Murine Models of Inflammatory Bowel Disease

Laura P. Hale, M.D. Ph.D.
Professor of Pathology
Duke University Medical Center

September 19, 2017

Outline

• Review of IBD in humans
• Murine models of IBD
• Tissue handling
• Scoring of colitis severity
• Examples

Inflammatory Bowel Disease

• IBD is characterized by chronic mucosal immune activation in response to intestinal bacteria in a genetically susceptible person
  – Ulcerative colitis (UC)
  – Crohn disease (CD)

Irritable Bowel Syndrome (IBS) is something different!
IBD Pathogenesis

- Mucosal immune responses
  - Activation of Th1 and Th17 cells
- Gut microbiota
  - Species, biofilms vs. planktonic
  - Defective epithelial barrier
- Genetics
  - Variants in ≥ 240 genes increase risk*
    - NOD2/CARD15, ATG16L1, IL10/IL10R


The “Three Factor” Model of IBD

- Defect in Intestinal barrier
- Defect in immune regulation/efficacy
- Colitigenic microbiota present

*Hygiene hypothesis*
Genetic susceptibility
Drugs
Infections

Modified from:

Crohn Disease

- Ulcerative Colitis

Can affect from mouth to anus

- Only affects colon

Granulomas
Fistulas
Fistulas
Increased risk of colon CA if colon is involved

Transmural inflammation
Increased risk of colon CA

Robbins 7th Ed.
Continuous Inflammation in UC

Severe left-sided UC

Inflammation in UC Affects Mucosa

Deep Fissuring Inflammation in CD

Danese & Fiocchi, NEJM 365:1713-1725, 2011
Murine Models of IBD

- Chemically-induced
  - DSS, 2,4,6-trinitrobenzene sulfonate (TNBS), oxazalone, acetic acid
- Biologically-induced
  - e.g. salmonella, *E. coli*, others
- Cell transfer
- Genetic

DSS Model of Colitis

- Administration of dextran sulfate sodium in drinking water damages mucosa, rapidly leading to colitis.
  - Rapid weight loss, bleeding
  - Generally, acute inflammation only
    - T cells not required
  - Can make chronic by multiple cycles
  - No need for genetic susceptibility
  - Enhances colon cancer after AOM,
    - But molecular changes not c/w human CA

DSS Colitis

- Advantages:
  - No breeding required
  - Easy to administer
  - Can control dose and duration to affect severity and healing
- Disadvantages:
  - Differs from both UC and CD
  - Inhumane??
**CD4+ T Cell Transfer Colitis**

- This model mimics the ↑ activation of CD4+ T effector cells and ↓ T regulatory cell activity via cell transfer into a T cell-deficient host.
- This also provides a way to specifically test T effector or T regulatory cell activity.

**CD4+ Transfer Colitis**

\[ \text{Colon length and colon weight correlate well with inflammation via histopathology.} \]


**Genetic Models of Colitis**

- Mutations in many genes have been reported to increase risk of colitis in mice.
  - Some of these models may be dependent on infections or exposures, although the specifics may not be known.
- Example: *Il10−/−* model
  - First reported in 1990’s
  - First used in Hale lab in 2004
  - First human relevance reported in 2008
**Il10⁻/⁻ Model**

Lack of Th2 cytokine IL-10 results in “spontaneous” Th1/Th17-mediated colitis in response to intestinal bacteria. Age of disease onset is dependent on both strain and environment.

- **Control**
- **IL-10 KO, C57BL/6**

Observe for clinical symptoms of colitis.

Euthanize and assess colitis histologically.

---

**“Spontaneous” Colitis in Il10⁻/⁻ Mice**

- **Distal colon at 29 wks**

Vehicle

Treatment

Occurred only after repeated oral administration of feces from sentinel mice

---

**Il10⁻/⁻ Colitis Triggered by Deliberate Helicobacter Infection**

- **H. typhlonius**
- **H. rodentium**

Cecum
NSAID-Triggered Colitis in *Il10*−/− Mice

Hale LP, et al. Inflamm Bowel Dis. 11:1060-1069, 2005

Colitis in *Il10*−/− Mice

Transmural Inflammation in *Il10*−/− Mice

Severities and extent of inflammation is typically:
- Cecum ~ prox ~ rectum >> other colon regions.
Colon CA in \textit{Il10}⁻/⁻ Mice

Depending on conditions, colon CA developed in 60-95% of mice by 32 wks (0–5 lesions/mouse)

IBD in \textit{Il10}⁻/⁻ Mice

- Grossly and histologically resembles Crohn colitis
  - Transmural inflammation
  - Skip lesions
  - Fistula formation
- Good model for IBD-associated colon cancer

UC-Like Colitis in “T/I” Mice

Severe spontaneous inflammation limited to mucosa begins in rectum and extends continuously proximally. ~94% (n=54) penetrance by 9 wks in T/I mice, with 72% (n=78) penetrance by 33 wks in T-het/I mice.
Colon CA in T/I Mice

CA was present in 25 of 38 mice (66%) ≥ 15 wks, mean 2 lesions/mouse, range: 0-4


Other Genetic Models of Colitis

CD-like
- SAMP1/Yit (term. ileum + colon)
- TNF-ΔARE
- N-cadherin (dom. neg. mutant)
- Stat3⁻⁻
- Smad3⁻⁻
- Il10⁻⁻
- Etc…….

UC-like
- Tcrα⁻⁻
- Wasp⁻⁻
- Mdr1a⁻⁻
- Il12⁻⁻
- Gαi2⁻⁻
- IL-7 Tg
- C3H/HeJ Bir
- Tbet⁻⁻ x Rag2⁻⁻
- Muc2⁻⁻
- Tnf⁻⁻ Il10⁻⁻
- Etc…..


Comparison with Human IBD Has Led to Additional Mouse Models

Dysbiosis drives colitis.
Requires norovirus in mice

Lees CW, et al. Gut 2011 (data from GWAS study)

Lees CW, et al. Gut 2011 (data from GWAS study)
Variables That Affect IBD Models

**General**
- Mouse strain
- Sex
- Age
- Genetic modifications
- Method and timing of acclimation to microbiota
- Source/Origin
- Housing density, light/dark cycle, temperature, humidity
- Food

**Model-Specific**
- Origin of chemicals, MW
- Dosing method & vehicle
- Clinical monitoring
- Species/strain & dose of introduced microbes
- Level of colonization
- Type, dose, strain, and genetic information for transferred cells

---

Original Article

Quality of Methods Reporting in Animal Models of Colitis
Michael Brantsaerd, MSc,* Oseogki Fling-Vargas, MSc,*, Robert Siemens, PhD,† Andy Blais, PhD,†
and Sheena Crowther, PhD*†

Background: Current understanding of the onset of inflammatory bowel disease relies heavily on data derived from animal models of colitis. However, the omission of information concerning the method used makes the interpretation of results difficult at best. Not assessed the current quality of methods reporting in 33 animal models of colitis that use a formal animal model to study the role of the intestinal microbiota in colitis. Methods: With the 33 published articles in the literature, Colitis models were identified in 33 reports. In total, all studies were included in the TNDs. Results: TNDs are not more than 3 colitis models. A total of 81.9% (23/28) of patients were reported across all models. The 17 of the 22 articles examined all similar outcomes as provided. Parallel and genetic differences between genotypes were noted. Conclusions: TNDs may include over 100 published articles. The way the data is treated has a large impact on the quality and comparability of published results. The technical information of a single published report may influence the quality, comparability, and final outcomes of the models. TNDs are not only more than 3 colitis models, but also provide information on the disease condition.

---

Tissue Handling

- To open or not to open?
  - Swiss roll/segments
  - “Sausages”

- Fixative
  - 10% neutral buffered formalin
  - Carnoy’s fixative
  - Others?

