The Adoptive T cell Transfer Colitis Model in Research and Drug Discovery

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Mouse Models as Tools for IBD Research

- Model Mechanism
  - Is the disease mechanism of the model compatible with the experimental goal?
- Model Validity
  - Predictive Validity
  - Face Validity
  - Construct Validity
- Reproducibility and Variability

"Every in vivo model has its strengths and weaknesses and the key to robust scientific investigation remains the selection of the correct model for the scientific question under inquiry with focus on mechanistic questions rather than direct translatability to efficacy in the clinic."

Pizarro, et al. Inflamm Bowel Dis. 2019;25(Suppl 2):S5-S12.

Constraints:

- Experimental
  - Efficacy vs. mechanistic
  - Animal availability
- Biological
  - Genetics
  - Breeding performance and health
  - Ability to produce cohorts
- Timeline and Budget
- Legal (Licensing)

Inflammatory Bowel Disease (IBD) Mouse Models

	Induced				Spontaneous	
Model	**DSS**	**TNBS/ oxazolone**	**Adoptive T cell Transfer**	**Anti-CD40**	**MDR1A KO**	**IL10 KO**
Recommended strain(s)	**C57BL/6 (B6, B6JBOM)**	**SJL, BALB/c**	**Rag2 knockout (RAGN12) at MPF health standard with C57BL/6 donors**	**Rag2 knockout (RAGN12)**	**Mdr1a knockout (MDR1A)**	**Il10 knockout (BALB/c background 15660)**
Alternative strain(s)	**Inbred strains:** BALB/c (BALB, BALJBO), SJL, C3H **GEMs:** NFκB-RE-luc (10499), Nod2 (16476) **Humanized immune system mice:** huNOG, huNOG-EXL	**Inbred:** C3H **GEMs:** NFκB-RE-luc (10499)	**Paired strains (donor / recipient)** BALB/c (BALB or BALJBO) and C.B-17 scid (CB17SC) BALB/c (BALB or BALJBO) and Rag2 knockout (601)	C.B-17 scid (CB17SC)	N/A	Il10 knockout (C57BL/6 background 16006)
Typical study duration	1-2 wk 2-4 mo	1-2 wk	5-10 wk	1-2 wk	1-3 mo	2-6 mo
Predictive validity*	+	+	++	++	+++	+++
Face validity*	+/-	+	+	+	++	++
Construct validity*	-	+/-	-	-	+/-	++/-
Available under Taconic's standard terms or GEMs label license†	✓					

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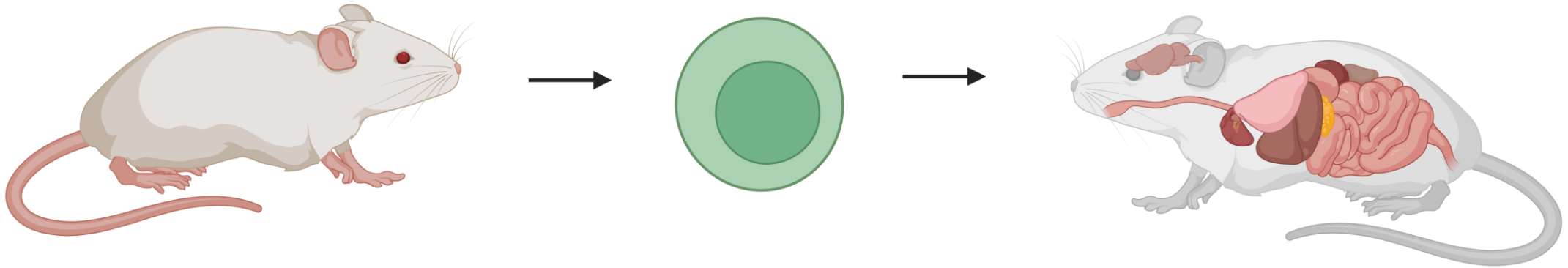
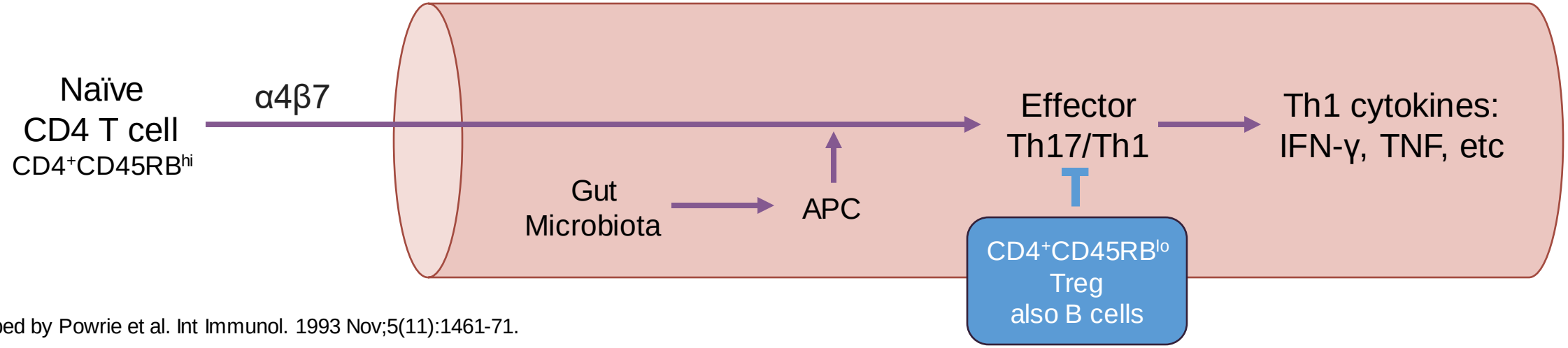
Adoptive T cell Transfer Colitis Model



