Investigations into the Role of NK Cell IL-10 Secretion in Human Malaria Disease

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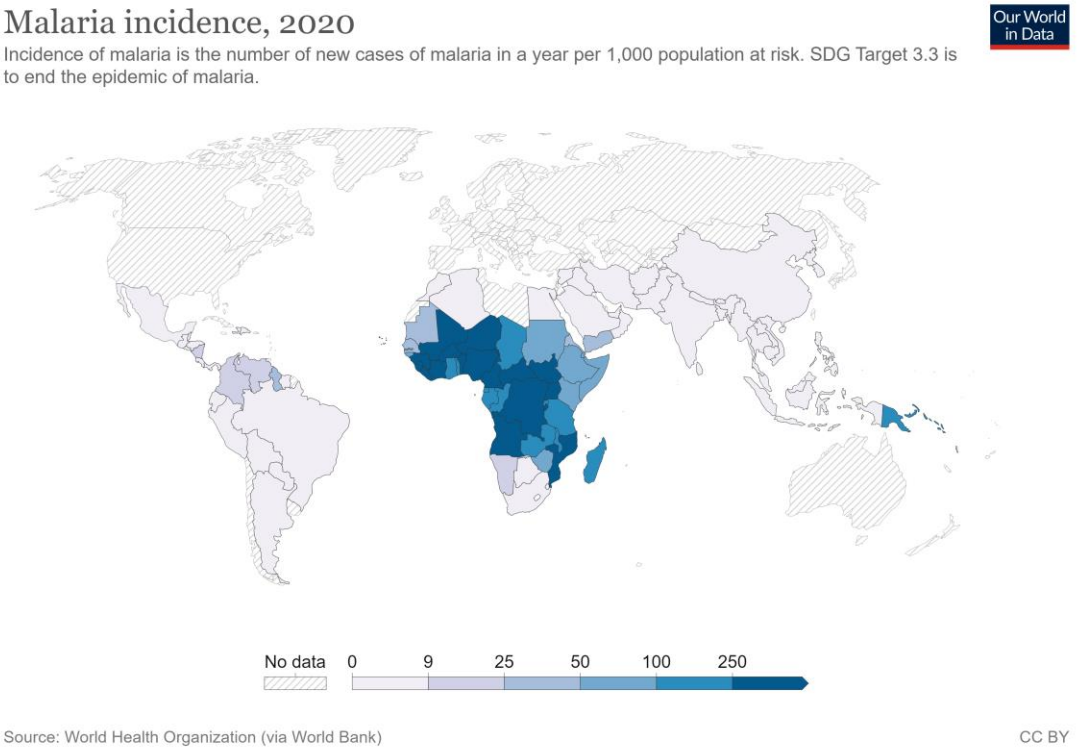
Malaria is a Global Health Burden

- *Plasmodium* species
  - *Plasmodium falciparum*
  - *Plasmodium malariae*
  - *Plasmodium vivax*
  - *Plasmodium ovale*
  - *Plasmodium knowlesi*
- 241 million malaria cases
- 627,000 deaths

James Gathany, Center for Disease Control, "*Anopheles*, the Malaria Mosquitoes" (2020). Published online at biogents.com. Retrieved from: 'https://eu.biogents.com/anopheles-malaria-mosquitoes/'



Max Roser and Hannah Ritchie (2020) - "Malaria". Published online at OurWorldInData.org. Retrieved from: 'https://ourworldindata.org/malaria'

Malaria infection spans a spectrum of clinical outcomes

James Gathany, Center for Disease Control, "*Anopheles*, the Malaria Mosquitoes" (2020). Published online at biogents.com. Retrieved from: 'https://eu.biogents.com/anopheles-malaria-mosquitoes/'

Infection ≠ Disease

Painting by Hamilton Banda

Asymptomatic Malaria

- Parasitemia without clinical symptoms

Uncomplicated Malaria

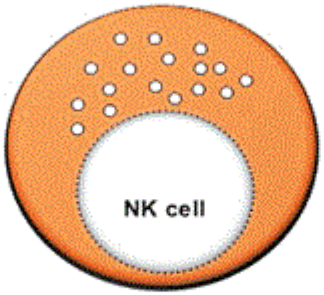
- Flu-like symptoms

Severe Malaria

- Severe malaria anemia
- Neurological complications (cerebral malaria)

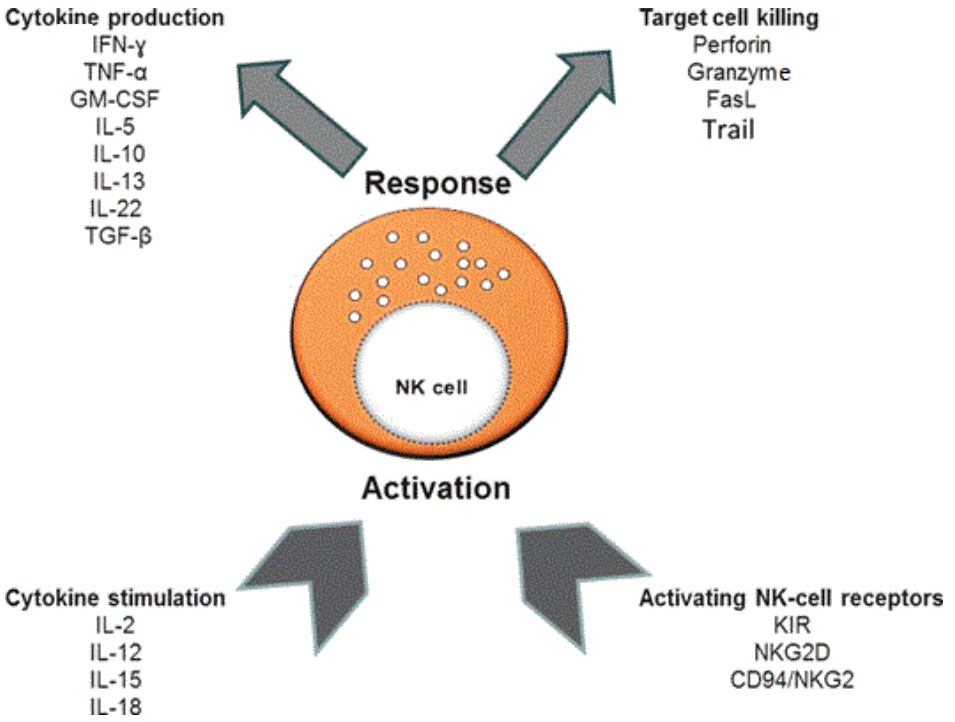
Natural killer (NK) are important immunomodulators during disease

