Introduction

• Overwhelming majority of initial antigen encounters occur at mucosal surfaces

• Surface bathed by a heterogeneous population of microorganisms

• Confronted by a large number of antigenic stimuli which must be deciphered for pathologic potential

• For the majority, a response characterized by either ignorance or active suppression is appropriate

• For a few, a robust immune response is in order
Introduction (II)

- Gut associated lymphoid tissue (GALT) is characterized by a regulated state of physiologic inflammation.

- GALT is poised for, but actively restrained from, full action and notable for a tendency to suppress responses, called oral tolerance.

- Certain microorganisms and food antigens elicit vigorous immune responses.

- The rules which govern these immunologic decisions are beginning to be clear and are important to the development of vaccines and the treatment of inflammatory bowel disease.
MUCOSAL BARRIER FUNCTION

Illustration by MIT OCW.
INNATE HUMORAL FACTORS

Lactoferrin, Lysozyme, Peroxidase, ITF, Complement, Defensins
TLR Ligands and their Receptors

Figure removed due to copyright reasons. Please see:

Commensal Bacteria Regulate Mucosal Gene Expression

Figure removed due to copyright reasons. Please see:

Figure 1 and Figure 2 in Hooper, Lora V., et al. "Molecular Analysis of Commensal Host-Microbial Relationships in the Intestine." Science 291 (2001): 881-84.
INNATE HUMORAL FACTORS: DEFFENSINS

Secreted by Paneth Cells, AEC
Luminal Factors:
Specific Extrinsic or Immunologic Barriers
Secretory Immunoglobulins
Isotype Distribution of Ig Production By Mucosal Plasma Cells

## Levels (µg/ml) of Immunoglobulins in Human Secretions

<table>
<thead>
<tr>
<th>Fluid</th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal Secretions</td>
<td>70-846 µg/ml</td>
<td>8-304 µg/ml</td>
<td>0 µg/ml</td>
</tr>
<tr>
<td>Broncho-alveolar fluid</td>
<td>3 µg/ml</td>
<td>13 µg/ml</td>
<td>0.1 µg/ml</td>
</tr>
<tr>
<td>Milk</td>
<td>470-1632 µg/ml</td>
<td>40-168 µg/ml</td>
<td>50-340 µg/ml</td>
</tr>
<tr>
<td>Duodenal fluid</td>
<td>313 µg/ml</td>
<td>104 µg/ml</td>
<td>207 µg/ml</td>
</tr>
<tr>
<td>Colonic fluid</td>
<td>162 µg/min</td>
<td>34 µg/min</td>
<td>17 µg/min</td>
</tr>
</tbody>
</table>

IgA2 is Enriched in Mucosal Secretions Relative to Peripheral Blood

### T Cell Independent IgA Secretion in the Intestine

(IgA-secreting cells, no. per 10^5 lymphocytes)

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Housing conditions</th>
<th>Intestinal lamina propria</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>SPF</td>
<td>11,600 ± 1,500</td>
</tr>
<tr>
<td>TCR^{β/-δ/-}</td>
<td>SPF</td>
<td>3,900 ± 1,600</td>
</tr>
<tr>
<td>C57BL/6 nu/nu</td>
<td>SPF</td>
<td>2,800 ± 1,700</td>
</tr>
<tr>
<td>CD4^{−/-}</td>
<td>Conventional</td>
<td>9,100 ± 930</td>
</tr>
<tr>
<td>TNFR-1^{−/-}</td>
<td>SPF</td>
<td>9,500 ± 540</td>
</tr>
<tr>
<td>aly/aly</td>
<td>SPF</td>
<td>&lt;1</td>
</tr>
<tr>
<td>LT^{α/-}</td>
<td>Conventional</td>
<td>&lt;10</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>Germ-free</td>
<td>1,600 ± 860</td>
</tr>
</tbody>
</table>
Enrichment of dimeric (d)IgA in Mucosal Secretions Relative to Serum Which contains monomeric IgA

Secretory dIgA is formed by Association With J Chain and proteolytic fragment of plgR or SC

STRUCTURE OF POLYMERIC Ig RECEPTOR (pIgR)

Intracellular Transport of plgA via plgR

Quantification of IgA Production In Mucosal Secretions

Image removed due to copyright reasons.
IgA is a Component of Bile via Expression of plgR in hepatocytes (rat) or bile duct epithelium (human)

Image removed due to copyright reasons.
IgA has complex effects in Mucosal Tissues Through interaction with Fcα-receptors

