

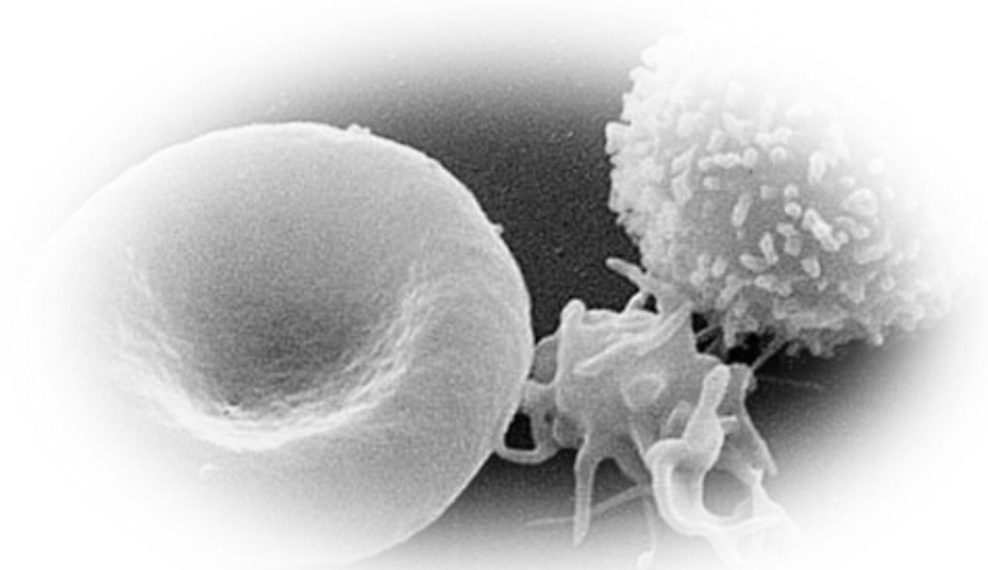
# Hidden immunological talents of platelets: Role in RSV infection

**Vesla Kullaya (BSc, MSc, PhD candidate)**



**Radboud Universiteit Nijmegen**

**23<sup>rd</sup> Nov 2014**



# Background.....

- Respiratory Syncytial virus (RSV) is major causative of acute LRTI in young children<sup>1</sup>.
- Wide range of symptoms: Cold-like to serious problems such as bronchiolitis and pneumonia which can be life-threatening.<sup>2</sup>
- Globally, 34 million episodes of acute LRTI, resulting in about 3.4 million hospitalizations per year.<sup>3</sup>

1. RSV| CDC: <http://www.cdc.gov/rsv/>. Accessed August 11, 2014.

2. RSV in Babies: <http://www.webmd.com/parenting/baby/rsv-in-babies>. Accessed August 13, 2014.

3. Nair H, et al. *Lancet*. 2010

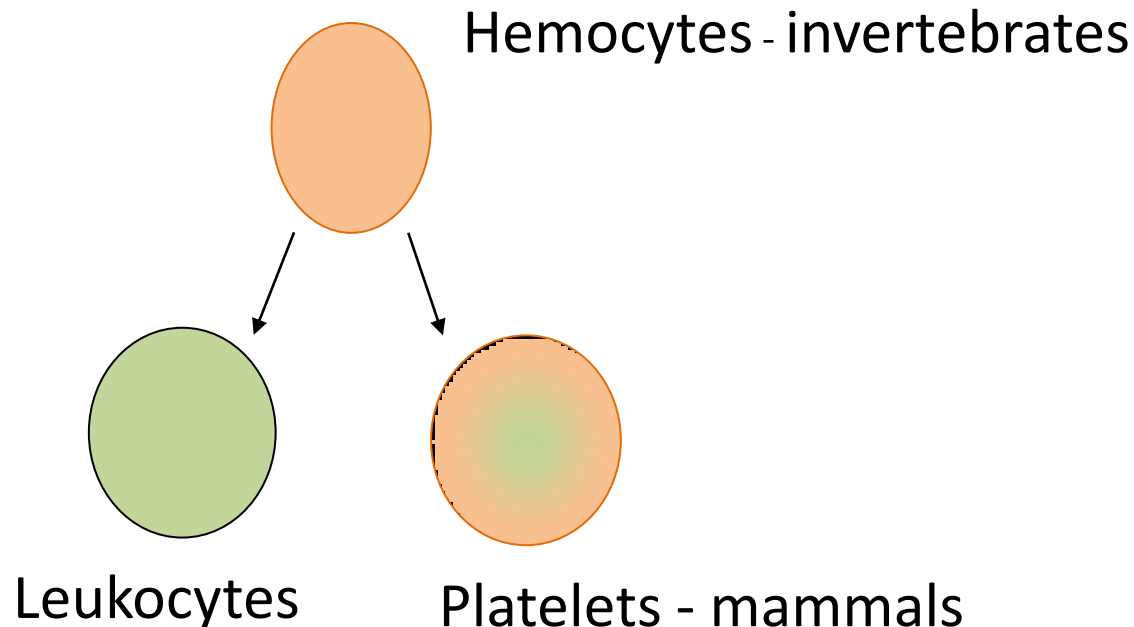
# Background.....

