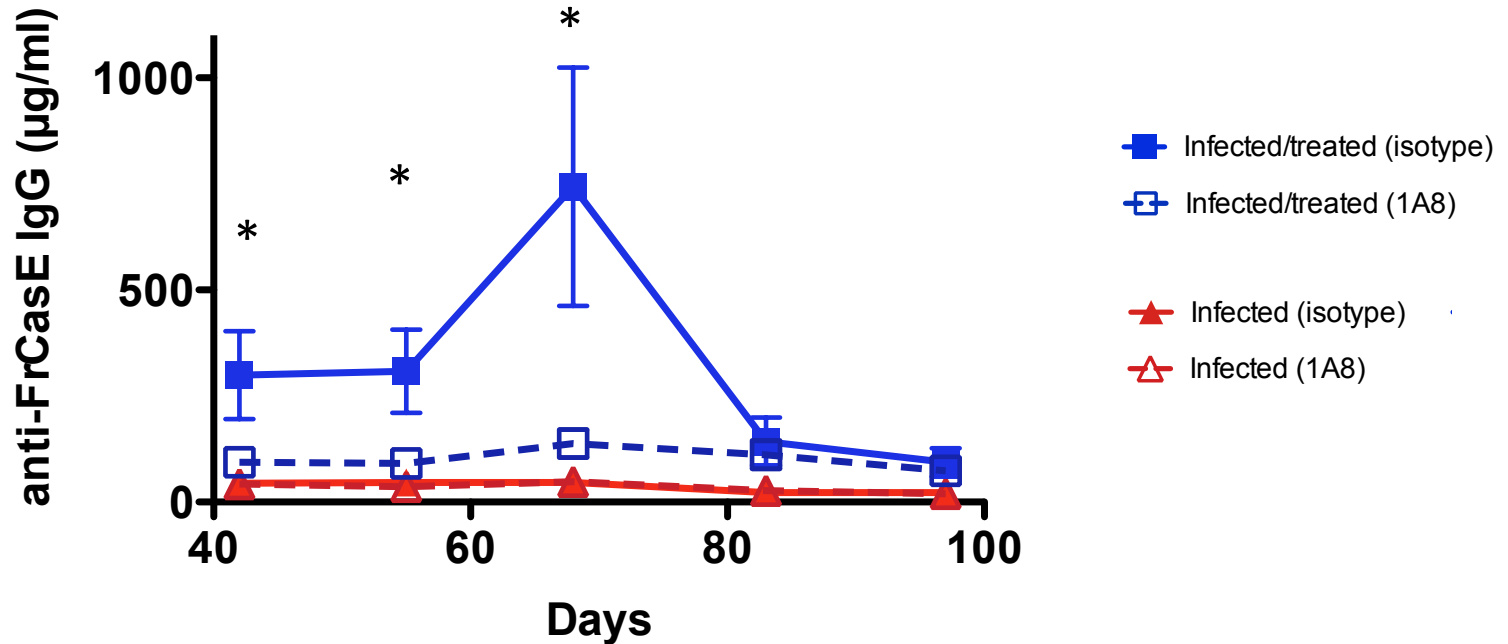
Viral propagation hardly affected by neutrophil depletion

➔ NK cells are crucial for mAb-mediated viral control

Immunomodulatory effects of neutrophils?



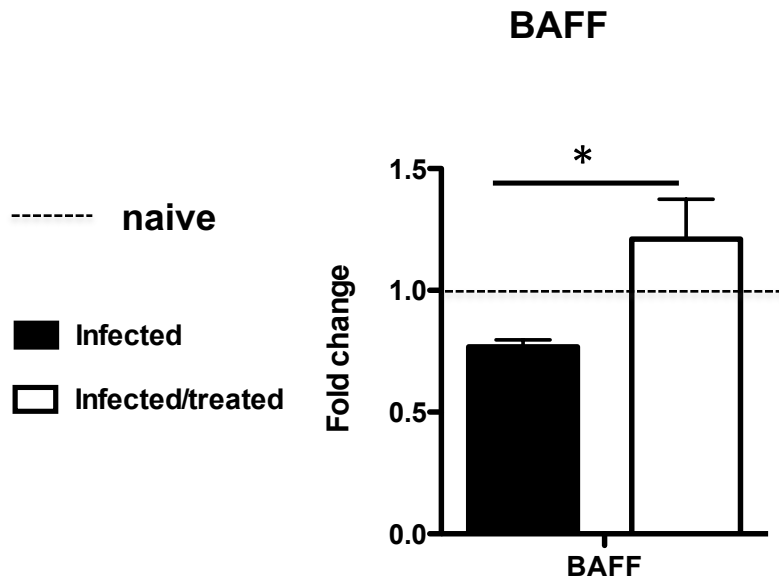
## Neutrophils are crucial for the enhancement of host humoral responses upon mAb treatment