- Innate immune response
- $CD3^{-}CD56^{+}$
- Play imp roles in intracellular infection and tumor defense

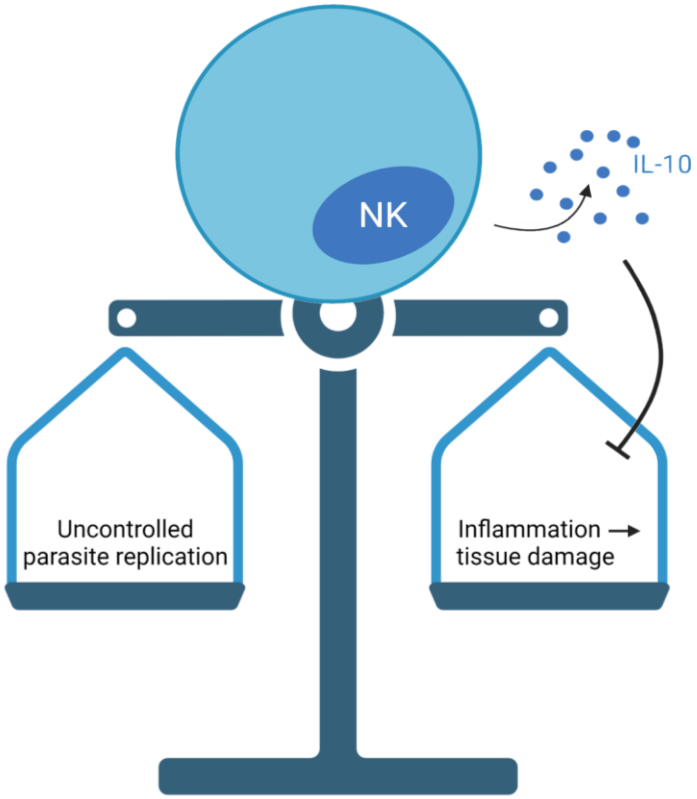


Scand J Immunol, Volume: 76, Issue: 5, Pages: 451-456, First published: 13 August 2012, DOI: (10.1111/j.1365-3083.2012.02769.x)

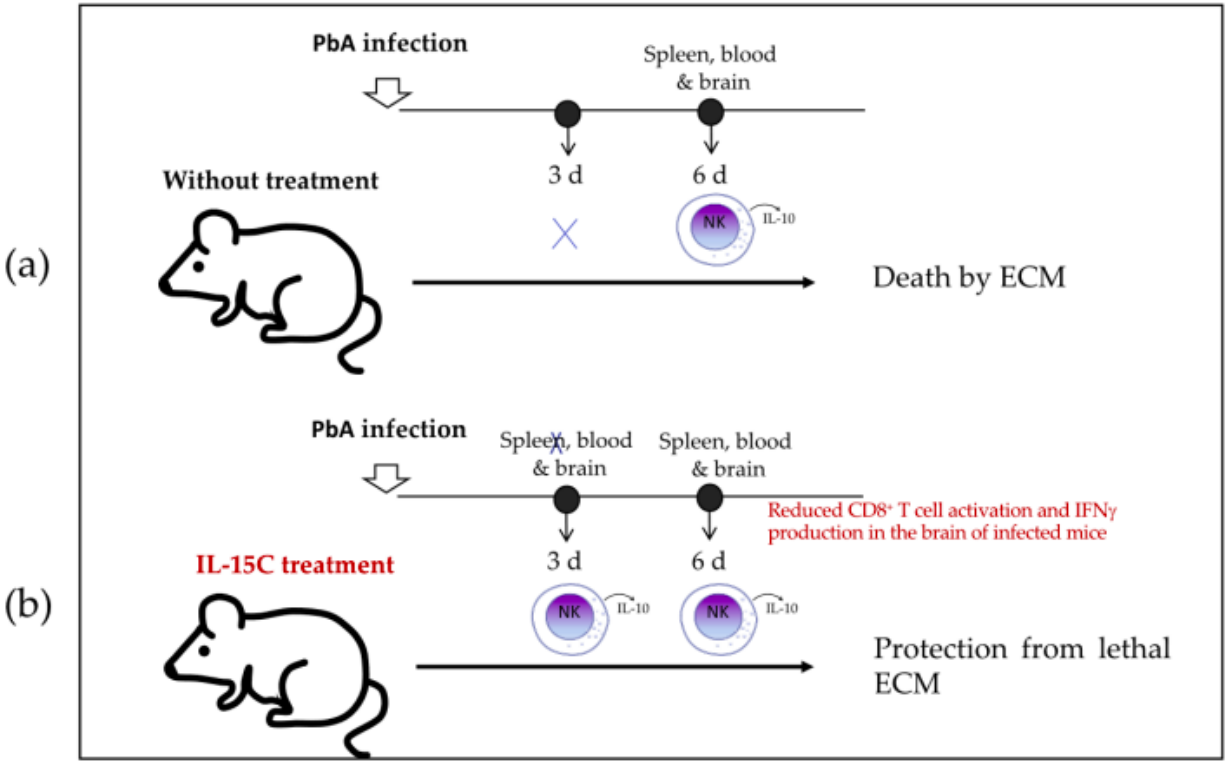
NK cells influence the immune response to malaria infection by secreting cytokines



Scand J Immunol, Volume: 76, Issue: 5, Pages: 451-456, First published: 13 August 2012, DOI: (10.1111/j.1365-3083.2012.02769.x)



NK cell derived IL-10 is necessary and sufficient for IL-15C-mediated experimental cerebral malaria (ECM) survival



Israel Martinez-Espinosa *et al.* Role of IL-10-Producing Natural Killer Cells in the Regulatory Mechanisms of Inflammation during Systemic Infection. Biomolecules. 2022