- No licensed vaccine, symptomatic treatment<sup>1</sup>
- Indicating that a full understanding of host immune responses to RSV is yet to be achieved.
- Research on the innate responses to RSV has been focused on epithelial cells and immune cells such as monocytes and dendritic cells. Others??

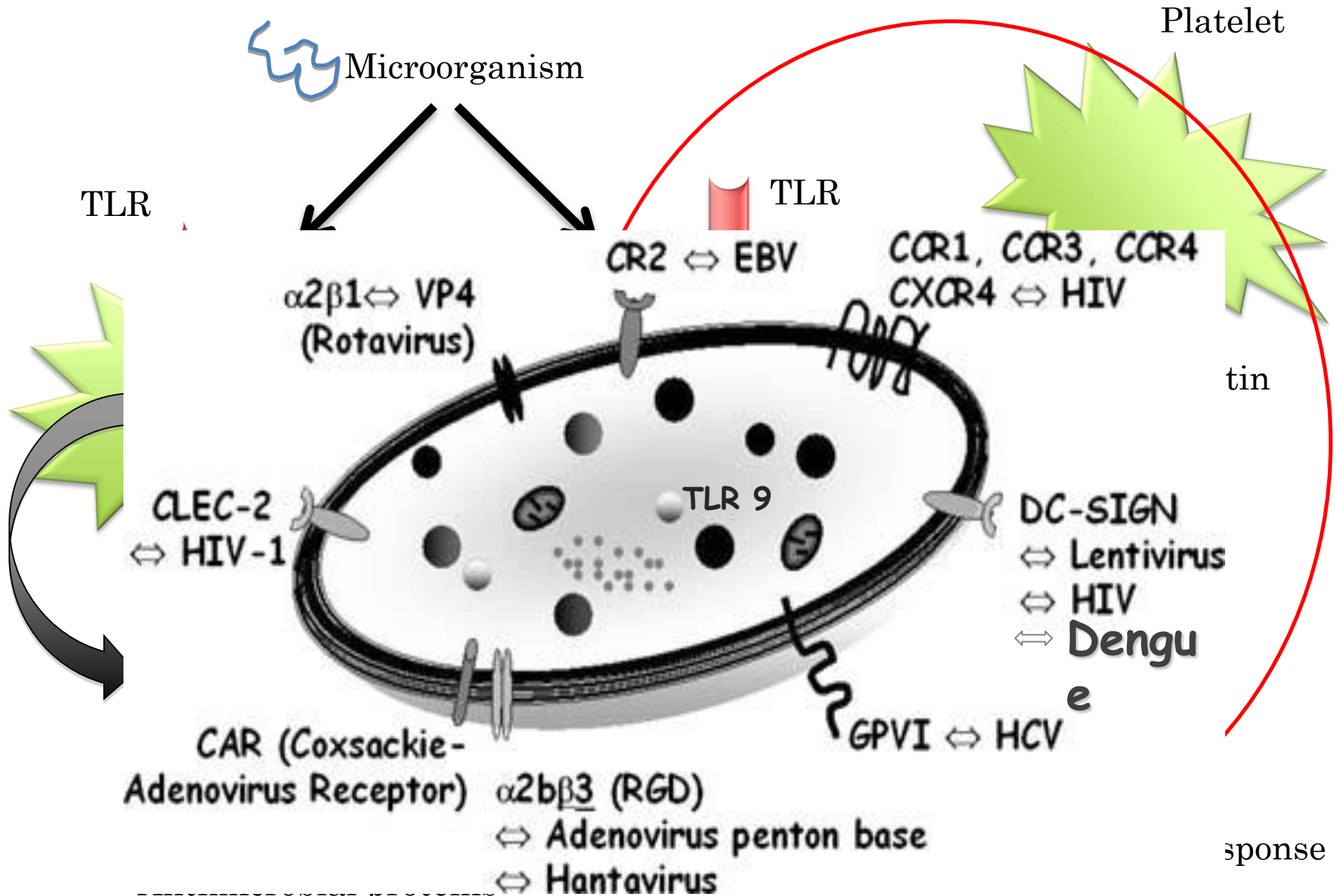


# Humans have 10x more platelets than required for hemostasis

- 150-400  $\times 10^9$  /L vs 20-30  $\times 10^9$ /L
- Also play role in inflammation, immunity / **host defense against infections.**



# Platelets in the immune system



# Background.....

- Despite these evidences that platelets are part of the innate immune system<sup>1,2</sup>, their immune function in RSV infection has not been explored.
- Studies on platelets in the context of RSV disease have reported the occurrence of thrombocytosis<sup>3-5</sup> and decreased mean platelet volume<sup>6</sup> in the course of infection.