High anti-FrCasE IgG seric levels correlated to disease protection

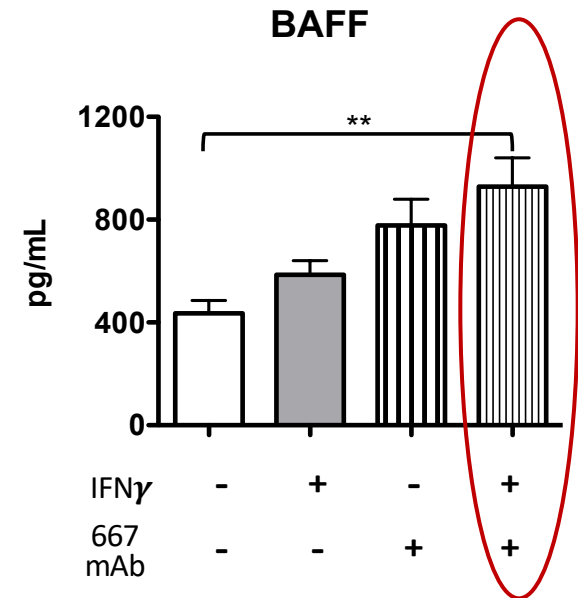
Crucial contribution of the humoral response in the long-term protection

## Neutrophils are differentially activated upon mAb treatment and acquire B-cell helper functions



8 d post-infection

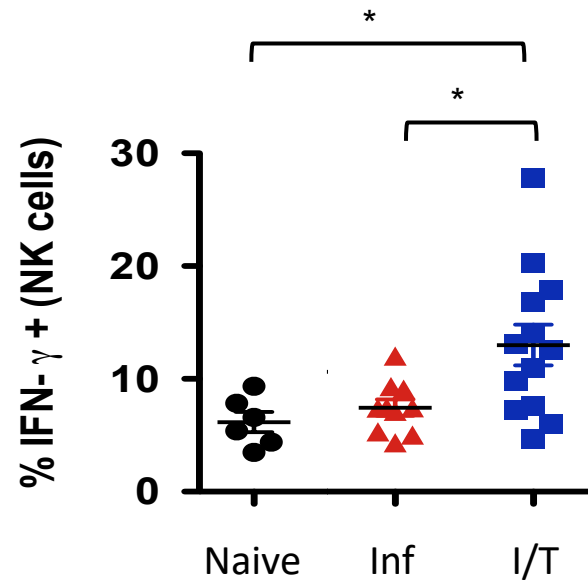
Neutrophils isolated from mAb-treated mice show enhanced B-cell activating factor (BAFF) expression



*In vitro* activation

Fc $\gamma$ R triggering and IFN $\gamma$  stimulation potentiate BAFF secretion by neutrophils

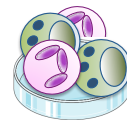
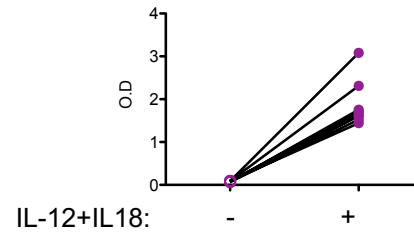
## mAb treatment enhances the IFN $\gamma$ secretion capacity of NK cells



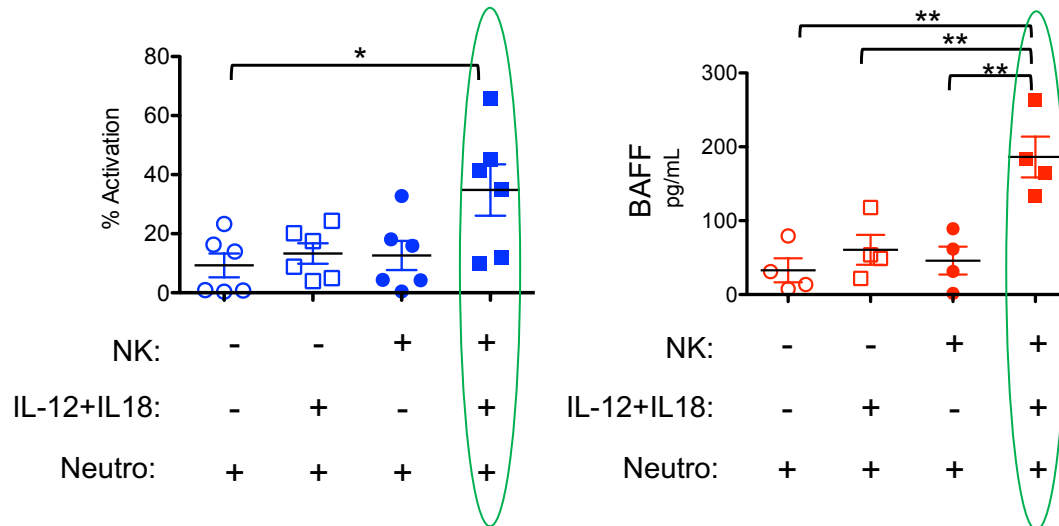
Do IFN $\gamma$ -secreting NK cells potentiate BAFF secretion?

