Molecular and Cellular Basis of Immune Protection of Mucosal Surfaces

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Introduction to Mucosal Immunity

- Mucosa represent a vast surface area
  - vulnerable to colonization and invasion
- Total amount of sIgA exceeds circulating IgG.
- Antigens are separated from mucosal immune tissue by epithelial barrier.
- Antigens must be transported across the epithelium.

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Significance of Mucosal Immunity

- Protection from microbial colonization (adherence)
- Prevention of environmental sensitization
- Focus of much vaccine work
- May have regulatory influence on systemic immunity
- May block allergic sensitization

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Secretory IgA

- >3 g of sIgA per day
- Structure of IgA
- Isotypes (A1 and A2) are tissue-specific
  - A1-
  - A2- mucosal plasma cells (has resistance to IgA1 proteases)
- J-chain
- Secretory component

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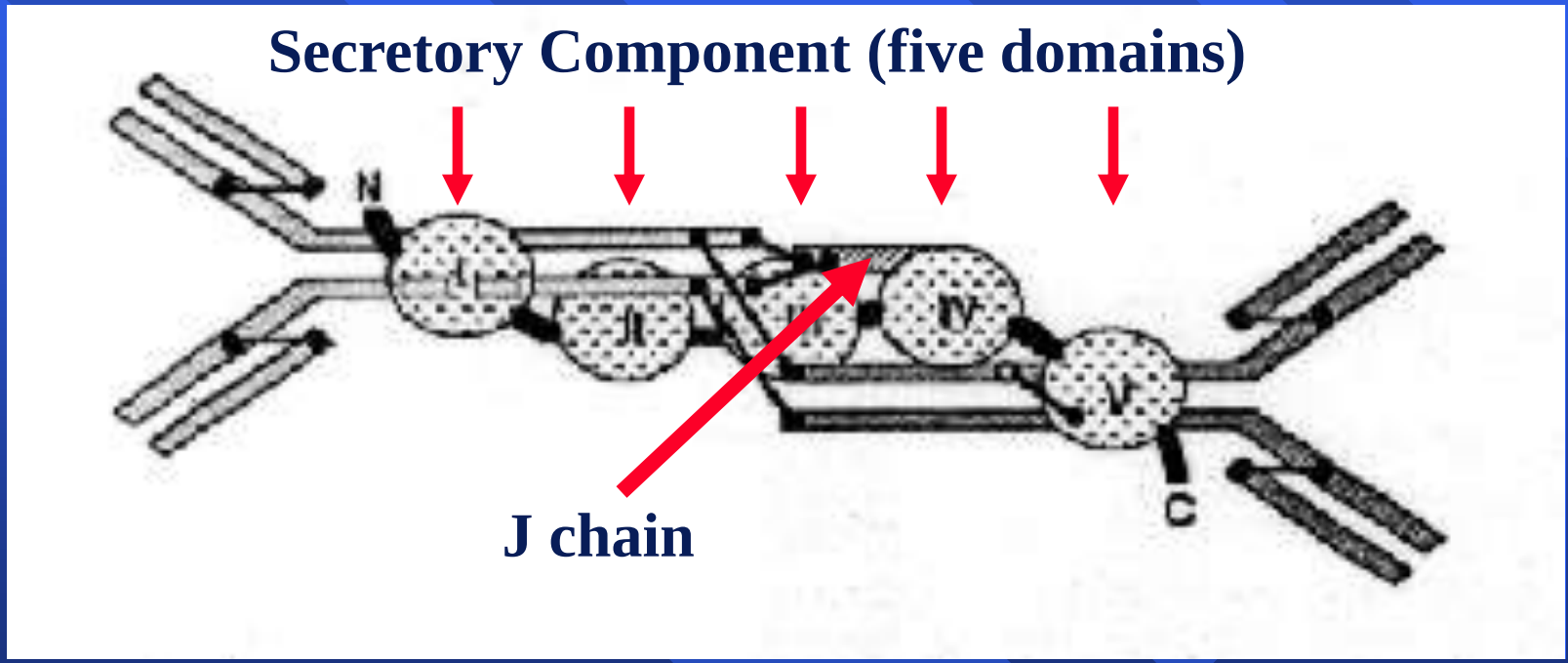
Structure of Secretory IgA (sIgA)



J chain

- 15,600 kDa
- Associated with polymeric Ig
- Synthesized by Plasma cell
- One J chain per polymer regardless of size
- Is probably associated with initiation of polymerization
- Induces confirmation that optimizes binding to SC

Secretory Component

- MW 80,000
- Synthesized by epithelial cells of mucous membranes
- IgA dimer binding sites per epithelial cell is approximately 260-7,000

Organization of Mucosal Lymphoid Tissue

- MALT cellular mass exceeds total lymphoid cells in bone marrow, thymus, spleen, and lymph nodes
- Other Terms
  - GALT
  - BALT
  - NALT

Organized lymphoid follicles at specific mucosal sites (O-MALT)

- Occur in tissues of digestive, respiratory and genital mucosal surfaces
- Light germinal centers
- Dark adjacent areas populated by B and T lymphocytes and antigen-presenting cells
- Site of antigen sampling and generation of effector and memory cells