1. Speth C, et al. *Future Microbiol.* 2013

2. Semple JW, et al. *Nat Rev Immunol.* 2011

3. Liu H, et al. *Zhongguo Dang Dai Er Ke Za Zhi.* 2014

4. Kubota M, et al. *Acta Paediatr.* 2005

5. Bilavsky E, et al *Isr Med Assoc J.* 2010

6. Renshaw AA, Gould EW. *Am J Clin Pathol.* 2013

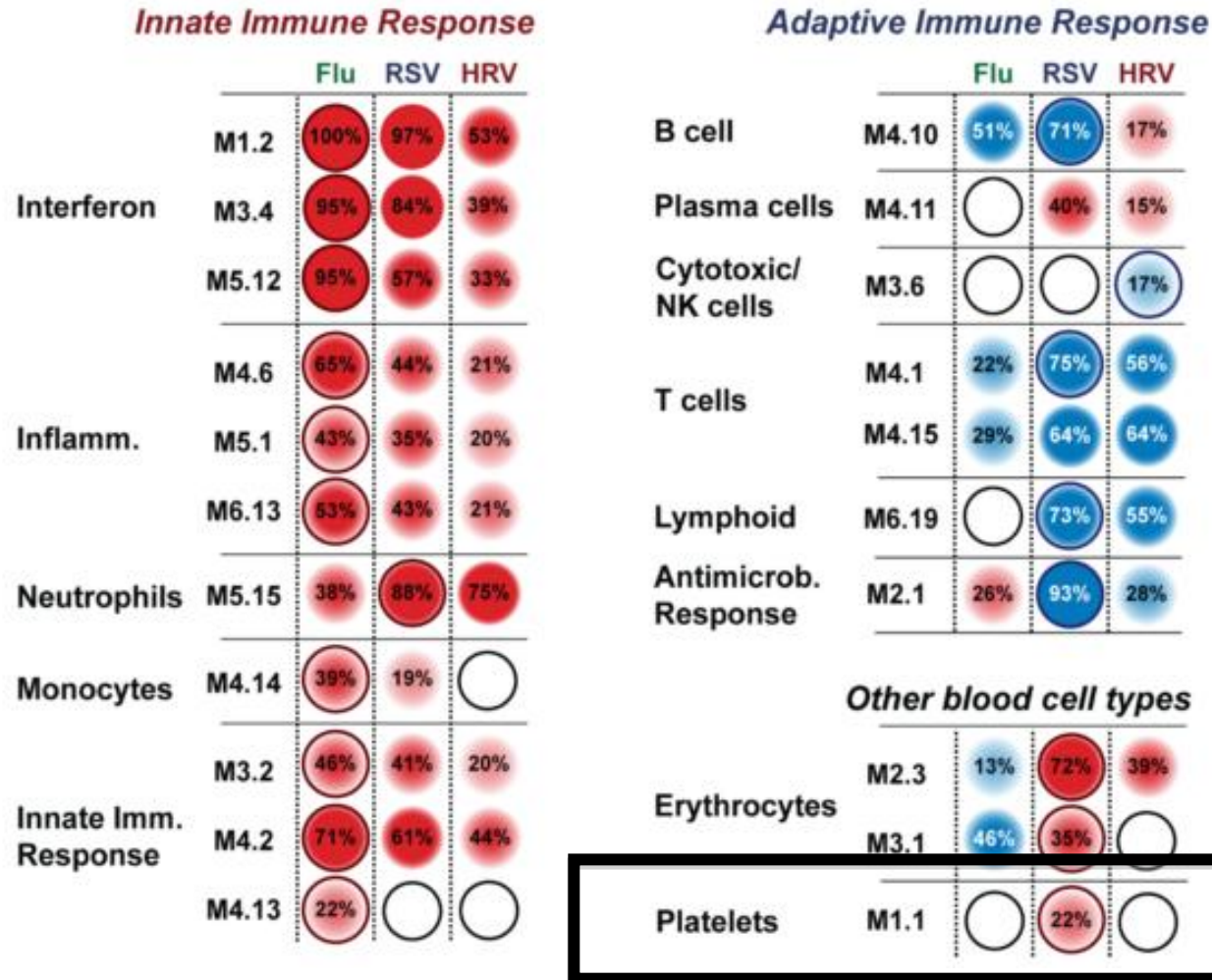
# Background.....