Syngeneic Donor

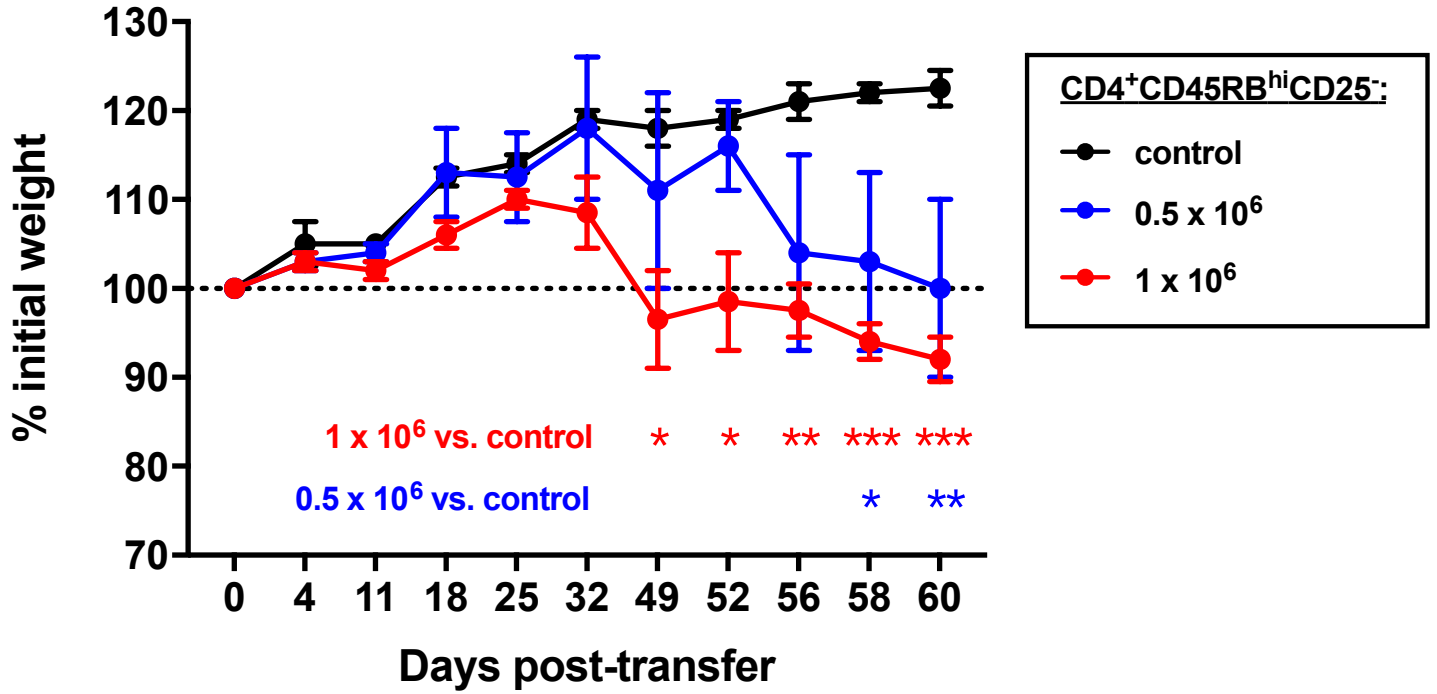
Naïve T cell
(e.g. $CD4^{+}CD45RB^{hi}$)

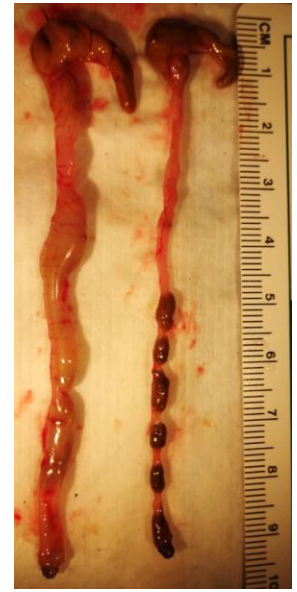
Lymphopenic Recipient
(lacking T and B cells)



First described by Powrie et al. Int Immunol. 1993 Nov;5(11):1461-71.