# IFN $\gamma$ produced by NK cells induces neutrophil activation and enhances their BAFF-secretion

IFN $\gamma$ -producing NK cells

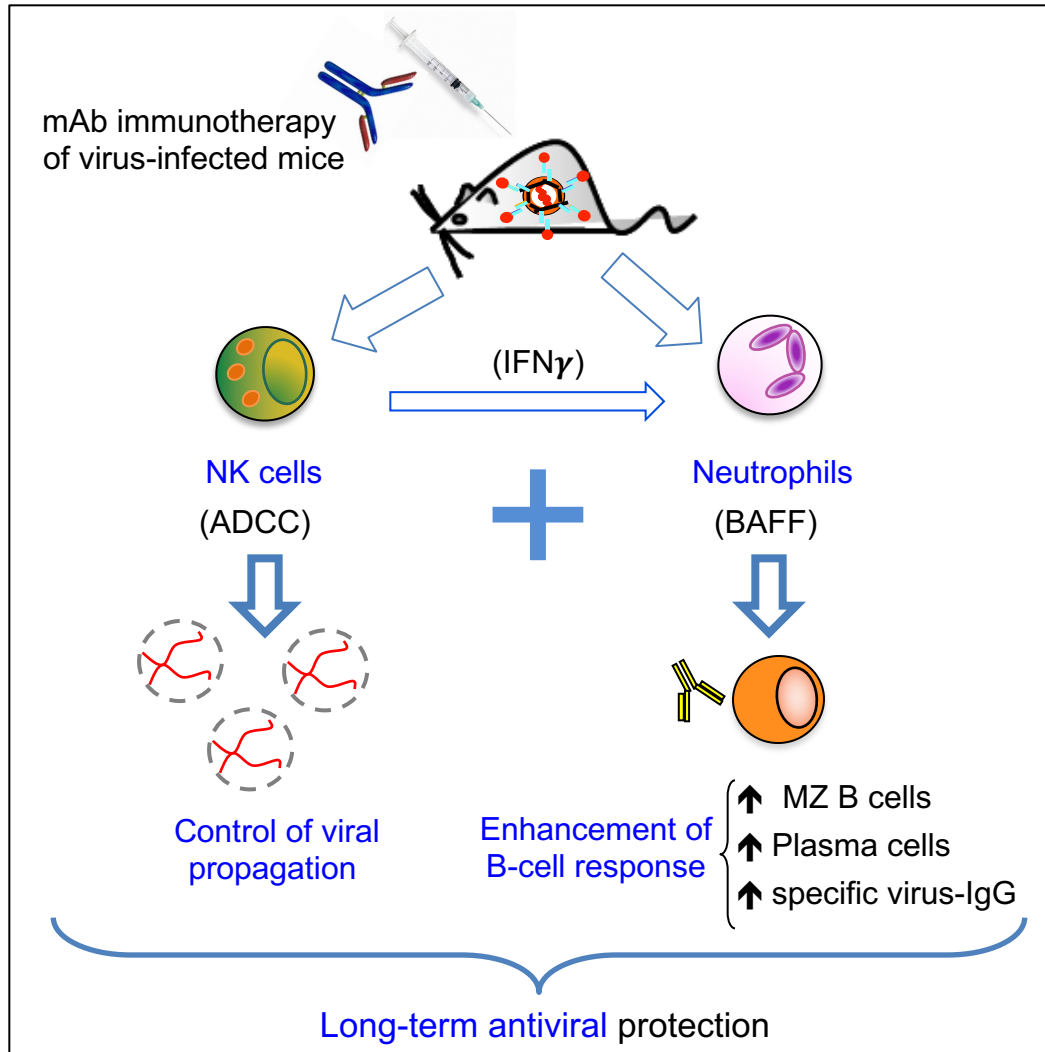


Neutro + IFN $\gamma$ - NK cells



IFN $\gamma$ -producing NK cells enhance B-helper function of neutrophils

# Key role of neutrophils in the induction of protective immunity by antiviral mAb

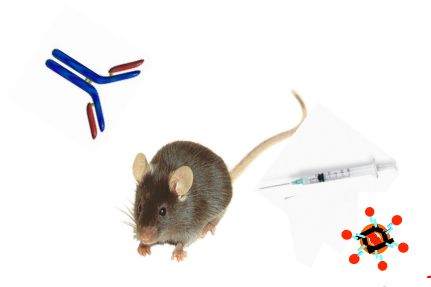


*Naranjo-Gomez M. et al, 2018, JCI Insight*

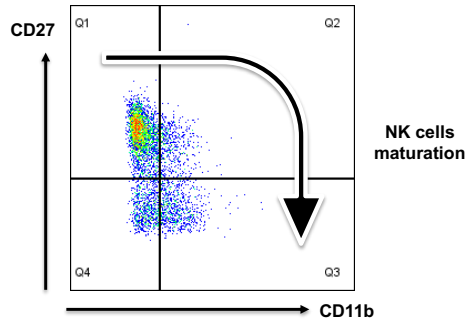
*Naranjo-Gomez M. et al, 2021, Vaccines*

Do NK cells have a key role in antiviral mAb therapy?