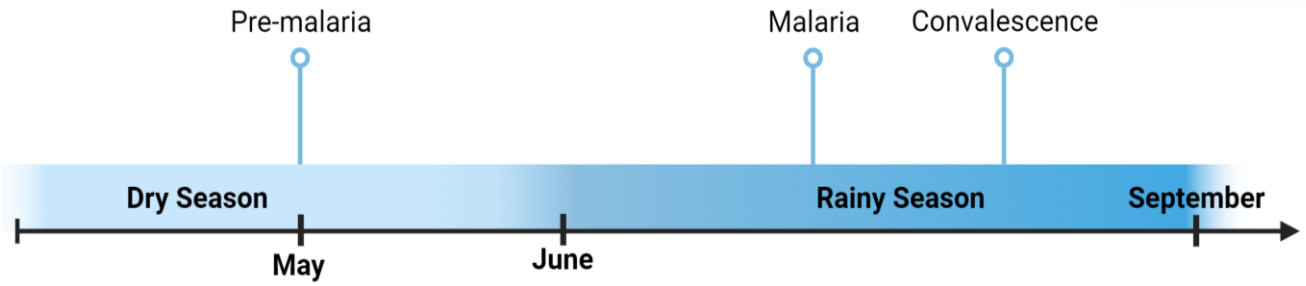
Is there a similar phenomenon in human malaria disease?

Hypothesis: Individuals more protected from severe malaria disease will have higher levels of NK cell IL-10 secretion in response to IL-15 stimulation compared to individuals more vulnerable to severe disease

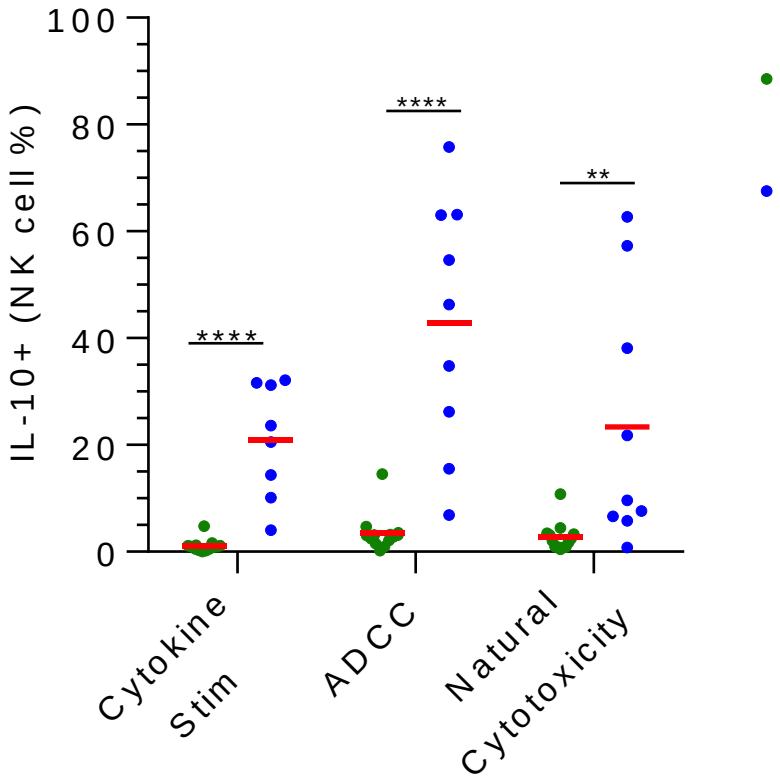
- Older children and adolescents (age 5-15 years) from malaria-endemic region → malaria exposed → "aged out" of the period of highest susceptibility to severe malaria → more protected from severe disease
- Individuals from regions without malaria → malaria-naïve → susceptible to severe disease

Malaria naïve (USA) (13)

Pre-malaria (9)
Malaria (6)
Convalescence (9)

