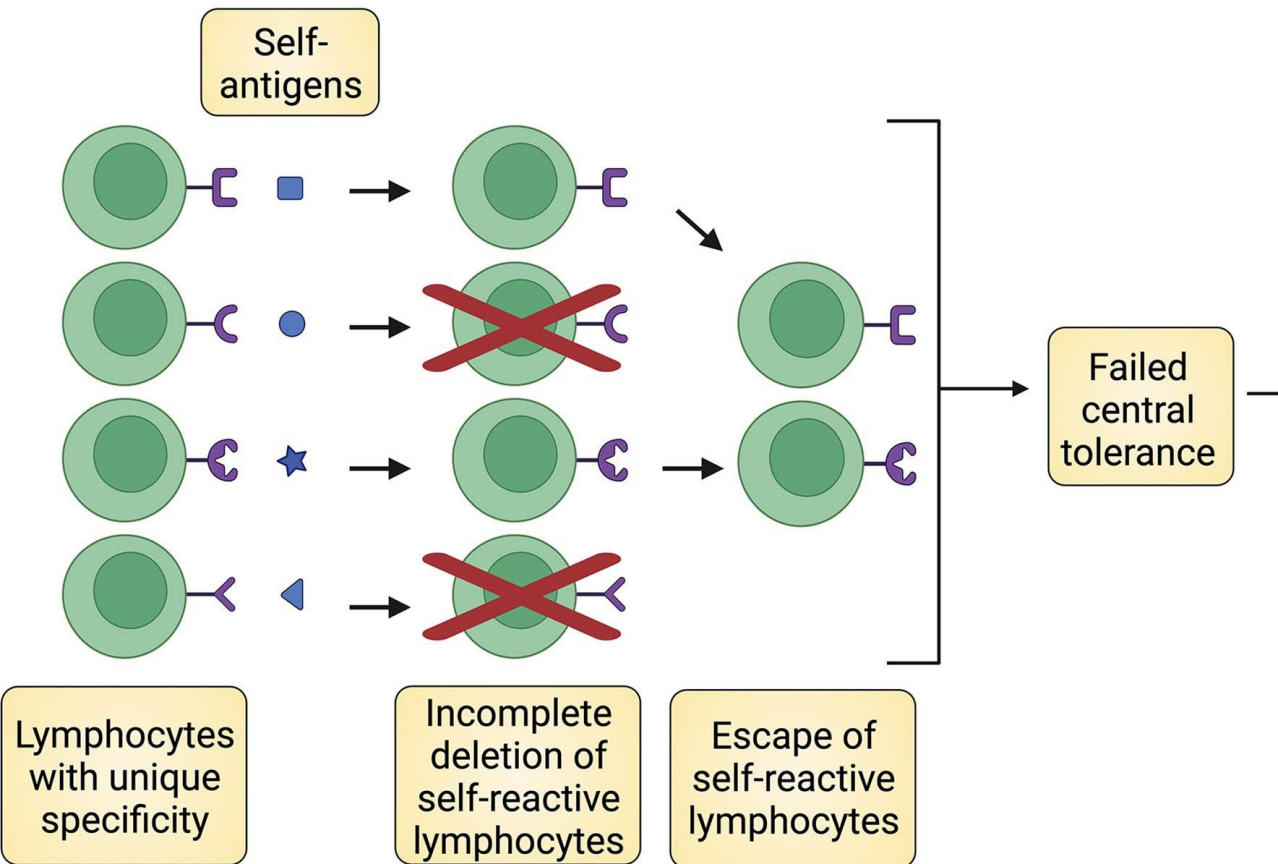


# *The role of anionic phospholipids in lipid nanoparticle inverse vaccines*

**Naomi Benne, PhD**

Assistant Professor Immunology Group  
Faculty of Veterinary Medicine

# Immune dysregulation in autoimmunity



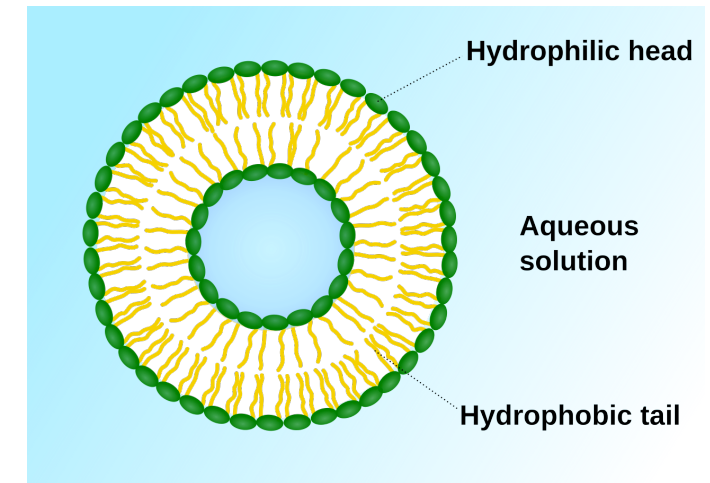
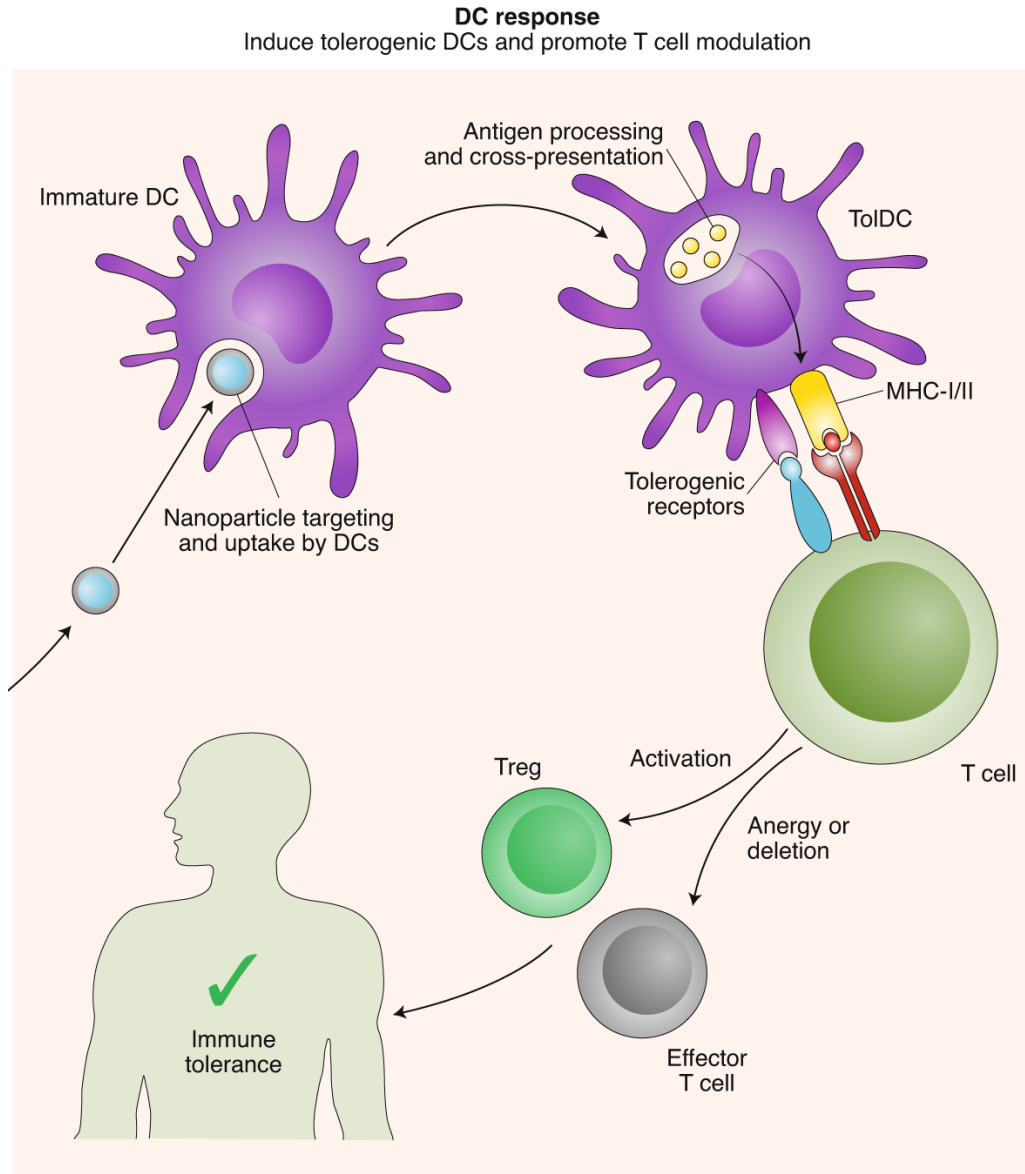
The immune system attacks the body's own tissues as though they are foreign pathogens

This results in irreversible tissue damage and chronic, debilitating and in some cases lethal disease

There is **no cure** for autoimmune disease

Patients rely on general immune suppression to relieve symptoms

# Inverse vaccines → nanoparticles for antigen-specific tolerance



Peptide delivery by liposomes