Diffuse MALT

- Lamina propria lymphocytes (primarily B cells) (LP major site of Ig synthesis)
  - Lamina propria: the layer of connective tissue underlying the epithelium of a mucous membrane
- Derived from O-MALT and represent effector and memory cells from cells stimulated by antigen
- Intraepithelial lymphocytes (IELs)
- Plasma cells producing dimeric IgA
- Antigen-presenting cells (macrophages and dendritic cells)

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Modes of Antigen Sampling

- Stratified, non-keratinized or parakaratinized epithelia (oral cavity, pharynx, esophagus, urethra, vagina)
  - Antigen sampling depends on Dendritic cells
    - Langerhans cells, phagocytic, antigen-presenting motile “scouts”)
  - Dendritic cells may then transport antigen to local and regional lymphoid follicles.
- Simple epithelia (bronchiole, intestine, bronchi)
  - Antigen sampling depends on M cells and Transepithelial transport
  - Dendritic cells may also participate in antigen transport

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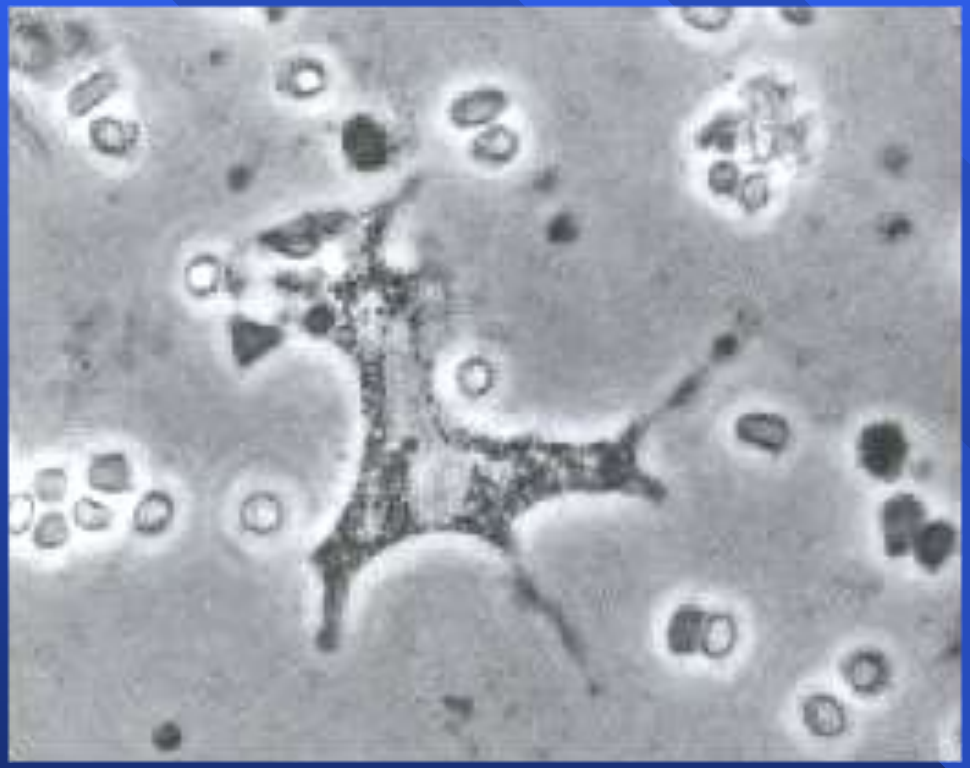
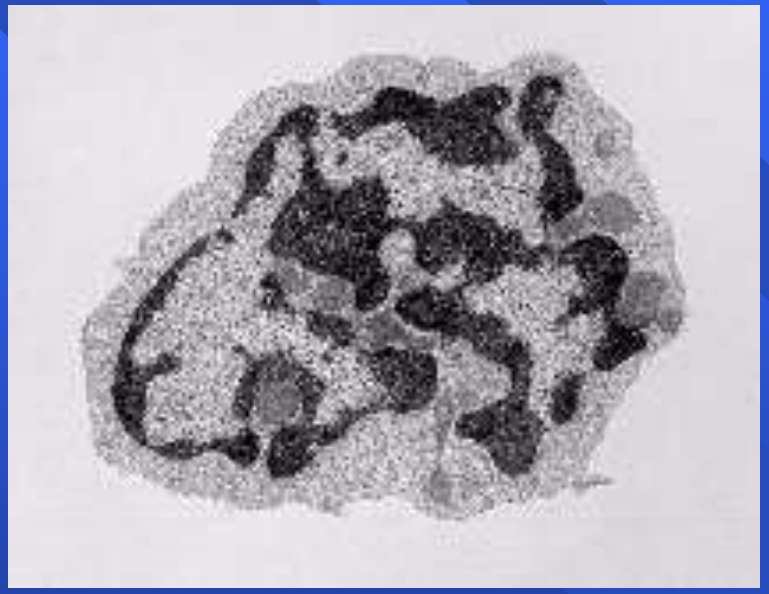
Dendritic cells



- Capture antigen in tissues
- Transport to secondary lymphoid organs
- Process and present to T cells
- An essential link between innate and adaptive immunity
- May also represent the "Achille's Heel" of the host? (Cutler et al. 2001)

