

## **Section 4**

### **LECTURE**

### **Mucosal Immunology of the GI Tract**

## MUCOSAL IMMUNOLOGY

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### I. INTRODUCTION

The intestinal epithelial cell surface represents a vast frontier of body surfaces that must be defended by the immune system. The intestinal immune system must defend against the many infectious and toxic assaults that may breach the epithelium and cause intestinal injury. The immune system also must recognize epithelial cell transformation, to which the intestine may be uniquely prone, because toxin exposure and the high proliferation rate of the epithelial cells increase the risk of cytogenetic error and malignant transformation. The intestinal immune system must simultaneously ignore the multitude of commensal organisms and dietary antigens that are not threats to the host.

Because specific immunity is driven by antigen recognition, the components of the immune system that protect the gut are presented with a significant challenge in differentiating foreign or nonself material from self-antigens by responding to the former and ignoring the latter. Except for a few other epithelial surfaces, no other organ system is presented with this combination of problems in such a dramatic fashion. The skin is covered by a horny layer of epithelial cells that effectively excludes most antigens. As a result of constant antigenic exposure, the gut possesses abundant lymphoid cells (i.e., B and T lymphocytes) and myeloid cells (i.e., macrophages, neutrophils, eosinophils, mast cells). The mucosal immune system of the gastrointestinal tract represents one of the largest immunologic compartments in the body.

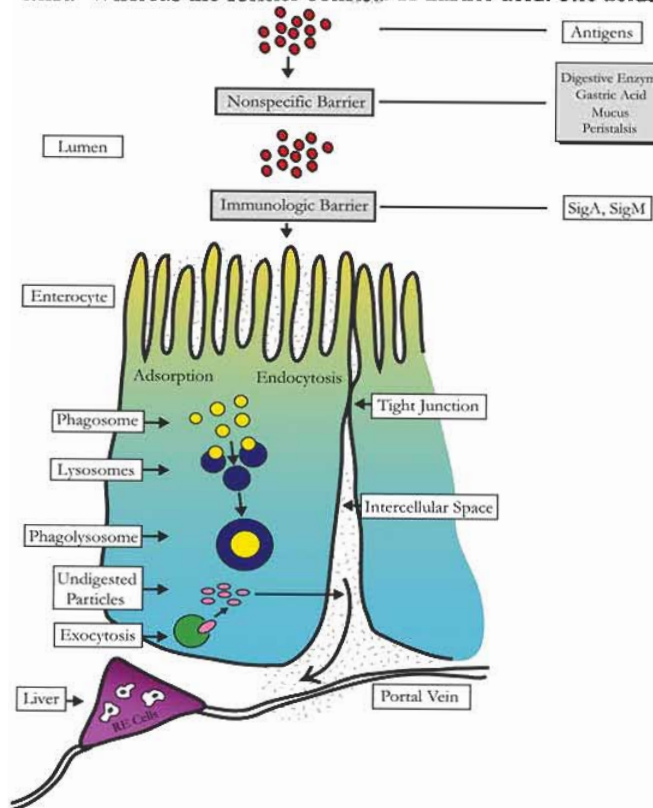
To deal with this challenge, the gut-associated lymphoid tissue (GALT) has evolved several important modifications of antigen processing, humoral immunity, and cellular immunity to cope with its organ-specific responsibilities. These include flattened epithelial microfold (M) cells that transport antigens, the specialized epithelial cells of the domes overlying lymphoid aggregates that sample luminal antigens selectively, the IgA system that helps to exclude and remove foreign antigens, and unique mechanisms of generating local specific secretory immunity in the context of systemic tolerance. The functional segregation of intestinal from systemic compartments reiterates the separate role of the intestine as a unique lymphoid organ that is linked to other mucosal surfaces such as those of the lung, breast, and genitourinary tract to create a common mucosa-associated lymphoid tissue (MALT). Functional linkage of the MALT is accomplished by the regulated traffic of lymphocytes between the affiliated tissues.

Advances in molecular biology have provided important insights into the general operation of the immune system, including the manner in which the immune system processes, presents, and recognizes antigens through the major histocompatibility complex, T-cell receptors, and immunoglobulins; the mechanisms by which the responsive cellular elements are stimulated after antigen contact through accessory molecules, adhesion molecules, and signal transduction; and the manner in which the activated immune cells carry out their functional destiny through the production of cytokines and other humoral factors and through cytotoxicity. The immune response generated by these events usually is self-limited after the foreign antigen is cleared. Without self-regulation or with persistent uncleared antigen, chronic inflammation results such as occurs in inflammatory bowel disease (IBD).

### II. MUCOSAL BARRIER FUNCTION

The dimensions of this problem are enormous. It has been estimated that, assuming 100 kilograms of food antigens are ingested per year by an average individual, approximately 0.1-1% of these antigens are absorbed, often intact. As a result, the normal host must deal with the uptake of approximately 100-1,000 grams of macromolecules per year. Moreover, within the lumen of the intestine  $10^{14}$  bacteria exist over a surface of approximately 200-400 square meters. In addition, epithelial cell turnover in this compartment is estimated to be 10 cells per day that are shed and replenished. The normal host therefore has established a variety of barriers to nonspecific macromolecular absorption. These barriers can be broken down into three

levels: luminal, enterocytes and liver. Within the lumen, both nonspecific and specific (immunologic) barriers exist. Whereas the former consists of gastric acid, bile acids, digestive enzymes, peristalsis, mucus and this is



peristaltic activity (motility), the latter consist of components of the innate immune system and specific, or adaptive, immune system (secretory IgA, secretory IgM, IgE and IgG). The enterocyte forms both a physical barrier to macromolecular absorption through itself and the structures associated with the tight junctions, which act as a physical gasket and limits passive absorption of macromolecules. Whereas the former process is considered a transcellular barrier, the latter is considered a paracellular barrier. Notably, most macromolecules that are taken up by the enterocyte are digested in lysosomes and only the minority are transported transcellularly in a transcytotic pathway across the epithelial cell. Those molecules that gain access across the epithelial cell are taken into the portal venous system and delivered to the liver, which is endowed with a rich reticulo-endothelial system, a major component of which is the Kupffer cells.