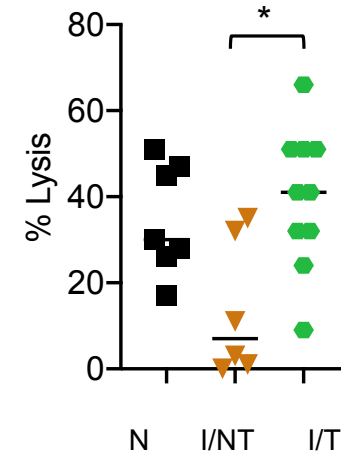
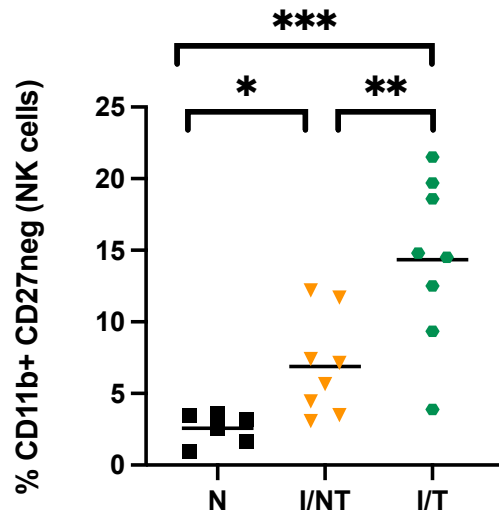
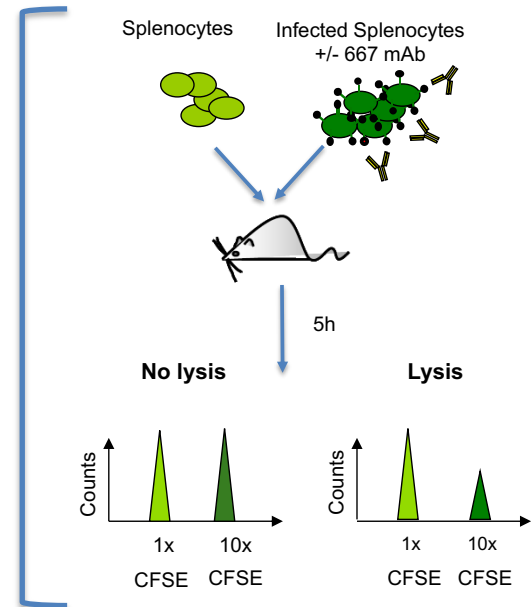
Effector and/or immunomodulatory functions?



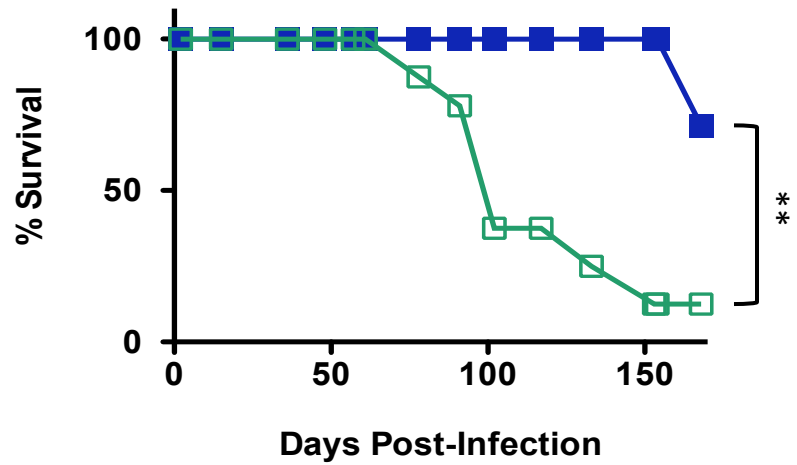
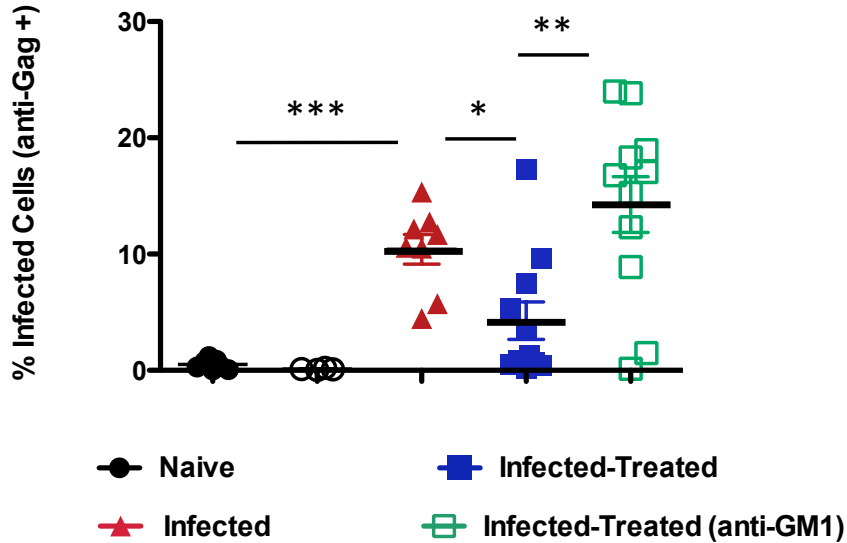
# mAb treatment enhances NK cells maturation and preserves their ADCC capacity