Maturation of Dendritic Cells



This "DC-precursor" found in TDL looks very much like a lymphocyte with several important exceptions. Mitochondria were far more numerous in the cytoplasm, and the DC nucleus was convoluted with more delicately distributed heterochromatin and lighter euchromatin than is normally found in lymphocytes. (From A. Anderson)

- **Loss of endocytic and phagocytic receptors**
- **Increased expression of MHC**
- **Up -regulation of co-stimulatory molecules (CD80 and CD86) required for T-cell stimulation**
- **Up-regulation of CD40 and adhesion molecules ICAM-1 and LFA-3**
- **Fc receptors (endocytosis) decrease**

Antigen Sampling across Simple Epithelia

- Mucosal surfaces generally lined by a single layer of epithelial cells
- Barrier sealed by tight junctions that exclude peptides and macromolecules
- Uptake of antigen requires active transepithelial transport (M-cells or Dendritic cells)
- Sampling is blocked by mechanisms such as local secretions, sIgA, mucins, etc.

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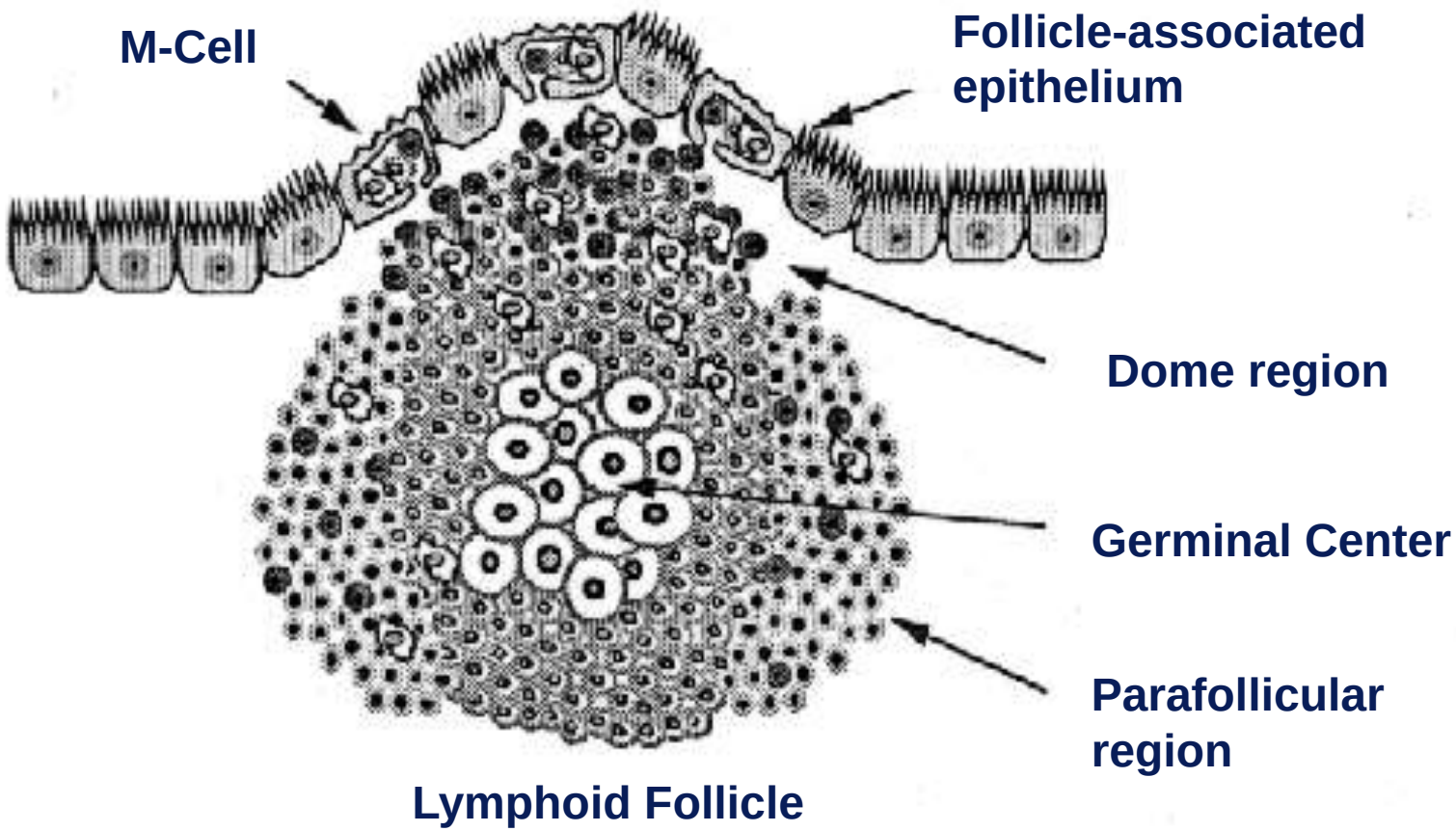
Organization of O-MALT



Antigen Adherence to M-Cells

- Adherence favors endocytosis and transcytosis
- Adherent materials tend to evoke strong immune responses
- Wide variety of pathogens adhere to M-cells
- Mechanism of adherence is unclear
- Many commensal microorganisms avoid adherence to M-cells