T cell transfer (TcT) in *Rag2*$^{-/-}$ mice





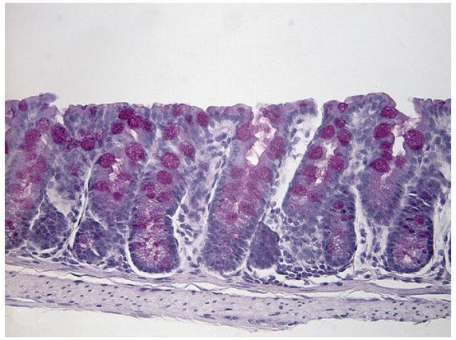
1 x 10^6 control $CD4^+$ $CD45RB^{hi}$ $CD25^-$

1 x 10^6 control $CD4^+$ $CD45RB^{hi}$ $CD25^-$

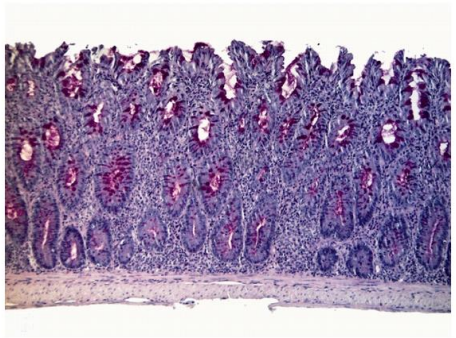
Female *Rag2*$^{-/-}$ mice on a C57BL/6NTac background (Model RAGN12) at the MPF™ Health Standard were used as recipients and female C57BL/6NTac mice were used as donors. Naïve $CD4^+$ T cells were isolated from peripheral lymph nodes, using a gating strategy to deplete regulatory T cells ($CD4^+CD45RB^{hi}CD25^-$), and injected into recipients at varying doses (0.5 x 10^6, 1 x 10^6 or saline, as a control). Data provided by an anonymous Pharmaceutical company.

Histopathological Features

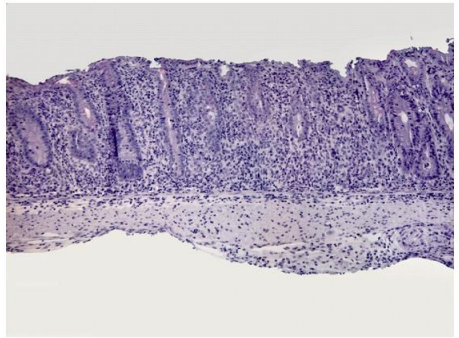
Increasing Severity



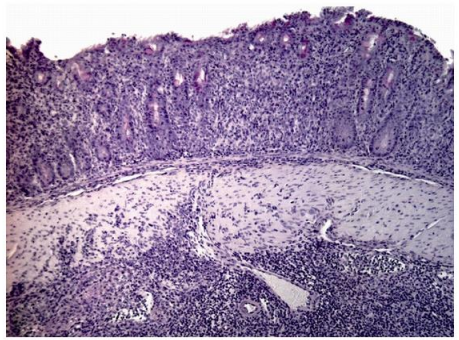
Normal colon



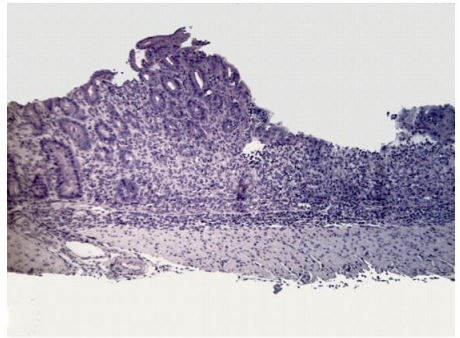
Moderate mucosal inflammation



Severe inflammation extending into submucosa; crypt degeneration, goblet cell dropout



Transmural inflammation; severe crypt degeneration



Ulceration

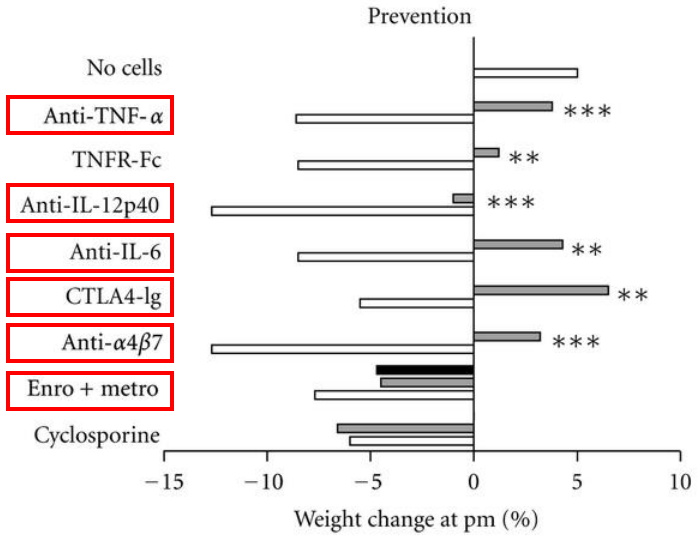
Donor Mice: BALB/cAnNTac females (8–10 weeks)
Recipient Mice: C.B-17 SCID (C.B-*Igh-1*^{b}/IcrTac-*Prkdc*^{scid}) females (8-10 weeks)
Transferred Cells: $CD4^{+}CD25^{-}$ splenocytes (3 x 10^{5}), intraperitoneal injection

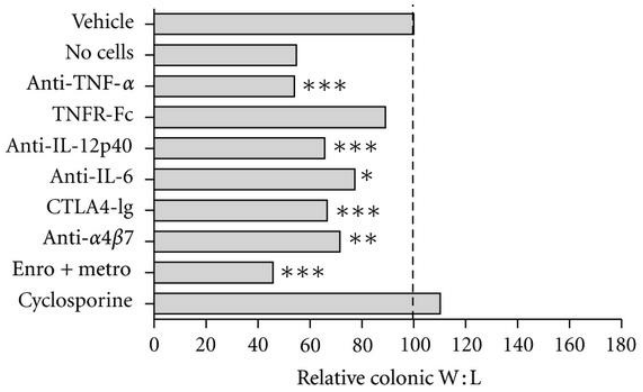
Lindebo Holm T, Poulsen SS, Markholst H, Reedtz-Runge S. Pharmacological Evaluation of the SCID T Cell Transfer Model of Colitis: As a Model of Crohn's Disease. Int J Inflam. 2012;2012:412178. doi:10.1155/2012/412178. (https://www.hindawi.com/journals/iji/2012/412178/)