*in vivo*  
killing assay  
(Guyre et al, 2008)



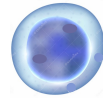
# NK cell depletion leads to an increase of viral propagation in infected/treated mice and abrogates mAb-mediated protection



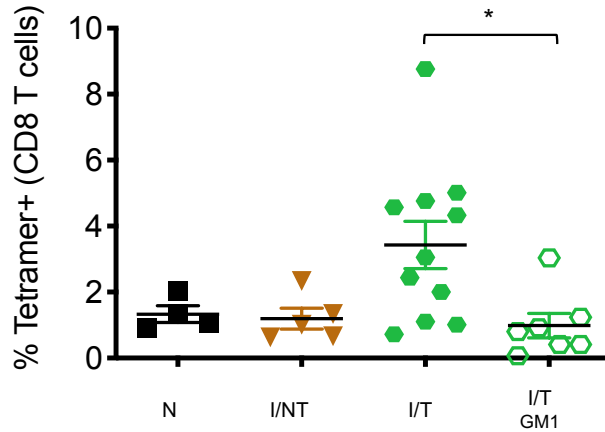
NK cells are crucial for mAb-mediated viral control



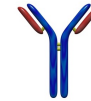
# Depletion of NK cells leads to reduced CD8 T-cell and humoral responses in infected/mAb-treated mice



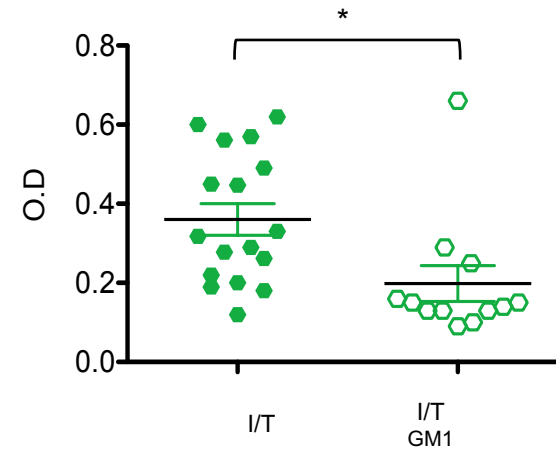
Virus-specific CD8<sup>+</sup> T cells



Day 14 p.i.



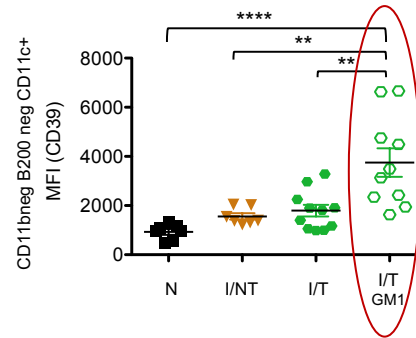
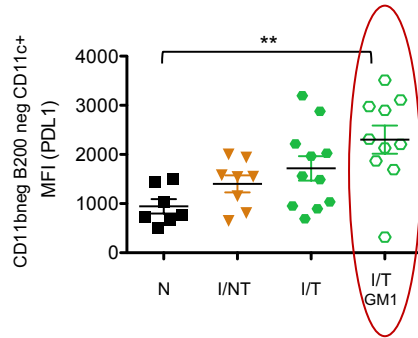
Virus-specific antibody titers



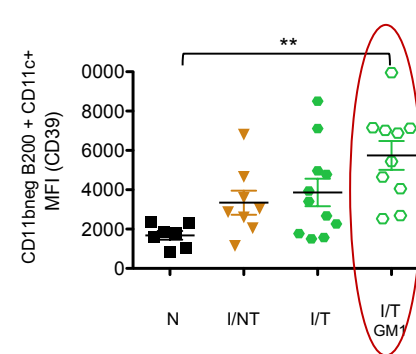
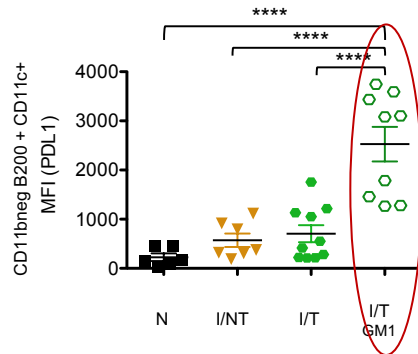
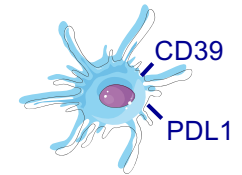
Day 67 p.i.