- Waghmare et al reported that there is an association between thrombocytopenia and increased mortality and detection of RSV in blood.<sup>1</sup>
- Mejias et al, whole blood gene expression profile to assess immune responses and biomarkers of disease severity in a cohort of infants with LRTI. <sup>2</sup>

1. Waghmare A, et al *Clin Infect Dis*. 2013

2. Mejias A, et al *PLoS Med*. 2013

# Background....





# Aim of the study

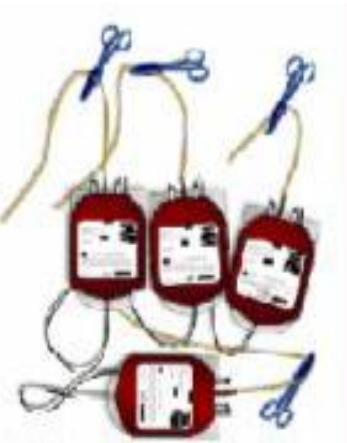
To explore the role of platelets in host response against RSV infection

1. Determined whether platelets could inhibit RSV infection in monocytes in vitro
2. Determined whether platelets modified immune response to RSV in vitro
3. Explored the importance of platelet-leukocyte interaction in these effects

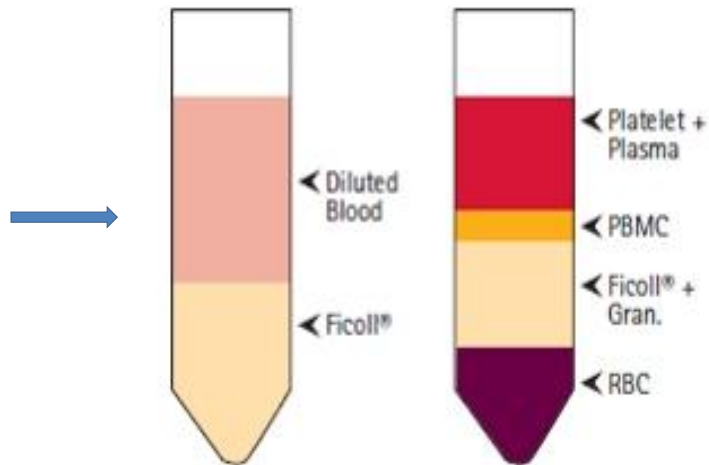
# Methods

## PBMC isolation

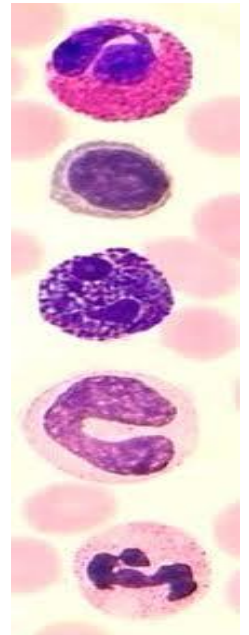
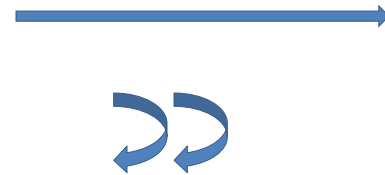
Buffy coat



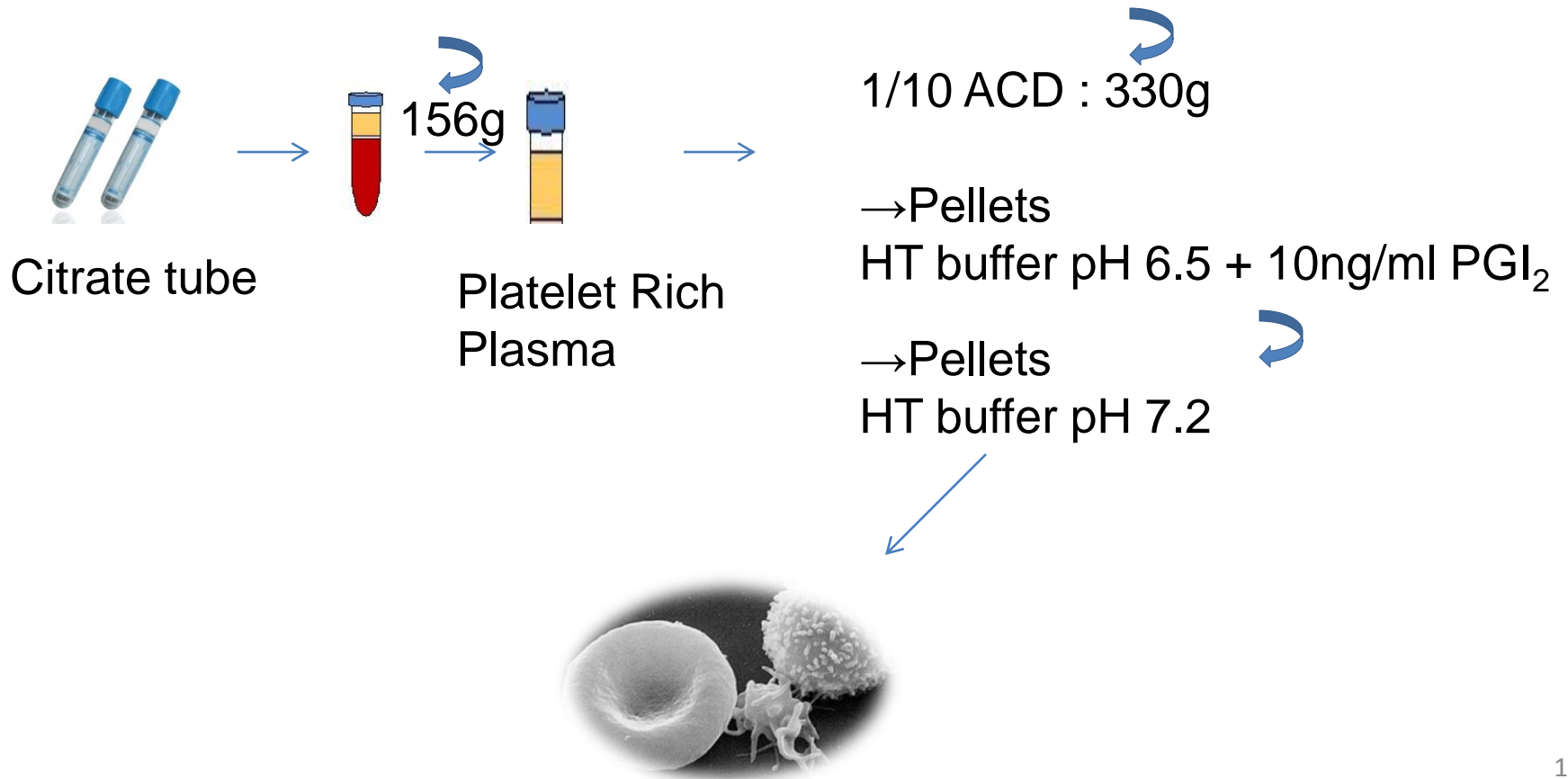
Density gradient  
centrifugation method