# The effect of liposomal composition on antigen-specific T cell responses

Physicochemical properties of OVA323-containing liposomes composed of 4:1:2 M ratio DSPC:charged lipid:chol.

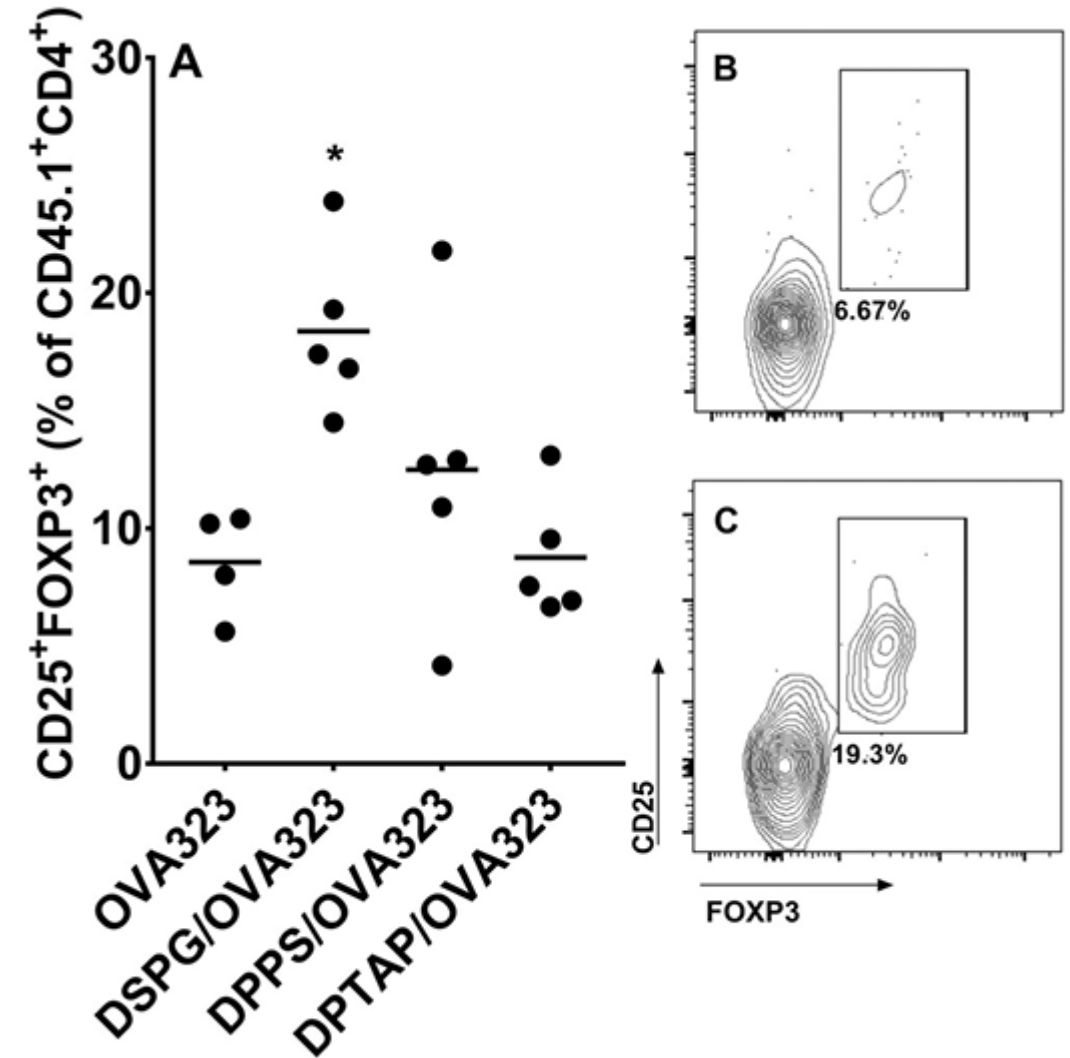
| Charged lipid | $Z_{ave}$ (nm) <sup>a</sup> | PDI         | Zeta-potential (mV) | % LE <sup>b</sup> |
|---------------|-----------------------------|-------------|---------------------|-------------------|
| DSPG          | 167.3 ± 11.8                | 0.08 ± 0.04 | −54.4 ± 5.5         | 10.6 ± 3.9        |
| DPPS          | 165.4 ± 15.7                | 0.12 ± 0.05 | −54.0 ± 6.4         | 14.7 ± 4.7        |
| DPTAP         | 166.9 ± 14.9                | 0.09 ± 0.04 | 33.7 ± 3.7          | 27.6 ± 8.5        |

