Pharmacologic Intervention Studies Using Mouse Models of IBD: Translating Preclinical Data into New Drug Therapies

Intervention Studies Using Mouse Models of IBD

>2,700 Studies

- ~800 DSS
- ~300 TNBS
- >200 IL-10⁻/⁻
- >100 T-Cell

>300 RX (+) >200 RX (+)
The literature is filled with numerous examples of drugs or biologics that attenuate intestinal inflammation using one specific animal model &/or therapeutic strategy that may not adequately mimic the clinical situation.

Many Targets but Few New Drugs: Why?

Pharmacologic Intervention Studies Using Mouse Models of IBD: Concepts and Considerations

- Animal Model
- Pharmacology
- Experimental Design and Data Analysis
Pharmacologic Intervention Studies Using Mouse Models of IBD: Concepts and Considerations

● Animal Model

a) Acute vs. Chronic inflammation
b) Similarities to human IBD
c) Availability

### Mouse Models of IBD

<table>
<thead>
<tr>
<th>Erosive/ Self-Limiting</th>
<th>Spontaneous</th>
<th>Genetically- Engineered</th>
<th>T-Cell Transfer</th>
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<tbody>
<tr>
<td>DSS</td>
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<td></td>
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<td>TCR(^{-/-})</td>
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<tr>
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</tr>
<tr>
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<td></td>
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<td>STAT3(^{-/-})</td>
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<td>STAT4 (\text{tg})</td>
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<td></td>
<td></td>
<td>CD40L (\text{tg})</td>
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<td></td>
<td></td>
<td>A20(^{-/-})</td>
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<td></td>
<td>TGFβ(^{-/-})</td>
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<td>N-Cadherin \DN)</td>
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### Models of *Erosive, Self-Limiting Colitis*

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### Misoprostol Attenuates Acetic Acid-Induced Colonic Mucosal Injury and Inflammation: *Prevent Injury and/or Enhance Repair?*

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<th>Control</th>
<th>Acetic Acid (AA)</th>
<th>AA + Miso</th>
</tr>
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Yamada et. al. AJP, 1991
Mucosal Permeability (ml/min/100g) 

Misoprostol Accelerates Mucosal Repair; No Effect on Initial Insult

Yamada et al, 1992

DSS Model of Colitis

DSS (3.0 %) in drinking water for 7 days
- Acute colitis of distal colon
- Weight loss
- Bloody diarrhea
- Histologic changes similar to ulcerative colitis

Histologic Damage Score
0 - 40
Epithelial Integrity vs. Inflammation During Progression of DSS-Induced Colitis

Preventing PMN Infiltration Enhances Repair of Colonic Epithelium

Abdelbaqui et. al, 2006
Choosing a Model: What is the Question?

**Erosive/Self-Limiting Models of Acute Colitis**
- Mechanisms of epithelial cell restitution and repair.
- PMN trafficking in response to tissue injury.
- Innate immune responses to tissue damage.

**T-Cell-Dependent Models of Chronic Inflammation**
- Role of adaptive immune system in chronic gut inflammation.
- Pathogenesis of small vs. large bowel inflammation.
- Role T-cell derived cytokines, chemokines, selectins and integrins in disease pathophysiology.
- Regulation of chronic intestinal inflammation.
- Relationship between the innate and adaptive immune system in the development of disease.

Models of **Chronic Small Bowel Inflammation, Colitis or Both**

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What Have We Learned From Mouse Models of IBD?

- Chronic inflammation is *T-cell mediated*.

- *Commensal enteric bacteria* drive gut inflammation by providing continuous antigenic and adjuvant stimulation.

- *Defective immuno-regulation* promotes chronic inflammation.

- Host *genetic background* modulates disease severity and phenotype.

Chronic gut inflammation develops as a result of a *dysregulated* immune response to components of the normal gut flora.
Adoptive Transfer of **Naïve CD4⁺ T-Cells** Induces **Chronic Colitis** in **Lymphopenic** Recipients

Incidence and Severity of Colitis

CD45RB<sup>low</sup> (Activated)

CD45RB<sup>high</sup> (Naïve)

Wild type

CD4⁺ T-Cells

No Colitis

Colitis

Incidence and severity of colitis (%)

0 20 40 60 80 100

CD45RB<sup>low</sup>

CD45RB<sup>high</sup>

Incidence and severity of colitis (%)

- No colitis
- Mild
- Moderate
- Severe
Does \(\alpha_4\) (CD49) Integrin Play an Important Role in Disease Pathogenesis?

T-cell

Endo

Deletion of \(\alpha^4\) Integrins from Adult Hematopoietic Cells Reveals Roles in Homeostasis, Regeneration, and Homing

Linda M. Scott, Gregory V. Priestley, and Thalia Papayannopoulou*

Division of Hematology, University of Washington, Seattle, Washington 98195-7710

\[\alpha_4^{\text{flox/flox}} \times \text{Mx.cre}^+ \rightarrow \text{Mx.cre}^+\alpha_4^{\text{flox/flox}} + \text{polyIC} \rightarrow \text{Mx.cre}^+\alpha_4^{\Delta/\Delta}\]
α₄-Deficient T-Cells Induce Little or No Disease

Histopathology Scores

α₄⁺

α₄⁻

CD45RB<sub>high</sub>

CD45RB<sub>high</sub>

Pharmacologic Intervention Studies Using the T-Cell Transfer Model of Chronic Colitis

- TNF-α mAb
- Steroids
- Anti-Angiogenic Peptide
- CD11a mAb
- CTLA-4 mAb
- MAdCAM-1 mAb
- Lymphotoxin mAb
- CD40L (CD154) mAb
Pharmacologic Intervention Studies Using Mouse Models of IBD: Concepts and Considerations

- **Animal Model**
  
  a) Acute vs. *Chronic inflammation*
  
  b) Similarities to human IBD
  
  c) Availability

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**Comparative Analysis of Colonic Gene Expression of Three Experimental Colitis Models Mimicking Inflammatory Bowel Disease**

Anje A. te Velde, PhD.* Floor de Kort, * Ellen Sterrenburg,† Inge Pronk, * Fiebo J.W. ten Kate, MD, PhD.‡
Daniel W. Hommes, MD, PhD,§ and Sander J.H. van Deventer, MD, PhD∥

The pattern of gene expression in **T-cell transfer** model *most closely reflects* the altered gene expression in human IBD when compared to the **DSS and TNBS** models.
A Disconnect Between Erosive and T-cell-Dependent Models

- DSS- or TNBS/ETHOH-induced colitis occurs in the absence of T-cells whereas most models of chronic disease require these lymphocytes (Dieleman, 1994; van Lierop, 2009).

- Disruption of CD40-CD40 Ligand attenuates DSS colitis but has no effect on the chronic inflammation in the T-cell transfer model (Vowinkel, 2007).

- Adiponectin deficiency attenuates DSS or TNBS colitis but does not affect onset or severity of chronic colitis in the IL-10−/− or T-cell transfer models (Fantuzzi, 2007; 2009).

- Selective inhibition of pathogenic angiogenesis attenuates T-cell induced colitis but has no effect in the DSS model (Chidlow, 2006).

- NO synthase inhibitors attenuate DSS and TNBS colitis but have no effect on T-cell –induced or IL-10−/− models of chronic colitis (MacCafferty, 1999; Kawachi, 1999).

Pharmacologic Intervention Studies Using Mouse Models of IBD: Concepts and Considerations

- Animal Model
  a) Acute vs. Chronic inflammation
  b) Similarities to human IBD
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“Availability” of Mouse Models of Chronic Gut Inflammation: *Really Check*

- Relatively few of the genetically-engineered and/or spontaneous models are readily available to most investigators.

- In some cases, a detailed characterization of the inflammation is lacking or the *penetrance* and/or *severity* of disease is *low or highly variable*.

- Utility of the model may be limited by the length of time for inflammation to develop and/or by the necessity to generate (via interbreeding) mice on appropriate backgrounds.

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Pharmacologic Intervention Studies Using Mouse Models of IBD: *Concepts and Considerations*

- Animal Model

- Pharmacology

- Experimental Design and Data Analysis
Pharmacologic Intervention Studies Using Mouse Models of IBD: 
*Concepts and Considerations*

● Pharmacologic Considerations

a) Route of administration of drug.
b) Unanticipated effects of drugs on induction of inflammation.
c) Prophylactic vs. therapeutic administration of drug.

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Pharmacologic Intervention Studies Using Mouse Models of IBD:  
*Concepts and Considerations*

● Pharmacologic Considerations

  a) *Route of administration.*
  b) Unanticipated effects of drugs on induction of acute or chronic disease.
  c) Prophylactic vs. therapeutic administration of drug.
Route of Administration vs. Bioavailability for Small Molecules

Problems with daily dosing for chronic studies

Route of Administration vs. Bioavailability

Blood Concentration vs. Time (hours)

iv (or ip)
sc
po

Blood Concentration vs. Time (Days)

Small Molecules
mAbs; Biologics

TNF-α mAb
Steroids
Anti-Angiogenic Peptide
CD11a mAb
CTLA-4 mAb
MAdCAM-1 mAb
Lymphotoxin mAb
CD40L (CD154) mAb
Pharmacologic Intervention Studies Using Mouse Models of IBD: Concepts and Considerations

- Pharmacologic Considerations
  a) Route of administration.
  b) Unanticipated effects of drugs on induction of acute or chronic disease.
  c) Prophylactic vs. therapeutic administration of drug.

