Physiology Conference
Gut Immunology, Dysentery, & IBS
November 18, 2004

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The patient states, “Ever since I had that terrible bout of diarrhea, I haven’t been quite right.”

The patient then asks, “Do you think that the food poisoning I had during my vacation caused my current condition?”
Objectives

- Overview of Innate and Adaptive immunity
- Bacillary dysentery (Shigella) and the immune response
- Is there a link to irritable bowel syndrome?
- Can an anti-inflammatory help post-infective irritable bowel syndrome?
Gut immunology: The challenge

- Discriminate between pathogens and benign organisms
- Stimulate effective protective immune response without causing excessive inflammation
- Provide a surface for absorption of nutrients while maintaining a barrier to harmful external pathogens
Gut immunology: Innate

- First line of defense
- Does not require prior exposure to antigen
- Acts immediately
Gut immunology: Adaptive

- Requires prior exposure
- Takes time to develop
- Highly specific response
- Provides lasting immunity
- Amplifies the initial protection provided by the innate immune response
Gut immunology

Gut defense mechanism

Innate
- Lumenal factors
- Antimicrobial factors
- Physical barriers
- Mechanical factors

Adaptive
- Gut-associated Lymphoid tissue
Innate: Lumenal factor

- **Saliva**
  - Flow 1500 cc/day
  - Specifically contains histatins (has activity against Candida albicans and Streptococcus mutans)

- **Gastric Acid**
  - Depends on hydrochloric acid
  - At a pH less then 4, 99% of bacteria are killed within 30 minutes
Innate: Lumenal factors

- Digestive secretions and enzymes
- Secretory IgA and IgM
- Lactoferrin from pancreas
- Lysozyme from Paneth cell
- Defensins

Fig. 11.40 Paneth cells.
Micrograph of the base of a small intestinal crypt from a paraffin section showing numerous Paneth cells (P) containing large numbers of bright red granules. A small endocrine cell (E) with ill-defined fine basophilic granules can also be seen.

Fig. 11.41 Enteroendocrine cell.
Micrograph of the base of a small intestinal crypt showing a typical pale-staining entero-endocrine cell (E). In this thin acrylic resin section the Paneth cell (P) granules are difficult to see.
What is a defensin?

- Best characterized antimicrobial peptide
- Two classes: α and β
- Made by neutrophils and Paneth cells
- Secrete chloride ions
- Provide chemotactic factors
- An important link between the two defense systems
What is a lysozyme?

- Antimicrobial protein found in many human secretions
- In normal intestinal tract in gastric and pyloric glands, duodenal Brunner’s glands, small intestinal Paneth cells and macrophages and granulocytes, but NOT in normal colon
- Predominantly active against Gram-positives
Gut immunology

Gut defense mechanism

Innate
- Lumenal factors
- Antimicrobial factors
- Physical barriers
- Mechanical factors

Adaptive
- Gut-associated Lymphoid tissue
Innate: antimicrobial factors

- Preventing colonization of pathogens
  - Immunological factors (complement, phagocytes, natural killer cells)
  - Indigenous flora (estimated population $10^{14}$ bacteria which outnumbers the population of host cells approximately $10^{13}$)

- Producing antimicrobial compounds
Innate: antimicrobial factors (the immunological components)

- Complement, NK cells, phagocytes
- Available systemically but are in the activated form in the lamina propria
Innate: Physical Barriers

- **Mucus layer**
  - Goblet cells store mucin
  - Secreted constitutively or in response to a variety of secretagogues (prostaglandins, microbial products)
  - Protect the epithelial layer against invasion through the binding of their carbohydrate moieties to various microbial receptors (adhesins)
  - Probiotics such as lactobacillus may exert a protective effect by stimulating mucin production
Innate: IECs

- Single columnar intestinal epithelial cell layer
- Consists of four main cell types
  - Absorptive enterocytes
  - Goblet cells
  - Enteroendocrine cells
  - Paneth cells
- Rapid turnover: $10^{11}$ per day in the human small intestine
Innate Immunity
What does the IEC do?

- Mechanical barrier: can produce a mucous coat of glycocalyx that covers the surface of the epithelium
- Purge pathogens by secreting water and electrolytes
- Can act as nonprofessional APCs using MHC class I to underlying macrophages
- May signal the presence of stimuli or pathogens to effector cells (GALT)

Jabbar et al.
IECs and Tight Junctions

- Another layer of protection
- Channels only allow specific peptides and molecules to pass
- Dynamic and well-regulated

Jabbar et al.

Chandran et al.
Innate: Mechanical factors

- Peristalsis
- Digestive secretions
- Desquamation
Gut immunology

- Gut defense mechanism
  - Innate
    - Lumenal factors
    - Antimicrobial factors
    - Physical barriers
    - Mechanical factors
  - Adaptive
    - Gut-associated Lymphoid tissue
GALT: Four compartments

- Intraepithelial lymphocytes (IEL)
- Lamina propria
- Peyer’s patches
- Mesenteric Lymph nodes
GALT: InTRAepithelial lymphocytes (IEL)

- Heterogeneous T cell population many of which are not found in the systemic lymphoid tissues

- Most are Mature T cells of CD8+ phenotype (suppressor/cytotoxic)
GALT: IEL

- Function in homeostasis between lumen, IEC, and lamina propria
- Induces apoptosis of “old” IEC and stimulates the proliferation of IEC (keratinocyte growth factor)
- Directly eliminate damaged cells or microorganisms (perforin and granzyme)
- Downregulate inflammatory response specifically secrete TGF-β and down regulate IgA or directly act as a suppressor T cell
What is homing? Process by which activated immune cells exit from original site to systemic circulation and then return which helps to increase the efficiency of immune surveillance

IEL use E-cadherin and αEβ7 (adhesion molecules) for homing and maintaining interaction with IEC
GALT: Lamina Propria

- Main cell population
  - CD 4 + T lymphocytes (T helper cells)
  - Dendritic cells
  - Macrophages
  - B cells
Lamina Propria: A closer look

- Dendritic cells and macrophages are professional antigenic presenting cells needed in the gut to expose naïve T helper cells (Th0) to antigens.

- This leads to the T helper cells to differentiate in one of three types of Th cells:
  - Th1
  - Th2
  - Th3 and Regulatory T cells (Tr1)
Dendritic Cell System

**Phase 1: Capture**
Dendritic cells wait at the body’s surface areas and traffic points to pick up antigens.

**Phase 2: Processing**
The dendritic cell develops its own chemical vocabulary with which it will inform other immune cells — the white blood cells known as B and T lymphocytes.

**Phase 3: Presentation**
Dendritic cells present antigens to T cells, which then expand in numbers to become helpers or killers for the immune system.

T cells are deaf and blind to the world of antigens, until the dendritic cell intervenes, enabling T's to detect antigen at their receptor sites.
What about Th3 and Tr1?

Tr1

IL-10
IF-γ

Th3

TGF-β

These cells produce suppressive cytokines
Balance of Th1/Th2

- Th1
  - Up-regulate the Inflammatory response
  - IL-10
  - IFN-γ

- Th3
  - Cellular immunity
  - Humoral immunity

- Th2
  - Down-regulate

- Up-regulate the Inflammatory response
  - Down-regulate
Role of B Cells, Th2, and IgA

B cell → Th2 (IL-5, IL-6, IL-10, TGFβ) → Plasma cell

IgA

IgM
Role of IgA

- Does not activate complement or an inflammatory response
- Directed against surface molecules
- Has its own enterohepatic circulation
Figure 2.2. Diagram of secretory mechanism of intestinal epithelial cells which delivers dimeric IgA to the intestinal lumen.
GALT: Peyer’s Patch
Peyer’s Patch: Contents

- Both CD4+ and CD8+ T cells
- B cells
- Macrophages
- Dendritic cells
- M cells (membranous cells)
GALT: Four compartments

- Intraepithelial lymphocytes (IEL)
- Lamina propria
- Peyer’s patches
- Mesenteric Lymph nodes
What is an M cell?

- Derived from stem cells in the crypts of lymphoid tissue
- Covers the Peyer’s patches
- Specialized cells which can efficiently take up antigens which are then taken up by APCs in the Peyer’s patch
- Susceptible to invasion and destruction
Peyer’s Patch: Homing

- Relies on homing to traffic undifferentiated immune cells and activated immune cells to and from systemic circulation and gut immune system.

- What are the components required for homing?
  - (LOCK) MAdCAM 1 on vascular endothelium
  - (KEYS) L-selectin and α4/β7 on immune cells
MAdCAM-1
(mucosal adressin cellular adhesion molecule -1)

GUT

L-selectin and α4/β7
GALT: Mesenteric Lymph Nodes (MLN)

- T and B cells, Macrophages, interdigitating cells, and Langerhans cells
- Function: cell population expansion
- Mechanisms: trapping and presenting antigen to lymphocytes; expansion of activated B and T lymphocytes

Jabbar et al
Shigella (Bacillary dysentery)
The immune system in action
Two places with infectious diarrhea
Two places with IBS
The question:

Does infection play a role in the pathogenesis of IBS?
In 1997, the prevalence of functional bowel disorders six months after infectious diarrhea was 25% 


A cohort study in 1999, indicated that during a one year follow up, the diagnostic rate of irritable bowel syndrome was 4.4% in patients after an episode of bacterial gastroenteritis compared with a rate of 0.3% in the general population cohort.

A UK study has shown that >50% of IBS patients remain symptomatic six years post-gastroenteritis. Also, Post-infectious IBS (PI-IBS) had more diarrheal features than non-infectious-IBS.

Immunological aspects in PI-IBS

- Dunlop S, Jenkins D, Spiller, R. Distinctive Clinical, Psychological, and Histological Features of Postinfective Irritable Bowel Syndrome
- Subjects: 75 consecutive IBS outpatients (ROME II) and 36 healthy control subjects
- Workup included symptom questionnaire and rectal biopsy, which included staining and quantification of lamina propria (intraepithelial T lymphocytes, IEL), serotonin-containing enterochromaffin cells (EC) and mast cells.
## Results

<table>
<thead>
<tr>
<th>Symptom</th>
<th>PI-IBS</th>
<th>Non-PI-IBS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>70%</td>
<td>42%</td>
<td>0.03</td>
</tr>
<tr>
<td>Previous tx for anxiety or depression</td>
<td>26%</td>
<td>54%</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Dunlop et al
# Cell quantification results

<table>
<thead>
<tr>
<th>Cell</th>
<th>PI-IBS</th>
<th>Non-PI IBS</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC/hpf</td>
<td>39.4 ± 2.9</td>
<td>31.1 ± 1.5</td>
<td>31.8 ± 1.6</td>
<td>0.012</td>
</tr>
<tr>
<td>LP T cells/hpf</td>
<td>120.5 ± 6.8</td>
<td>118.5 ± 4.6</td>
<td>101.6 ± 5.9</td>
<td>0.042</td>
</tr>
<tr>
<td>Mast cells/hpf</td>
<td>41.9 ± 3.0</td>
<td>53.0 ± 2.4</td>
<td>45.9 ± 2.8</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Dunlop et al
T Lymphocyte counts and frequency of diarrhea

**Figure 3.** Lamina propria (LP) T lymphocyte counts per high power field (hpf) in 52 IBS patients with diarrheal symptoms. Lymphocyte scores increased with increasing frequency of diarrhea. *p = 0.04 vs 2 days/wk of loose stools. **p = 0.012 vs 2 days/wk of loose stools.
PI-IBS and interleukin 1:

- Gwee et al. examined sequential rectal biopsy samples from patients with PI-IBS and infectious control group during and after infection

- Measured
  - Expressions of interleukin 1 beta (IL-1β)
  - Expression of receptor antagonist (IL-1ra)
Changes in Interleukin 1(beta)mRNA expression

*Figure 2.* Changes in interleukin 1[beta] (IL-1[beta]) mRNA expression after infection in INF-IBS patients (who developed irritable bowel syndrome after acute gastroenteritis) and in INF-CON patients (who returned to normal bowel habits after acute gastroenteritis).

- Wang et al performed a cohort study in 295 patients recovering from acute gastroenteritis (controls were 243 siblings or spouses who had not been infected with Shigella) in Beijing, China

- During a 1-2 year follow-up, 8.1% incidence of IBS (Rome II) among all patients compared with 0.8% of controls, and an incidence of 10.2% of IBS in those patients with documented Shigella infection.

- 22.4% of patients (versus 7.4% of controls) exhibited functional gastrointestinal symptoms that did not meet the Rome II criteria.
Shigella and Immune response

- Wang et al. also found that expression of IL-1β mRNA in the terminal ileum and rectosigmoid mucosa was higher in PI-IBS (p<0.01).
- Mast cell numbers in the terminal ileum were higher in PI-IBS and non-PI-IBS patients compared with control subjects (p<0.01).
Lastly, an increased density of 5-hydroxytryptatimine and substance P immunoreactive nerves surrounding mast cells in the ileum and colon of IBS patients. (48% with infection hx and 52% without infection hx)

Confirms another study done by Barbara et al demonstrating a correlation between nerve to mast cell interactions with the severity of abdominal pain.
Limitations

- The studies varied on the infectious agent (Campylobacter, salmonella, shigella)
- Definition of IBS
- Differences in patient recruitment
- Tissue sampling
- Use of antibiotics
Can an anti-inflammatory help?

Dunlop SP, Jenkins D, Neal KR, Naesdal M, Borgaonker, Collins M, and Spiller RC. Randomized, double-blind, placebo-controlled trial of prednisolone in post-infectious irritable bowel syndrome
# Study characteristics

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Intervention</th>
<th>Outcomes measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI-IBS: new bowel symptoms developing in a previously asx individual immediately after an acute illness characterized by two or more of the following: D, V, F or positive stool culture</td>
<td>Placebo or prednisolone 30mg/day for 3 weeks</td>
<td>Mucosal enterochromaffin cells, T lymphocytes and mast cells in rectal biopsies before and after treatment and bowel symptoms</td>
</tr>
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</table>
Results

- No significant change in enterochromaffin cell counts
- T lymphocyte counts decreased significantly after prednisolone (p=0.003), BUT not associated with improvement in symptoms
Conclusions

- Gut immunology has multiple components to carefully maintain a balance between protective inflammatory states and non-inflammatory absorptive function.
- Studies from very different regions of the globe provide evidence for increasing recognition that infection may contribute to the pathogenesis of diarrhea predominant IBS.
- Low grade inflammation may contribute to diarrhea predominant IBS. The severity of the acute infection before PI-IBS is a strong risk factor.
- Currently, there is no role for prednisolone for treatment, but other medications may need to be explored.
Special Thanks

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- Ivy Tiu, Pharm D who drew the IgA and allowed me to use her scanner