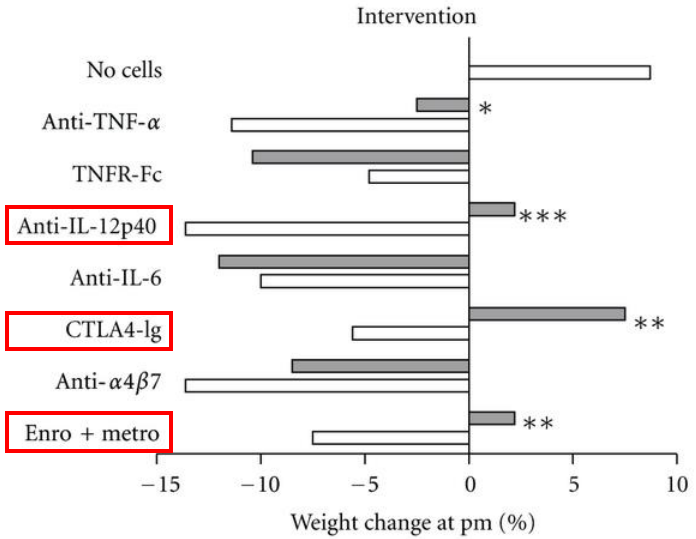
Pharmacological Evaluation of the T Cell Transfer Colitis Model

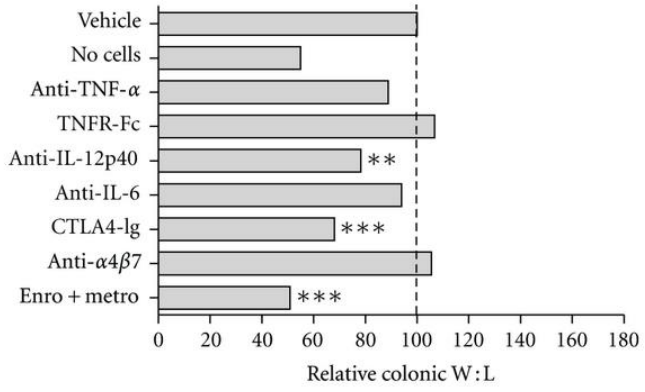
Prevention:
Treatment from d0

Significant histological improvement









Intervention:
Treatment from wk 3

Translational Utility of T Cell Transfer Colitis

Predictive Validity

Clinically-relevant pathways:

- Anti-IL12p40 and IL-23 pathway
- Anti-TNF
- Anti-integrin

Face Validity

Model features:

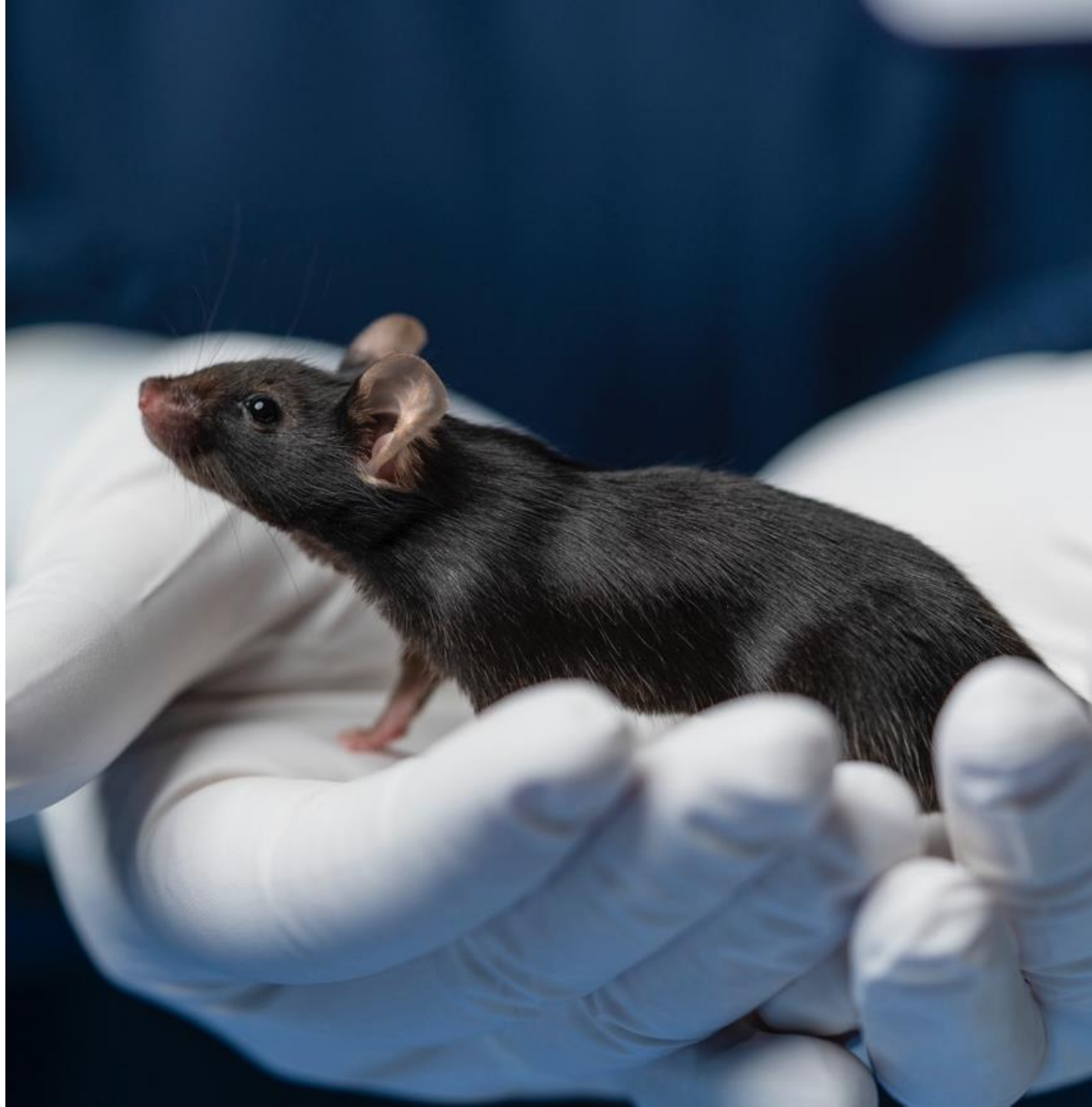
- Moderately chronic, immune-mediated colitis
- Largely colon involvement but may involve small intestine
- T_H1/T_H17 inflammatory pathways
- Absence of Treg, CD8 T cells, B cells, NKT cells