<sup>a</sup> Z-average diameter ( $Z_{ave}$ ), mean ± SD,  $n = 12$ .

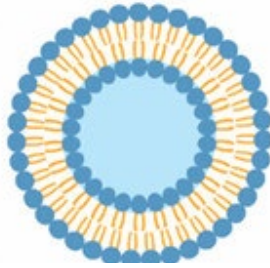
<sup>b</sup> %LE was calculated as the total amount of peptide before extrusion/total amount of peptide after purification \* 100%.

# DSPC:DSPG:CHOL liposomes induce Tregs

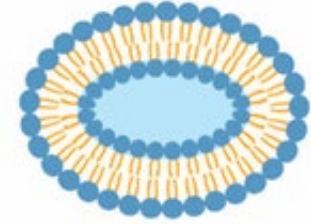
- CD4<sup>+</sup> T cells isolated from the spleens of CD45.1<sup>+</sup> OT-II mice (TCR for OVA antigen)
- Injected IV into CD45.2<sup>+</sup> WT mice
- IV injection of OVA-containing liposomes
- After 7 days, isolate spleens and measure antigen-specific (CD45.1<sup>+</sup>) CD25<sup>+</sup>FOXP3<sup>+</sup> regulatory T cells using flow cytometry



## Formulation of liposomes



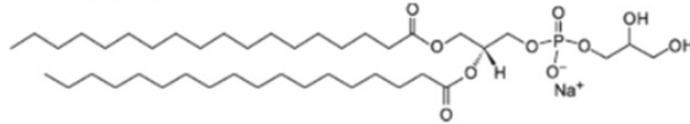
# Rigid



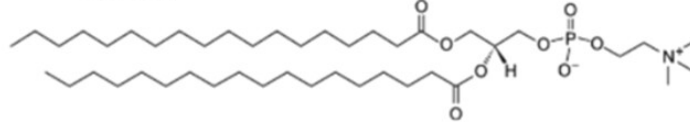
## Non-rigid

Size → <200 nm for APC uptake  
Charge → anionic for APC uptake  
Rigidity → ?

