Engineering Tolerance: Acetalated Dextran Microparticles in the Generation of B-regs and T-regs

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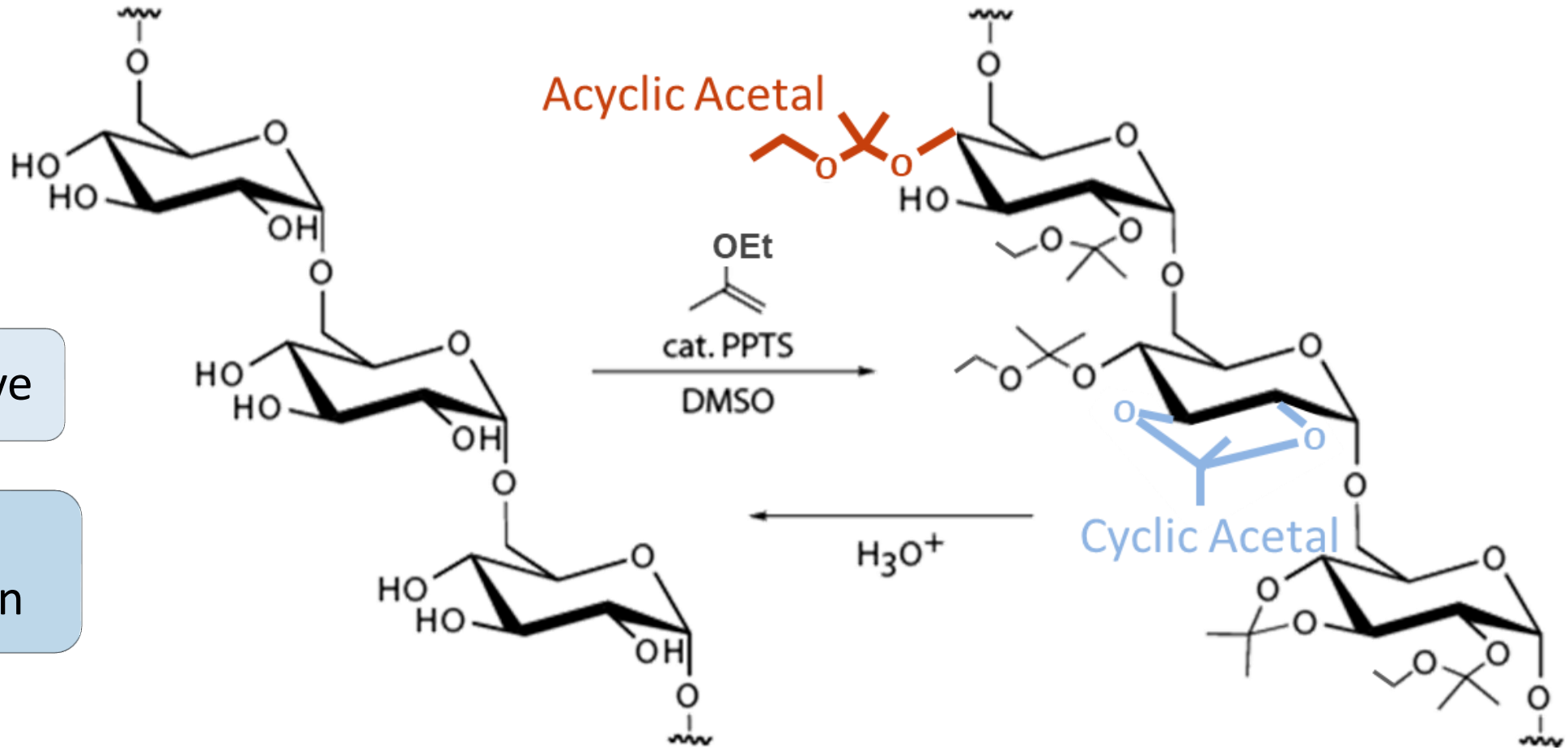
Ainslie Lab @ UNC

UNC ESHELMAN SCHOOL OF PHARMACY

Acetalated Dextran is an Acid Sensitive & Tunable Biopolymer

Acid Sensitive

Tunable Degradation



Dextran (DEX)

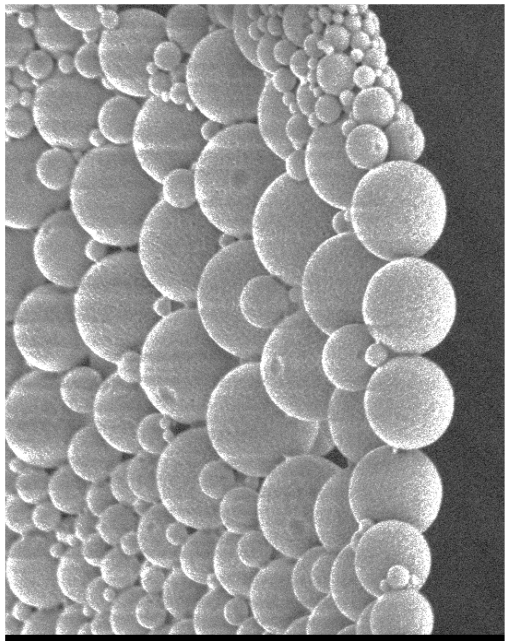
Acetal-modified dextran (Ace-DEX)

Bachelder et al., JACS 2008
Kauffman et al. ACS Appl Mater Interfaces 2012

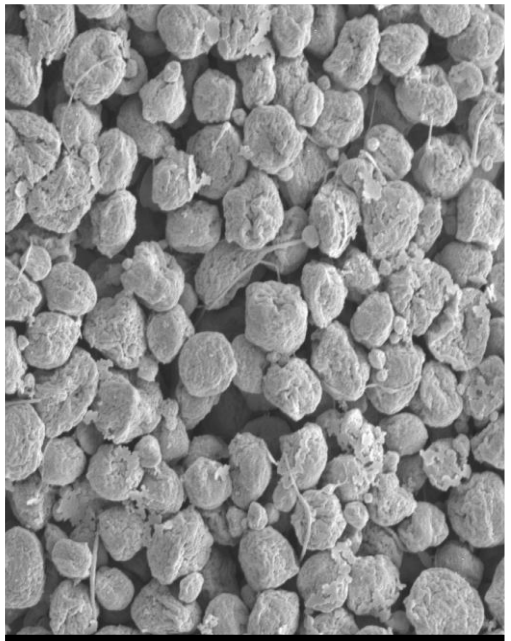
Ainslie Lab Ace-DEX Platforms

Drug Delivery Applications

Immunomodulation



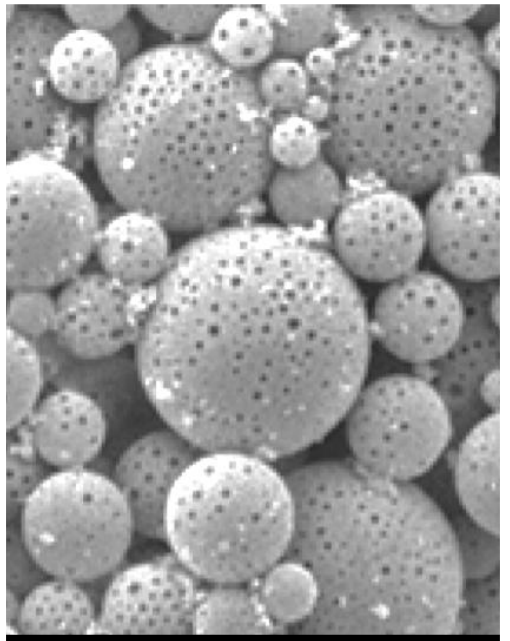
Emulsion Particles



Sprayed Particles

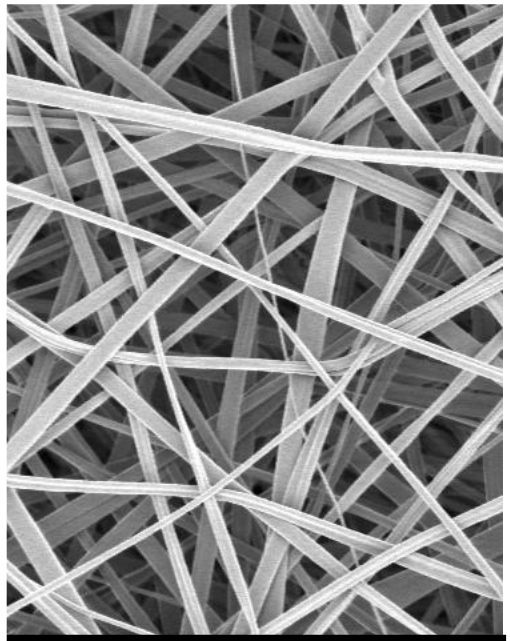
Microconfetti

Pulmonary Delivery



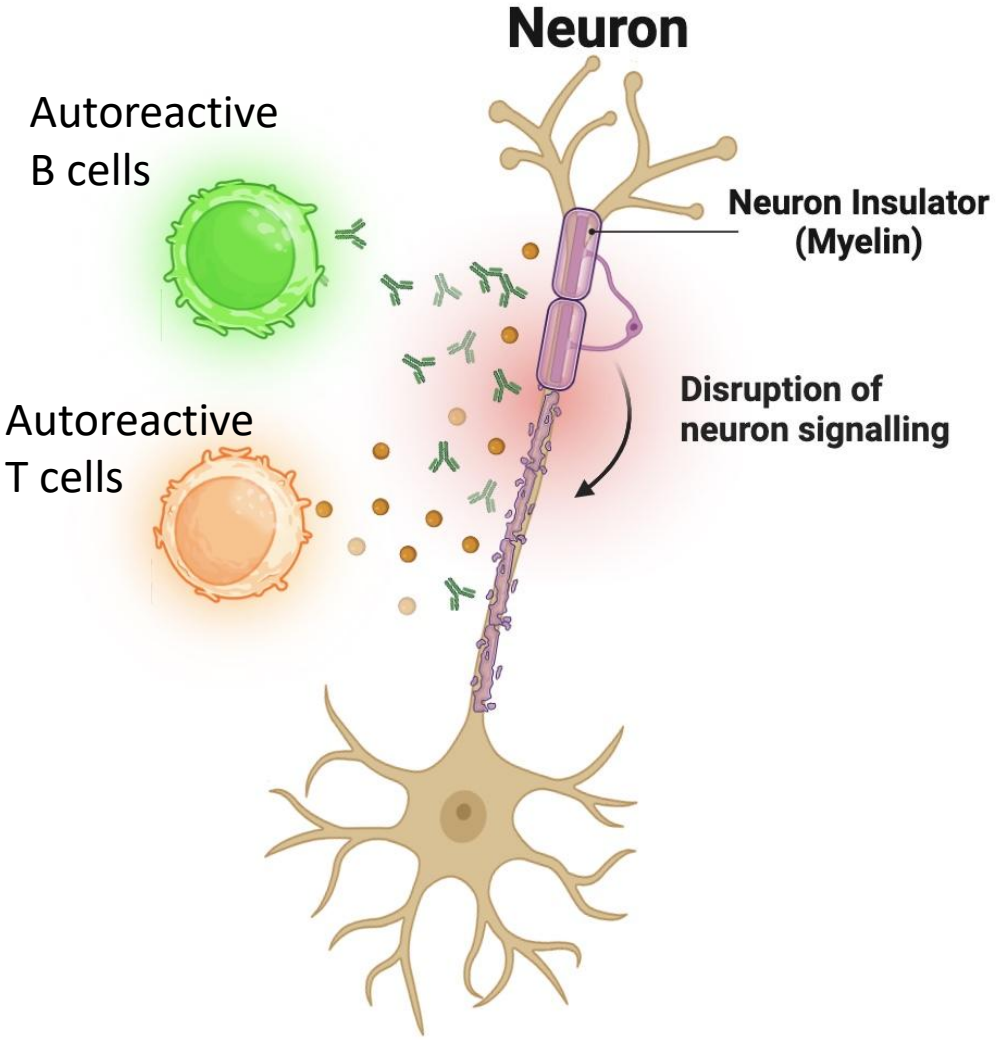
Porous Particles

Stem Cell Delivery



Electrospun scaffolds

Central Nervous System Breakdown in Multiple Sclerosis (MS)