NK cells have immunomodulatory effects during mAb therapy

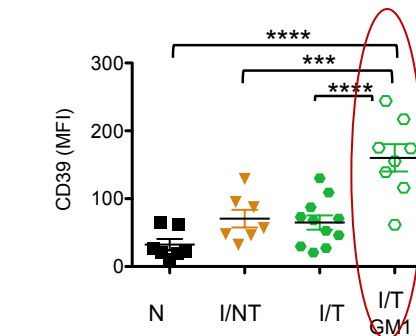
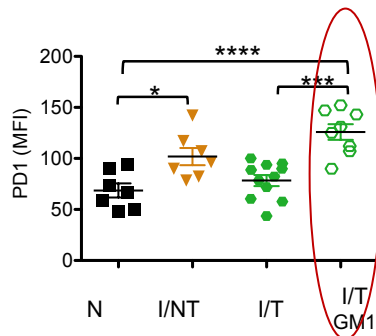
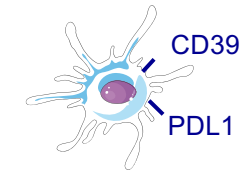
# Depletion of NK cells leads to enhanced expression of immunosuppressive molecules in infected/treated mice



cDC



pDC



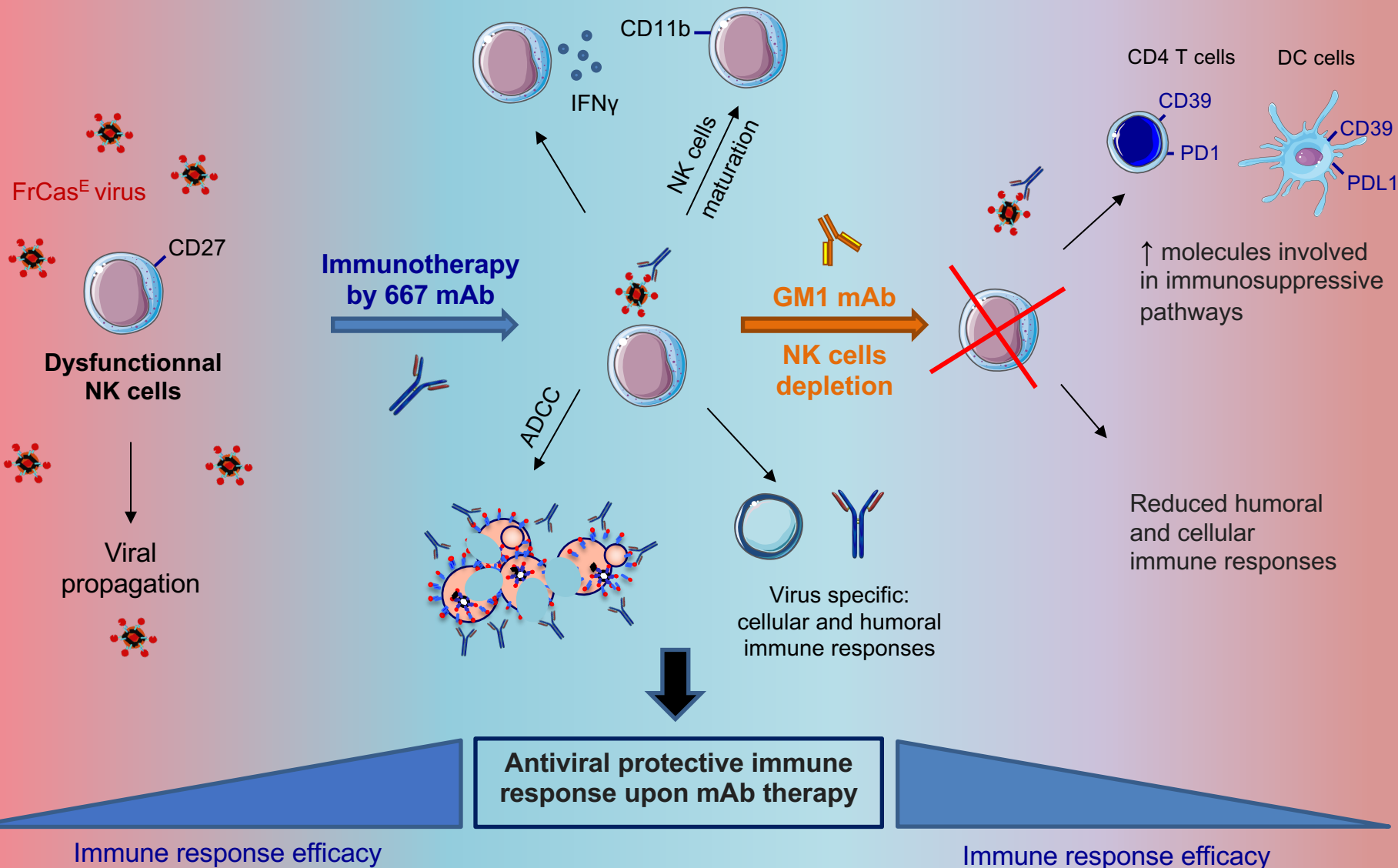
CD4<sup>+</sup> T cells



### Viral Infection

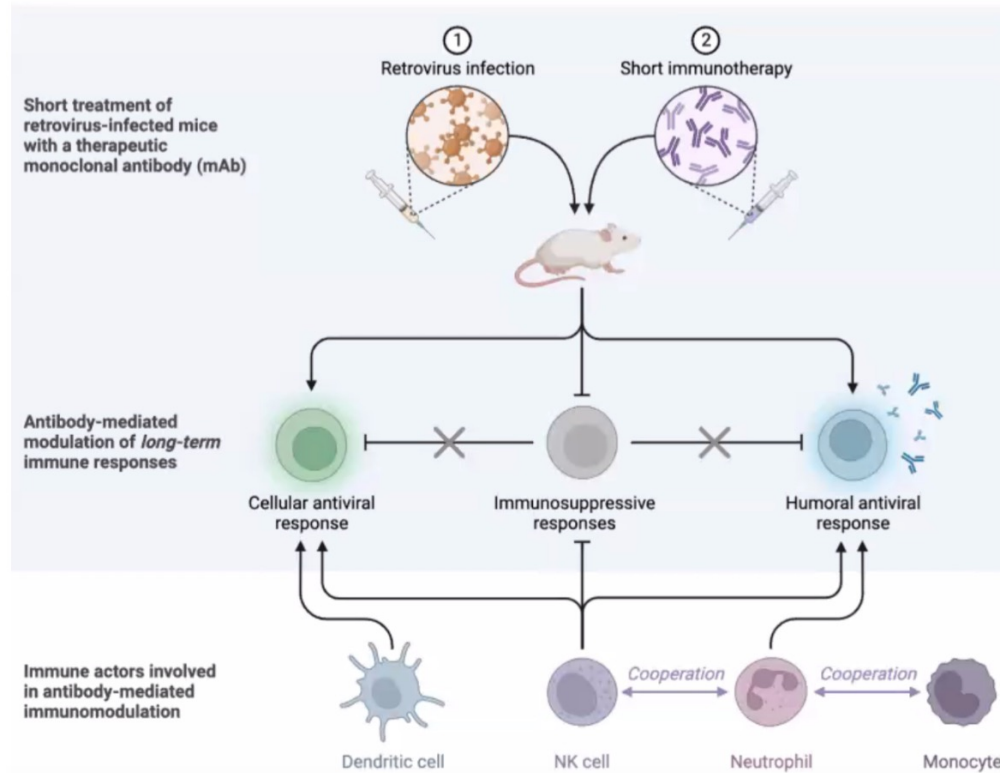
### Viral Infection and mAb treatment

### Viral infection and mAb treatment in absence of NK cells



## Immunomodulatory Role of NK Cells during Antiviral Antibody Therapy

# Immunomodulatory effects of antibody based immunotherapy : Multiple FcγR-expressing cells involved



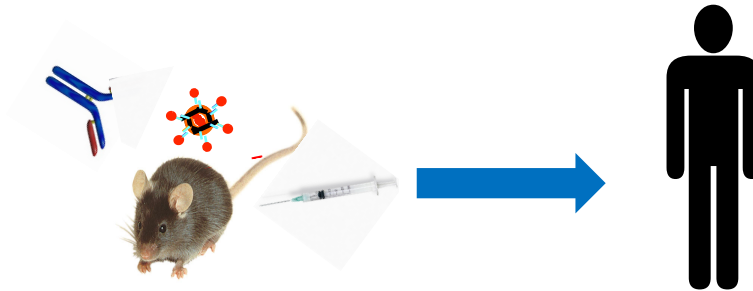
*Review*  