Construct Validity

Clinical relevance of T cell transfer:

- Essentially unheard of in patients, except in rare cases following pregnancy
- Overactivity of T cells towards microbial antigens

Key Factors for T cell Transfer



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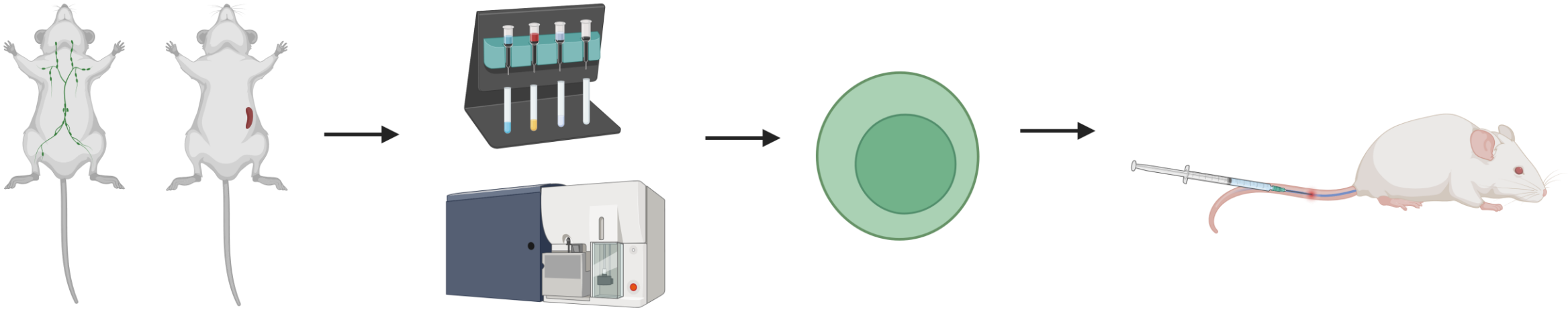


Syngeneic Donor
Spleen vs. Lymph Node

FACS vs. MACS

Naïve T cell Isolation Strategy
Cell Viability

Lymphopenic Recipient
Cell Dose ($0.2 – 2 \times 10^6$)
Route of Administration

Recommended Reference:
Ostanin et al. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. Am J Physiol Gastrointest Liver Physiol. 2009 Feb;296(2):G135-46. doi: 10.1152/ajpgi.90462.2008.

T Cell Transfer Recipients

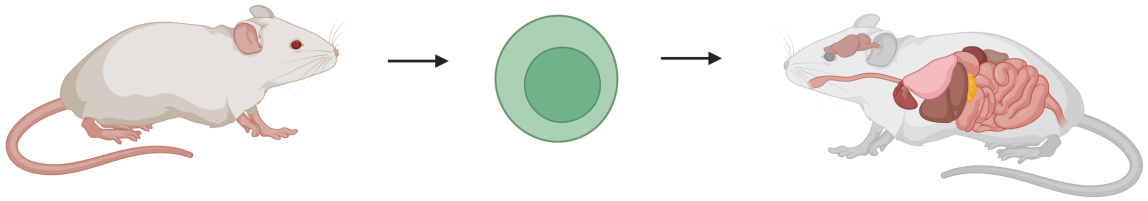
C.B-17 Scid

- Severe combined immunodeficiency mutation in DNA-dependent protein kinase (*Prkdc*^{scid} mutation)
- Defective DNA repair preventing V(D)J recombination
- Lacks B and T cells
- Mutation is “leaky” meaning animals will develop mature B cells over time
- Susceptible to DNA damaging agents
- Largely on a BALB/c genetic background except is carries the *Igh-1b* allele from the C57BL/Ka strain