Most common CNS Inflammatory disease

- 350,000 in USA

Damage caused by autoreactive T cell attack myelin and B cells

Results in axonal and neuronal loss

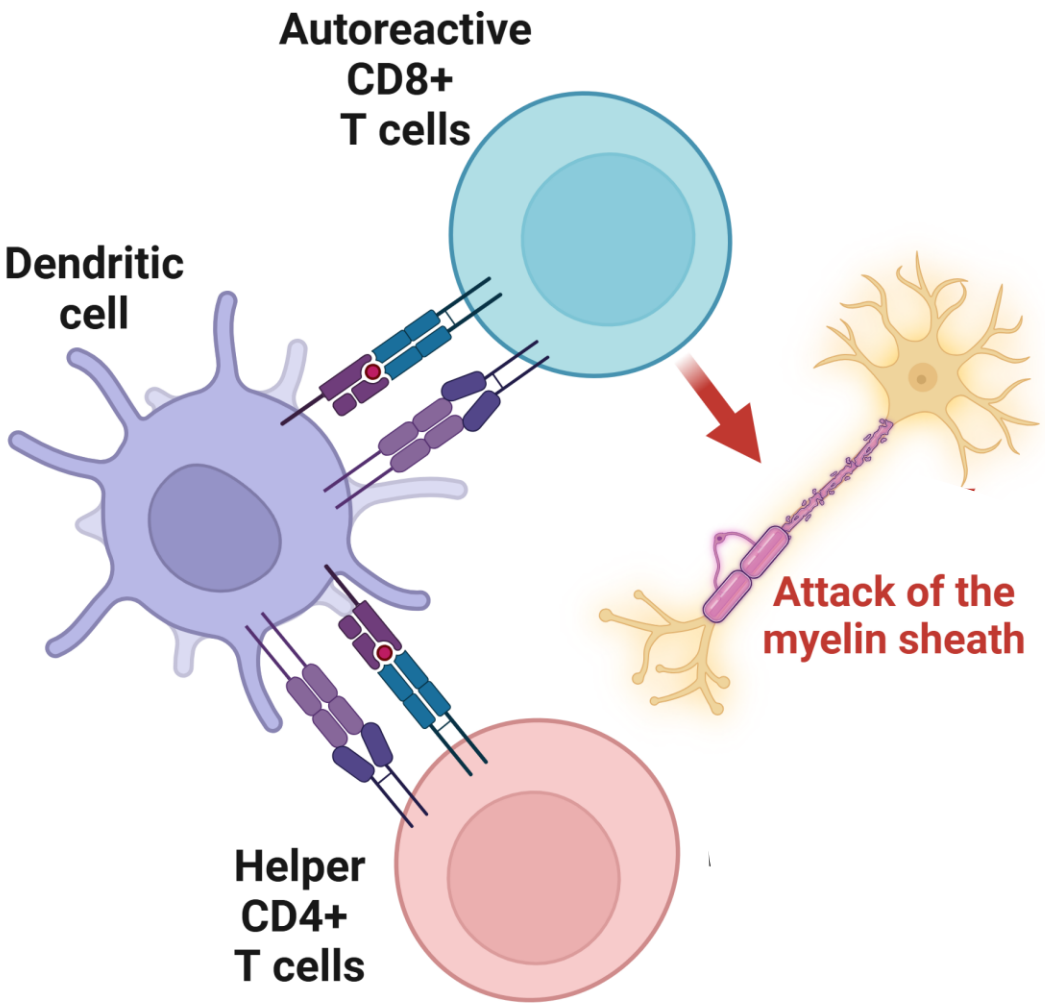
Currently drugs are administered systemically

- Steroids
- Anti-Interferon

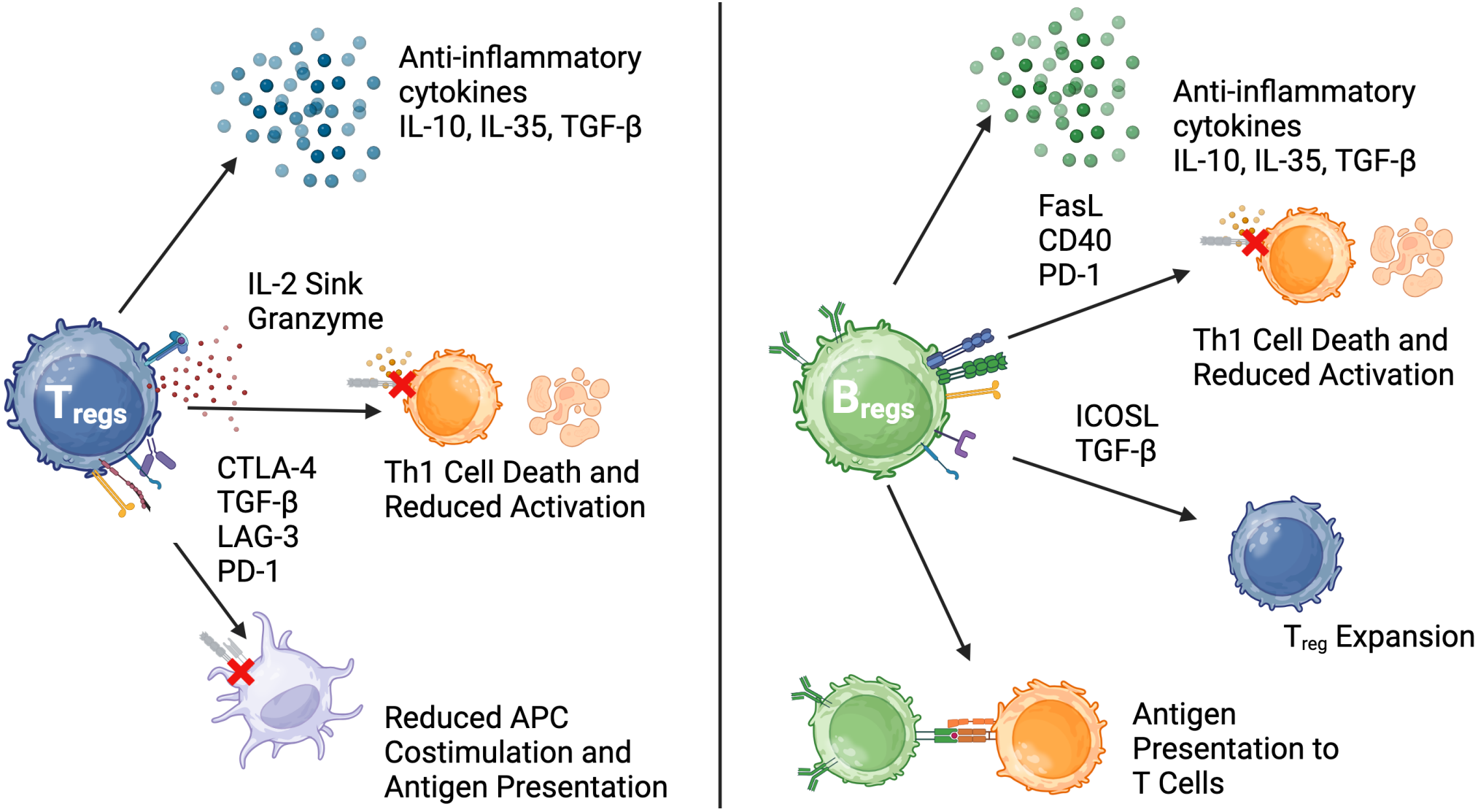
Causes non-specific immune dampening

- Opportunistic infections

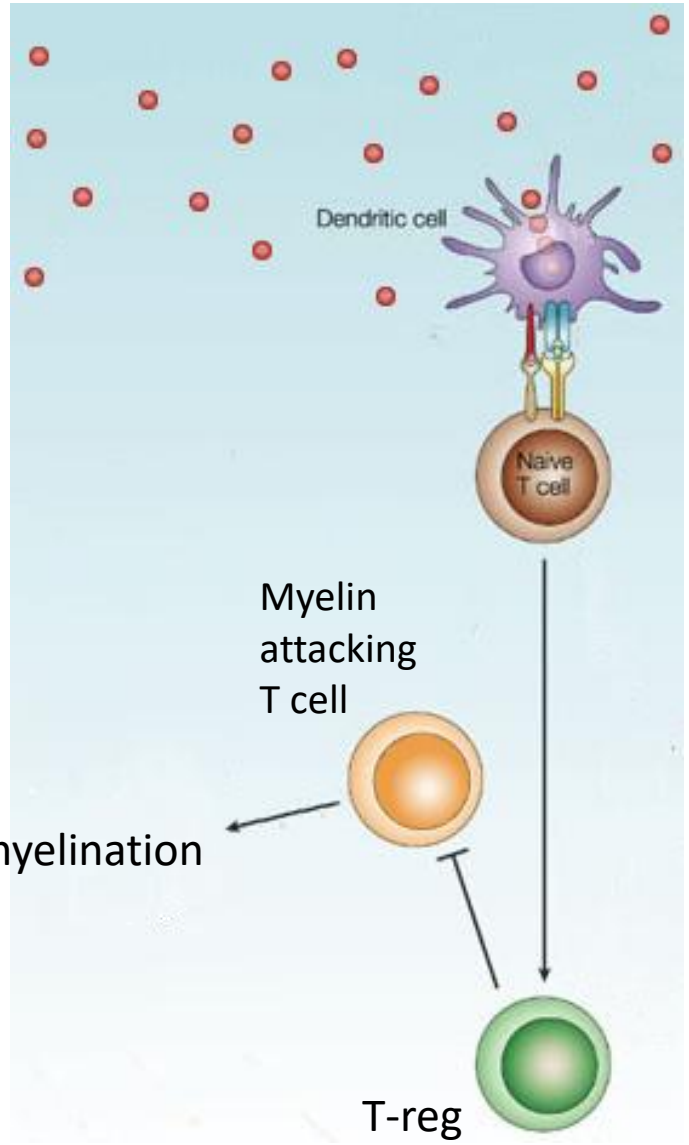
Both Autoreactive T cells and B cells contribute to MS pathogenesis

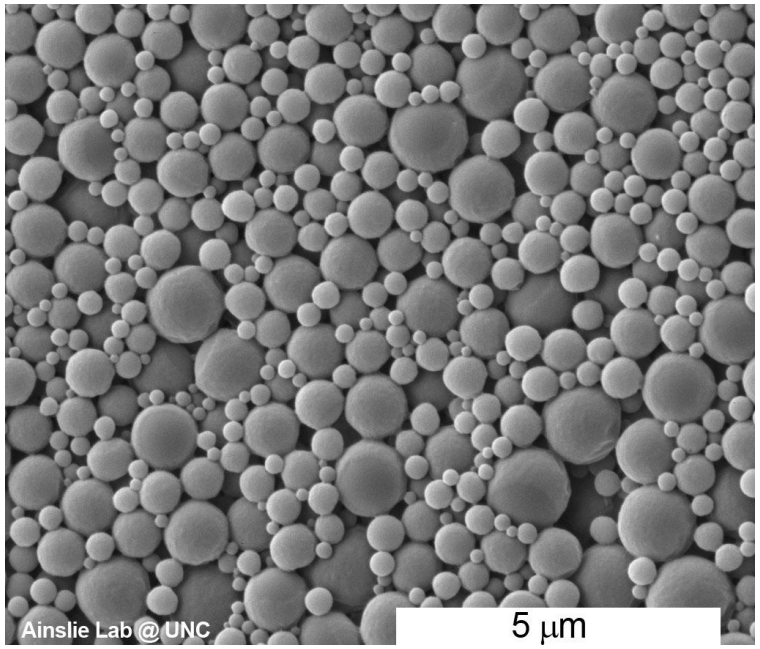


Targeting Antigen-Specific T_{regs} and B_{regs} May Improve Outcomes



Generation of T Regulatory Cells (T_{regs})





A tolerogenic agent (e.g. dexamethasone, rapamycin) is co-delivered with antigen to dendritic cells in Ace-DEX microparticles