**DSPG**



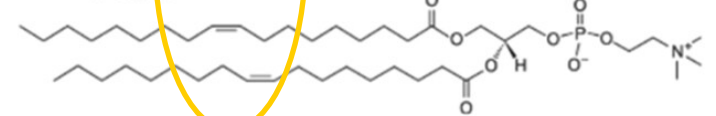
**DSPC**



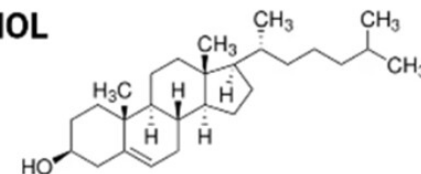
**DOPG**



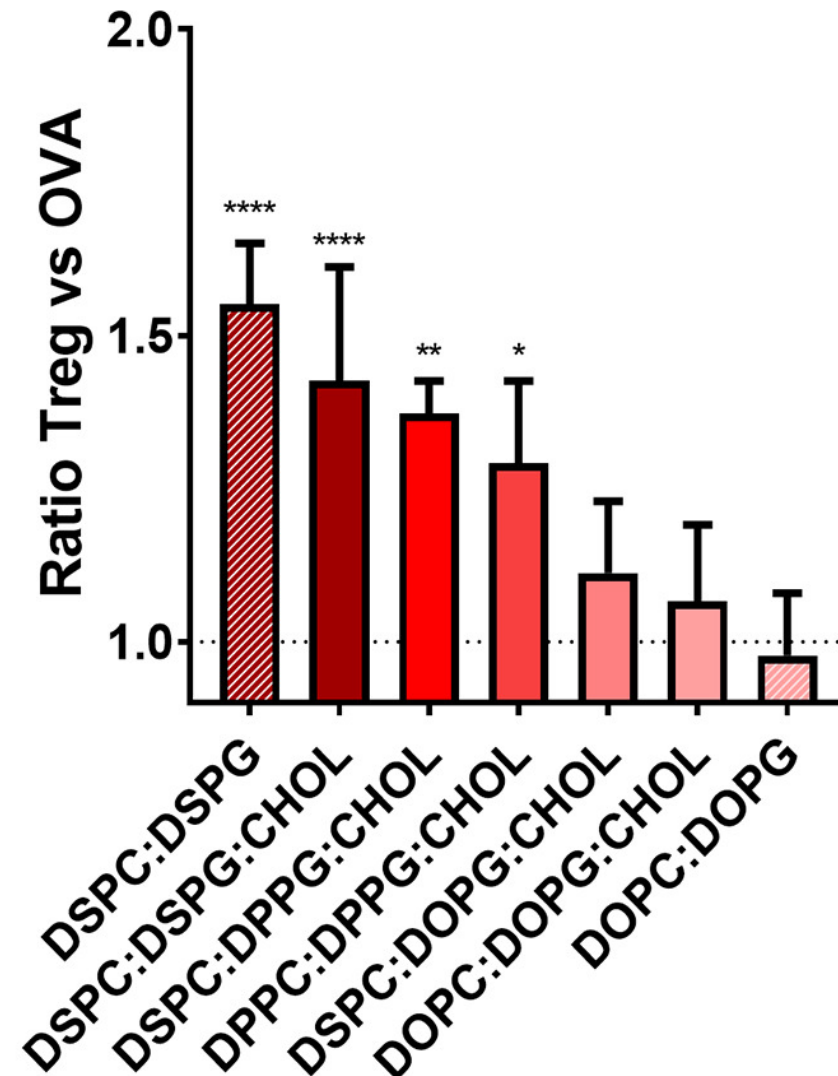
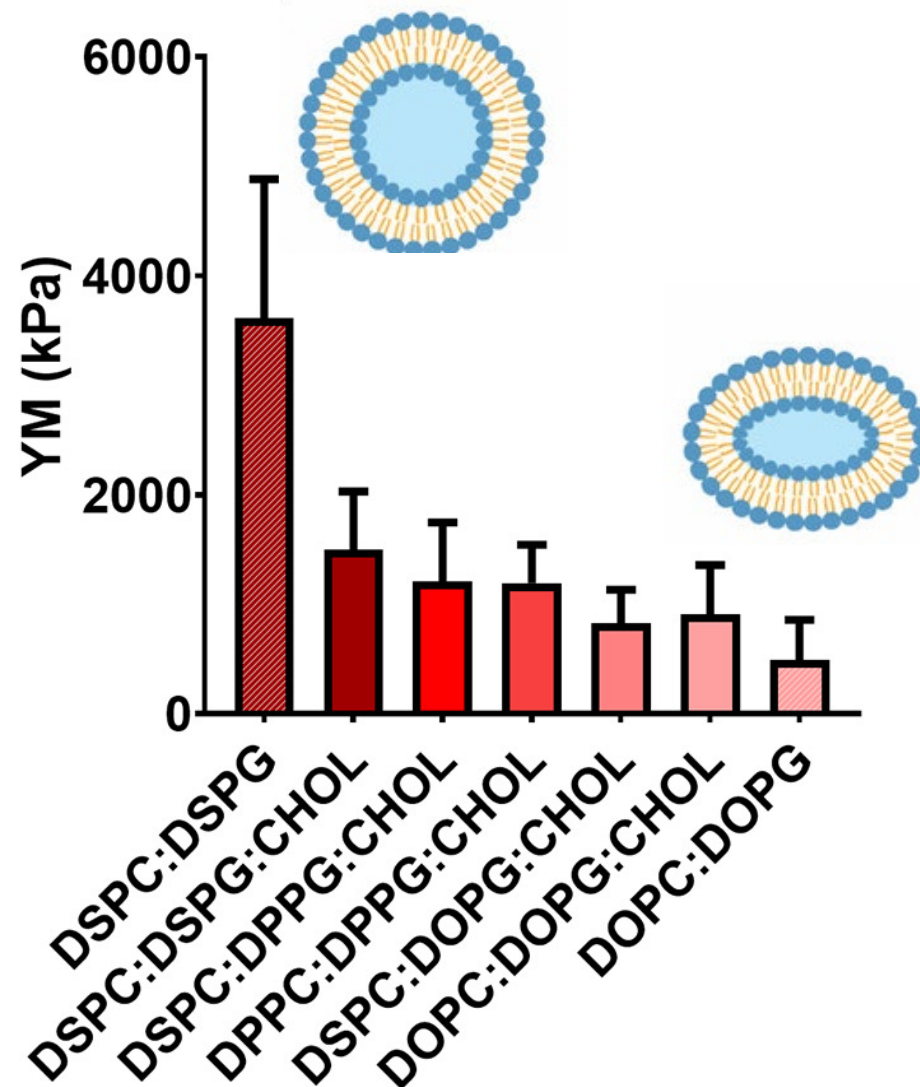
DOPC