Image removed due to copyright reasons.
The **Neonatal** Fc Receptor for IgG, FcRn

- MHC I-like structure/$\beta_2$m associated

- Closed cleft/no defined role in antigen presentation

- Binds overlapping region of IgG as Protein A

- Binds IgG with a 2:1 stoichiometry

- Binds IgG at pH 6.0 ($K_d = 10$ nM) but negligibly at physiologic pH 7.4

Figure removed due to copyright reasons. Please see:

**FcRn Plays a Role in the Uptake of Lumenal Antigens**

*Polymeric IgR-mediated IgA Transport*

*FcRn-mediated IgG Transport*

- IEC
- DC
- IgG
- CD4+ T cells
- B cell or Plasma cells
Intrinsic Barrier Function of Epithelium

TJ

Desmosome

IEL

BM
ASSOCIATION OF DENDRITIC CELLS WITH MUCOSAL EPITHELium

**STRATIFIED EPITHELIA**
- Vagina
- Tonsil

**SIMPLE EPITHELIA**
- Bronchiole
- Intestine
- Bronchi

**Lymphoid Tissue**
- O-MALT

**Peripheral Lymph Nodes**

**DC**

Dendritic Cell

Figure by MIT OCW.
Pathways for antigen-uptake from the lumen

1. Antigen uptake by M cell
   - M cell
   - Mφ
   - DC

2. Antigen uptake by IEC
   - IEC
   - DC
   - MHC class II

3. Antigen uptake by FcRn
   - FcRn
   - APCs
   - MHC class II
   - B7-1/2

Mucosal Tolerance/Activation?
Subtypes of Epithelial Cells in Intestinal Mucosa
M (MICROVILLOUS FOLD) CELLS

M (Microvillous Fold) Cells

- M cell
- B & T Lymphocytes
- Enterocyte
- Mφ (Macrophage)
- Dendritic cell

Image by MIT OCW.
M CELLS TRANSPORT PARTICULATE Ag AND ASSOCIATE WITH MONONUCLEAR CELLS

Image removed due to copyright reasons.
Absorptive epithelial cells take up Ag by Receptor and non-Receptor mediated mechanisms sorting Ag to either a degradative or absorptive fate.
Epithelial Transport of Macromolecules

**Luminal Protein**

- **Intact Protein**
  - 50-200 ng/h.cm²
  - Direct pathway: 10%

- **Processed Protein**
  - 500-2500 ng/h.cm²
  - Indirect pathway: 90%

*adapted from Martine Heymann*
Absorbed antigens may enter an antigen presenting pathway such as that associated with MHC class II.
THE IEC AS AN APC

• Ability to acquire and/or transport antigen

• Ability to process and/or present antigen

• Ability to provide costimulatory and/or regulatory second signals to T cells
Molecules expressed by IECs possibly associated with antigen presentation

**Induced**
- CD86
- ICAM-1
- Enhanced class II
- MHC class I-related molecule A and B (MICA and MICB)

**Constitutively expressed**
- MHC class I
- MHC class II
- MHC class Ib (CD1d, T1)
- E-cadherin (aEb7 ligand)
- IL7R
- gp 180 (CD8 ligand)
- Common gamma chain (IL2R, IL4R, IL7R, IL9R, IL15R)
- LFA-3 (CD2 ligand)

Absent - CD80, CD40

Image by MIT OCW.
Antigen Presentation by Absorptive Epithelial Cell

- Antigen Uptake (fluid phase pinocytosis)
- Soluble Antigen
- Insoluble or Carbohydrate Antigen
- FcRn (? uptake of IgG complexes)
- Tight junction
- CD1d/gp 180 Complex

IEC projection through the basement membrane expressing class Ib, class I, or class II MHC

Image by MIT OCW.
AEC secrete and respond to a wide variety of cytokines and chemokines.

Absent or Controversial:
- IL 1
- TNF
- IL10R
- LPS - R (CD14)
- TNFR

Suppressor:
- TGF-b (rat)
- PGE2

Regulatory:
- IL-6
- IL-1RA
- GM-CSF
- IL-15
- IL-7
- SCF
- TSH

Chemokines:
- IL-8
- ENA-78
- MCP-1
- GRO-alpha
- GRO-beta
- RANTES

Image by MIT OCW.
AEC Respond to cytokines and inflammatory mediators
With increased chloride and mucus secretion and paracellular Permeability resulting in diarrhea clinically

Figure by MIT OCW. After Yamada, Atlas of Gastroenterology, 2003.
Peyer’s patches