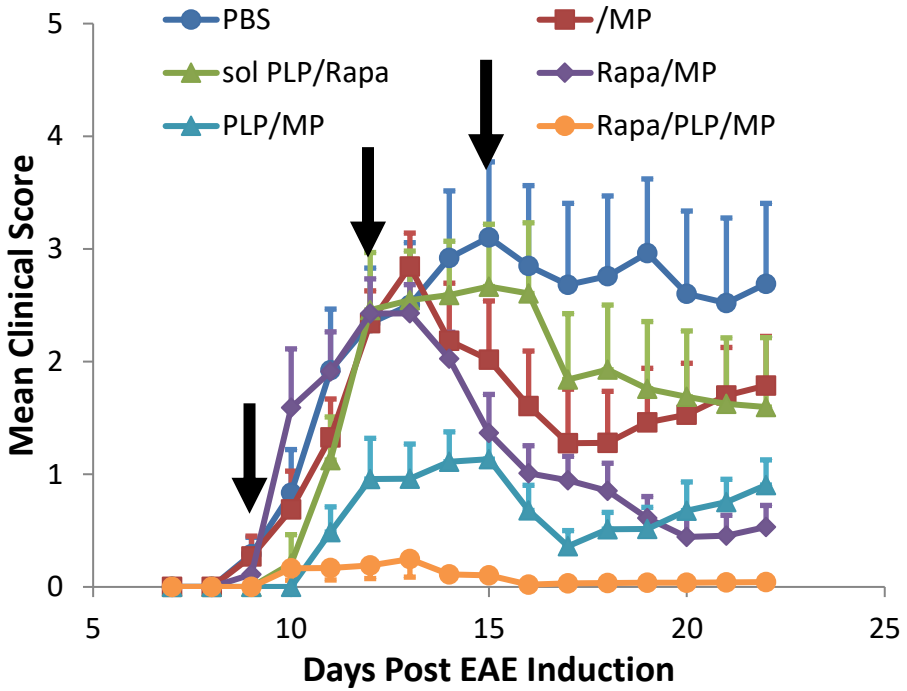
Ace-DEX MPs Have Been Previously Applied to MS Mouse Model: Experimental Autoimmune Encephalomyelitis (EAE)

Relapsing-Remitting Model

Tolerogenic agent: Rapamycin (Rapa)

Peptide epitope: $PLP_{139-151}$ (PLP)

SJL mice (I-A^s)



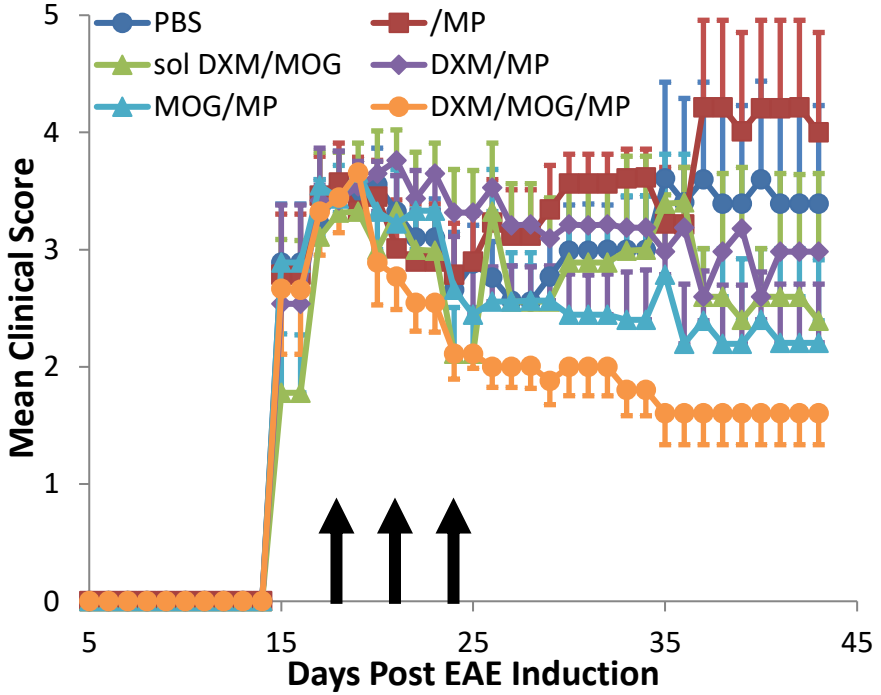
Chen *et al., Adv. Biosys.* 2017

Primary Progressive Model

Tolerogenic agent: Dexamethasone (DXM)

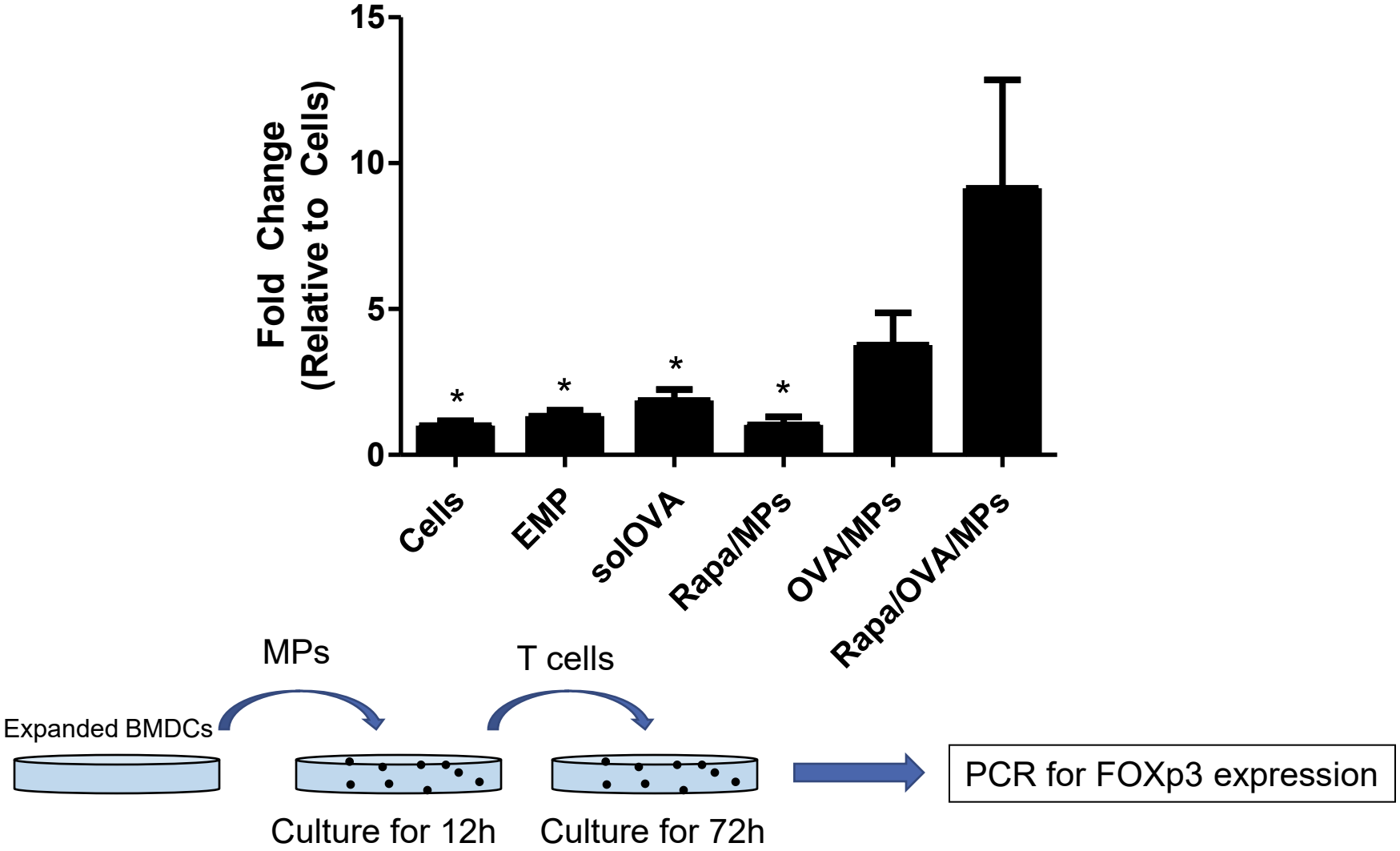
Peptide epitope: MOG_{35-55} (MOG)

C57BL/6 mice (I-A^b)