M-Cells May Serve as Entry sites for Pathogenic Microorganisms

Bacteria

- *Vibrio cholerae*
- *Escherichia coli*
- *Salmonella typhi*
- *Salmonella typhimurium*
- *Shigella flexneri*
- *Yersinia enterocolitica*
- *Yersinia pseudotuberculosis*
- *Campylobacter jejuni*

Viruses

- Reovirus
- poliovirus
- HIV

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Antigen Recognition

- Antigen transport is effected by M-Cells which occur over Organized Mucosa-Associated Lymphoid Tissue (O-MALT)
- After antigen stimulation, effector B-lymphocytes leave O-MALT and migrate to distant mucosal or glandular sites

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Migration and Homing of Lymphocytes

- **Distribution of Homing Specificities in Mucosal Tissues**
  - Epithelial cells lining postcapillary venules (HEV's) display organ-specific recognition sites called "vascular addressins"
  - Recognized by cell adhesion molecules "homing receptors"

High Endothelial Venules (HEV)

- Contain specialized endothelial cells lining post capillary venules.
- Display organ-specific recognition sites called “vascular addressins” that are recognized by specific cell adhesion molecules on lymphocytes.
- HEV cells are characterized by:
  - Elongated shape and prominent glycocalyx on luminal surface
  - Polarized, with a domed luminal surface separated from the basolateral surface by adherent junctions, but not tight junctions
  - Cells rest on a basal lamina that constitutes the rate-limiting barrier to migrating lymphocytes

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HEV (continued)

- In O-MALT, HEV's are present in T-cell areas between B cell follicles
- In D-MALT, venules have flat endothelial cells that share many features with HEV's
- HEV's produce sulfated glycolipids and glycoproteins into the vascular lumen (not known whether these products play a role in homing or extravasation)

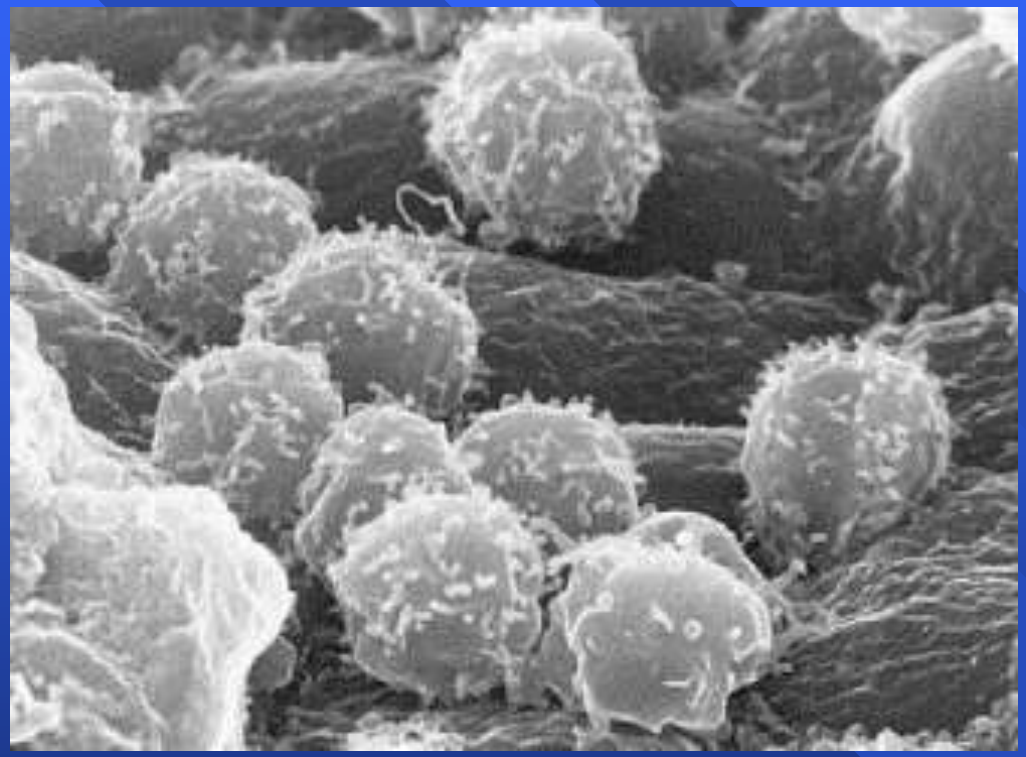
Adhesion molecules cloned so far belong to four main protein families

- Integrins
- Selectins
- CAMs (cell adhesion molecules)
- Proteoglycan-link.core proteins