**Fc-Dependent Immunomodulation Induced by Antiviral Therapeutic Antibodies: New Perspectives for Eliciting Protective Immune Responses**

*Pelegri et al, 2022, Antibodies*

**Differential and sequential immunomodulatory role of neutrophils and Ly6C<sup>hi</sup> inflammatory monocytes during antiviral antibody therapy**

*Lambour et al, 2021, Emerg Microbes Infect*

The mouse model allowed to characterize several mechanisms involved in the induction vaccinal effects by antiviral mAbs



Can mAb directed to human viruses also induce vaccine-like effects?

mAbs enhance enhance host immune responses in:

- Pre-clinical models of respiratory infection (Influenza, SARS-CoV2)

*Bournazos et al, 2020; Winkler et al, 2021*

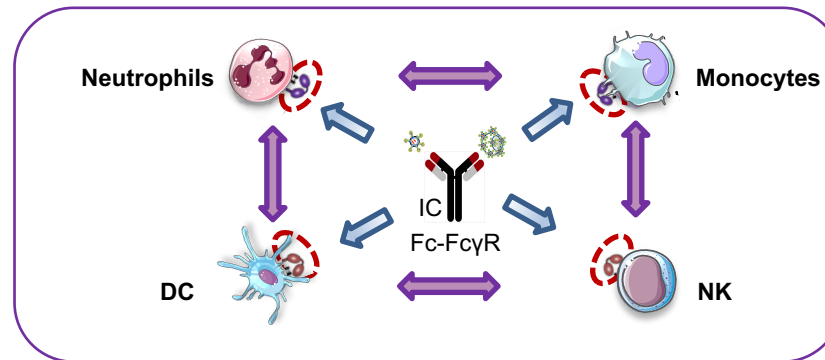
- In HIV-1 infected patients

*Schoofs et al, 2016; Niessl et al, 2020;  
Rosas-Umbert et al, 2022; Gunst et al, 2023*

Long-term protection?

Mechanisms involved?