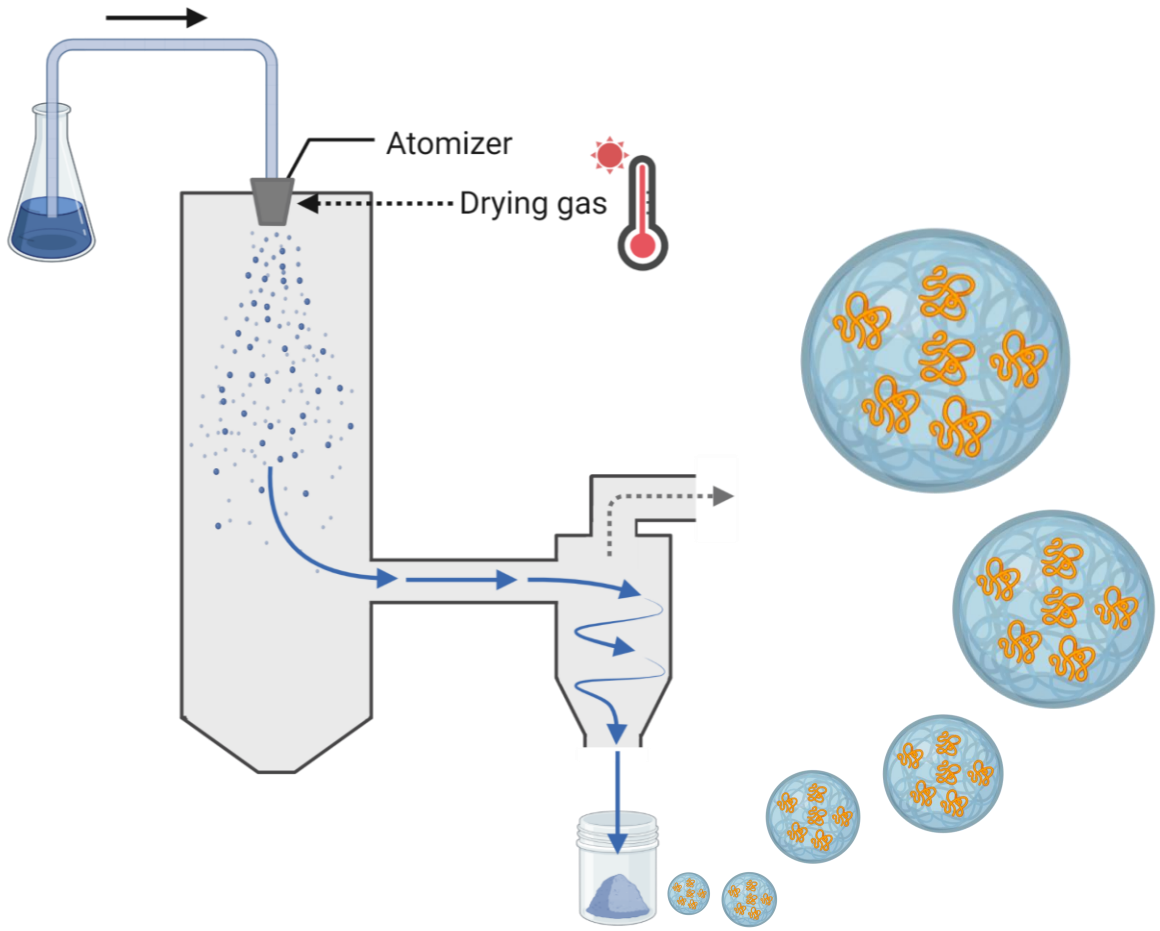
Peine *et al., Mol Pharmaceutics* 2014

In vitro FOXp3 expression in T cells co-cultured with Treated Dendritic Cells



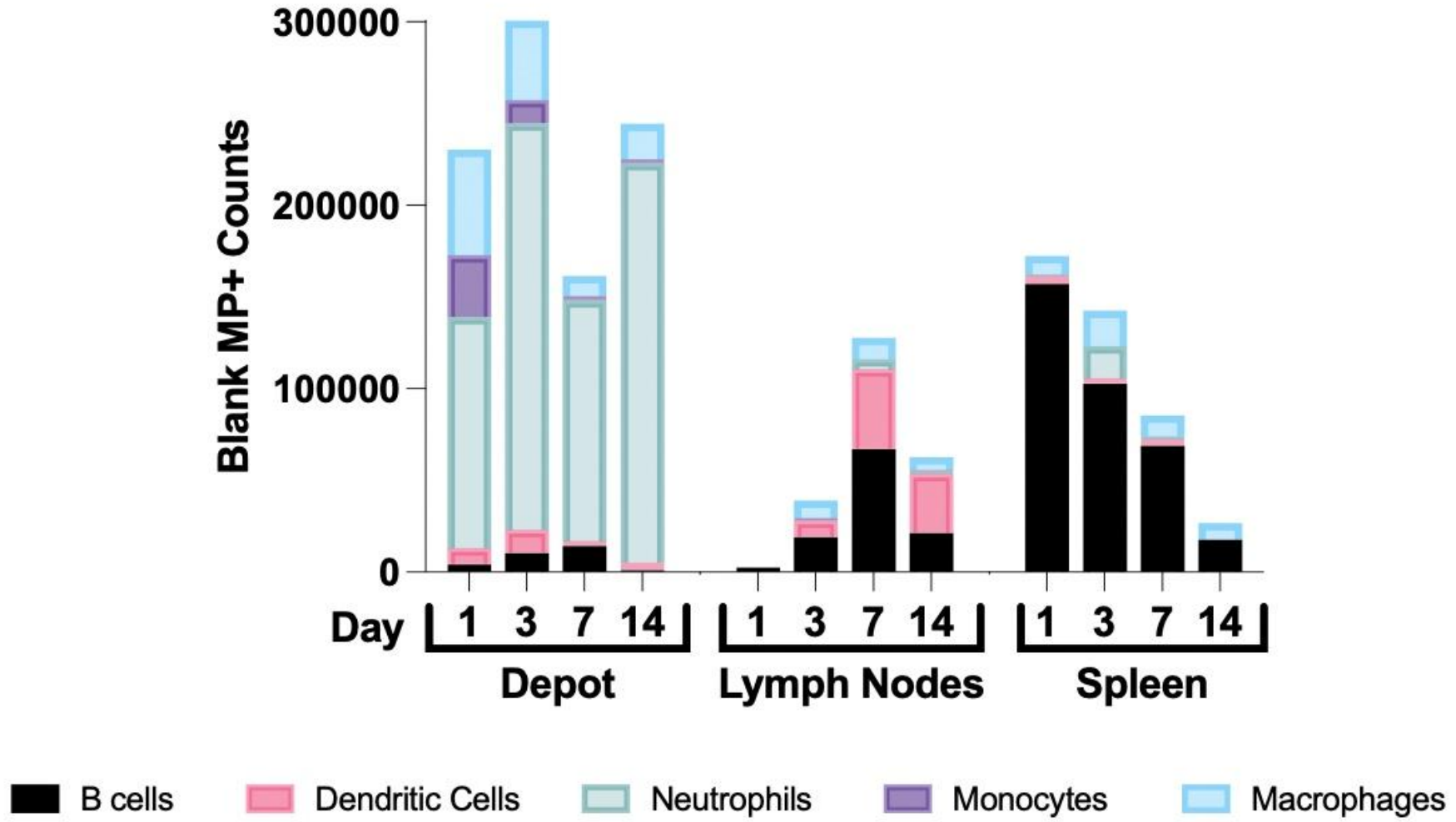
Chen et al. Advanced Biosystems 2017

Spray Dried Ace-DEX MPs versus previous emulsion particles



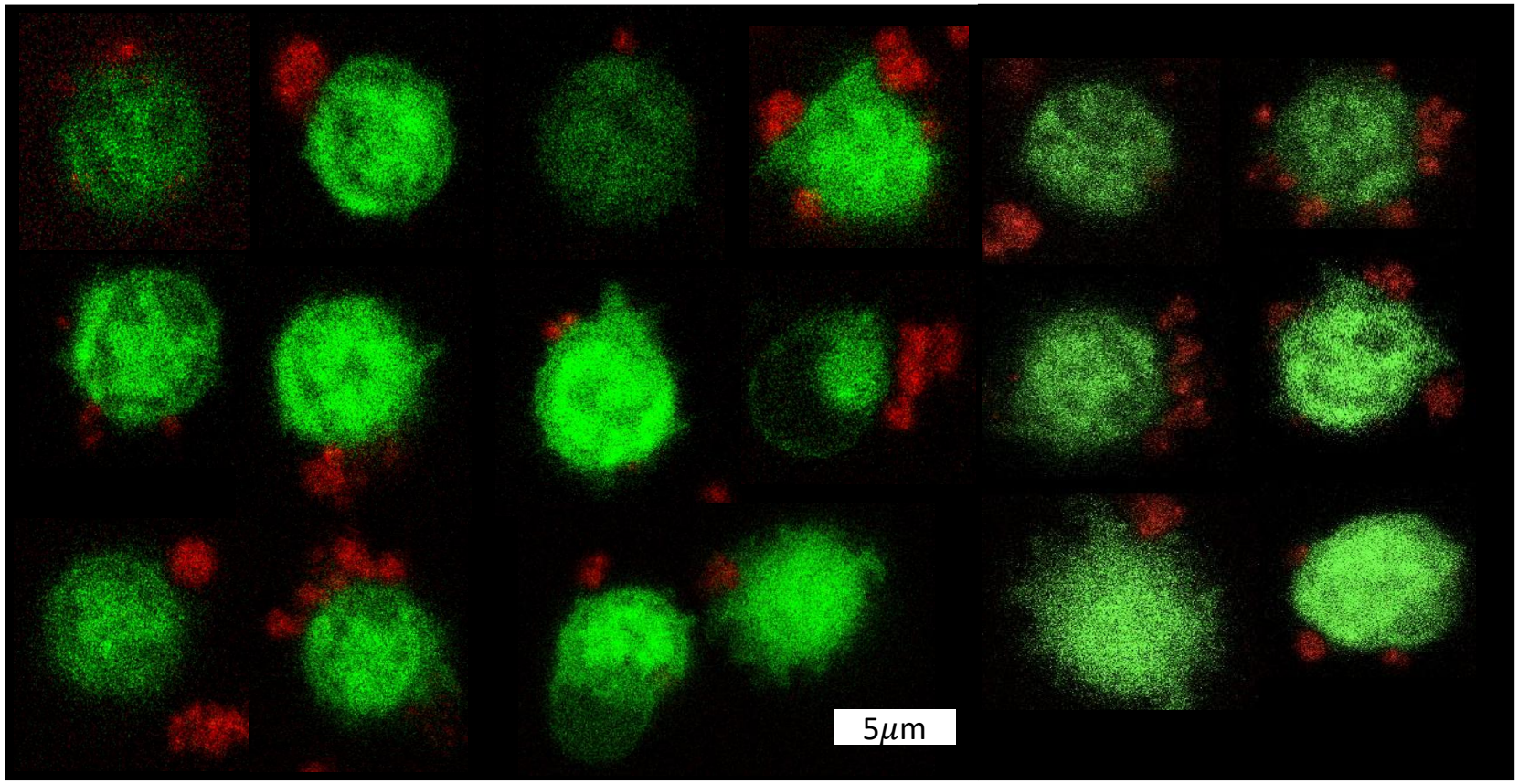
Acetalated Dextran Microparticles (AMPs) encapsulating myelin oligodendrocyte peptide (MOG_{35-55})

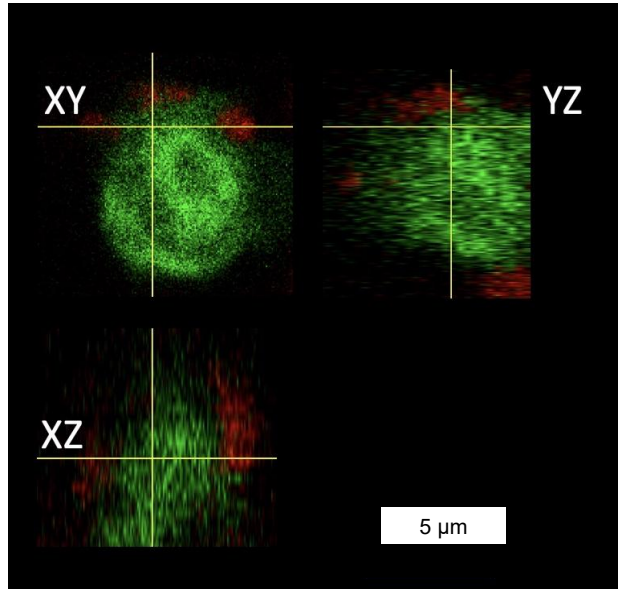
B Cells are MP+ via Flow in the Subcutaneous Depot, Draining Lymph Nodes, and Spleen



Data shown as mean±SEM

Acetalated Dextran MPs (AMPs) Surface-Associate with B Cells

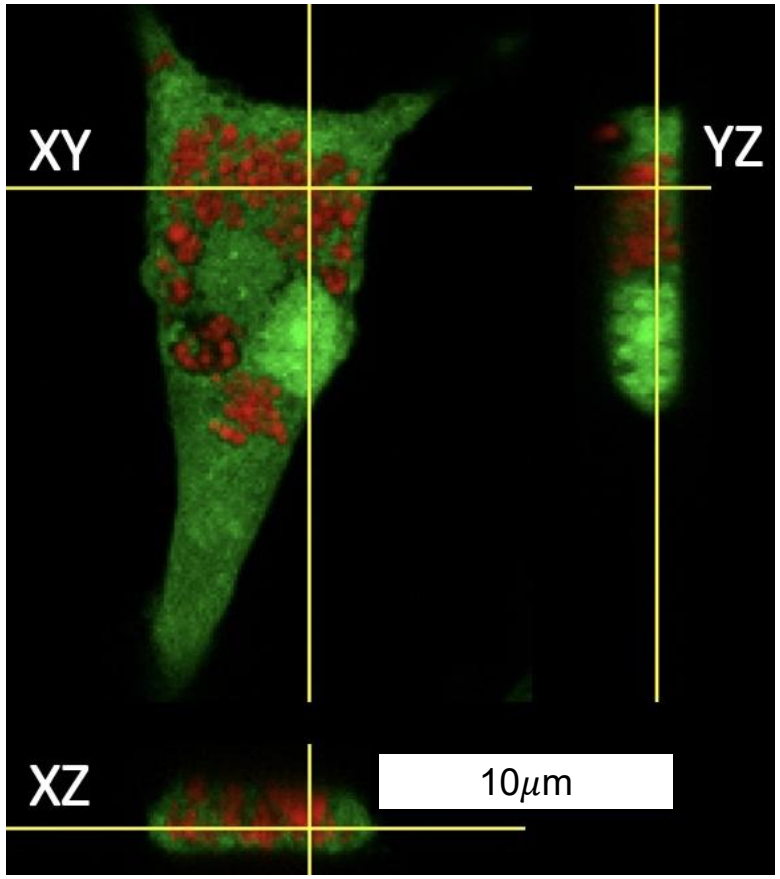




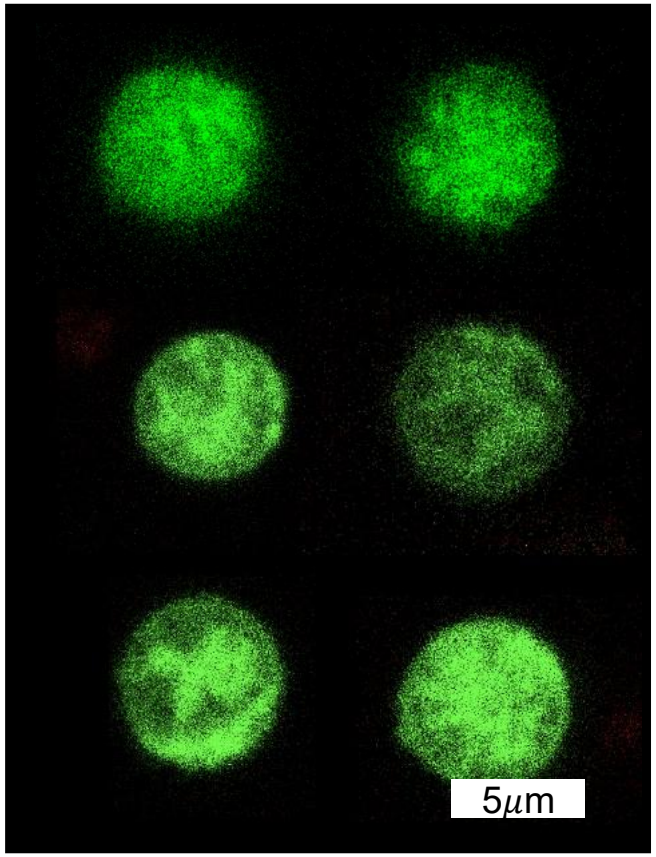
Red is DiD labeled AMPs. Green is CellTracker Green