60% EOH
30% CHCl3
10% acetic acid
Describes a variety of validated scoring systems, based on the type of model being evaluated.

Histologic Scoring of Colitis in Mice

Assign a value ranging from 0 to 3 based on severity of:
- Glandular hyperplasia
- Goblet cell loss
- Leukocyte density in mucosa & submucosa
- Extent of lesion
- Degree of vasculitis
- Number of crypt abscesses, mucinous lakes
- Extent of ulceration

Swiss roll: 1 = mild, 2 = moderate, 3 = severe
Max = 21
Histologic Scoring of Colitis in Mice

- **Mucosa (M)**
  - Mild epithelial hyperplasia 1
  - Moderate epithelial hyperplasia 2
  - Severe hyperplasia with crypt branching &/or herniation 3
- **Inflammation (I)**
  - Mild, limited to mucosa 1
  - Moderate, in mucosa and submucosa 2
  - Severe, with obliteration of architecture &/or crypt abcesses 3
  - Level 3 changes plus ulceration 4
- **Extent (E), area involved**
  - < 5% 1
  - 5 - 30% 2
  - 31 - 60% 3
  - >60% 4
- **Segment score = M + I + E1 + E2 (with Level 3 or 4 changes)**
- **Total score for 5 segments = 75 max; >12 indicates colitis**

Hale LP, et al. Inflamm Bowel Dis. 11:1060-1069, 2005

### Tissue Handling

Cecum
Proximal
Mid
Distal
Terminal colon/Rectum

Cecum, DSS

### Histology of Normal Murine Colon

The mucosa (M) is composed of a single layer of simple columnar epithelium, the lamina propria (LP), and the muscularis mucosae (*). Underlying the mucosa are the submucosa (Sub), muscularis propria (MP), and serosa (obscured by black marking dye).
Mild epithelial hyperplasia, defined as less than 2-fold increase in height and/or mitoses present in the lower half of the gland.

Mucosa Score = 1

Moderate epithelial hyperplasia, with both >2-fold increase in crypt height and mitoses (arrow and circle) in the upper half of the gland.

Mucosa Score = 2

Severe epithelial hyperplasia with crypt branching (an example of architectural distortion).

Mucosa Score = 3
Inflammation Score = 1

“Inflamed” inflammation, limited to mucosa. Neutrophils must be present, as mononuclear cells are common in normal colon tissues.

Inflammation Score = 2

Moderate inflammation, with neutrophils present in mucosa and submucosa. Score does NOT increase if transmural.

Inflammation Score = 3

Severe inflammation, with crypt abscesses. Obliteration of architecture by inflammation also scores as 3.
Inflammation Score = 4

Severe inflammation, with crypt abscesses and/or obliteration of normal architecture, PLUS ulceration.

Scoring Extent

Extent 1 = area with any changes
Extent 2 = area with changes scored as 3-4

Final score = M + I + E1 + E2, summed for 5 segments

0 = Not involved
1 = < 5%
2 = 5 – 30%
3 = 31 – 60%
4 = >60%

Chronic DSS colitis, mid-colon

Mucosa score 3 (hyperplasia with crypt branching); Inflammation score 4 (ulcer).
Mucosa score 2 (moderate hyperplasia); Inflammation score 3 (severe, with crypt abscesses), based on the most severe lesion, even though inflammation is limited to the mucosa.

### Advantages

- Scores entire colon, but allows taking samples for other assays.
- Sub-scores can distinguish between continuous inflammation limited to the mucosa (UC-like) and scattered focally severe lesions (CD-like).
- Extent score is particularly sensitive to treatment effects.

### Final Thoughts

- Many murine IBD models are currently available.
- Any single genetic model is unlikely to fully recapitulate human IBD, which is genetically heterogeneous.
- Choose models, tissue handling, and scoring systems wisely to maximize reproducibility and translatability.