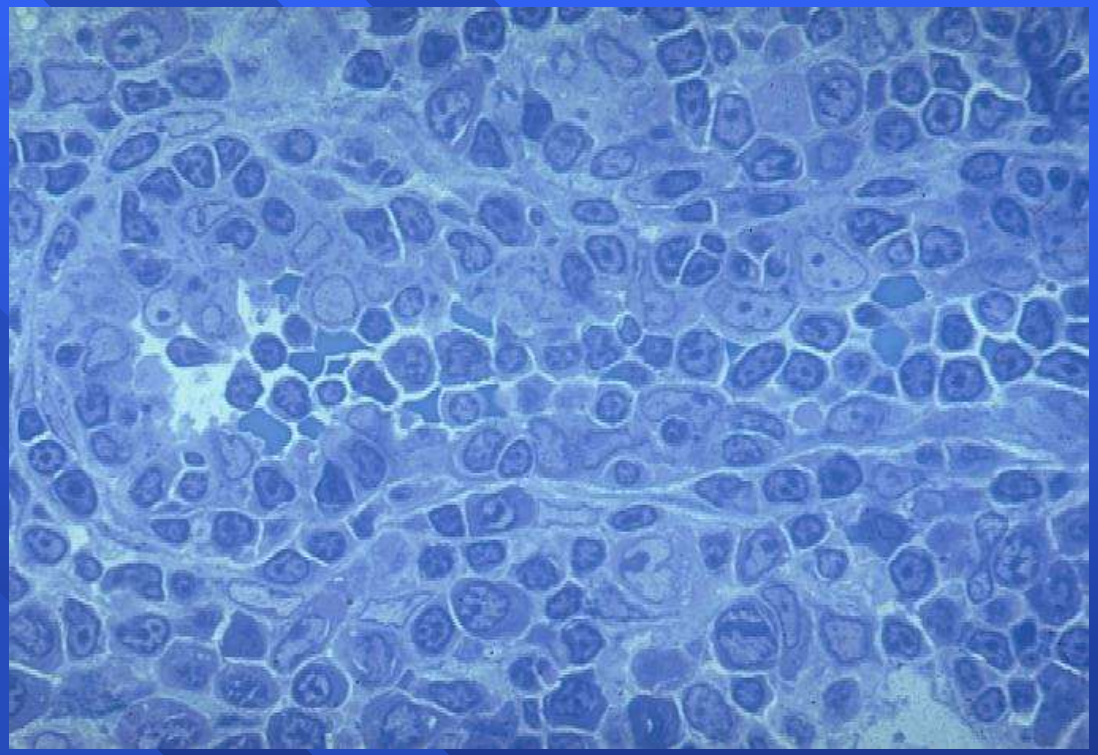
Modulation of Homing Specificities

- Naive lymphocytes prior to antigenic stimulation demonstrate no migration preference
- Following antigenic stimulation, lymphocytes acquire homing specificities

Lymphocytes in HEV

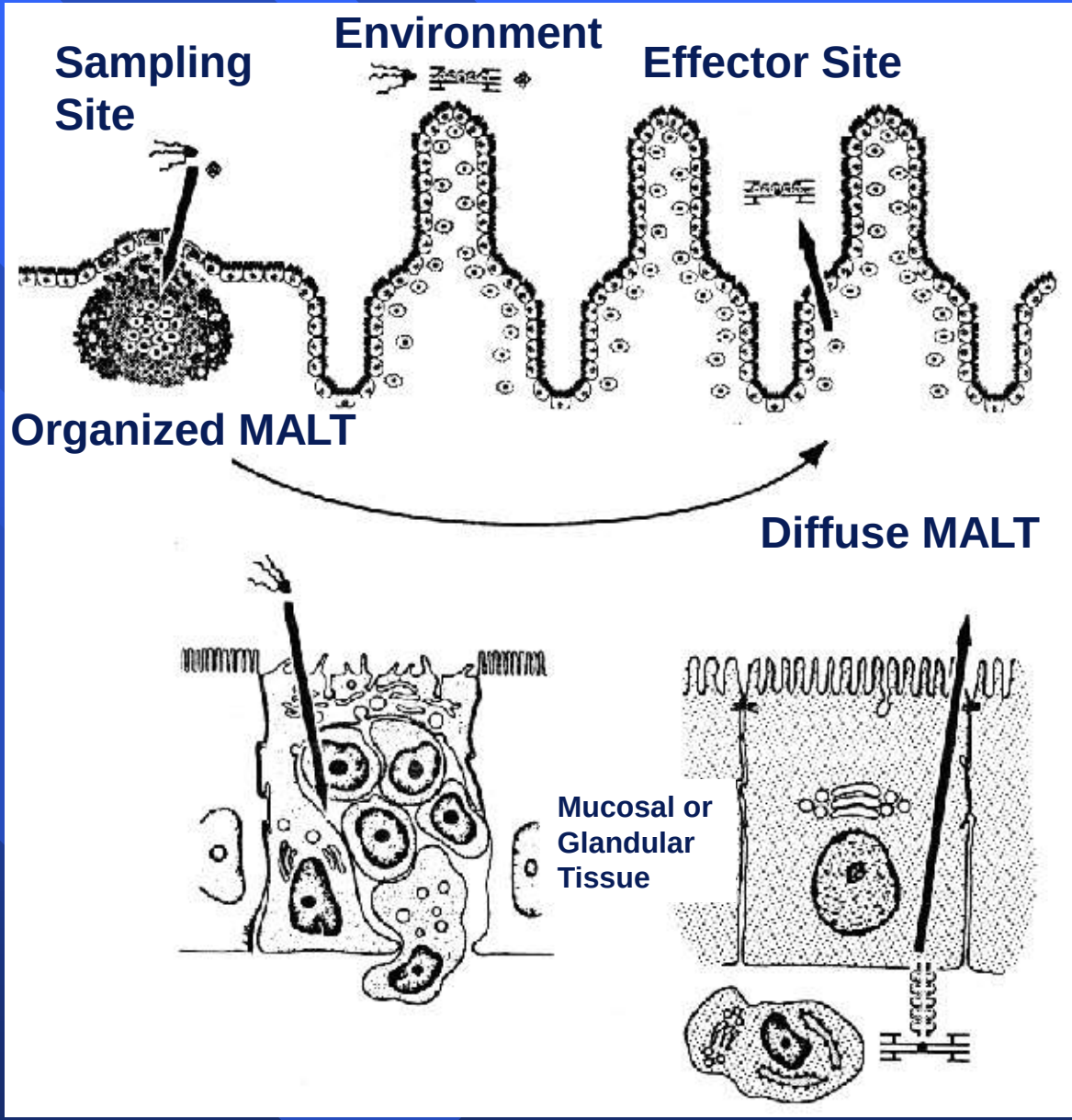


Lymphocytes adhering to luminal surfaces of HEV endothelial cells. Note microvilli on surface of lymphocytes.



Cross-section of HEV

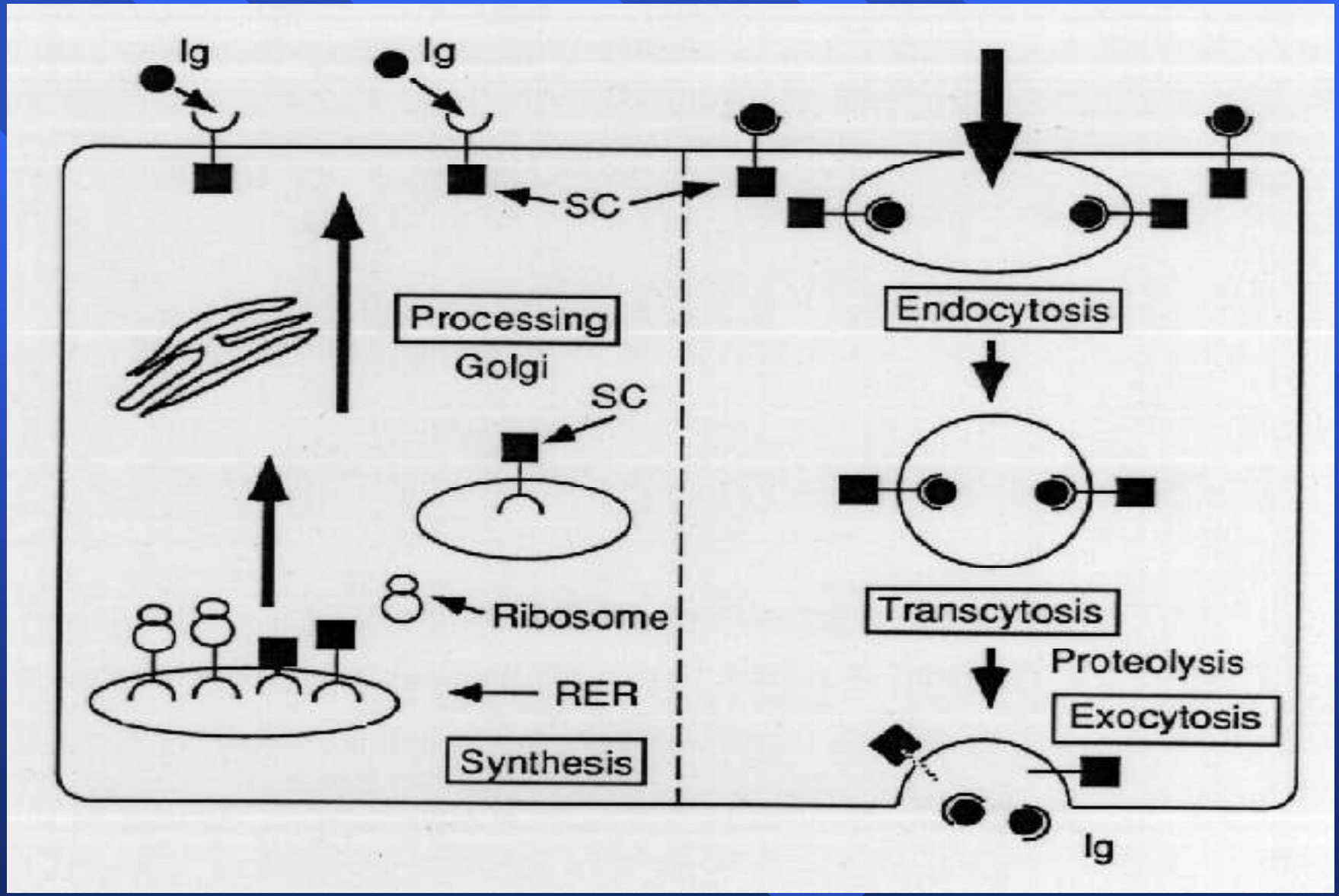
Transepithelial Transport in Mucosal Immunity



Transepithelial Transport of IgA Antibodies

- Polymeric immunoglobulin receptor and its intracellular trafficking
  - poly-Ig receptor
- Binding of IgA to polymeric immunoglobulin receptor

Transport and Distribution of IgA Antibodies



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Effector Functions of Mucosal Antibodies

- IgA antibodies are not good mediators of inflammatory reactions
  - complement activation
  - neutrophil chemotaxis
  - phagocytosis
- Immune Exclusion/Serve “escort" function
- Beneficial not to induce inflammation
- Intra-epithelial virus neutralization by IgA
- Excretory function for IgA

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Relationship between Systemic and Mucosal Immunity

- Oral tolerance (anergy)
  - Oral administration of antigen suppresses systemic immunity
- "Mucosal Internet"
  - Epithelial cell-Immune Cell Interactions
  - May be critical for induction of adaptive response
  - Danger theory

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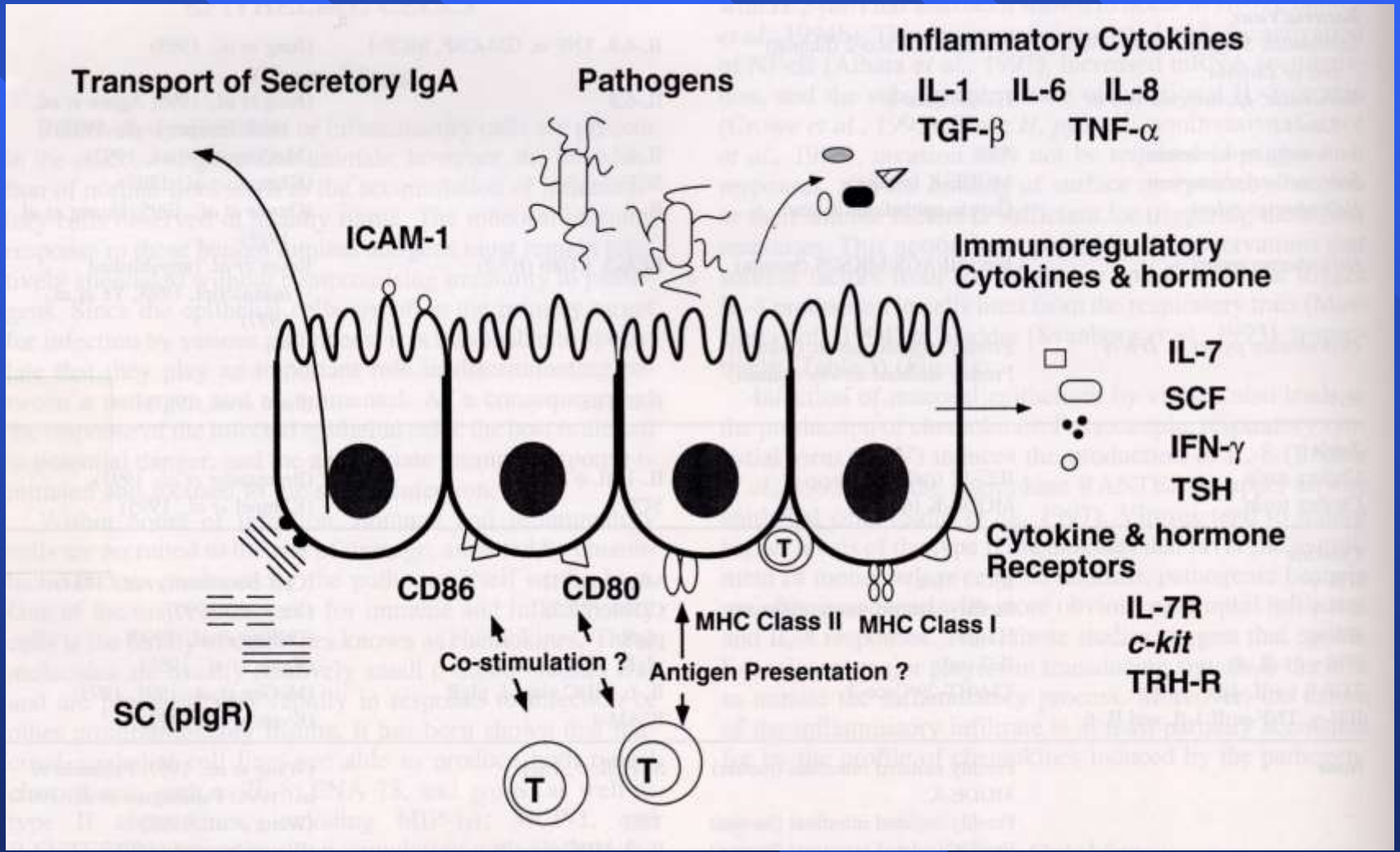
Epithelial Cell Response to Pathogens



Requirements of Protective Vaccines

- Block adherence of microorganism to host
- Facilitate clearance from host
- Neutralize toxin
- Must recognize “virulence” epitopes
- Must be immunogenic
- Must not induce autoimmune disease
- Should induce long-lasting immunity
- Must induce the type of response that is effective to eliminate pathogen (eg. T_{H1} or T_{H2})

Rational Strategies for Mucosal Immunization

- Requirements
  - Safe taken orally
  - Long-lasting due to continued maintenance of memory
  - Survive in gastric and intestinal environments
  - Must escape normal clearance mechanisms
  - Must compete for inclusion within M-Cell transport
  - Must arrive intact to antigen-processing cells
  - Must induce dimeric sIgA reactive with cell surface

Rational Strategies for Mucosal Immunization (continued)