Rag Knockouts

- Recombination activating genes (Rag1 and Rag2) are both required for V(D)J recombination
- Rag-2 and Rag-1 knockouts are often used interchangeably
- Lacks B and T cells
- Not “leaky” and not susceptible to DNA damaging agents
- Available on multiple different genetic backgrounds, for example:
  - C57BL/6 (Taconic model RAGN12)
  - BALB/c (Taconic model 601)
  - 129S6 (Taconic model RAG2)

Donor and Recipient Requirements



Donor:

BALB/cAnNTac

C57BL/6NTac

BALB/cAnNTac

Recipient:

C.B-17 scid

B6.129S6-*Rag2*^{tm1Fwa} (Model RAGN12)

C.129S6(B6)-*Rag2*^{tm1Fwa} (Model 601)

Male Donor → Male or Female Recipient

Female Donor → Female Recipient only

Other important factors:

8-10 wk of age (naïve T cells may decrease with age)

0.5-1 spleens required for each recipient (varies)

Microbiome (especially of the recipient)

Naïve T cell Isolation

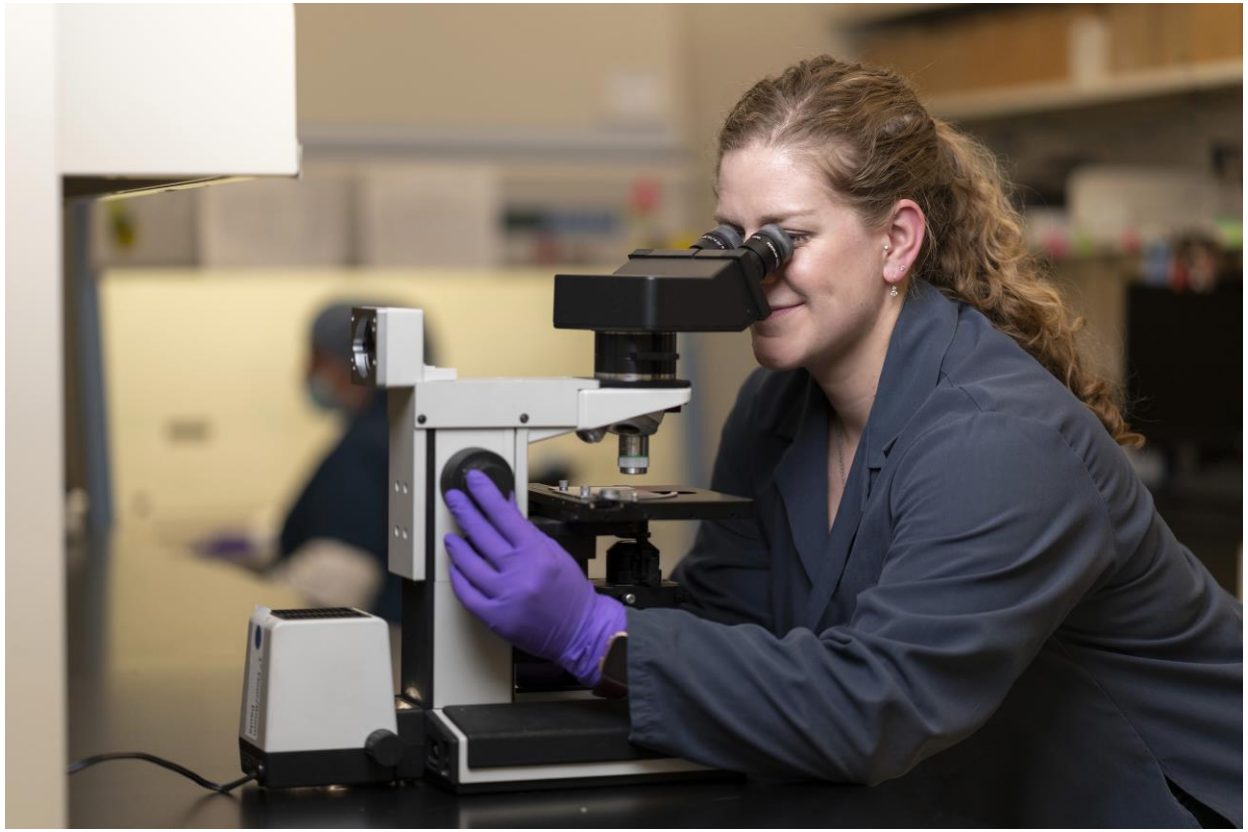
Cell population options:

- $CD4^{+}CD45RB^{hi}$ (brightest 40%)
- $CD4^{+}CD45RB^{lo}$ (negative control)
- $CD4^{+}CD45RB^{hi}CD25^{-}$ or $CD4^{+}CD25^{-}$
- $CD4^{+}CD62L^{+}CD44^{-}$
- *Il10*$^{-/-}$ $CD4^{+}$
- $CD8^{+}CD62^{+}CD44^{-}$

Techniques:

- FACS vs MACS
- Touched vs. untouched

Analysis of Colitis Severity



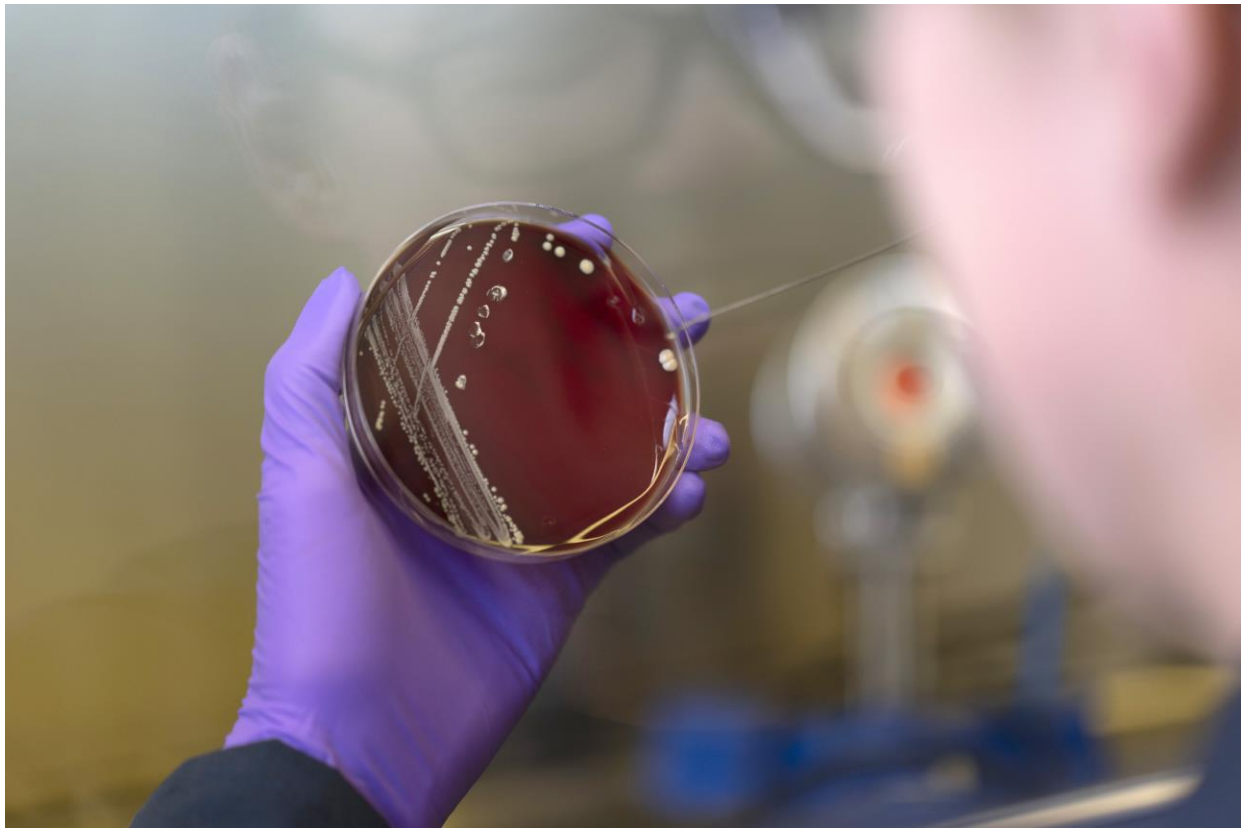
In-life measurements:

- Body weights
- Stool scoring
- Body condition scoring
- Endoscopy
- Fecal lipocalin-2 or calprotectin

Endpoint measurements:

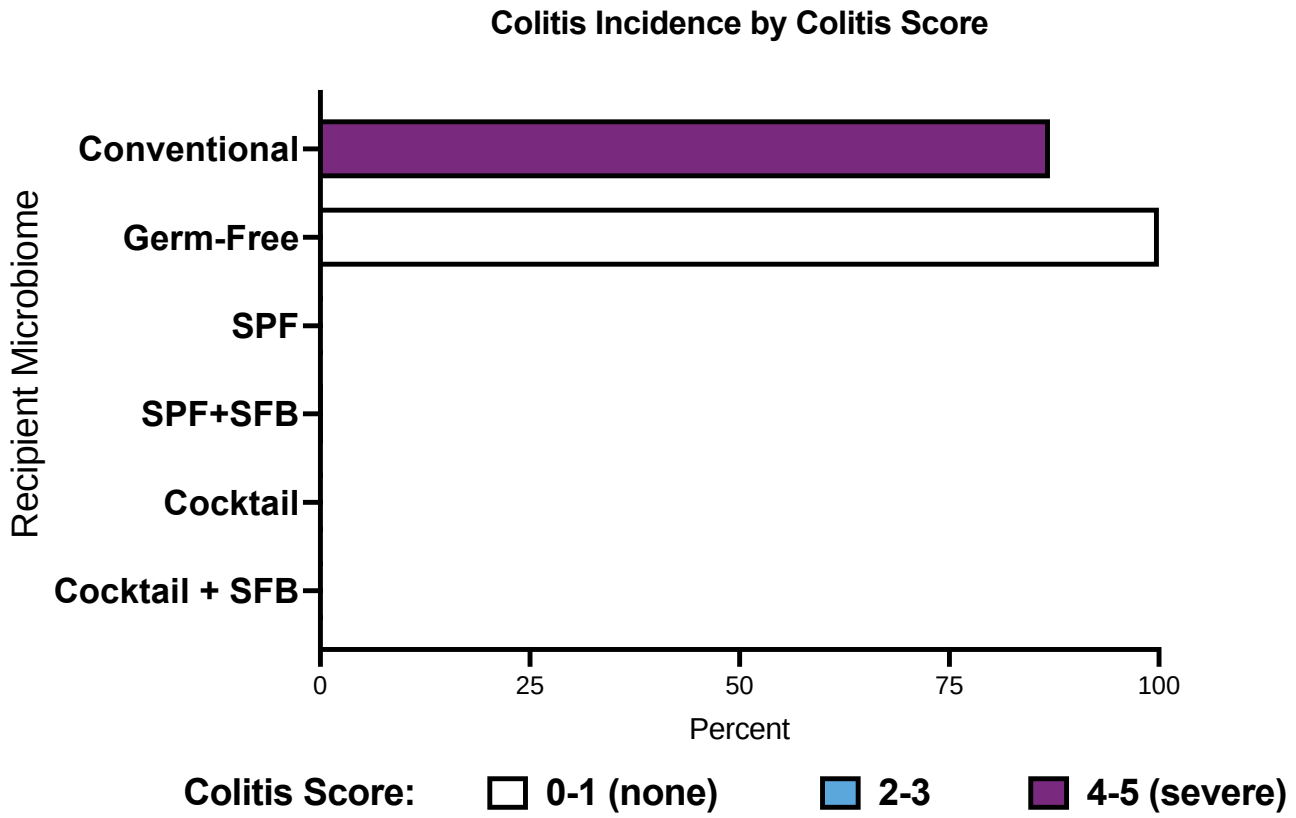
- Colon weight:length ratios
- Histopathology scoring
- Lamina propria/mLN FACS analysis
- Colon cytokine levels
- Colon explant cytokine production