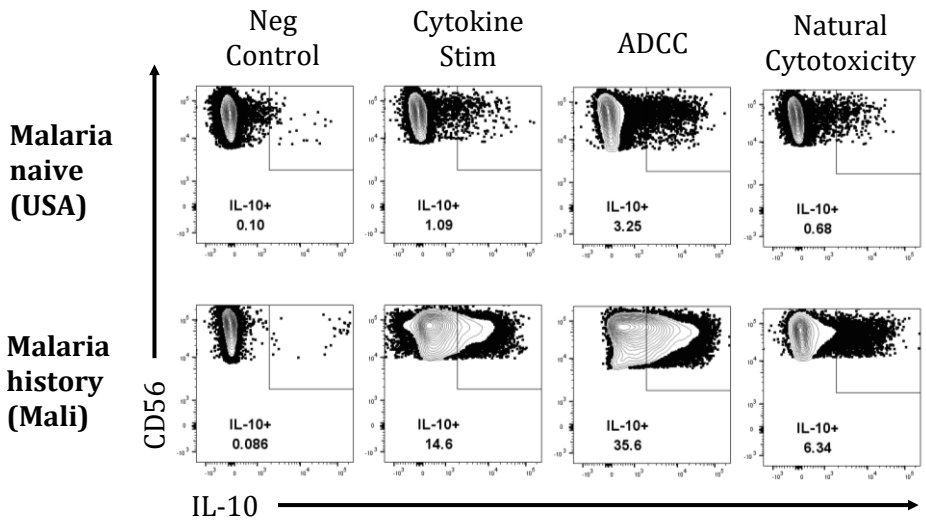


NK cells from Malian individuals secreted more IL-10 compared to US donors

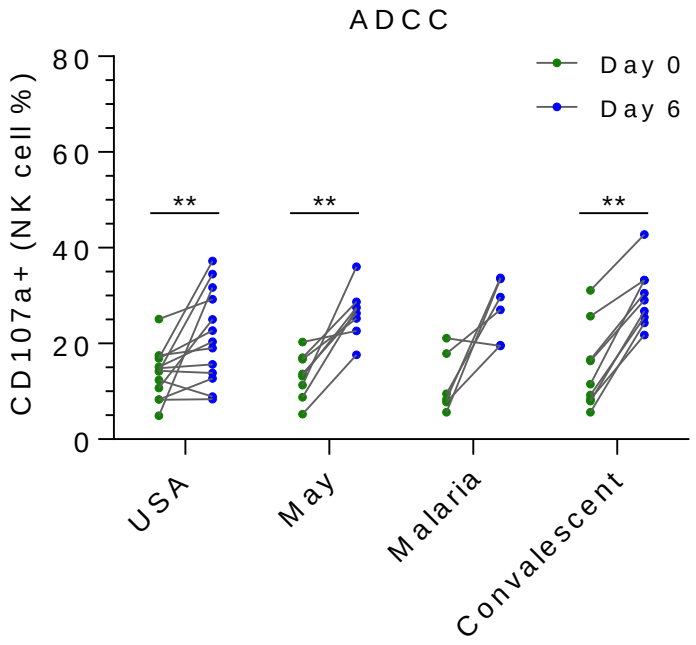


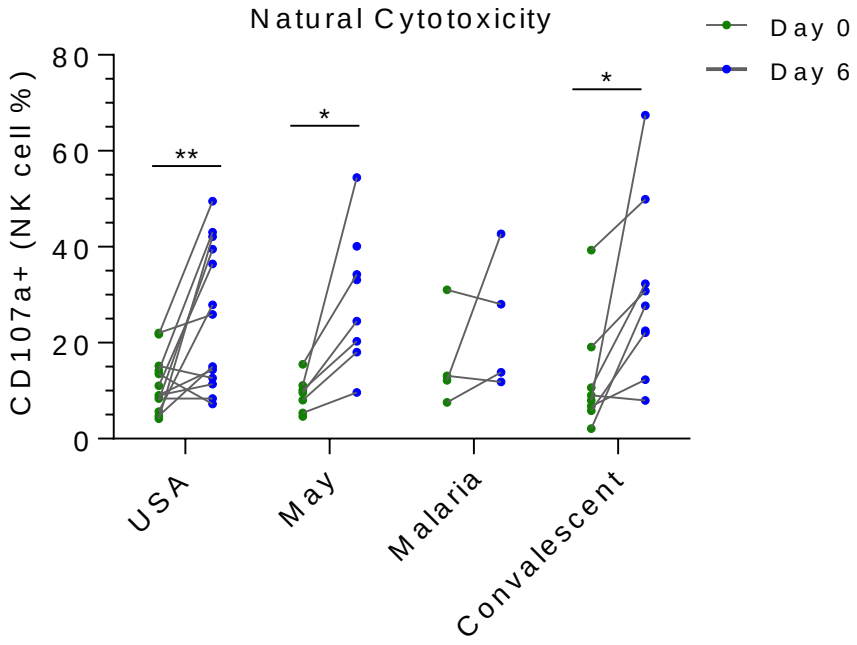
Malaria naive (USA)

Malaria history (Mali)



Cytokine stimulation enhances NK cell cytotoxicity





Can we identify an immunophenotype associated with IL-10 secreting NK cells?

Immunophenotyping results indicate a cytotoxic profile associated with IL-10 secreting NK cells

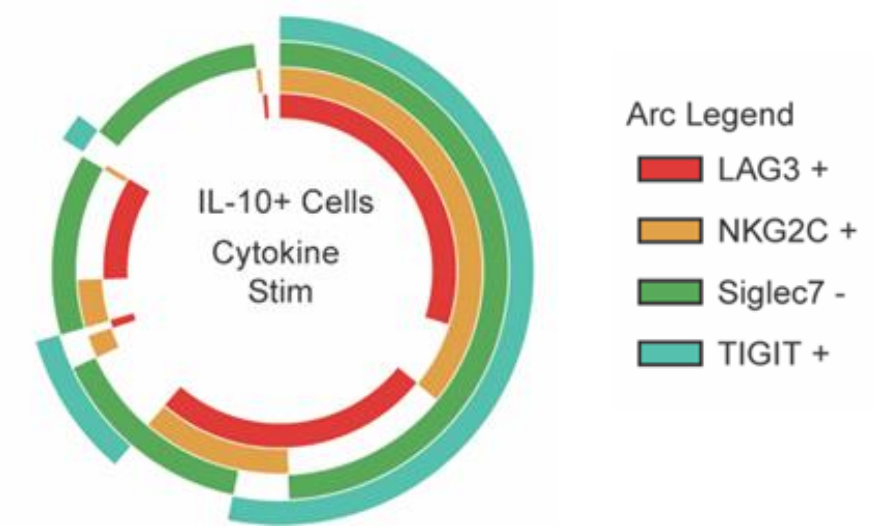
Utilized LEGENDScreen™ (Biolegend)

- 354 cell surface marker antibodies

Single Cell RNA-sequencing

Several surface markers were differentially expressed on IL-10^{pos} NK cells compared to IL-10^{neg} NK cells

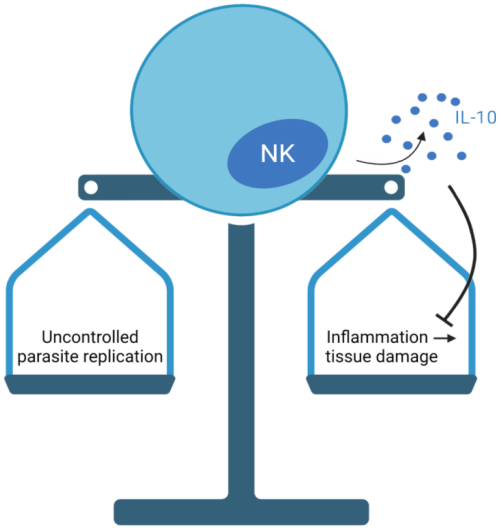
- Several immune checkpoint molecules
  - LAG3
  - TIGIT
- Several adaptive NK cell markers
  - NKG2C
  - Siglec-7^{neg}

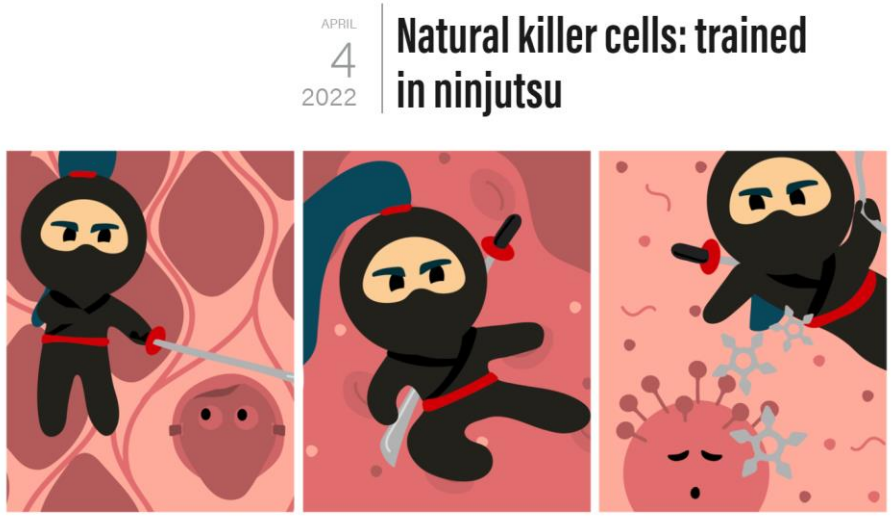


NK cell IL-10 secretion may be a protective mechanism for limiting inflammation that follows target cell lysis

1. NK cell IL-10 secretion Malian >> USA
2. Cytokine exposure is necessary for IL-10 secretion and also stimulates greater NK cell degranulation.
3. IL-10 secreting NK cells express variety of markers associated with increased NK cell cytotoxic capabilities.

❖ Cell lysis → inflammation (cell death, granzymes, intracellular components)





Comic by Sarah Nersesian. https://blogs.dal.ca/openthink/natural-killer-cells-trained-in-ninjutsu/

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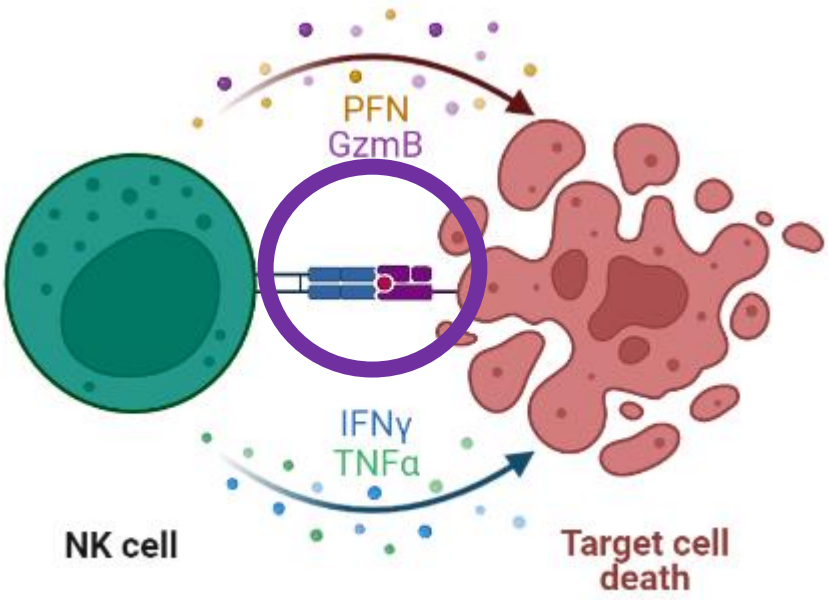
Thank you ☺

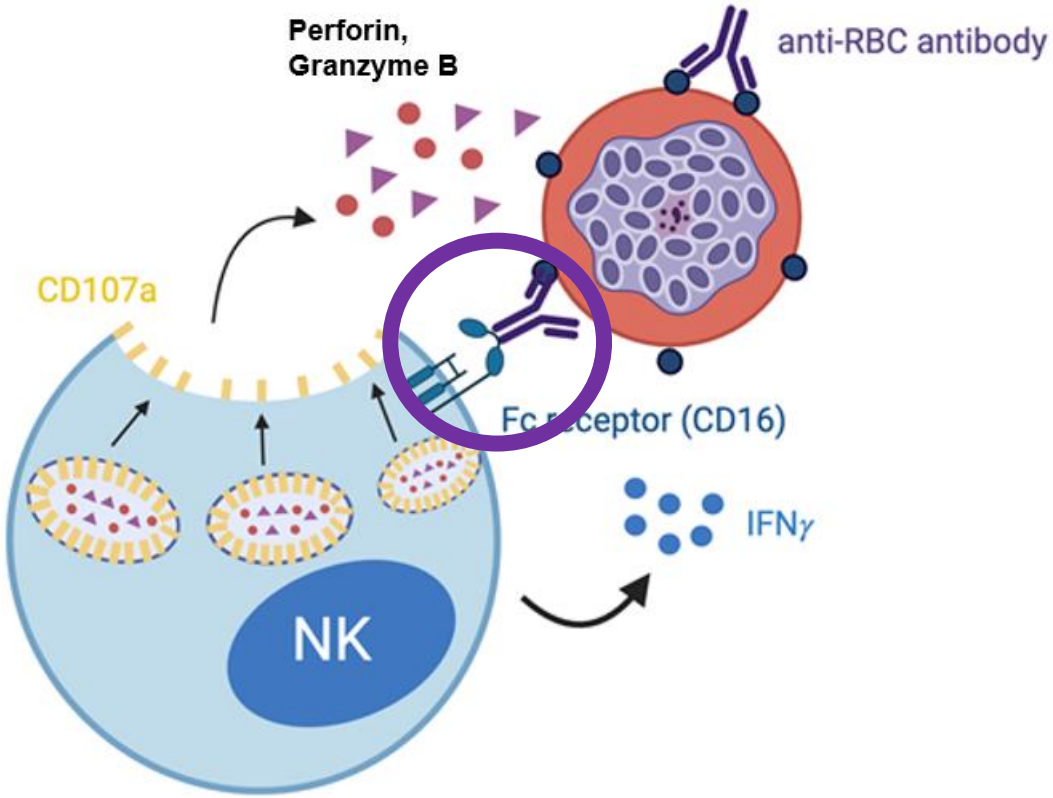
Mechanisms of NK cell-mediated target cell lysis: Natural Cytotoxicity

Binding of ligand to Natural Cytotoxicity Receptors → degranulation (natural cytotoxicity)