Lukesh et al. Nat Comm *in revision*

Dendritic Cells Internalized AMPs, While T Cells Do Not



Dendritic Cell



T Cells

Red is DiD labeled AMPs. Green is CellTracker Green

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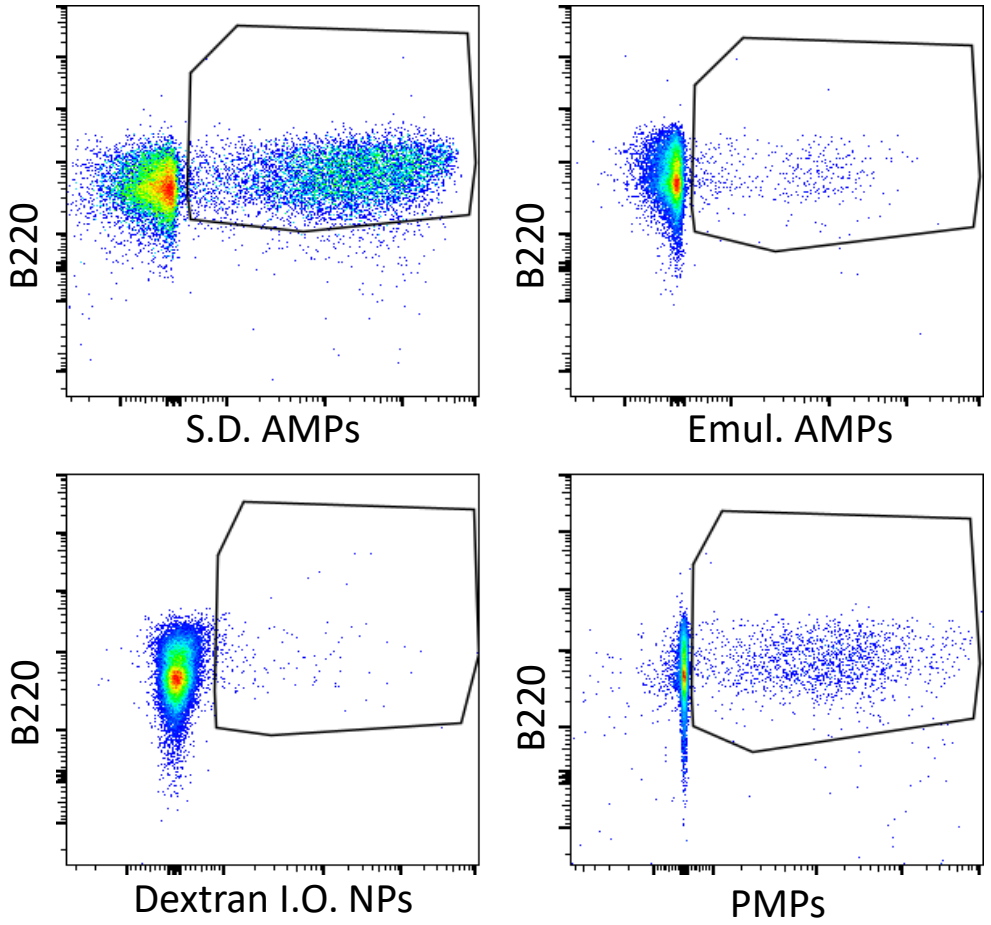
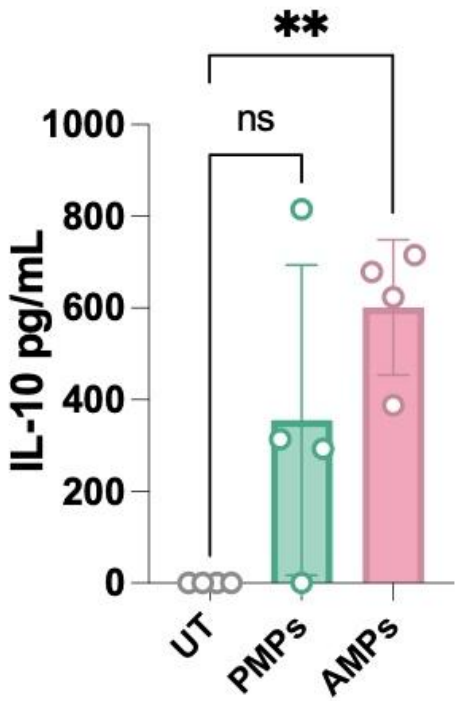
B cell Association was Unique to Spray Dried AMPs and Led to IL-10





EMP = empty
S.D. = spray-dried
Emul = emulsion
I.O. = iron oxide
PMPs = PLGA MPs.

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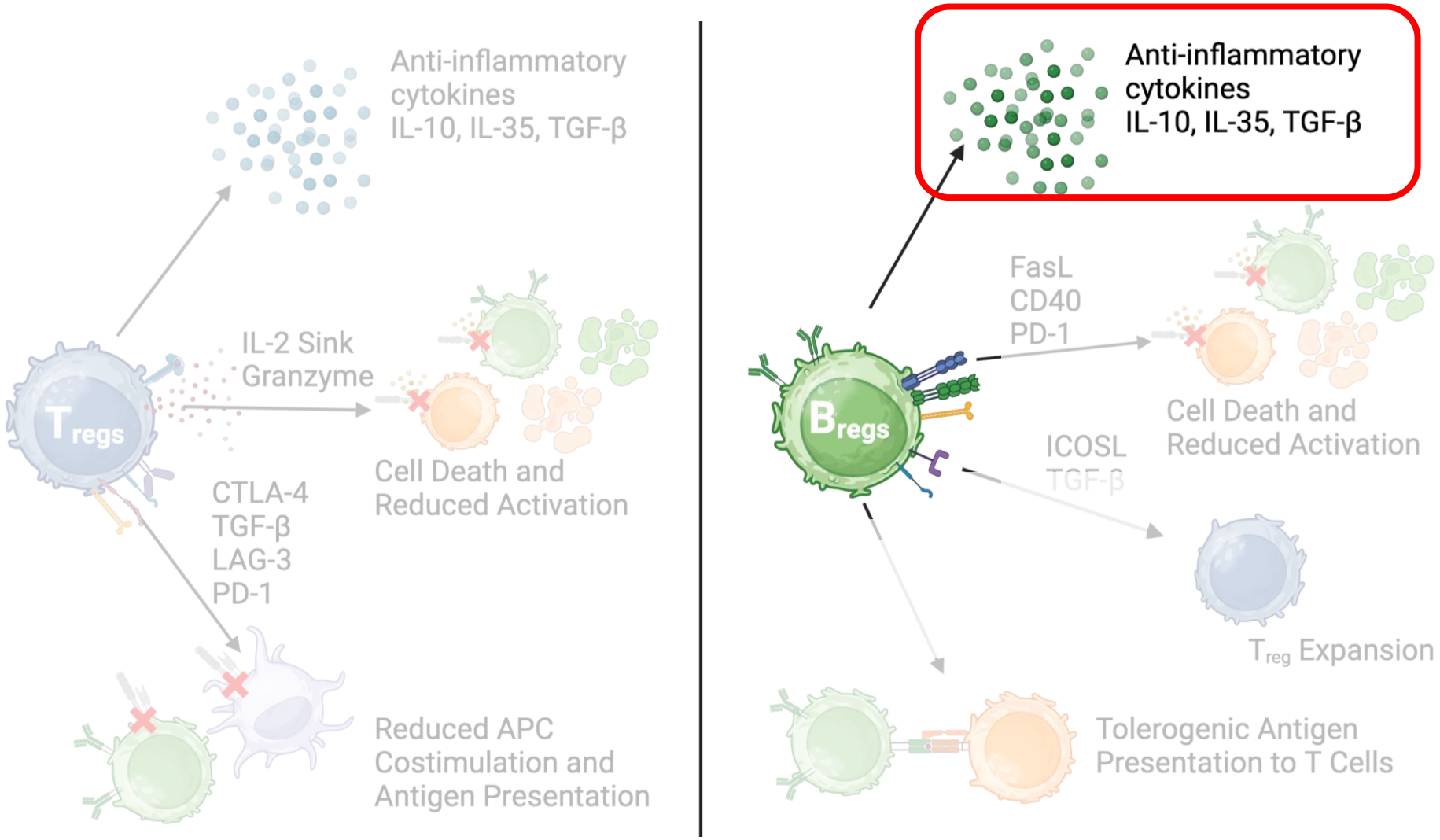
Conclusion: AMPs Associate with B Cells Leading to IL-10 Production



Can AMPs Facilitate B Cell Antigen Presentation to T Cells Tolerogenically?

B Cells Tolerogenically Present Antigen to T Cells

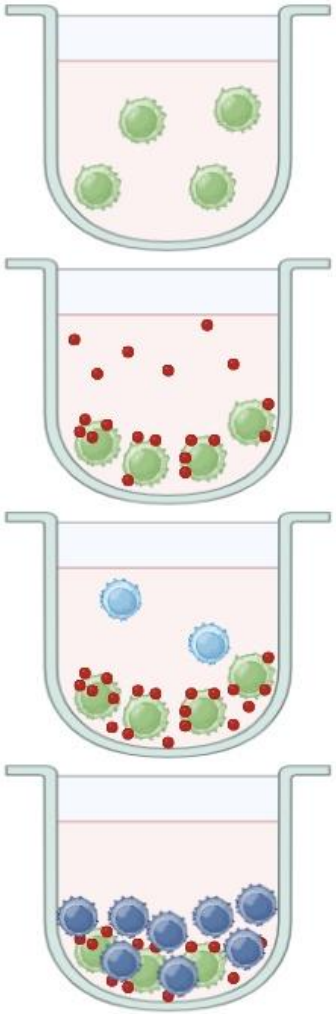
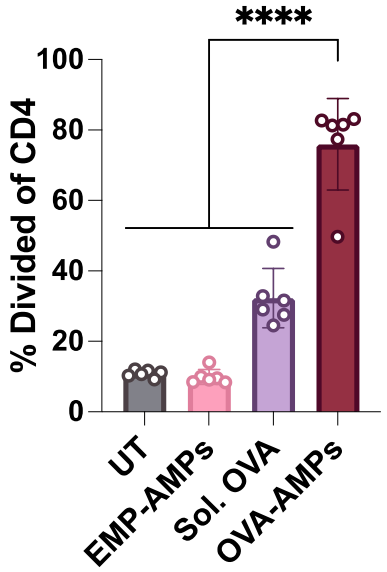


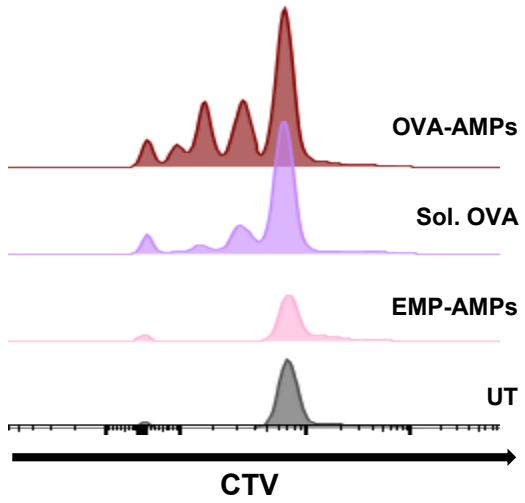
Plate B cells
for 30min

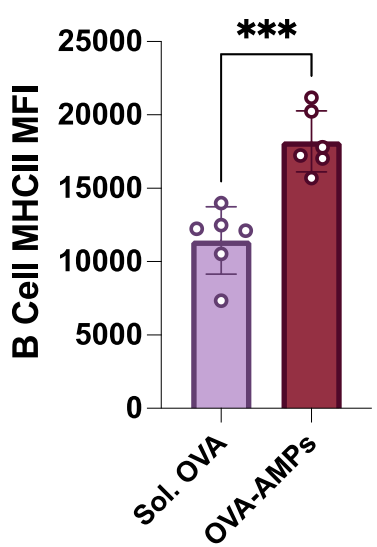
Treat B cells with
AMPs for 2hr

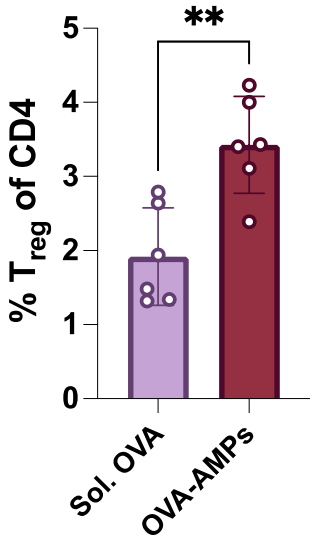
Add stained T
cells for 72hr

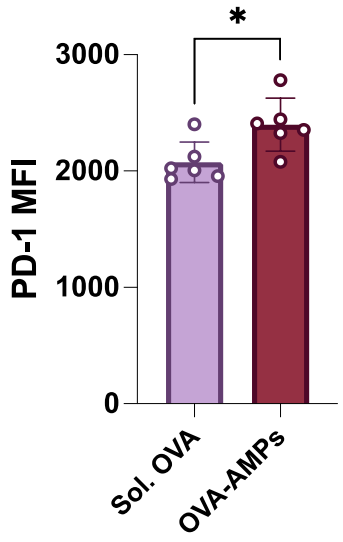
Assess T cell proliferation,
phenotypes, and cytokine
production