Microbiome



- Microbiome is absolutely required for colitis development
- Major factor in reproducibility and variability
- Overall microbial diversity/richness vs. a specific agent
- Worsens colitis: Segmented filamentous bacteria (SFB), *Helicobacter hepaticus*
- Ameliorates colitis: *Akkermansia muciniphila*

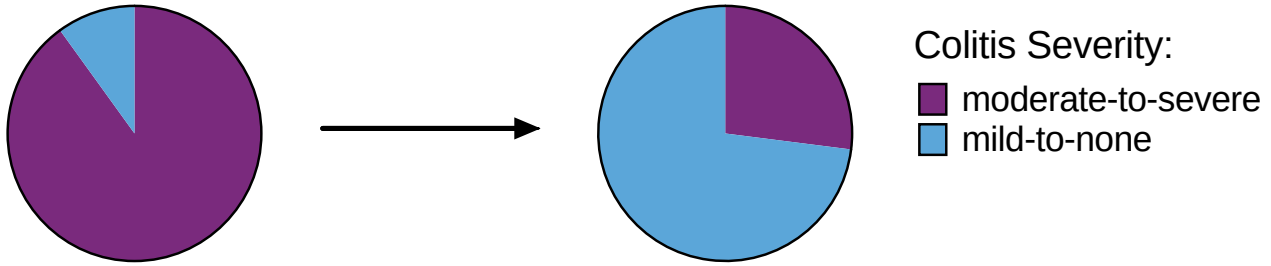
How does the microbiome impact T cell transfer colitis?



- $CD4^{+}CD45RB^{hi}$ T cells transferred from BALB/c donors to C.B-17 scid mice with varying microbiota or germ-free

Stepankova et al. Inflamm Bowel Dis. 2007 Oct;13(10):1202-11. doi: 10.1002/ibd.20221.

TTUHSC vs. LSUHSC Case Study



Louisiana State University Health Sciences Center (LSUHSC)

Texas Tech University Health Sciences Center (TTUHSC)

- Both used *Rag-1*$^{-/-}$ and C57BL/6J mice obtained from the Jackson Laboratory and housed for 2 weeks prior to T cell transfer
- TTUHSC: Less microbial diversity, dominated by fewer but highly abundant taxa
- Phenotype at TTUHSC could be restored by:
  - Performing T cell transfer at LSUHSC and shipping to TTUHSC after 2 weeks
  - Gavaging recipients with fecal material from colitic mice (sourced from LSUHSC)

- Chow was different between institutions:
  - LSUHSC - Teklad 7012 LM-485 (sterilization not specified)
  - TTUHSC - irradiated Prolab Isopro RMH 3000
- Notable differences in formulation:
  - RMH 3000 contains Porcine Animal Fat Preserved with BHA and Citric Acid, Fish Meal (nitrosamine source)
  - Teklad 7012 LM-485 contains only soybean oil and no fish meal

Webb et al. Inflamm Bowel Dis. 2018 Jan 18;24(2):361-379. doi: 10.1093/ibd/izx014.

Husbandry Considerations

- Validate model with standardized housing, bedding, cage type, source of mice, feed, water
- Maximize microbial diversity in T cell recipients:
  - Avoid antibiotics and acidified water
  - Select appropriate Health Status:
    - Murine Pathogen Free (MPF™) – less restrictive
    - Opportunist Free (OF™) – more restrictive
  - Alternate approaches:
    - Fecal microbiota transplantation
    - Exposure to dirty bedding
- Co-housing and randomization:
  - If selecting to use only female recipients, be sure to only use donor females