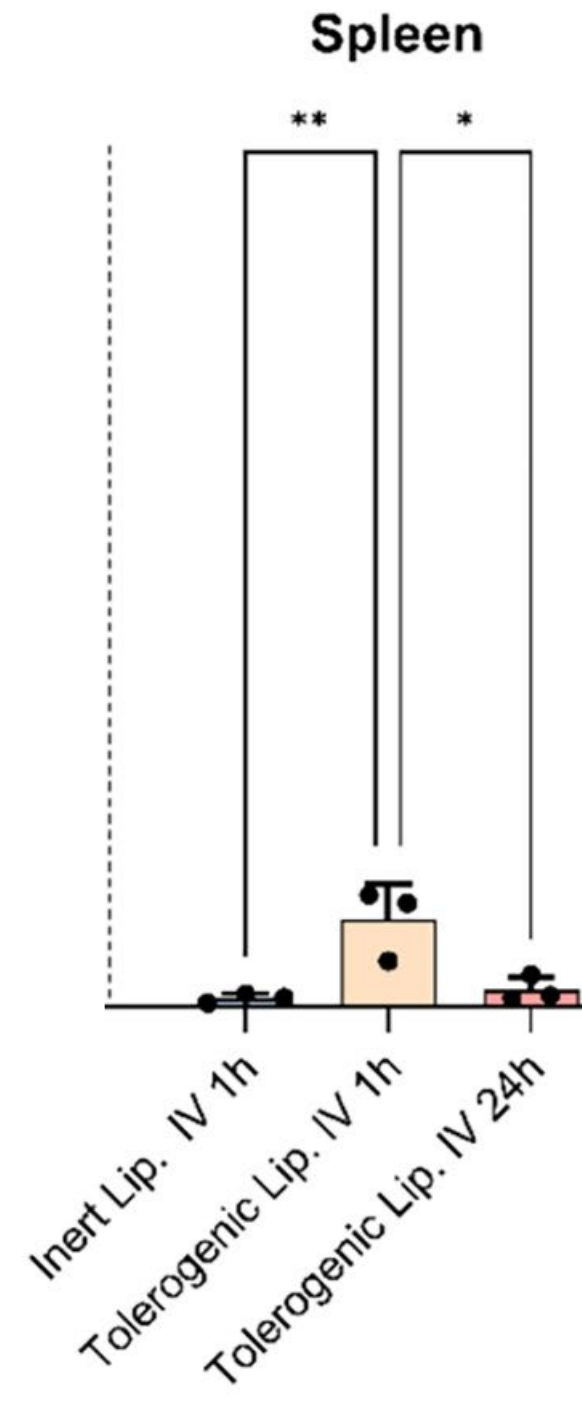
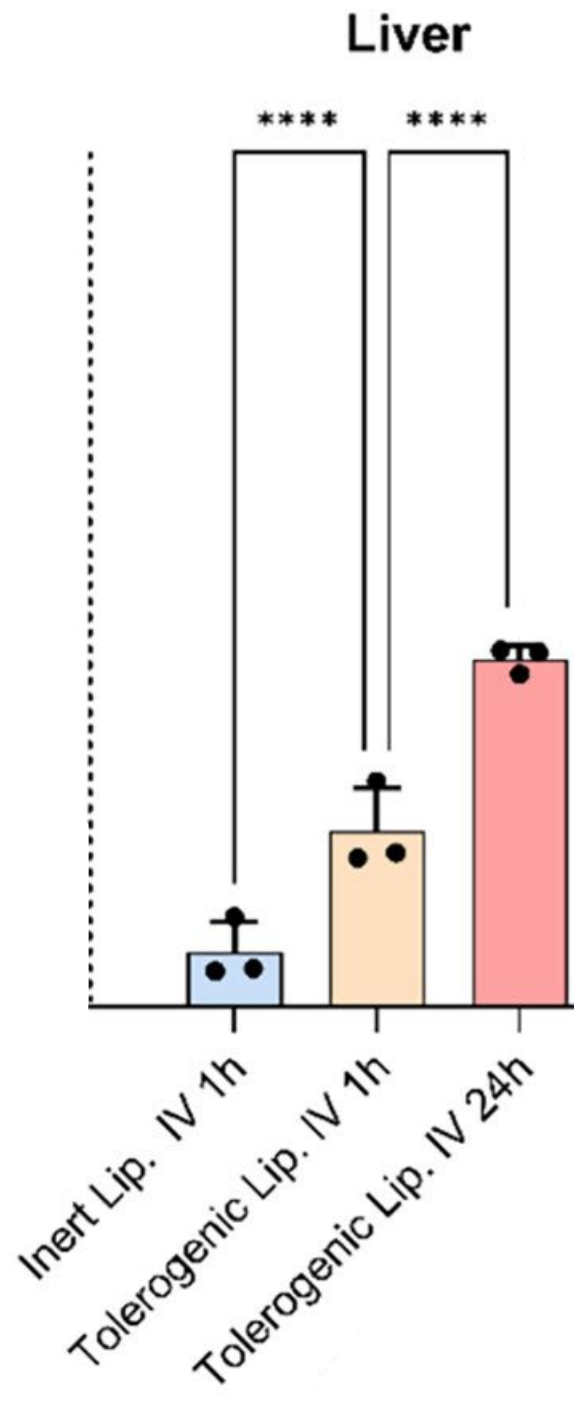
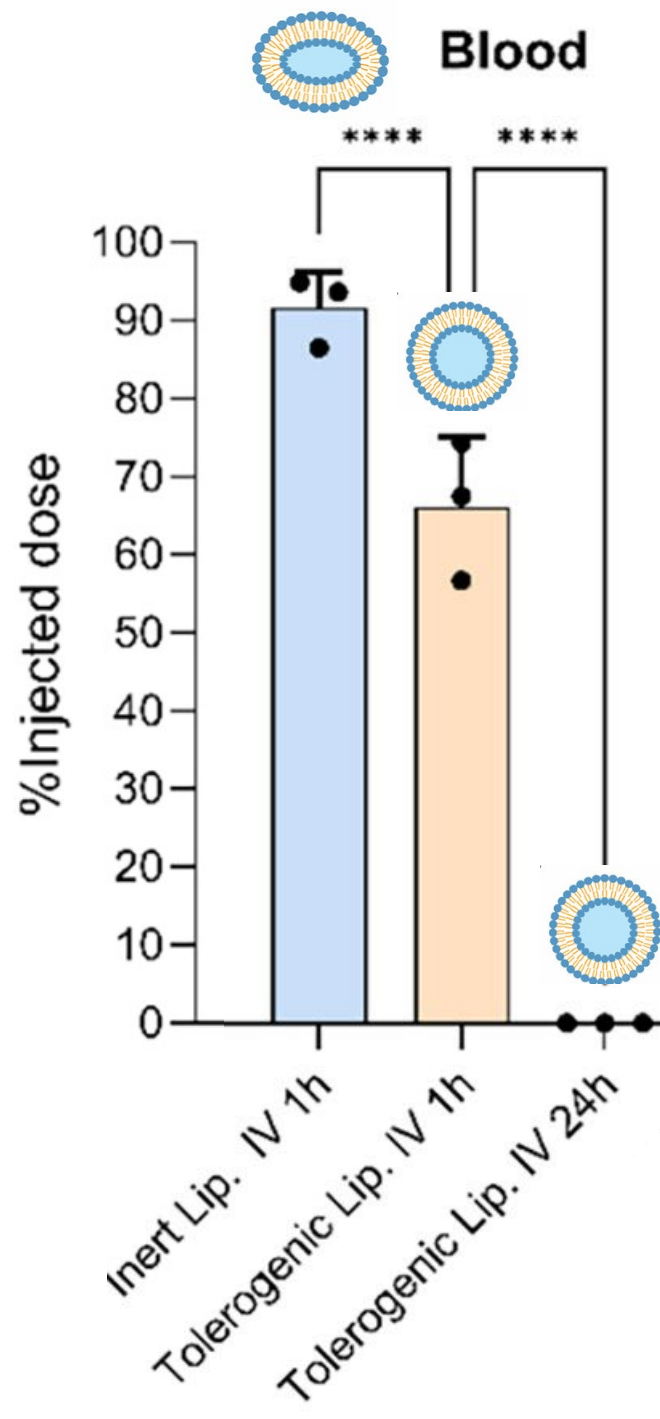
**CHOL**



# Rigid liposomes induce antigen-specific Tregs



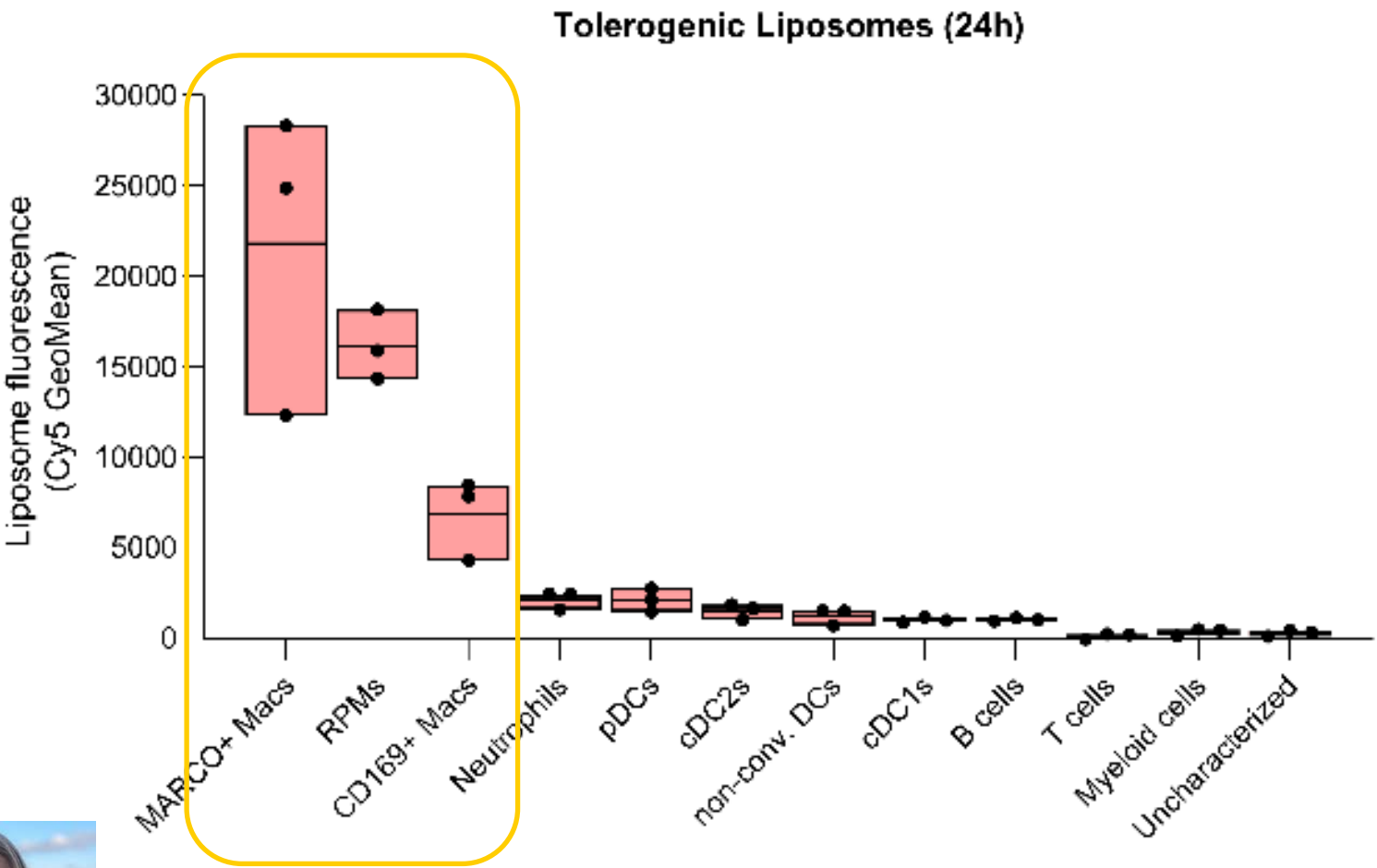
Biodistribution?



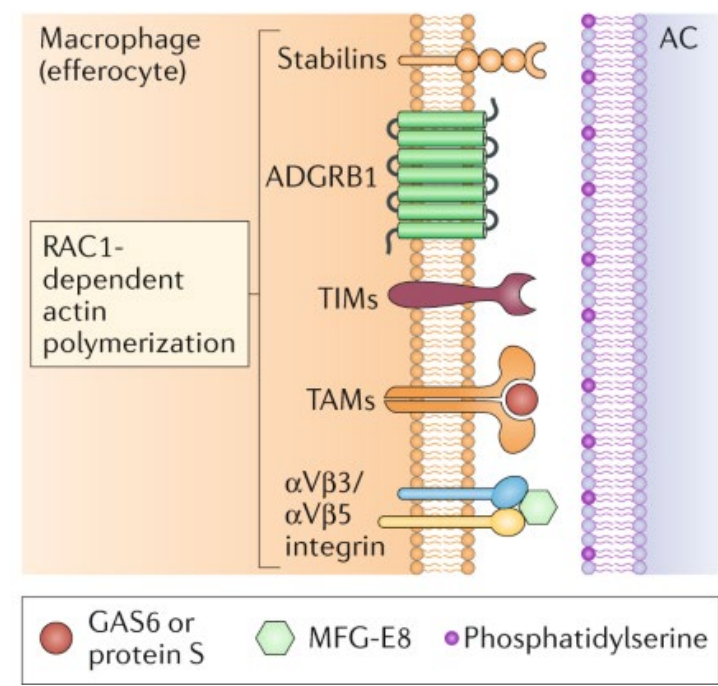
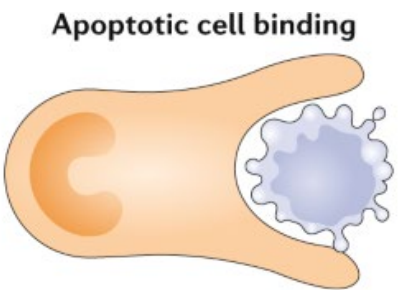
Signal/mg  
equal in liver  
and spleen