IEL

IEC

M-cell

Ag

IEL

IFR

HEV

FO

GC

B

MLN

IEL

T

B

T

B

IgA

Peyer’s patches Lamina propria

Lamina propria

IEL

B

M

IgA

MLN

MALT

PCV
Peyer’s Patch Development

IL-7 → IL-7R → CD4+ CD45+ CD3- → LTα2β LTβR NIK → Stromal Cell

CCL19 CCL21 CXCL12 CXCL13

T and B cell Recruitment
Concept of the Common MALT

Image removed due to copyright reasons.
Heirarchical Linkage of MALT Component Tissues

Image removed due to copyright reasons.
### Molecular interactions during lymphocyte trafficking

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Endothelial Cells</th>
<th>Contact ➔ Rolling ➔ Arrest ➔ Diapedesis</th>
</tr>
</thead>
</table>
| **Naive B or T cells**<br>\( \alpha 4 \beta 1 \)\, \( \alpha 4 \beta 7 \)<br>L-selectin\(^{++} \), LFA-1\(^{++} \)<br>\( \alpha 4 \beta 1 \) \( \alpha 4 \beta 7 \)<br>L-selectin\(^{hi} \), Lselectin\(^{\pm} \)<br>**Gut homing blasts or memory cells**<br>\( \alpha 4 \beta 1 \)\, \( \alpha 4 \beta 7 \)<br>L-selectin\(^{++} \), LFA-1\(^{++} \)<br>\( \alpha 4 \beta 1 \) \( \alpha 4 \beta 7 \)<br>**Peripheral lymph node**<br>\( \alpha 4 \beta 1 \)\, \( \alpha 4 \beta 7 \)<br>L-selectin\(^{++} \), Lselectin\(^{++} \)<br>**Skin homing memory cells**<br>CLA\(^{++} \), LFA-1\(^{++} \)<br>\( \beta 7^- \), L-selectin\(^{+} \)<br>**Skin**<br>CLA\(^{++} \), LFA-1\(^{++} \)<br>\( \alpha 4 \beta 1 \) \( \alpha 4 \beta 7 \)<br>**Vesicular Adhesion Protein 1 (VAP1)**
| Peyer's patch<br>MAdCAM CHO<br>MAdCAM-1<br>ICAM's<br>**L-selectin** | **L-selectin**<br>\( \alpha 4 \beta 7 \)<br>LFA-1<br>**L-selectin**<br>**LFA-1?**<br>**L-selectin**<br>??<br>LFA-1<br>**CLA**<br>\( \alpha 4 \beta 1 \)<br>LFA-1<br>**LFA-1**<br>**VCAM-1**<br>ICAM's<br>**E-selectin**<br>ICAM's<br>**E-selectin**<br>ICAM's<br>**E-selectin**<br>ICAM's

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Figure by MIT OCW. After Kiyono, Essentials of Mucosal Immunology.
Intraepithelial Lymphocyte

small intestine

- TCR\(\alpha\beta\) +
- CD8 +
- 45RO +
- \(\alpha E\beta 7\) +
- CD69 +
- CD25 -
- CD28 -
- CD101 +
- BY-55 +

large intestine

- 1/3 CD8 +
- 1/3 CD4 +
- 1/3 CD4-CD8 -

TCR\(\gamma\delta\) < TCR\(\gamma\delta\)
## Potential Functions of iIELs

<table>
<thead>
<tr>
<th>Function</th>
<th>Mouse</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Tolerance</td>
<td>TCR_{γδ}</td>
<td>???</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>TCR_{αβ}</td>
<td>TCR_{αβ} &amp; γδ</td>
</tr>
<tr>
<td>Regulation of B cell immunoglobulin production</td>
<td>TCR_{γδ}</td>
<td>???</td>
</tr>
<tr>
<td>Anti-microbial immunity</td>
<td>TCR_{αβ}</td>
<td>???</td>
</tr>
</tbody>
</table>
Mast Cells: Stimuli and Mediators

**Stimuli**
- Allergen-IgE
- T cell factor (antigen specific)
- Polypeptide histamine releasing factors
- Neuropeptides
- Cytokines (e.g. SCF, IL-8)
- Complement anaphylatoxins
- Cationic agents

**Mediators**

**PREFORMED/STORED**
- Histamine
- Proteoglycans
- Proteinases
- Chemotactic factors

**NEWLY SYNTHESIZED**
- PGD2, LTC4
- PAF, NO

**CYTOKINES**
- IL3, 4, 5, 8, 10, 13
- TGF-β, TNF-α
Concept of Oral Tolerance

No oral feeding \[\rightarrow\] Immunize subcutaneous \[\rightarrow\] T cells from regional lymph nodes respond

