Food and intestinal gene expression

Ian R. Sanderson
Centre for Digestive Diseases

NSKE, Oslo, 14. Januar 2010

Gut-Associated Lymphoid Tissue

Synopsis

- Clinical relevance
- Evidence for enterocyte signaling in vivo
- Developmental regulation of MHC class II
- Developmental changes in lumen (SCFA)
- SCFA and signaling genes

Diet and gastrointestinal disease

Crohn’s disease
Necrotising enterocolitis

Enteral feeding

Sanderson et al., 1987
Changes in ESR

Sanderson et al., 1987

ΔΔΔΔΔΔ
ESR

3 7 14 21 28 56 112

-75
-50
-25
25
0

Enteral feeds reduce inflammation rapidly in Crohn’s disease

Bannerjee et al. 2004

Day

3 7 14 21 28 56 112

Δ ESR

Endoscopic score was significantly better after treatment in the diet group

Borrelli et al. 2006

DIFFERENT LUMINAL FACTORS INFLUENCE GENE EXPRESSION IN THE ENTEROCYTE

Groove Factors

Nutrients

Bacteria

Antigens

Short chain fatty acids

Protein +

Protein +

INTERACTIONS OF MOLECULES IN THE INTESTINAL LUMEN WITH THE EPITHELIUM

A) Epithelial transfer

B) Change in epithelial responses

C) Epithelial regeneration

Borrelli et al. 2006

The epithelium transduces afferent stimuli to efferent signals

From: Sanderson, MJ (1990)
**Afferent limb:** Effect of the lumen on the epithelial cell

**Efferent limb:** Effect of the epithelial cell on the immune system

---

**Epithelial cell signaling**

- NF-κB MAPK
- TLR
- MyD88
- TIRAP
- IRAK
- TRAF6


**Signaling via TLRs**

- TRIF
- TRAM
- Co-stimulation Interferon α/β

Inflammatory cytokines (e.g., MIP-2)

---

**Plasmid with Fabpi promoter and MIP-2**

- EcoR 10.31 kb
- Fabpi
- Xho I 1.54 kb
- Fabpi-MIP2
- Xba I 1.92 kb
- MIP-2
- Nsi I 2.68 kb
- SV40 and I + pA

Ohtsuka et al., 2001

---

**Epithelial cell chemokine (MIP-2) increases neutrophil recruitment**

- Wild-type
- MIP-2 transgenic

Ohtsuka et al., 2001

---

**Construct used to generate transgenic mice**

- AMP-R
- Fabpi-MIP2
- Xho I
- SV40
- Ohtsuka et al., 2001
Phenotype of TLR transgenic mice
No difference in growth or weight
Equivalent stool production
No difference on intestinal histology
Intestinal morphometry: similar numbers of neutrophils, LP lymphocytes and IELs

Superarray of intestinal epithelial cell RNA
Many transcripts of TLR signaling products examined simultaneously (against internal standards)

Tollip is up-regulated in TLR4 transgenic mice
Tollip (protein) is expressed in greater amounts in TLR transgenic mice

Tollip

β-actin

Transgenic mice Wild-type mice

Signaling via TLRs


IL-6 increases IL17 cells and decreases Treg (Korn et al., 2007)
TLR transgenic mice have fewer circulating IL17 cells

- IL17+ in CD3+CD4+
- IL17 in blood (not stimulated)
- average WT, average TG
- n=4 WT, 6 TG/ p=0.03

TLR transgenic mice sustain greater body weight during DSS inflammation

- IL17 in stimulated blood
- average WT, average TG
- n=4 WT, 6 TG/ p=0.02

TLR transgenic mice have less inflammation after DSS

- Persistent TLR activation:
  - does not alter intestinal morphology
  - decreases circulating T17
  - protects against inflammation

Luminal regulation of epithelial cell gene expression

- Luminal changes in infancy
  - In utero
  - Birth
  - Milk
  - Weaning
Surface molecules include MHC class II.

Signaling occurs through surface molecules or secreted proteins.

Antigen presentation: MHC class II and invariant chain.

Elemental diet on enterocyte MHC Class II expression:

MHC class II is expressed on the normal mouse intestinal epithelium after weaning.

Elemental vs complex diet on enterocyte MHC class II ontogeny:

Mice weaned at 18 days and litters split.

Normal diet → elemental diet

Enterocytes isolated Compared from both groups at subsequent time points.

Invariant chain and MHC class II induced on weaning with a complex (normal) diet.

Class II MHC is regulated by three isoforms of the class II transactivator.
Weaning mice onto normal diet induces type IV MHC ontogeny.

Class II MHC ontogeny
Type III regulated by time (independent or diet)
Type IV regulated by diet (independent of time)

Appearance of butyrate in the large intestine of weanling mice

Appearance of butyrate in the large intestine of children

Diet
Bacteria

Unabsorbed Carbohydrate (e.g., Fiber)

Bacteria

Short Chain Fatty Acids
Butyrate regulation of signaling molecules

Secreted proteins include:
- Chemokines
- IGF binding proteins

Signaling occurs through surface molecules or secreted proteins

Dose response at 18 hours Caco-2 + sBLP

DN-TLR2 inhibits NFκB production in response to sBLP in Caco-2 cells

Signal integration

C = f (A) (B)

Short chain fatty acid modulation of signaling chemokine

1. Bacterial lipoproteins
2. IL-1

IL8 response to BLP + Butyrate in Caco2 cells
Hypothesis

Butyrate up regulation of gene expression: histone acetylation

Butyrate down regulation of gene expression: transcription factor acetylation

Trichostatin A Inhibits Histone Deacetylase

Butyrate and TSA increase histone acetylation and alter chemokine expression

Butyrate up-regulates through histone acetylation

Stromal cells enhance epithelial cell chemokines
Proposed model of MMP action

Kruidenier et al. (2006)

(Co-)culture system

Butyrate also affects stromal cells

Kruidenier et al. (2006)

Caco-2 response to colonic fibroblasts

CCD-18co cells induce Caco-2 cells to attract neutrophils. This response is inhibited by doxycyclin and anti-MMP-2 antibodies.

Kruidenier et al. (2006)

SCFA enhance MMP-3 expression from stromal cells (Pender et al., 2000)

SCFA and TSA increase histone acetylation in stromal cells (Pender et al., 2000)

Pender et al. (2000)
Conclusions

Diet plays a critical role in the developing intestine in health and disease

Luminal molecules regulate gene expression by promoter and epigenetic pathways

Epithelial signaling can orchestrate the mucosal immune system in vivo

ACKNOWLEDGEMENTS

Bob Fusunyan
Demetra Stamm
Jessica Quinn
Laurens Kruidenier
Rachel Levi
Sven Petterson
Sylvia Pender
Tom MacDonald
Yoshi Ohtuska