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AMPs Reduce Cytokine Production in B-T Cell Co-Culture

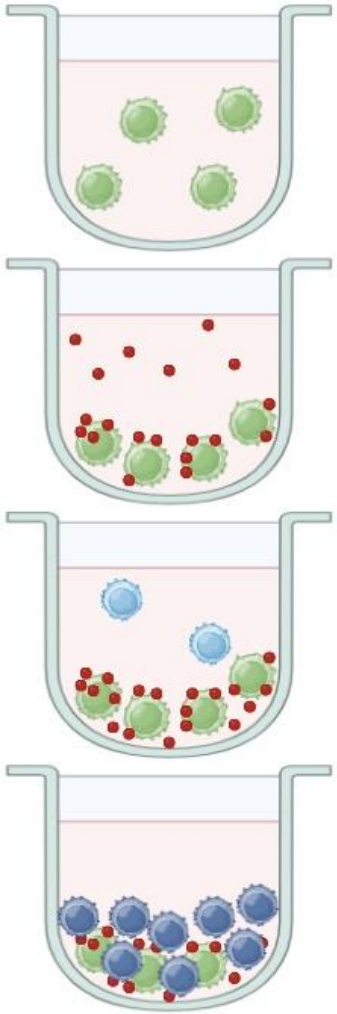

Plate B cells for 30min

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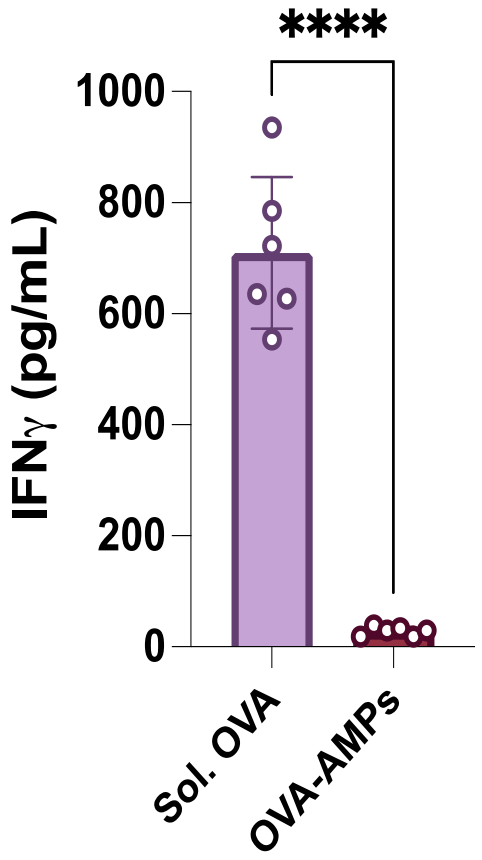
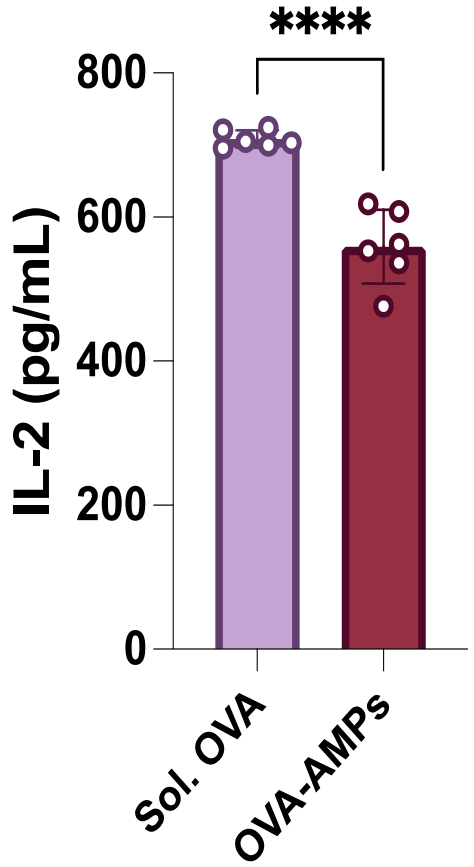
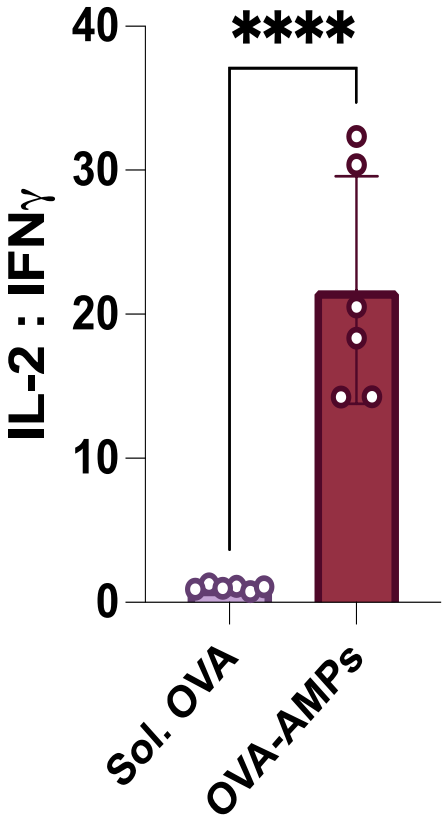
Add stained T cells for 72hr

Assess T cell proliferation, phenotypes, and cytokine production







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AMPs Increase Tr1 Cells in B-T Cell Co-Culture

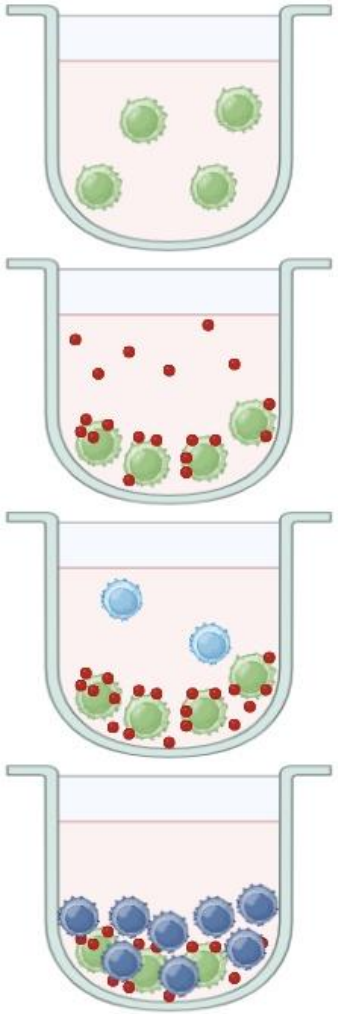

Plate B cells for 30min

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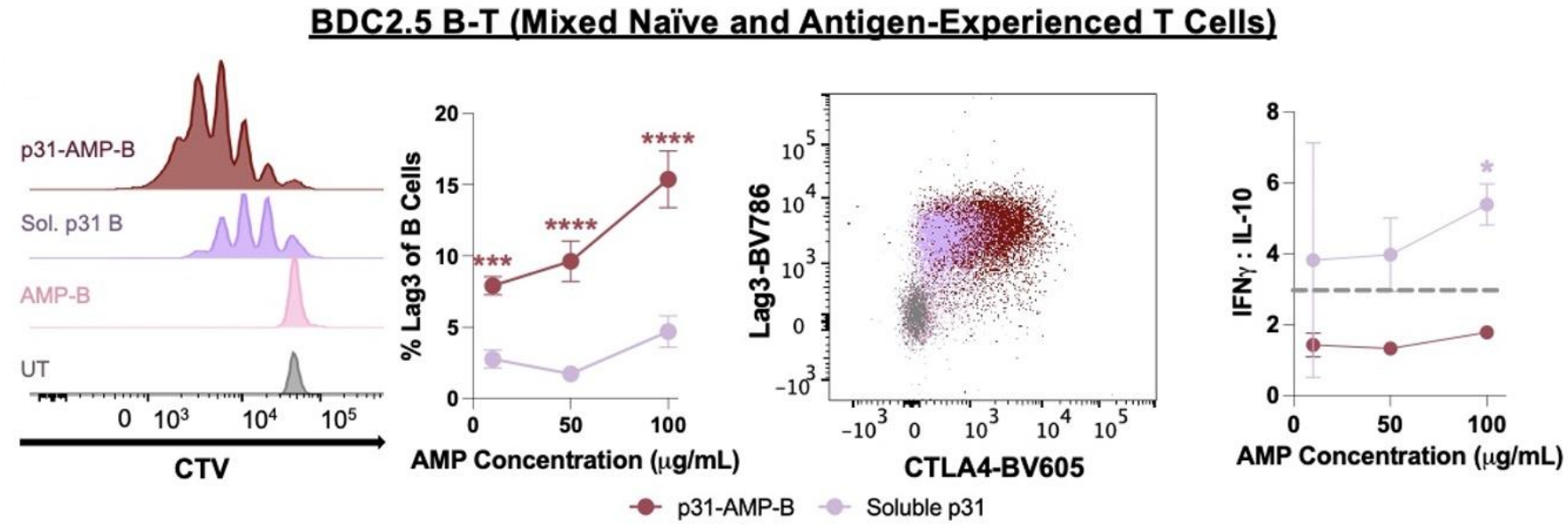
Assess T cell proliferation, phenotypes, and cytokine production



Lag3 and CTLA4 expression and IL-10 production is indicative of Type-1 regulatory T cells (Tr1) that are FOXP3^{-} regulatory T cells