Feed oral antigen \[\rightarrow\] Immunize subcutaneous

1. T cells from regional lymph nodes do not respond.
2. Specific IgA is measurable in gut.

from Challacombe and Tomasi, J Exp Med, 1980
Mechanisms of Oral Tolerance

Oral administration of antigen

GALT

Low dose (1 mg X 5)
- Induction of Th2 (IL-4/IL-10) and Th3 (TGF-β) secreting regulatory cells
- Active suppression

High dose (20 mg)
- Deletion or anergy of Th1 and Th2 cells
- Clonal deletion/anergy
Inflammatory Bowel Disease

Modifying Environmental Factors (e.g. tobacco)

Mucosal Immune Response

Commensal Microbial Antigens

T Regulatory Response

Th1 or Th2 Inflammatory Response

Tissue Injury

Clinical Symptoms

Genetics (e.g. chr. 5 & 16)

Regulation Immune Response?

Regulation Of Barrier Function?
# Summary of IBD Susceptibility Loci

<table>
<thead>
<tr>
<th>Locus</th>
<th>Chromosomal Region</th>
<th>Comments</th>
<th>Variation identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBD1</td>
<td>16q12</td>
<td>CD-specific CARD 15</td>
<td>YES</td>
</tr>
<tr>
<td>IBD2</td>
<td>12p</td>
<td>Possibly UC specific</td>
<td>No</td>
</tr>
<tr>
<td>IBD3</td>
<td>6p</td>
<td>IBD HLA region</td>
<td>Potential HLA alleles</td>
</tr>
<tr>
<td>IBD4</td>
<td>14q11-12</td>
<td>Possibly CD specific</td>
<td>No</td>
</tr>
<tr>
<td>IBD5</td>
<td>5q31</td>
<td>OCTN§</td>
<td>YES</td>
</tr>
<tr>
<td>IBD6</td>
<td>19p13</td>
<td>IBD</td>
<td>No</td>
</tr>
<tr>
<td>IBD7</td>
<td>1p36</td>
<td>IBD</td>
<td>No</td>
</tr>
<tr>
<td>IBD8</td>
<td>10q30</td>
<td>Scaffold protein</td>
<td>YES</td>
</tr>
</tbody>
</table>

§ OCTN: organic cation transporter

adapted from Rioux J 2003
IBD-1: Nucleotide binding oligomerization Domain (NOD)2 or CARD15: Intracellular Bacterial Sensor

Muramyl Dipeptide

Oligomerization with NOD2

R702W  G908R  Δ33

Mutations in CD (loss of function)

LRR = leucine rich repeats

RICK

PO₄

IKKγ

IKKα

IKKβ

NF-κB Activation

LRR = leucine rich repeats

CARD

CARD

NOD

LRR

LRR = leucine rich repeats

CARD

CARD

NOD

LRR

LRR = leucine rich repeats
Luminal Bacteria Stimulate Colitis

Germ Free Housing → Addition of Bacteria

Mice
- IL-2 -/-
- IL-10 -/-
- CD45RB hi
- SCID
- SAMP1-Yit
- TCRα -/-

Rats
- HLA-B27 Transgenic

Addition of Bacteria

No immune activation → No Colitis

Mφ
- IL-1
- TNF
- IFNγ

Th1

Severe Colitis
Crohn’s Disease

- Ileocolitis
- Transmural Granulomas
- Th1 Inflammation: IL-12, IFN-γ, TNF-α

Ulcerative Colitis

- Colitis
- Superficial Crypt Abscesses/Ulceration
- Th2-like Inflammation: EBI3, IL-5, IL-13, IL-6
Bacterial Antigens

IFN-γ

TNF

IL-12(+)

APC

Th1

Th1

Th1

(t-bet)

IL-10

TGF-β

Treg

IFN-γ

TNF

IL-12(+)

Th1

Th1

Th1

APC

Neurath et al, JEM 2002
Blumberg et al, Ann Rev Immunol 2002
Bacterial Antigens

Breg
IL-10

Th2

NKT
CD1d
EBI3 (+)

IL-4
IL-13

Mizoguchi et al, Immunity 2001
Heller et al, Immunity 2001
Nieuwenhuis et al, PNAS 2002
Van de Waal et al, Gastroenterology 2003
Celiac Disease

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Figure removed due to copyright reasons. Please see: