MUCOSAL IMMUNITY

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- CHALLENGES FACED BY THE MUCOSAL SYSTEM
- SPECIALIZATION OF CELLS INVOLVED IN MUCOSAL IMMUNITY
- ORGANIZATION OF THE MUCOSAL IMMUNE SYSTEM
- CLINICAL IMPLICATIONS

DEFINITIONS

MALT= MUCOSA-ASSOCIATED LYMPHOID TISSUE

MALT is the highly specialized immune system which protects mucosal surfaces. The lymphoid elements associated with different mucosal sites share organizational as well as functional similarities. It is the largest mammalian lymphoid organ system and in an adult it comprises approximately 80% of all lymphocytes.

SUBDIVISIONS OF THE MUCOSA

Distinct features of type I and type II mucosal surfaces

<table>
<thead>
<tr>
<th>Feature</th>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelia</td>
<td>Simple</td>
<td>Stratified</td>
</tr>
<tr>
<td>Presence of MALT</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Presence of pIgR</td>
<td>IgA</td>
<td>IgG</td>
</tr>
<tr>
<td>Major Ig isotype</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Goblet cells</td>
<td>*</td>
<td>-</td>
</tr>
</tbody>
</table>

pIgR=polymeric Ig receptor

ILF=Isolated lymphoid follicle
PP=Peyer's Patches
THE CHALLENGES

- MOST FREQUENT PORTAL OF ENTRY FOR HARMFUL SUBSTANCES. THUS THE MALT HAS TO MOUNT AN EFFECTIVE RESPONSE AGAINST A VAST NUMBER OF POTENTIAL PATHOGENS.
- THE MUCOSAL MEMBRANES OF THE DIGESTIVE TRACT MUST ALLOW FOR THE ABSORPTION OF NUTRIENTS BY THE HOST. THUS THE MALT MUST REMAIN HYPORESPONSIVE TO AN ENTIRE ARRAY OF HARMLESS SUBSTANCES.

SPECIALIZED COMPONENTS OF MALT

B CELLS

- HUMORAL RESPONSES ARE CENTRAL TO AN EFFECTIVE MUCOSAL IMMUNITY.
- THE MAIN HUMORAL MEDIATORS OF SPECIFIC MUCOSAL IMMUNITY ARE SECRETORY IgA AND, TO A LESSER EXTENT, SECRETORY IgM.
- THE NORMAL INTESTINAL MUCOSA CONTAINS AT LEAST 20 TIMES MORE IgA+ THAN IgG+ LYMPHOCYTES.

CRITICAL FEATURES OF SECRETORY IgA

- RESISTANCE AGAINST COMMON INTESTINAL PROTEASES
- INABILITY TO INTERACT WITH COMPLEMENT OR CELLS IN A WAY TO CAUSE INFLAMMATION

MECHANISMS OF PROTECTION BY sIgA AT MUCOSAL SURFACES

- INHIBITION OF ADHERENCE
- VIRUS NEUTRALIZATION
- NEUTRALIZATION OF ENZYMES AND TOXINS
- IMMUNE EXCLUSION AND INHIBITION OF ANTIGEN ABSORPTION

FACTORS CONTROLLING IgA ISOTYPE SWITCHING
**FACTORS CONTROLLING THE SECRETION OF IgA: THE J CHAIN**

- The J chain is a 15 kD polypeptide that is disulfide-bonded to the tail-pieces of both IgM and IgA.
- IgA-secreting B cells in the bone marrow do not express the J chain and thus secrete IgA monomers.
- The majority of IgA-producing B cells in the mucosa express the J chain and thus produce dimeric IgA.
- The J chain stabilizes the multimers and it appears to determine the polymeric IgA and IgM structure which allows polymeric IgA to complex with the secretory component.

**LAMINA PROPRIA LYMPHOCYTES**

- Lymphocytes which are scattered diffusely throughout the lamina propria of the intestine. (Lamina propria = layer of connective tissue between the epithelium and the muscularis mucosa).
- Largest single T-cell site in humans. Most of the T cells within the lamina propria are CD4+.

**T CELLS**

**TH1, TH2 or TH17?**

**INTRAEPITHELIAL LYMPHOCYTES (IELs)**

- IELs are lymphocytes which are interspersed between the columnar epithelial cells of the villi in the small and large intestine.
- In humans, most of the IELs are CD8+ T cells. Approximately 10% of IELs are γδ T cells.
- Both the γδ and the αβ TCR+ IELs show limited diversity of T cell receptor.
FUNCTIONAL PROPERTIES OF IELs

- First immune cell line of defense in the intestine
- Display cytotoxic activity
- Secrete large amounts of cytokines especially IFN-γ and TNF-α
- Modulate the kinetics of epithelial cell renewal

REGULATORY T CELLS

- TH3 cells: a population of CD4+ T cells that produce TGF-β. Isolated from mice fed low dose of antigen for tolerance induction
- TR1 cells: a population of CD4+ T cells that produce IL-10. Can produce suppression of experimental colitis in mice
- CD4+CD25+ regulatory T cells: a population of CD4+ T cells that can prevent autoreactivity in vivo.

REGULATORY T CELLS

- CD8+ suppressor T cells: the first identified population of regulatory T cells thought to be involved in oral tolerance.
- γδ T cells: studies in mice indicate that they have an important role in some models of oral tolerance.

ORAL TOLERANCE

- Oral administration of a protein antigen may lead to suppression of systemic humoral and cell-mediated immune responses to immunization with the same antigen.
- Possible mechanisms:
  - Induction of anergy of antigen-specific T cells
  - Clonal deletion of antigen-specific T cells
  - Selective expansion of cells producing immunosuppressive cytokines (IL-4, IL-10, TGF-β)

The relative balance between effector T cells and regulatory T cells determines intestinal immunity vs tolerance.
**EPITHELIAL CELLS**

Scanning electron microscopy of a single microdissected dome (a) of a murine Peyer's patch. The M cells are identified by their relatively short, dark brush border; they are restricted to the dome epithelium (upper half in b). Crypts (arrows) are opening to the cleft between the dome and the neighboring villi.

From: Gebert et al., *Am. J. Pathol.* 154:1573, 1999

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**CHARACTERISTICS OF M CELLS**

- M (“membrane-like”) cells are specialized epithelial cells which overlie lymphoid follicles domes along the length of the small and large intestine.

- Structural features include:
  - Few short irregular microvilli
  - Abundant endocytic vesicles
  - Low lysosomal content
  - Distinctive glycocalix
  - Binding sites for secretory IgA but no SC
  - Pockets in the basolateral surface

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**FUNCTIONS OF M CELLS**

- Antigen sampling

- Portal of entry for selected pathogens

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**FUNCTIONS OF FAE ENTEROCYTES**

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**DENDRITIC CELLS**
**Dendritic cell-mediated transport of commensal bacteria in the gut**

Dendritic cells can sample antigen indirectly via M-cell transcytosis (**right**) or directly via processes that extend across the epithelial barrier (**left**). DCs present antigen to B- and T-cells, either directly within the lamina propria or following trafficking to the regional lymph nodes.


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**Role of CX3CR1 in Luminal Sampling by Gut DCs**

CX3CR1 is a chemokine receptor whose ligand is an unusual chemokine; rather than secreted, it is membrane-bound.

From: Neus et al., *Science* 307:254, 2005

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**ORGANIZATION OF MALT**

- Organized mucosal lymphoid follicles which lack afferent lymphatics
- Peyer's patches are found in the small intestine
- Follicles similar to Peyer's patches are found in the appendix, in the rest of the GI tract and in the respiratory tract


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**INDUCTIVE SITES = PEYER'S PATCHES**

**CELLULAR TRAFFIC IN ORGANIZED MUCOSAL LYMPHOID TISSUES**

- Response to harmless pathogens
- Response to harmful pathogens

From: Neutria et al., *Nature Reviews Immunology* 8, 100-118 (February 2008) | doi:10.1038/nri2177
**CLINICAL IMPLICATIONS**

**IgA DEFICIENCY**
- It is the most common primary immunodeficiency
- It is usually defined by a serum IgA concentration of less than 50 µg/ml
- IgA deficient individuals often appear perfectly healthy and are identified:
  - upon serving as blood donors
  - upon undergoing anaphylactic shock when receiving blood transfusions

**CLINICAL MANIFESTATIONS OF IgA DEFICIENCY**
- Increased incidence of infections
  - upper and lower respiratory tract
  - gastrointestinal
- Higher incidence of autoimmune diseases
- Higher incidence of allergic diseases
- Higher incidence of celiac disease

**CELIAC DISEASE**
- Celiac disease is a T cell mediated immune disease of the small intestine triggered by gluten
- Major features:
  - villous atrophy with a lymphocytic infiltrate
  - increased epithelial proliferation with crypt hyperplasia
  - Malabsorption

**CELIAC DISEASE: IMMUNOLOGIC FEATURES**
- Antigen: gluten (gliadin and glutenins)
- It is associated with HLA-DQ2 or HLA-DQ8 restricted lamina propria CD4+ T cells that recognize gluten and secrete interferon-γ (98% of people will carry these haplotypes)
- Gliadin is a substrate of tissue transglutaminase (transforms positively charged glutamines to negatively charged glutamic acid)
- Increased B cell activity
  - antibodies against gliadin (IgA-AGA, IgG-AGA)
  - endomysial antibody (IgA-EMA)
  - tissue transglutaminase (IgA-tTG)

**CELIAC DISEASE PATHOGENESIS**
- Updated and uncorrected. Copyright ©2007 American Society for Clinical Investigation

INFLAMMATORY BOWEL DISEASE (IBD)

- IBD is a chronic, relapsing and remitting inflammatory condition
- Two overlapping phenotypes:
  - Crohn's disease (CD), which affects the distal small intestine as well as the colon in a transmural manner
  - Ulcerative colitis (UC), which predominantly affects the colon in a superficial manner

CELL-MEDIATED IMMUNITY (ACTIVE CD):
- Increased number of activated mucosal T cells secreting IFN-γ (TH1)
- Increased mucosal production of cytokines that activate TH1 cells (IL-12 and IL-18)
- Defects in regulatory (IL-10 producing) T cells

HUMORAL IMMUNITY: Massive increase in the number of plasma cells and in IgG production (IgG2 in CD and IgG1 in UC)

IMBALANCE OF PRO-INFLAMMATORY (TNF-α, IL-1, IL-8, IL-12) AND ANTI-INFLAMMATORY CYTOKINES (IL-10, IL-4, IL-13)

THE ETIOLOGY IS UNKNOWN: Due to a dysregulated mucosal immune response to unknown antigens present in commensal flora

IBD PATHOGENESIS


MUTATIONS IN NOD2 (a cytosolic receptor for pathogenic bacterial signals) increase the risk of Crohn’s disease by a factor of 20-40.

NOD2 AND IBD PATHOGENESIS

MDP = muramyl dipeptide, a portion of bacterial cell wall


INHIBITORS OF PROINFLAMMATORY CYTOKINES
- Anti-TNF therapies: infliximab

ANTIINFLAMMATORY CYTOKINES
- IL-10
- IL-11

ANTI-LEUKOCYTE ADHESION THERAPIES
- Anti-α4 integrin: Natalizumab

INHIBITORS OF TH1/TH17 POLARIZATION
- Anti-IL-12/IL-23
- Anti-IL-18
- Anti-IFN-γ

IBD: EMERGING BIOLOGIC THERAPIES

MUCOSAL IMMUNIZATION
**MUCOSAL VACCINES**

- Vaccines against mucosal infections must stimulate the MALT in order to be efficacious.
- Because of subcompartmentalization within the MALT, vaccines must be administered by the appropriate route.
- Nonreplicating antigens are often relatively inefficient in yielding strong and long-lasting mucosal antibody responses.

**NEW STRATEGIES FOR ANTIGEN DELIVERY:**

- Live attenuated recombinant bacteria and viruses with known mucosal tropism.
- Protective vehicles, e.g., liposomes and biodegradable microparticles.
- Mucosal lectin-like molecules endowed with immunostimulatory properties, e.g., cholera toxin.

**EFFECT OF IMMUNIZATION ROUTE ON LOCAL AND DISTAL ANTIBODY RESPONSE**

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>Route</th>
<th>Specific antibody response (specific IgG)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small</td>
<td>Large</td>
<td>Carotid</td>
</tr>
<tr>
<td>Cholera toxin</td>
<td>Oral</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Nasal</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cholera toxin</td>
<td>For</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>OR</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>OR</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

*Responses are based on experimental mouse models and may not be applicable to human responses. Corresponding symbols: ++, ++, ++, ++, ++, ++.*

**MUCOSAL IMMUNOTHERAPY**

- Strategy to attempt to treat illnesses resulting from immune reactions against autoantigens encountered in nonmucosal tissues.
- Human trials have been conducted in multiple sclerosis, rheumatoid arthritis, uveoretinitis, and type I diabetes.

**MUCOSAL IMMUNOTHERAPY**

- Potential problems:
  - Limited success in suppressing the expression of an already established immune response.
  - Massive amounts of tolerogens are required.
  - Immunosuppressive effect is of short duration.

**ILLUSTRATION**

- Graph or chart showing the effect of different routes of immunization on mucosal antibody response.

**Table:**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Oral antigen</th>
<th>Disease progression</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>Collagen</td>
<td>Type 1 diabetes</td>
<td>Transient</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type 1 diabetes</td>
<td>Transient</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Type 2 diabetes</td>
<td>Transient</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type 2 diabetes</td>
<td>Transient</td>
<td></td>
</tr>
</tbody>
</table>

*The table shows the effectiveness of oral administration of different antigens in type 1 and type 2 diabetes.*