- Strategies for Delivery of Vaccine Into O-MALT
  - Inert particulate carriers
    - Biodegradable copolymers
    - Immune-stimulating complexes (ISCOMs)
    - Hydroxyapatite crystals
  - Live vaccine vectors (recombinant)
    - Vaccinia virus
    - *Salmonella*
    - *Mycobacterium bovis*

Rational Strategies for Mucosal Immunization (continued)

- Strategies for Enhancing Mucosal Immune Response
  - Co-delivery with cytokines
  - Co-immunogens (Cholera toxin)
  - Peptides presented with potent T-cell epitopes

Oral Vaccines

ITHACA, N.Y. -- The Boyce Thompson Institute for Plant Research Inc. (BTI), an affiliate of Cornell University, announced that clinical trials will begin today (July 7) at Roswell Park Cancer Institute (RPCI) in Buffalo, N.Y., to test the safety and immunogenicity of the world's first potential oral vaccine against the **hepatitis B virus**. The vaccine will be delivered simply by eating potatoes genetically designed to contain the vaccine.

Oral Vaccine Protects Infants from Severe Rotavirus Diarrhea
First Success in a Developing Country

An oral vaccine against **rotavirus** -- the most important cause of life-threatening diarrhea in children under age 2 -- reduced severe diarrheal illness by 88 percent in a study of more than 2,000 infants in Venezuela. This is the largest and most successful trial to date of a rotavirus vaccine among children in a developing country.

Oral Vaccines (cont'd)

Vaccine Now Available as an Oral Series or a Single Dose Injection

Typhoid fever immunization is recommended for all travelers to lessor developed countries especially those in Central and South America, Africa, Southeast Asia, and The Indian Subcontinent. The highest risk countries are Peru, India, Pakistan, and Chile. However, about half of all cases of typhoid fever reported in American tourists are acquired from travel to Mexico even though the risk of disease is lower there. Typhoid fever is generally spread person to person especially by food handlers who do not wash their hands adequately after bowel movements. Visitors who stray off the beaten path and eat meals prepared at foodstands or by street vendors are at highest risk. Carefully selecting restaurants with close attention to their sanitation standards can reduce the risk.

There now is an oral typhoid vaccine and a new single dose injectable vaccine that produces fewer side effects that the older two dose injectable vaccine. Both vaccines are equally effective and offer 65-75% protection against the disease.

Alzheimer's vaccine looks promising
Brain deterioration slowed by nose drops
Medical researchers have successfully treated Alzheimer's disease in mice by putting drops of vaccine in their noses. They think it will ultimately be possible to do the same with people. "We plan to begin human trials next year," says Howard Weiner, a neurologist at Harvard Medical School who has pioneered the use of oral and nasal vaccines.

Oral Vaccines (Cont'd)

AVANT RECEIVES PATENT LICENSE ON ORAL TYPHOID FEVER VACCINE
NEEDHAM, MA (August 22, 2000): AVANT Immunotherapeutics, Inc. (Nasdaq: AVAN) announced today the signing of a cross-licensing agreement with Megan Health Inc. for exclusive rights to a patent portfolio supportive of AVANT's single-dose, oral vaccine candidate against typhoid fever, called Ty800. The agreement allows AVANT to further its clinical development of Ty800 in expanded Phase II studies, while Megan Health gains non-exclusive rights to use AVANT's high-level expression system for human and non-human vaccines.

Agreements Set Innovative AIDS Vaccine on Fast Track to Developing Countries

Oral vaccine would be delivered by "bacterial robot" and be
produced and sold for far less than other AIDS vaccine candidates

Baltimore **--**The development of an innovative, orally administered AIDS vaccine by the Institute of Human Virology (IHV) will be funded by the International AIDS Vaccine Initiative (IAVI) under terms of a new multi-year, multi-million dollar vaccine development partnership agreement between the organizations.

IAVI and the IHV, a center of the University of Maryland Biotechnology Institute founded by Robert Gallo, co-discoverer of HIV, estimated that the new vaccine could be produced and sold for far less than other AIDS vaccine candidates currently in the pipeline. An inexpensive AIDS vaccine is a desperate need for developing countries, where 95% of new HIV infections occur.

Oral Vaccines (Cont'd)

Birth control vaccine in the works

A dose or two a year would halt pregnancy, researchers hope
(CNN) -- Family planning experts say what's needed to prevent unwanted pregnancies among women of all ages is a method of birth control that's foolproof and easy to use. Researchers think they may be on track towards developing just that: a birth control vaccine, something that could prove to be the most effective birth control method ever.

Common bacteria, which sometimes causes food poisoning or typhoid fever, could be the key to the vaccine. Researchers are taming salmonella and genetically altering it into a protein factory of sorts. The goal is to produce proteins that will cause the body to have an immune reaction to sperm, thus blocking fertilization. In simple terms, scientists want to treat fertilization like a disease.

Roy Curtiss, a professor at Washington University, says the concept makes sense because the interaction between a sperm and an egg is sort of like the interaction between a virus and a cell. Curtiss hopes to use proteins unique to sperm and eggs to make vaccines that could be used by men or women.

He says an oral vaccine that would be taken only once or twice a year could have many advantages when it comes to birth control. "It's very, very inexpensive and safe," Curtiss said. "There's no need for refrigeration, which makes its use in the developing world attractive. And you don't need to remember to do something 21 days in a row."

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