No uptake in  
other organs

# IV injected rigid liposomes are taken up by splenic MZMs

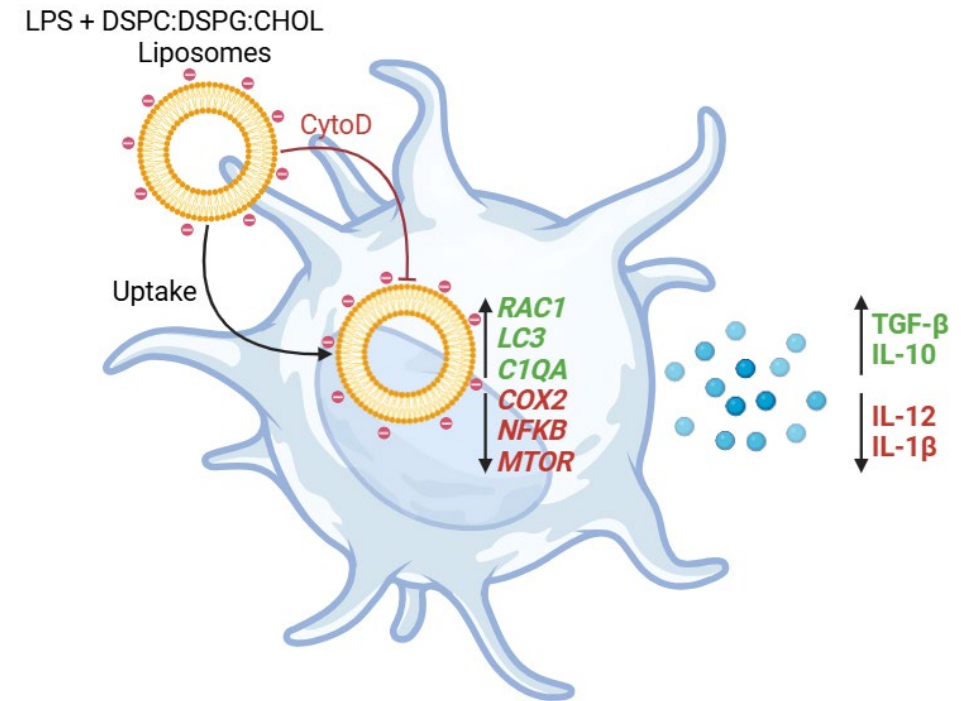
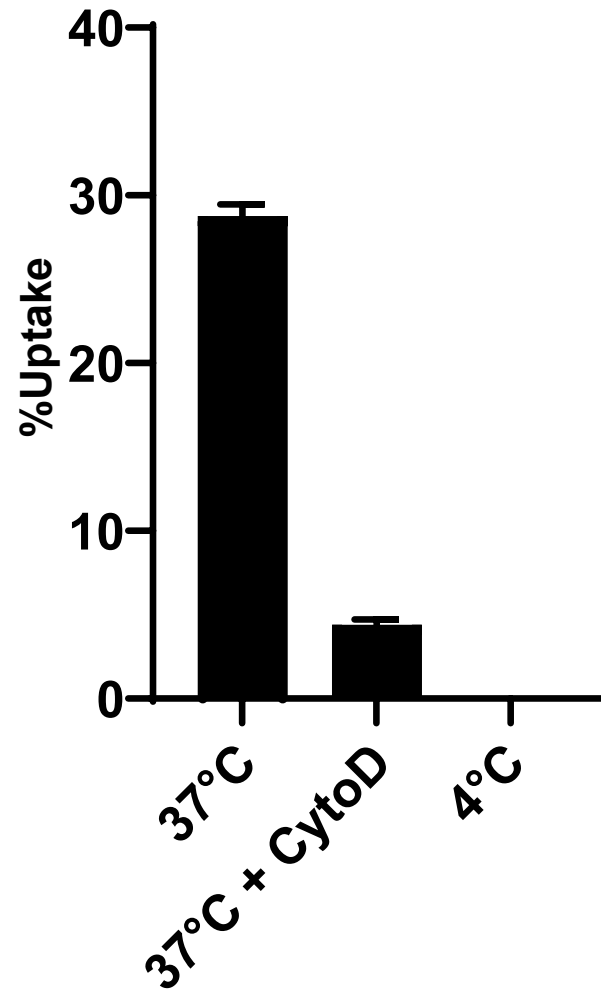