Proliferation not observed with AMP-Dendritic cell and T cell co-cultures

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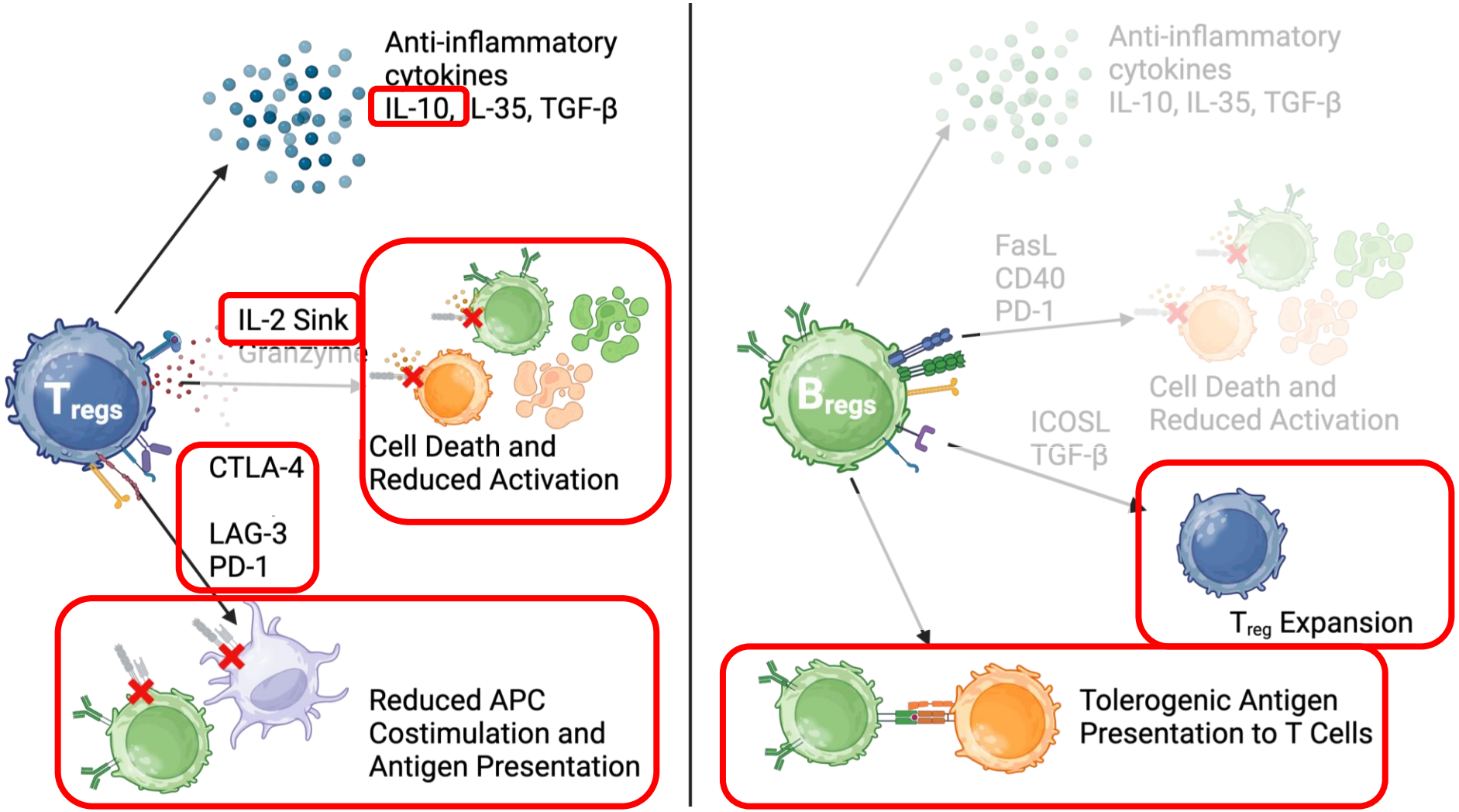
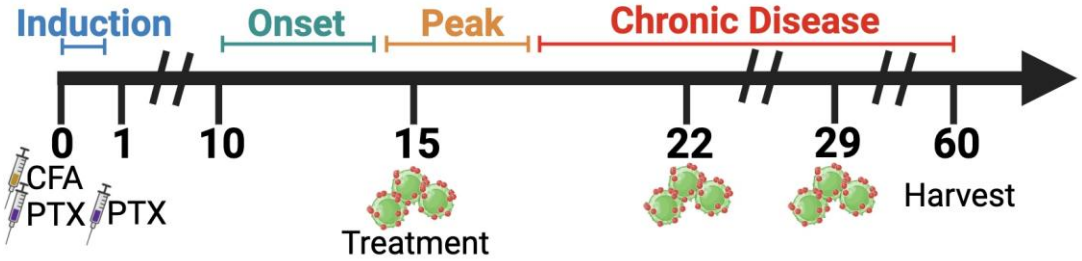
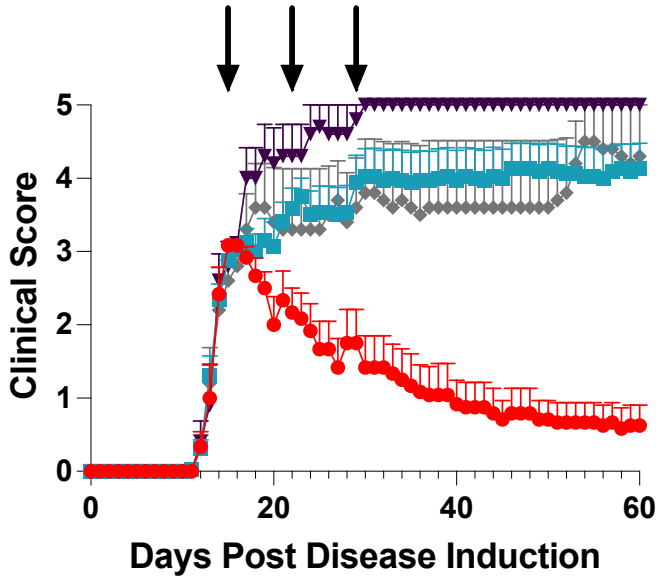
Conclusion: AMPs Led to Multiple Regulatory Mechanisms In Vitro



Can AMP-Bs Lead to a Therapeutic Effect in EAE when Loaded with a MOG_{35-55}?

MOG-AMP-B Treatment Reduces EAE Clinical Score





Clinical Score Guide:

5 – Moribund
4 – Full hind limb and partial front limb paralysis
3 – Full hind limb paralysis
2 – Partial hind limb paralysis
1 – Limp tail
0 - Healthy

PBS MOG-AMP-B AMP-B MOG Controls

MOG controls = pooled group of MOG-AMPs administered i.p. and s.c. and MOG-PMPs with B cells. (n=16 total)

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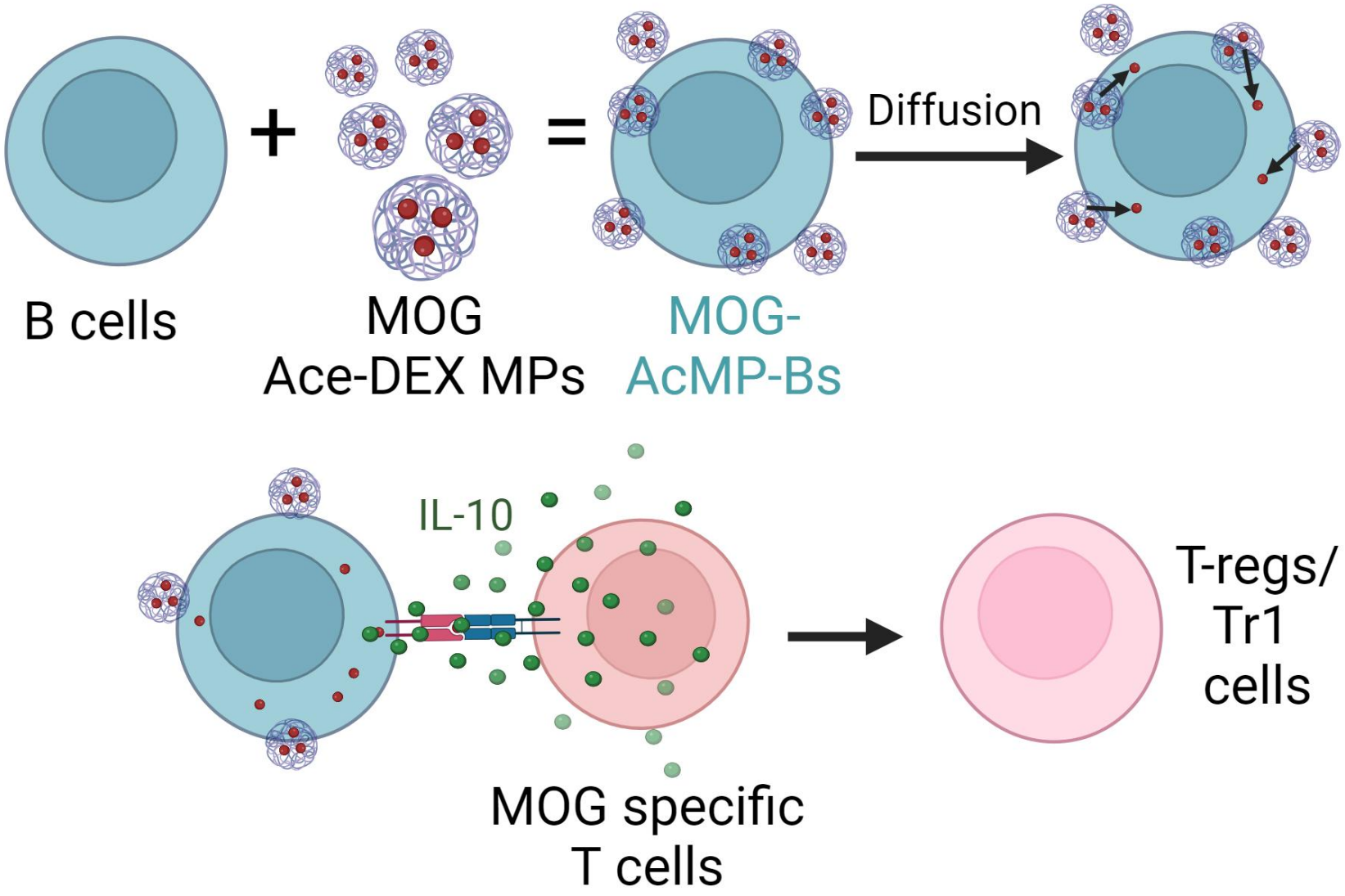
Our likely mechanism of AMP mediated tolerance



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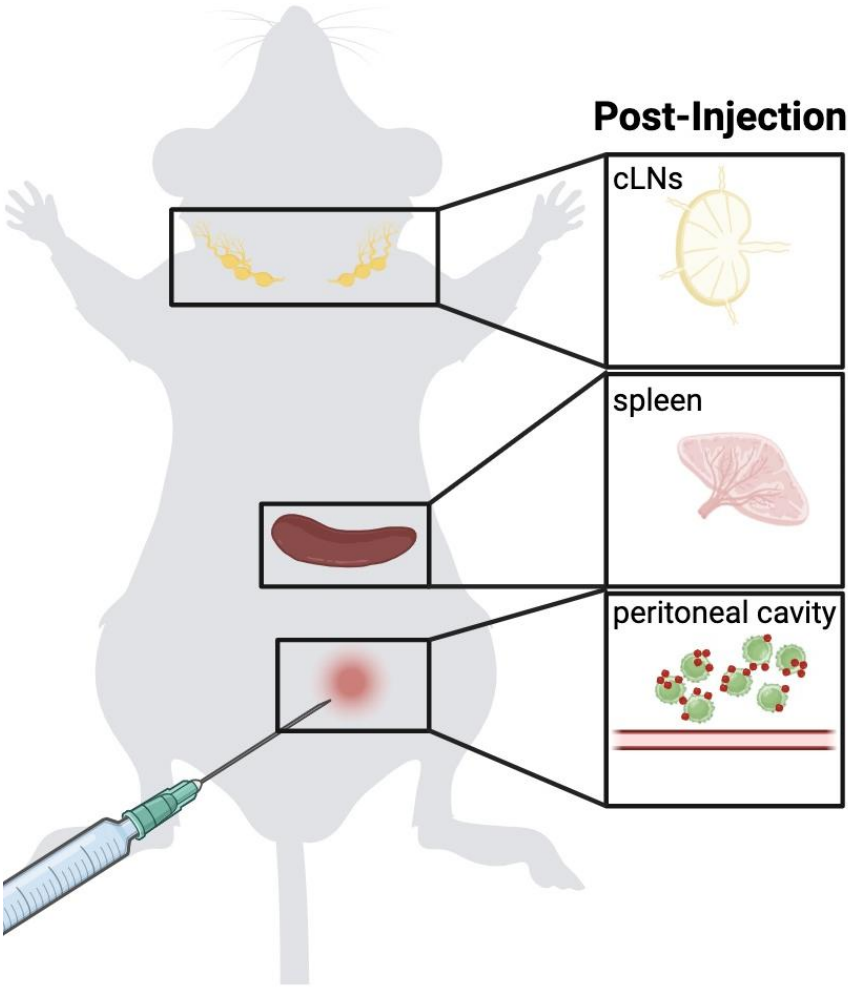
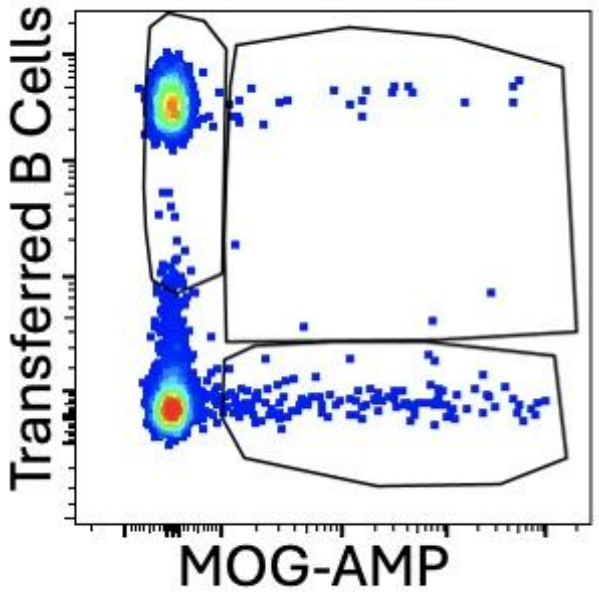
How do MOG-AMP-Bs Traffic Post-Injection in EAE Mice?