Neutralizing mAbs are not just simple blocking agents  
They might also help patients to develop their own antiviral immune response

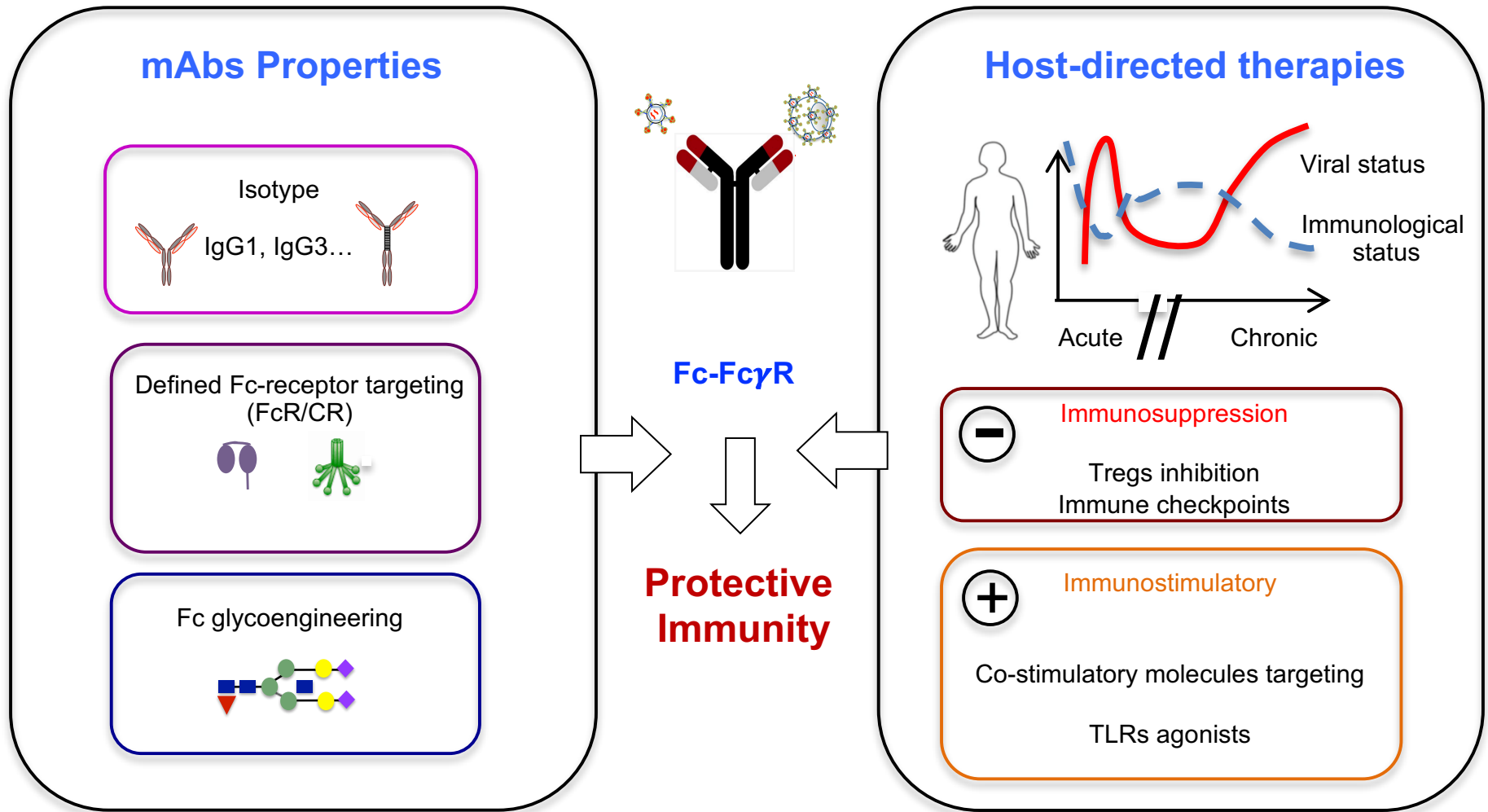


### Antibody-mediated immunomodulation

1. Multiple FcγRs-expressing cells involved
2. Cooperation between FcγRs-expressing cells
3. Need to counteract virus-induced immunosuppressive responses
4. Need to preserve/restore the function of main immune cells involved

Elucidating and harnessing the mechanisms involved in the induction of protective immunity is key to improve mAb-based immunotherapies

# Potential therapeutic interventions to improve the vaccinal effect induced by antiviral mAb therapy



Important to « step on the gas » but also « take off the brake »

# Acknowledgements

## « Antibody and Immunomodulation » Team

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## KEY QUESTIONS

- Can Antiviral mAb induce **protective immunity** against human viral infections?
- What are the cellular types and molecular effectors involved in the induction of vaccine-like effects by mAbs?
- Which Fc-dependent effector functions is/are needed or involved in the induction of vaccine-like effects?
- Can genetic engineering improve vaccine-like effects of antiviral mAbs?
- Can FcγR polymorphisms be used as a predictive factor for vaccine-like effects of antiviral mAbs?
- When should mAb therapy be commenced?
- Can combination therapies improve vaccine-like effects of antiviral mAbs?
- What is the best approach to counteract immunosuppressive responses?