Efferocytosis

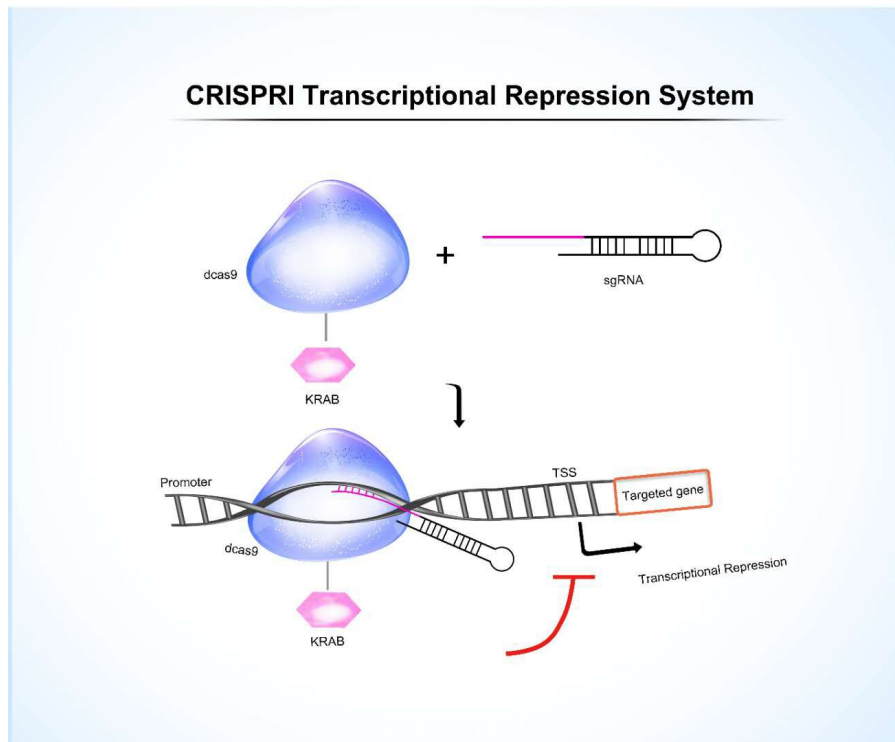


Deja Porenta

# Liposome uptake by antigen-presenting cells is an active, actin-mediated process



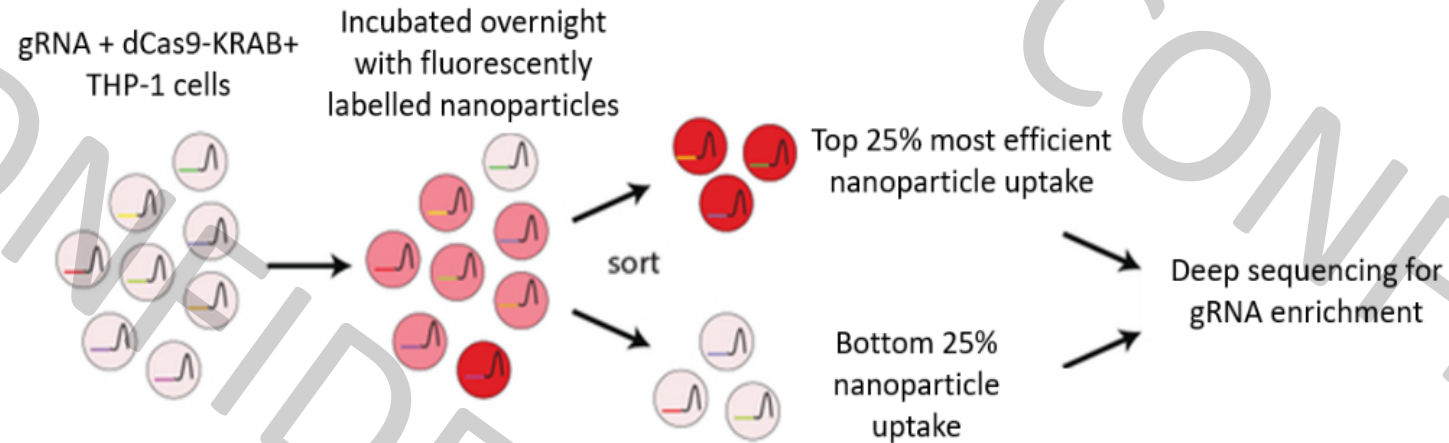
## CRISPR interference



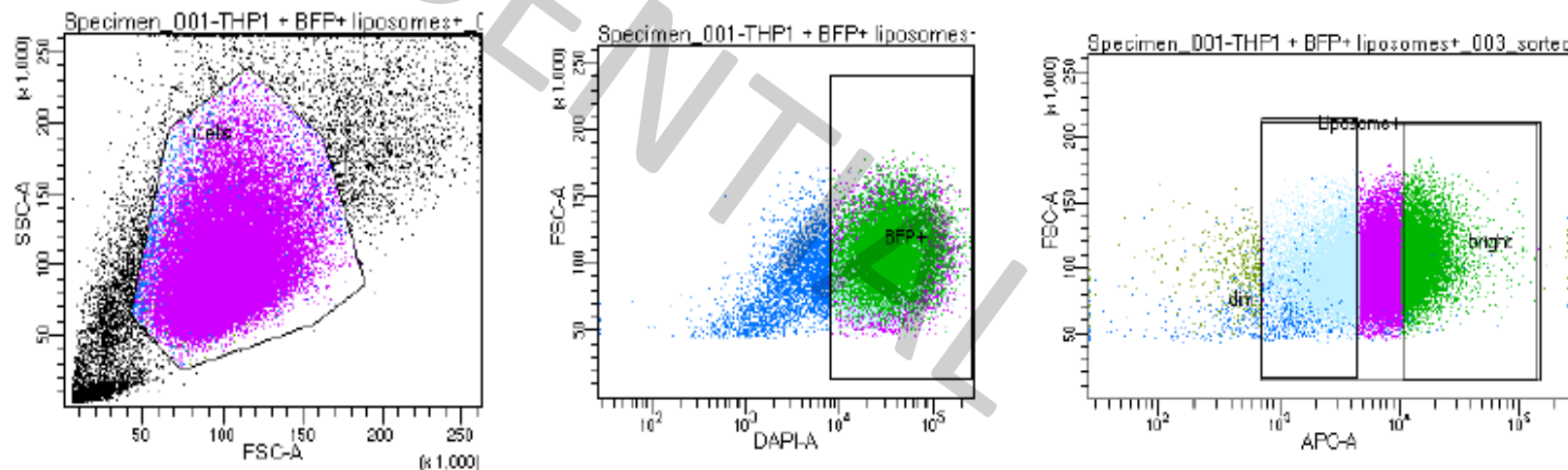
- THP-1 cells transduced with a lentiviral library covering entire human genome
- Low Multiplicity of Infection (MOI) to ensure that cells integrate just one viral particle for gene editing of a single gene
- ~500x coverage

# Identifying receptors for uptake of liposomes by immune cells

**A**



**B**



**C**

| Gene   | KD in     | Significance |
|--------|-----------|--------------|
| LTA4H  | Dimmest   | p < 0.01     |
| IGFBP5 | Dimmest   | p < 0.01     |
| EHBP1  | Dimmest   | p < 0.01     |
| PSMA1  | Dimmest   | p < 0.01     |
| CCR3   | Dimmest   | p < 0.01     |
| SIGIRR | Dimmest   | p < 0.01     |
| MS4A2  | Dimmest   | p < 0.01     |
| TFRC   | Brightest | p < 0.01     |

## Conclusion

- 🕒 Phospholipid choice for liposomes affects immune responses
- 🕒 Phosphatidylglycerol-containing lipid nanoparticles carrying autoantigens can be effective inverse vaccines
- 🕒 CRISPR interference is an unbiased screen to identify receptors involved in nanoparticle uptake

# Acknowledgements

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Bram Slütter





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*shaping tomorrow*

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Contact me: [n.benne@uu.nl](mailto:n.benne@uu.nl)

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