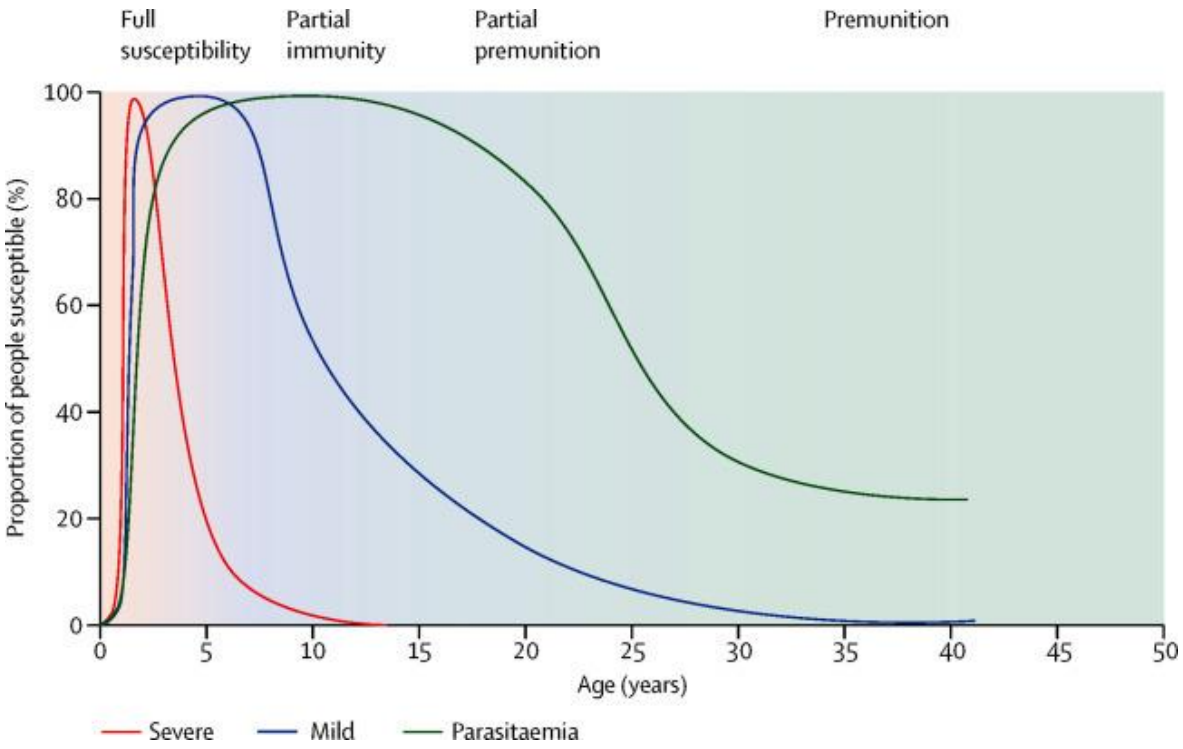


Mechanisms of NK cell-mediated target cell lysis: Antibody-dependent Cellular Cytotoxicity (ADCC)

Antibody-dependent cellular cytotoxicity (ADCC) relies on CD16 activation



Parasite genetic diversity and antigen variation contribute to immune evasion



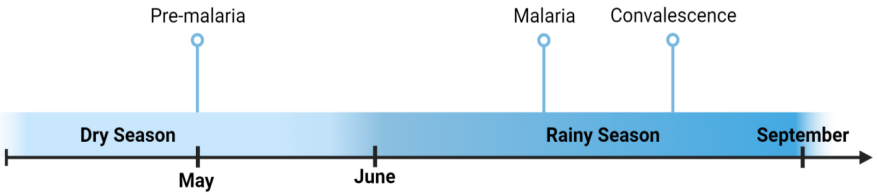
Relation between age and malaria disease severity in an area of moderate malaria transmission intensity. Reprinted from "Malaria" by White, NJ, 2014, Lancet, 383, p. 724.

Experimental Set-up

US controls (13)

Thaw PBMC's

Pre-malaria (9)
Malaria (6)
Convalescence (9)

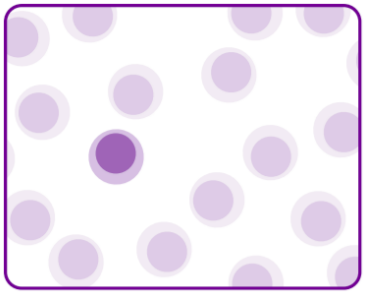


Readout measurements:

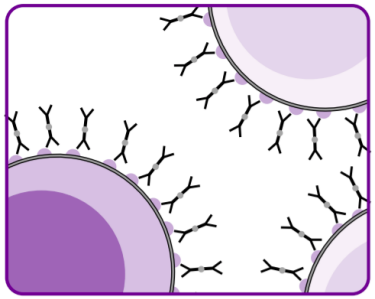
- CD107a
- IL-10

Overview of IL-10 detection

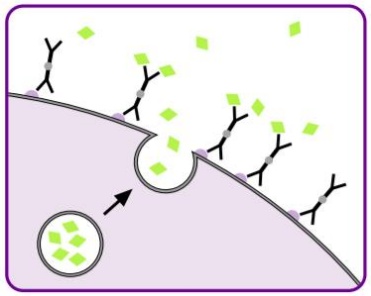
1. In vitro stimulation of cells



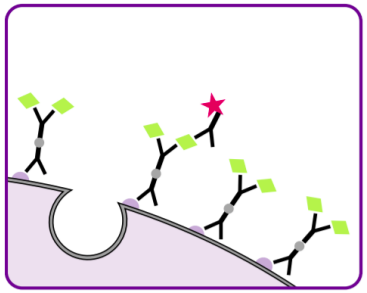
2. Label cells with cytokine catch reagent



3. Cytokine secretion period

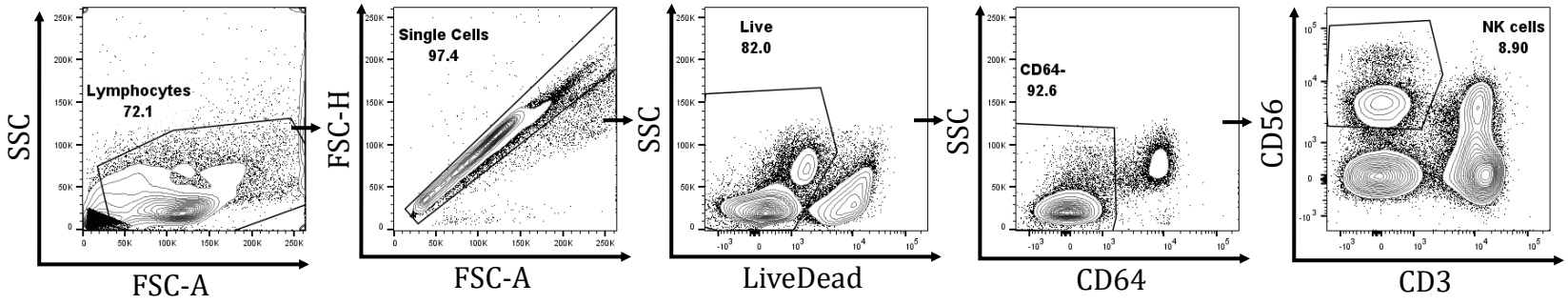


4. Label cells with detection antibody



https://www.miltenyibiotec.com/US-en/products/il-10-secretion-assay-detection-kits-human

Gating Strategy



Markers for identifying NK cells:

- LiveDead (negative)
- CD64 (negative)
- CD3 (negative)
- CD56 (positive)

Functional markers:

- IL-10
- CD107a (degranulation)
- CD16 (ADCC)
- CD8 (poor NK cell effector functions)
- CD45RO (degranulation)
- CX3CR1 (chemokine R)

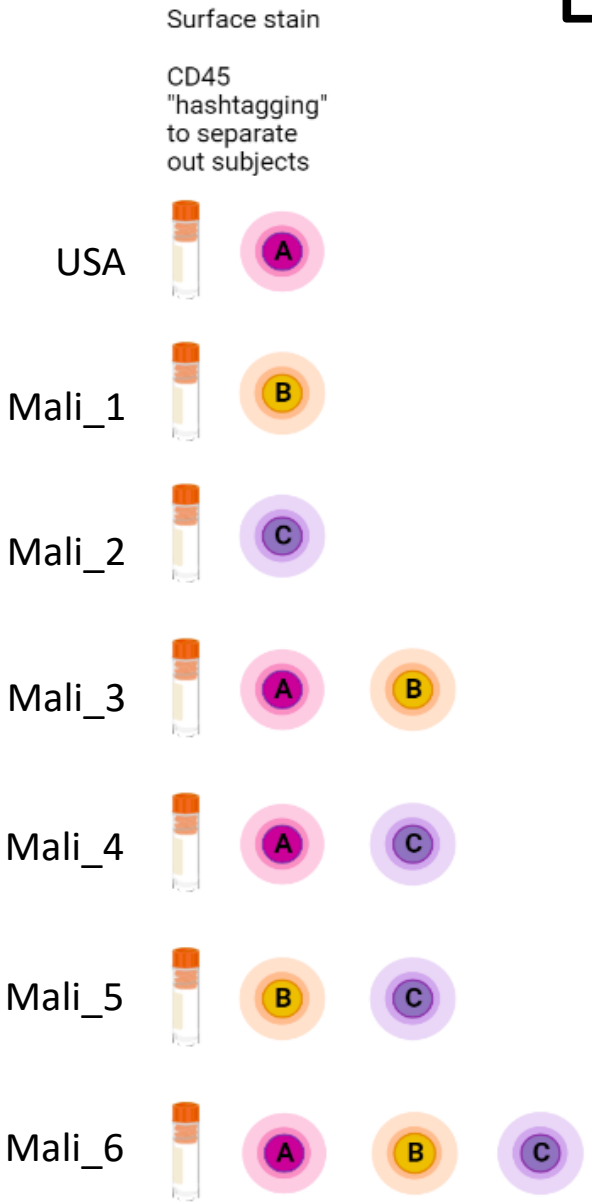
Adaptive NK cell markers:

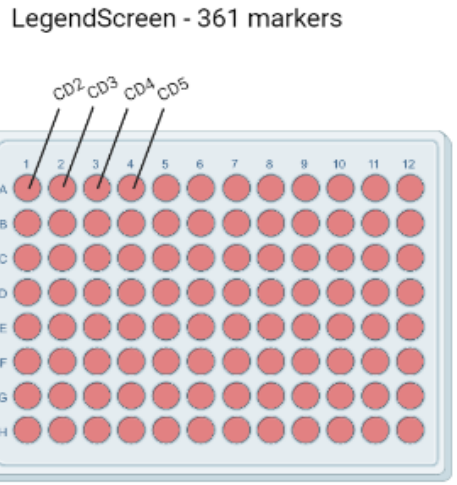
- NKG2C
- Siglec-7 (?)

Immune checkpoint molecules:

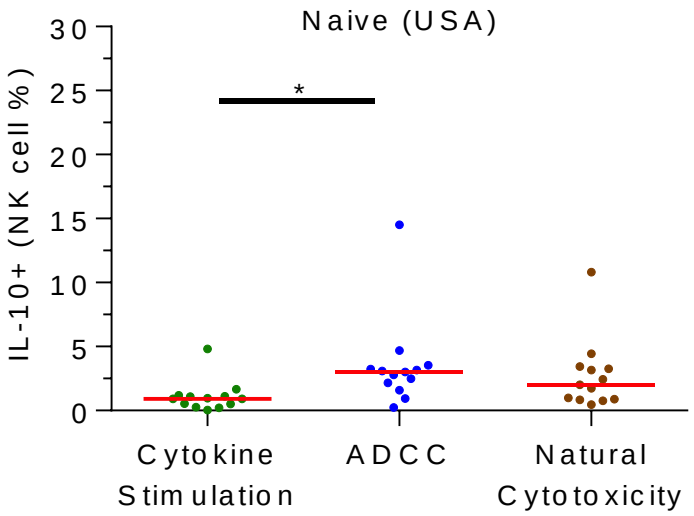
- 4-1BB
- CRTAM
- CTLA-4
- KLRG1
- PD-1
- TIGIT
- TIM-3

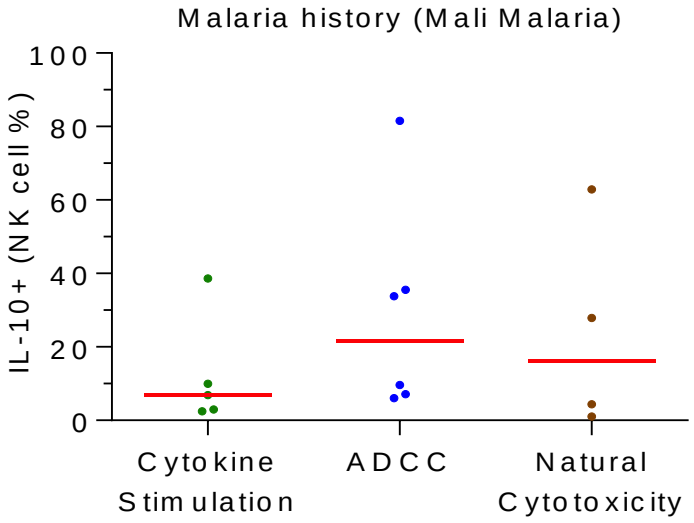
Biolegend Screen





More IL-10 is released from NK cells during ADCC than cytokine stimulation





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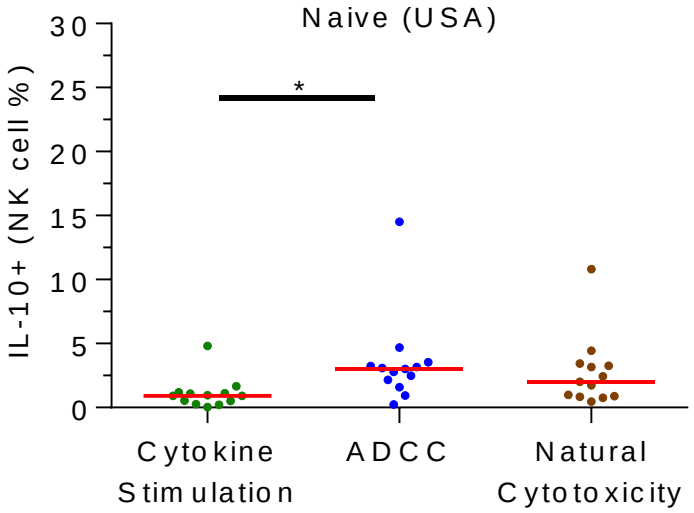
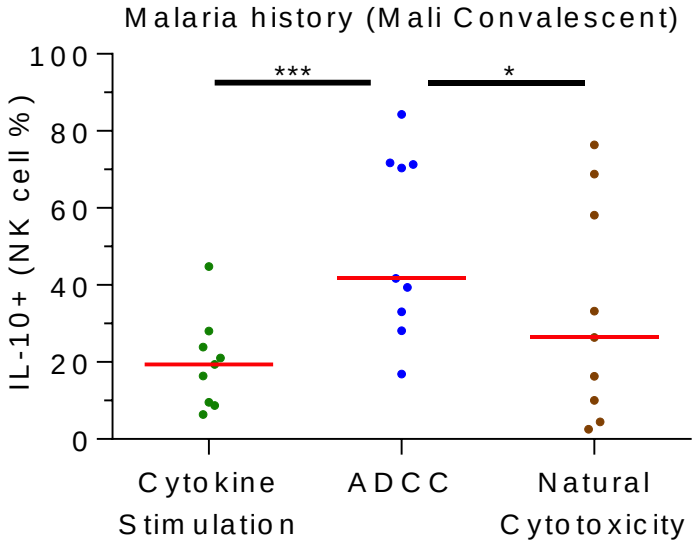
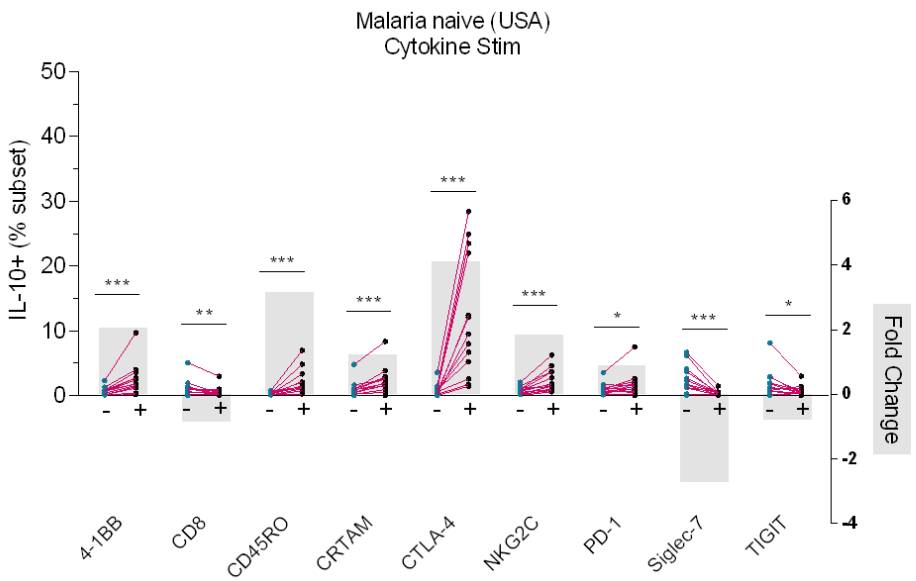
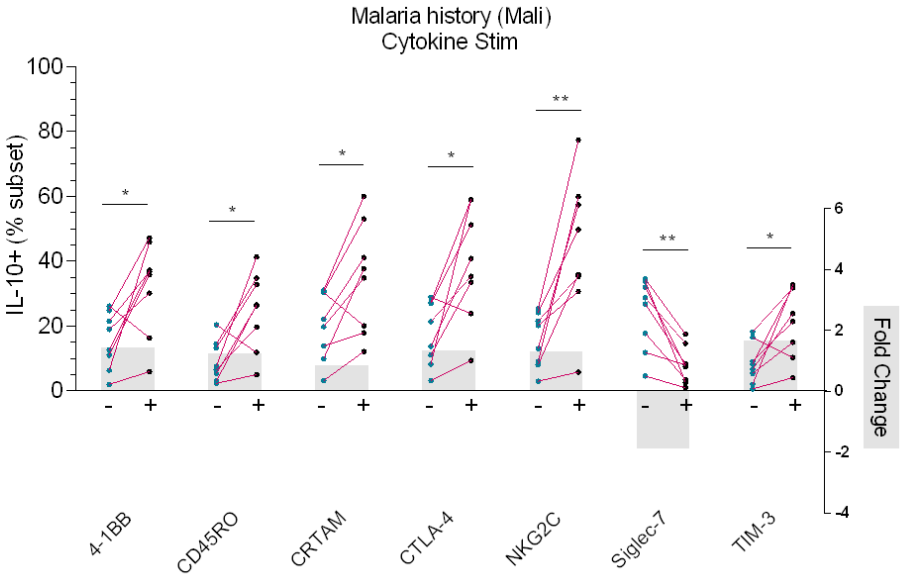




NK cell subsets expressing immune checkpoint molecules and adaptive markers had higher percentages of IL-10 releasing NK cells





Cytokine stimulation elicits IL-10 release from NK cells