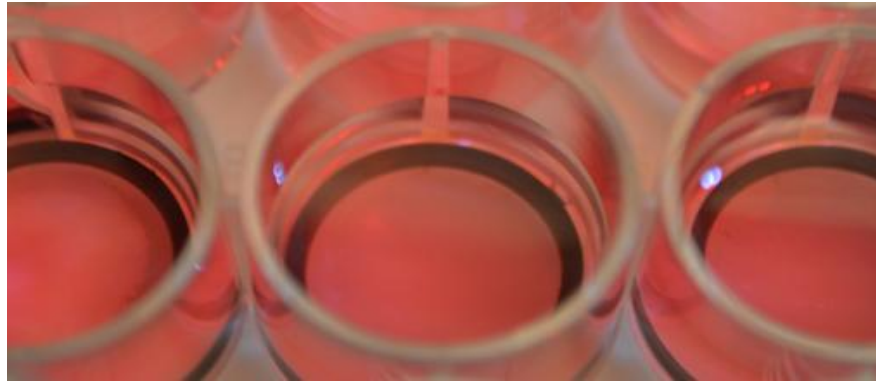
PBMCs



# Platelet isolation



# PBMC stimulation assay



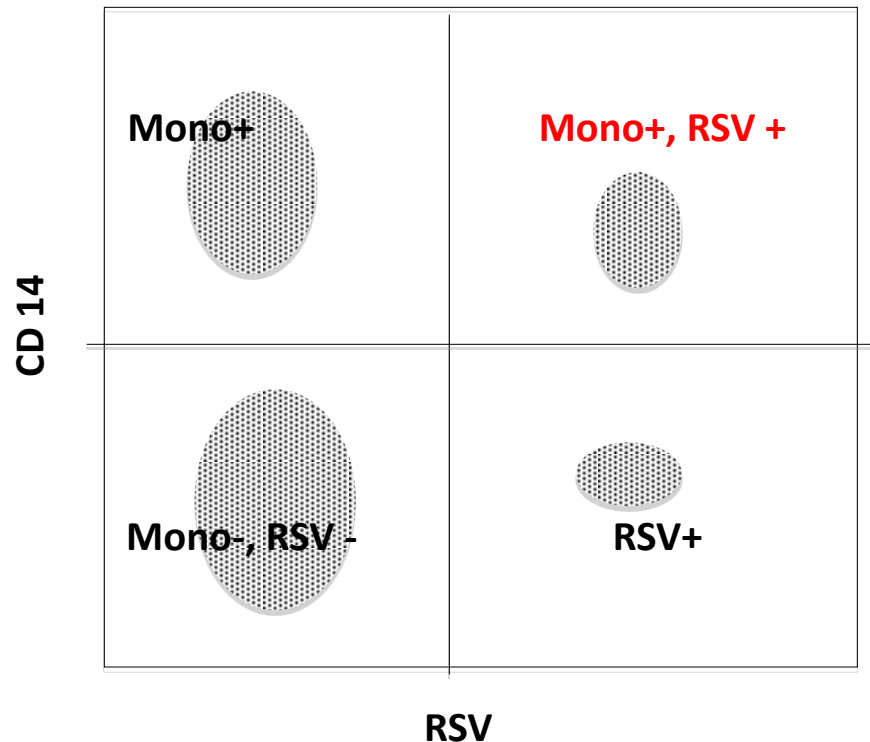
PBMCs +  
RSV

PBMCs +  
RSV  
**Platelets**

- PBMC:platelet ratio = 1:150
- FITC-GFP labeled RSV
- MOI of 10
- Incubated for 48 hrs at 37°C, 5% CO<sub>2</sub> humidified atmosphere

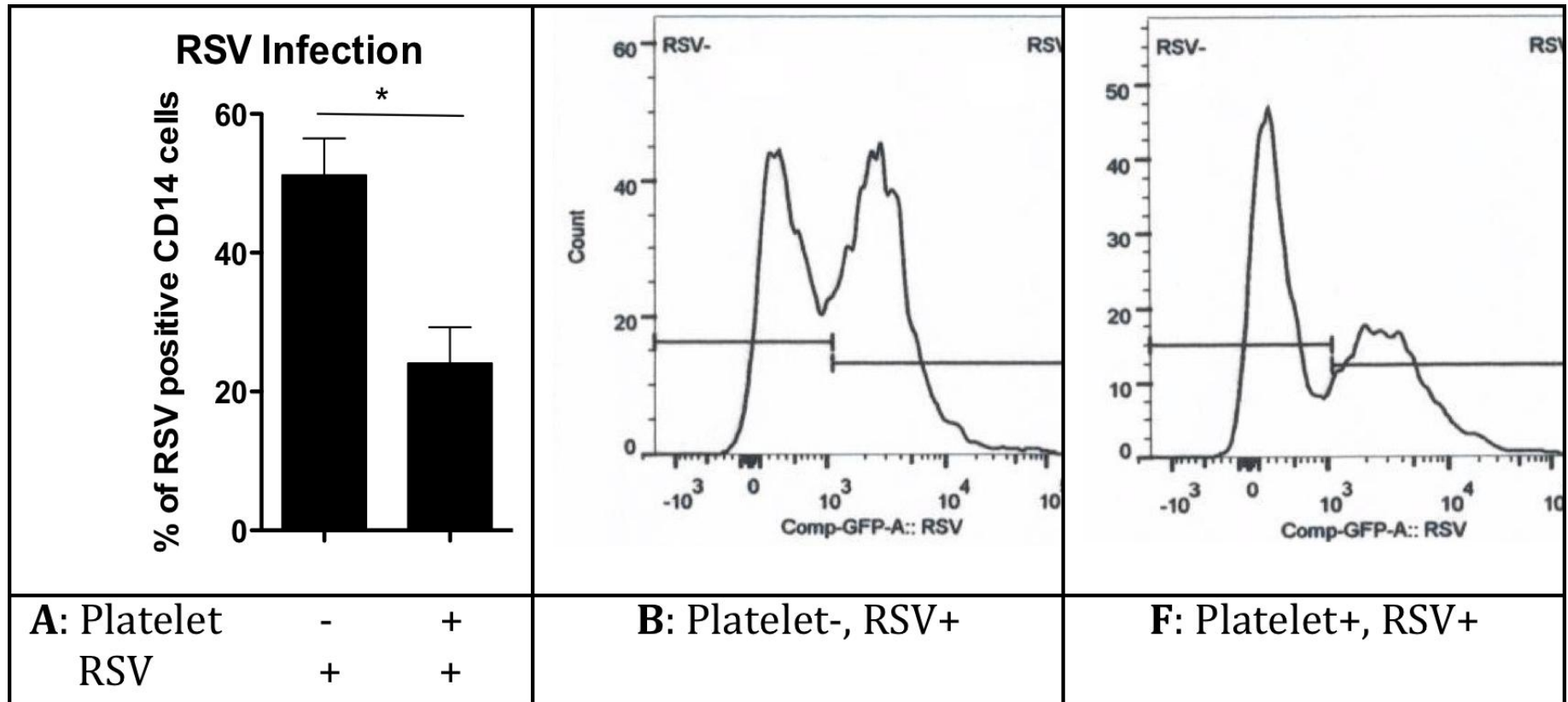
# Methods.....

- Monocyte marker - CD14
- The **percentage of monocytes infected with RSV** was analyzed by FC500 flow cytometer.



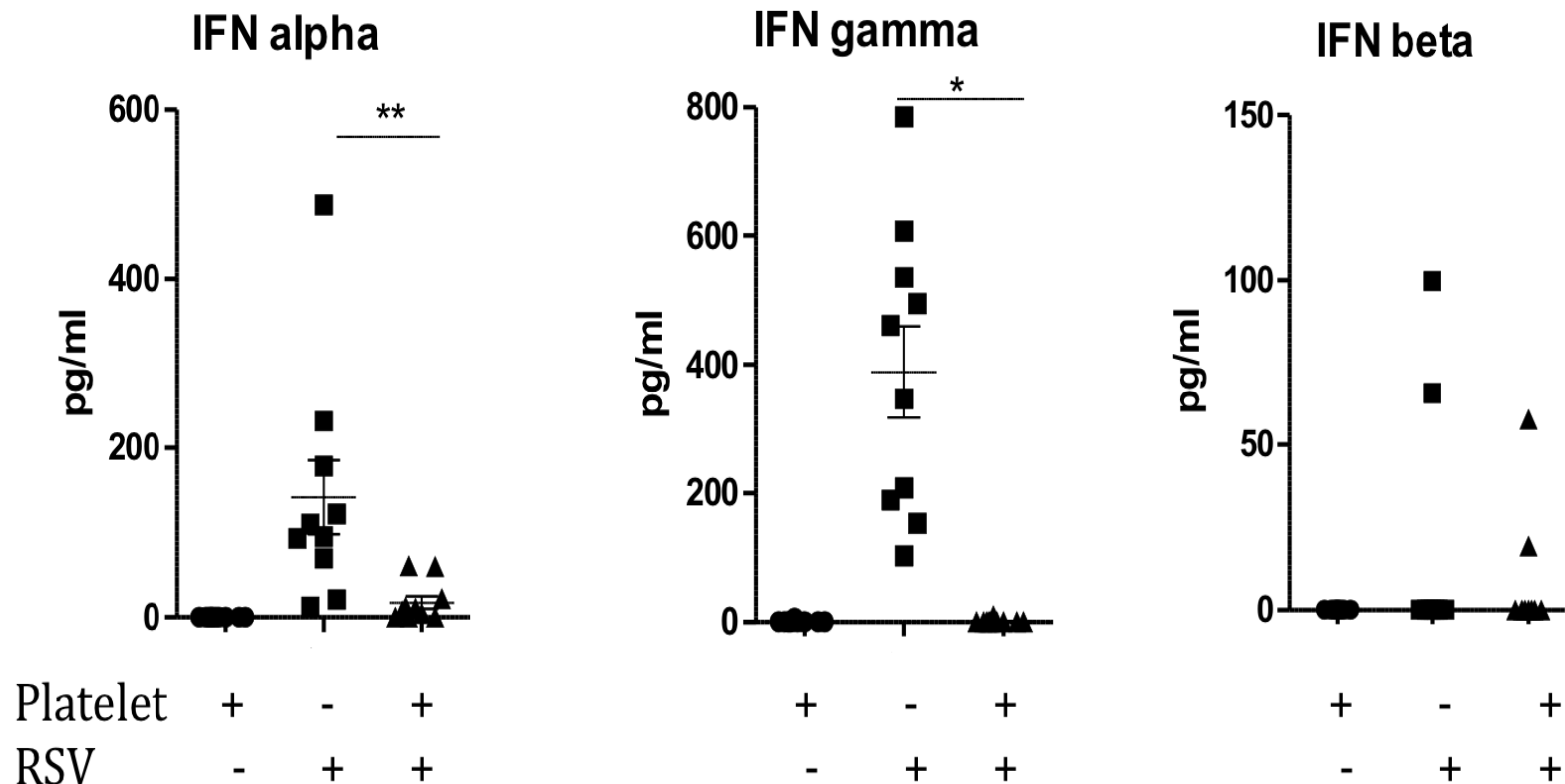
# Results 1

- Platelet reduce RSV infection in monocytes



# Results 2

- Platelet reduce IFN production from PBMCs following stimulation with RSV



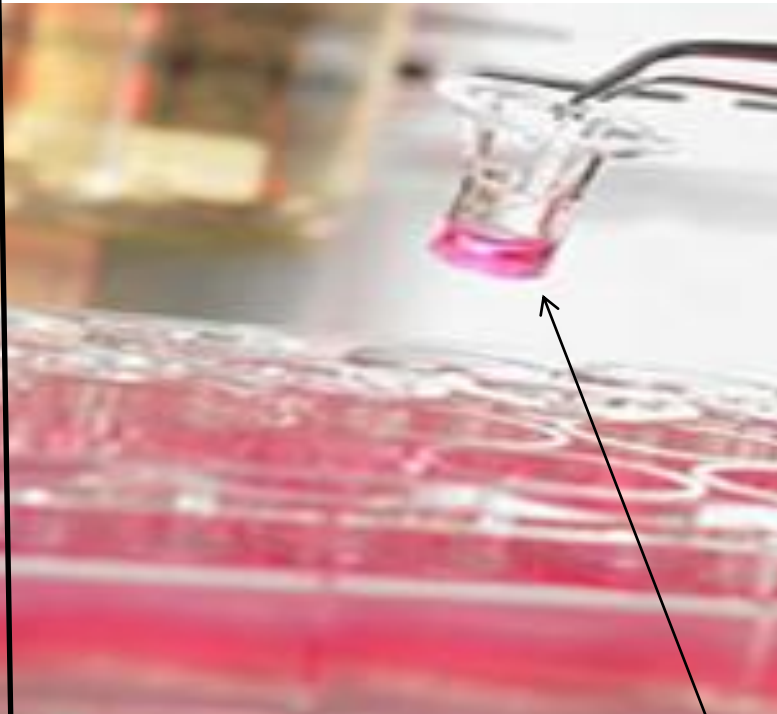
## RSV vs Purified agonist



1. PBMCs (with and without platelets) + TLR 3 agonists

- Purified TLR 3 agonist – 100  $\mu\text{g/ml}$  Poly IC
- Transwell system

## Platelet-leukocyte interaction



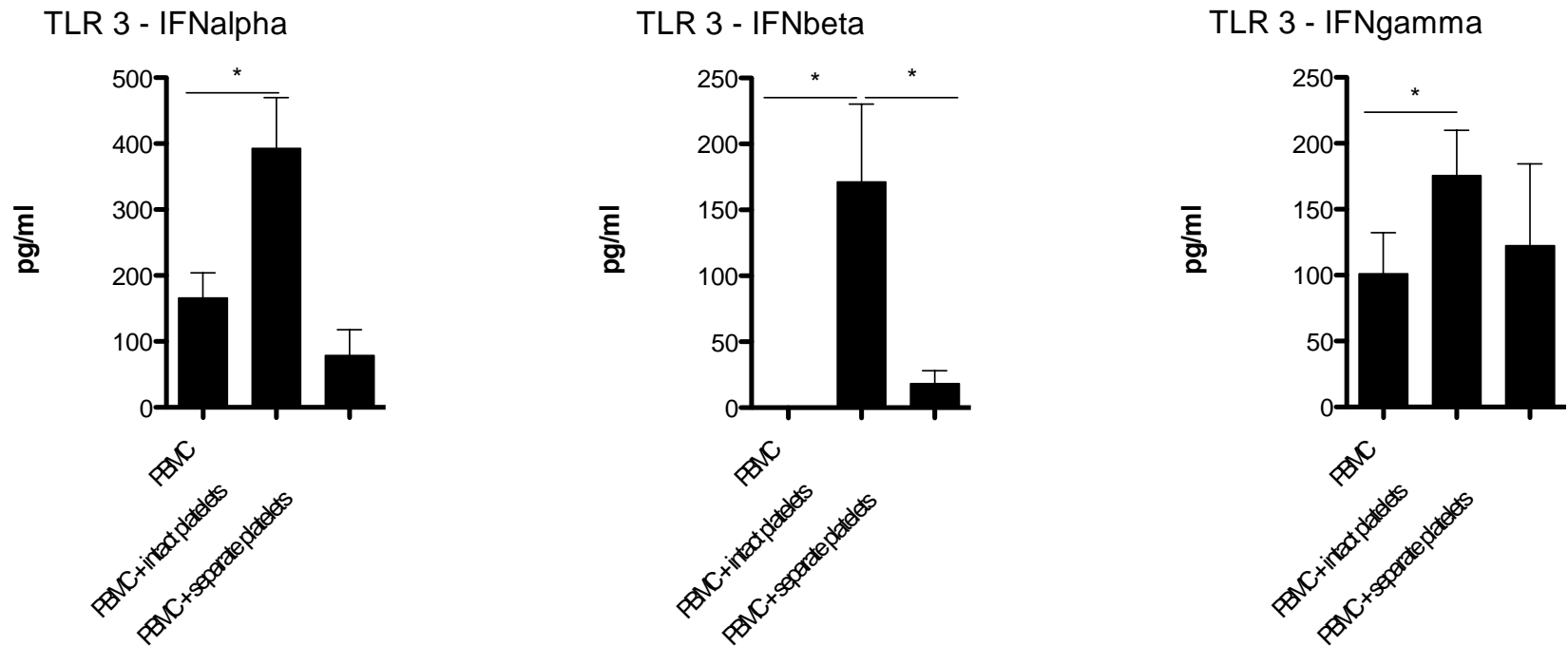
2. PBMCs + TLR agonists + Platelets separated by a 0.4  $\mu\text{m}$  filter



# Results 3

- Platelet increase IFN production from PBMCs following stimulation with RSV
- Platelet-leukocyte direct interaction is important for these

A



# Conclusion

- Results indicate that platelets are important in host innate defenses against RSV infection, and that direct platelet-monocyte interaction may be crucial for these effects.
- It can be speculated that platelets may be important in preventing vascular dissemination of the virus.
- Further studies need to be done to broaden the understanding of defense mechanisms of platelets during RSV infection.

# I acknowledge,



Radboud Universiteit Nijmegen



## ASANTENI KWA KUNISIKILIZA!!