Erosive Models of Colitis Require Oral or Rectal Delivery of Damaging Agent: Potential Pitfalls!

- Drug-induced alterations in water (or food) consumption may dramatically alter severity of disease.
- Drug-induced fluid, bicarbonate and/or mucus secretion may dilute &/or neutralize noxious luminal insult resulting in reduced disease.
- Drugs with vasoactive properties (ie vasoconstrictor) may reduce inflammation by decreasing blood flow-dependent delivery of inflammatory cells and mediators.
- Luminal interaction between inducing agent and drug.
Any Drug that Alters Water Consumption Will Affect Severity of DSS-Induced Colitis

\[
\text{"DSS Load"} = WC \times [\text{DSS}] \times BW
\]

(WC=water consumption)

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Protein Leukocytes Mediators

Inflamed

Delivery = Blood flow \times [\text{concentration}]

Vasoconstriction

NOS Inhibitors!

\downarrow BF = \downarrow Delivery

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Prophylactic vs. Therapeutic Administration Lessons Learned from the IL-10 Studies

- Parental administration of recombinant IL-10 prior to onset of inflammation prevents the development of acute or chronic colitis induced in rats or mice but does not reverse established disease in these animal models.

- Placebo-controlled randomized clinical trials in patients with active CD failed to demonstrate therapeutic efficacy of IL-10.

The ability of a drug/biologic to reverse established disease is a much better predictor of clinical efficacy than is prevention of injury & inflammation.
Adoptive Transfer of Ex vivo-Generated iTregs Reverses Established Colitis

Karlsson et al., Meth Mol Biol, in press

Pharmacologic Intervention Studies Using Mouse Models of IBD

Concepts and Considerations

• Animal Model

• Pharmacology

• Experimental Design and Data Analysis
**Study Design and Data Analysis**  
*Lessons Learned from the Stroke Community*

- >1,000 drugs have been tested in different animal models of ischemic stroke.
- Of those tested, > 450 drugs have been reported to have efficacy in the different animal models.
- Currently, only thrombolysis (TPA) and aspirin have been shown to convincingly improve outcome in patients with ischemic stroke!

Why is there such a dramatic disconnect between preclinical studies and clinical efficacy?

**Stroke Therapy Academic Industry Roundtable (STAIR)**

- Originally organized in 1999 to standardized preclinical stroke studies for both academia and industry.
- Systematic review and meta analysis of numerous putative neuroprotective agents revealed that the presence or absence of randomization to treatment group, blinded drug assignment during stroke induction and blinded outcome assessments were the most powerful determinants of outcomes.
- Sample size calculations were reported in only 3% of the studies; many studies are underpowered.
- Publication Bias: “If positive rather than neutral studies are more likely to be published (and many journals favor positive results), then any conclusion drawn from the published study will overstate the magnitude of the effect seen” *(Sena et. al. 2007).*
Recommendations

- **Randomization of animals:** picking animals at random is not good enough.

- **Inclusion and Exclusion Criteria:** all randomized animals should be accounted for in the presented data.

- **Allocation Concealment:** the method for concealment should be clearly stated.

- **Blinded Evaluation of Outcome:** Need I say more!

- **Reporting of positive, neutral and negative data:**
  All randomized and allocated animals should be part of the blinded assessment outcome.

Summary and Conclusions

- Erosive self-limiting models of acute colitis are ideal for evaluating pharmacologic agents thought to promote epithelial cell restitution and/or mucosal repair; these models have also proven useful in evaluating the role that innate immune cells/genes play in the healing process.

- T-cell-dependent models of *chronic* ileitis and/or colitis are useful for evaluating the anti-inflammatory properties of a wide range of small molecules/biologics directed towards various mediators, cells and pathways associated with innate and adaptive immune systems.

*The ability of a pharmacologic agent to reverse established disease in two different, T-cell-dependent models of chronic gut inflammation appears to be the best predictor of therapeutic efficacy in human IBD.*

(Adapted from Sartor)
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Yuta Abe
Koichi Takabayashi
Robert Chervenak
Songlin Zhang