One Day Post-Injection, MOG-AMPs Separated from B Cells in EAE Mice





(Collected from the injection site on day 1)

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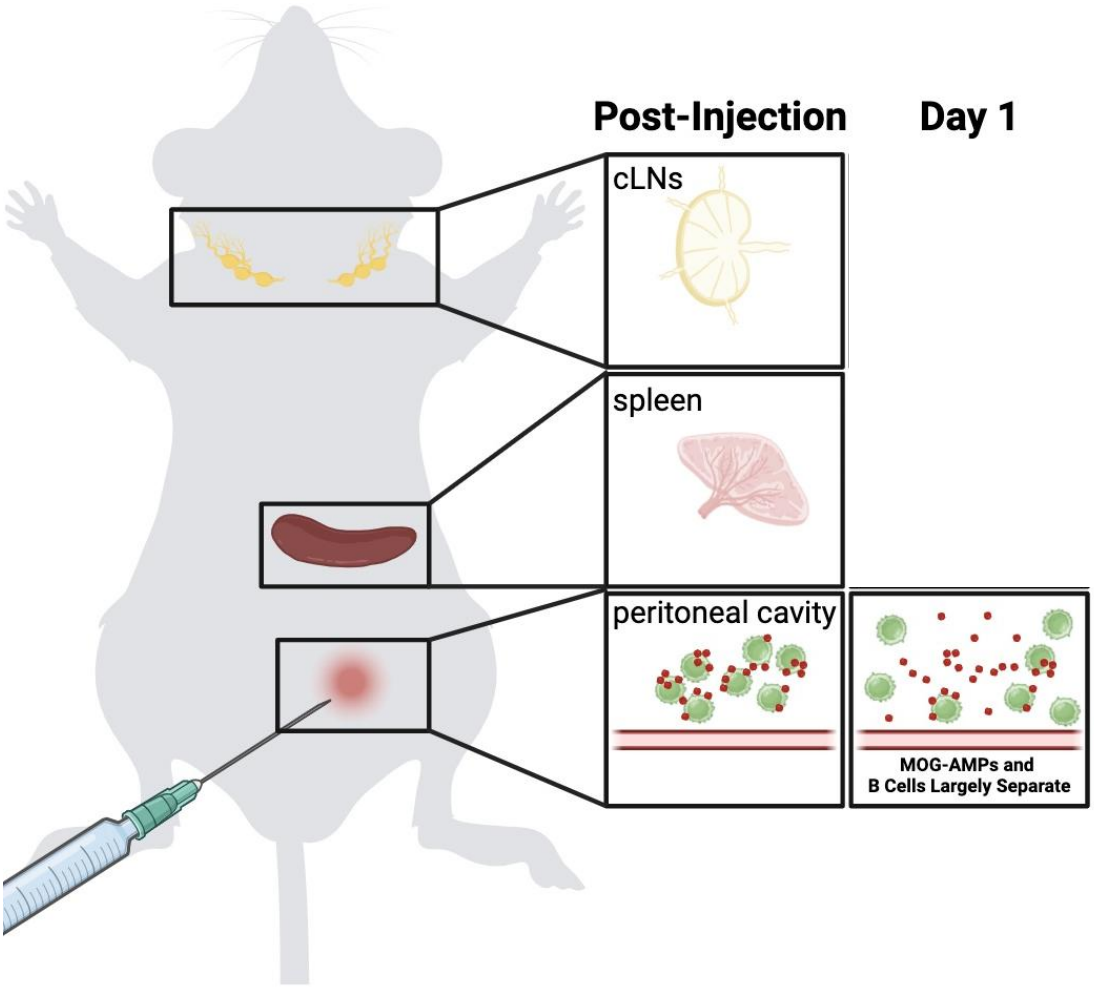
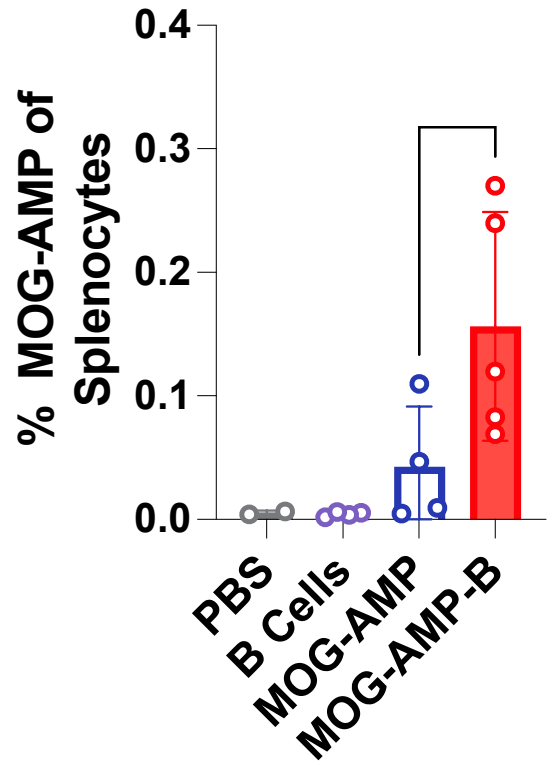
AMPs Primarily Trafficked to Spleen by Day 1





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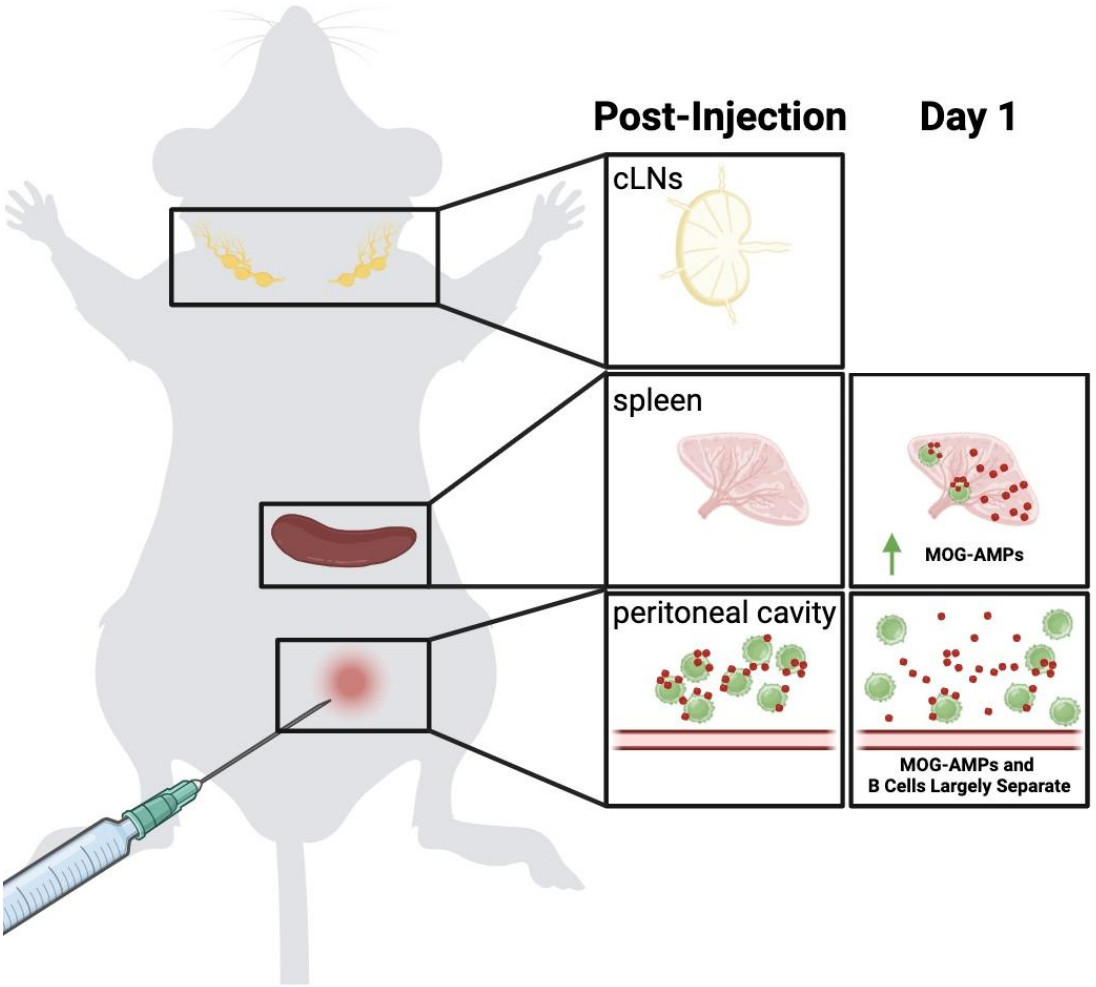
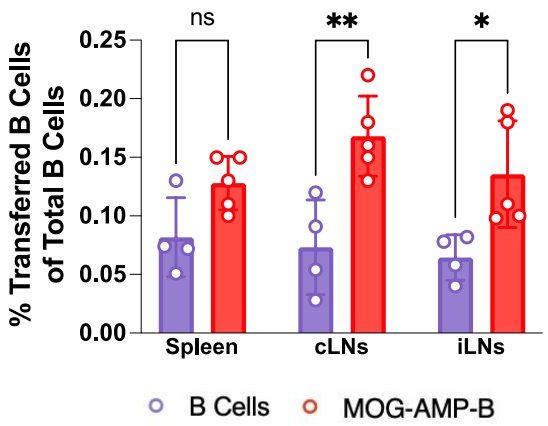
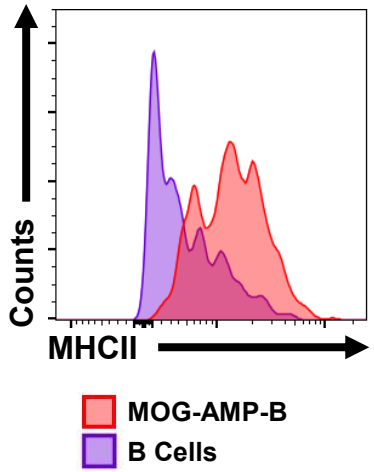
B Cells Trafficked to Cervical Lymph Nodes with Increased MHCII by Day 3







MOG-AMPs have primed B cells for accumulation in the disease-relevant lymph nodes.

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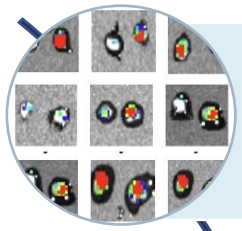
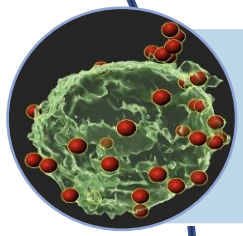
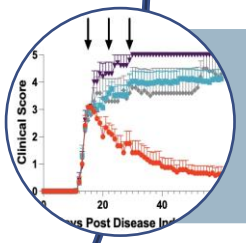
Overall Conclusions



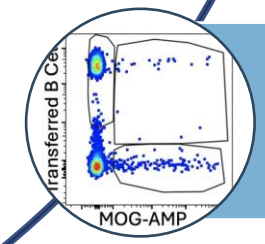
Acetalated dextran microparticles (AMPs) associate to B cells.



AMPs facilitate B cell antigen presentation to T cells tolerogenically.



MOG-AMP-Bs led to a therapeutic effect in EAE.



MOG-AMPs and B cells separate post-injection and traffic to the spleen and cLN respectively.

Acknowledgements – Ainslie Lab

Tolerogenic Vaccines and Therapies

- Drs. Eric Bachelder,
- Nicole Rose Lukesh, George (Brian) Barbery, Kiersten Clark
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NIH NATIONAL CANCER INSTITUTE

NIH National Institute of Diabetes and Digestive and Kidney Diseases

NIH National Institute of Allergy and Infectious Diseases

CIVICs Collaborative Influenza Vaccine Innovation Centers