Figure 1: Components of mucosal barrier function: luminal (nonspecific and immunologic barriers), epithelial and hepatic. (Figure by MIT OCW.)

#### A. Luminal factors (nonspecific extrinsic barriers)

1. **Proteolysis and gastrointestinal acidity:** Luminal proteolysis from a wide variety of digestive enzymes derived primarily from the pancreas and enterocyte leads to macromolecular digestion thus limiting the access of full macromolecules to the epithelial cell surface and uptake. Consistent with this, in diseases such as cystic fibrosis in which pancreatic enzyme activity is affected, incidence of cows milk allergy, presumably due to increased antigen uptake, is increased. In a similar manner, gastric acidity has important effects on the uptake of macromolecules through activation of certain digestive enzymes as well as through denaturation of macromolecules. For example, neutralization of gastric acid through sodium bicarbonate has been shown to promote increased absorption of bovine serum albumin.

2. **Peristalsis:** Peristaltic activity limits the time for contact between antigens and the epithelium as well as regulates the levels of luminal microbiota. Diminution in peristalsis as may occur in neurodegenerative diseases and short bowel syndrome will result in increased microbial colonization of the upper gastrointestinal tract resulting in degradation of



important luminal factors associated with preventing macromolecular absorption as well as increasing potential contact time between the antigen and the epithelial cell surface.

3. **Mucus:** The mucus blanket consists of three components: the glycocalyx, the mucus blanket itself, and a lipid monolayer appended to the top of the mucus blanket. The glycocalyx, which is closest to the gastrointestinal epithelial cell, has a thickness of between 10-500 nanometers. The glycocalyx consists of nonsecreted, mucinous fibers that are anchored into the cell membrane forming a dense glycoprotein coat. The function of the glycocalyx is unknown but clearly regulates the access of microparticles to the apical membranes of the intestinal epithelial cell. Above the glycocalyx is a very thick mucus blanket that is composed of

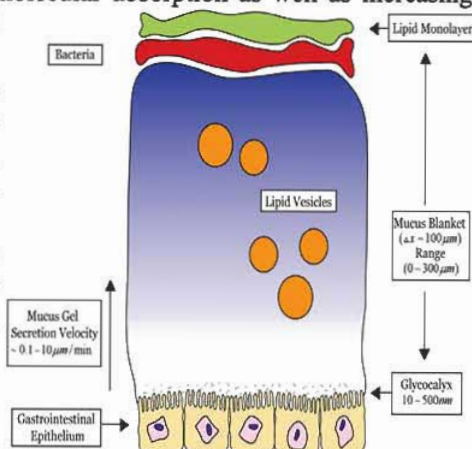


Figure 2: Components of the nonspecific barriers associated with the glycocalyx, mucous blanket and lipid monolayer. (Figure by MIT OCW.)

the secreted mucins. The mucus appended to the apical cell surface of the epithelium is composed of 1% mucin, 1% free protein, 1% dialyzable salts and more than 95% water within which exist albumin, immunoglobulins,  $\alpha$ 1-anti-trypsin, lysozyme, lactoferrin and growth factors such as EGF. Mucins are highly glycosylated fibrous peptides that are derived from eight different potential human mucin genes (MUC 1-8). Epithelial cells express various combinations of the MUC genes. The soluble mucins are secreted at different rates from the epithelial cell varying from approximately .01-10  $\mu\text{m}/\text{min}$  from the epithelial cell such that different thicknesses of the mucus coat are achieved. The mucus blanket in the gastrointestinal tract varies between the stomach (50-400  $\mu\text{m}$ ) and colon (110-160  $\mu\text{m}$ ) and in the small intestine where the thickness can vary greatly depending upon digestive activity. Under certain circumstances, for example, the mucus coat may be nonexistent in the small intestine due to the fibrous nature of the diet. The molecular weight of the mucins varies from 1 to several million daltons and can be composed of up to 70-80% bound carbohydrate. As a result of this molecular composition, mucus is highly visco-elastic. Adherent to the top of the mucus blanket is a thin lipid layer that helps to impede the passive diffusion of acid and other injurious substances. The major functions of mucus are to act as a physical barrier for preventing penetration of organisms (e.g., *Entamoeba histolyca* trophozoites), competitively binding carbohydrate moieties of bacteria to prevent their invasiveness (e.g., *Shigella flexneri*) and, finally, to provide a substrate for binding immunoglobulins and thus link the immune system to these extrinsic factors.

4. **Mucosal microbiota:** The human intestine is colonized by large quantities of bacterial microorganisms that are considered a part of the normal microbiota. The numbers of these organisms is enormous such that it has been estimated that the numbers of bacteria in the normal intestine are greater than the total number of cells in the human body. As such, many individuals studying these organisms have suggested that they are an extension of self given that they are resident over long periods of time as if they were a normal organ of the host. Therefore, an extremely dynamic relationship exists between these organisms and the human host. Most of these organisms are congregated in the large intestine, where the total population is predicted to exceed  $1 \times 10^{14}$  cells or 99.9% of the total indigenous microbiota of the intestine. However, microbes can also be identified in the stomach and small intestine. Some organisms, such as *Helicobacter pylori*, are considered to be normal residents of the stomach and under certain conditions, presumably in the context of genetic predisposition, can cause immunopathology or even cancer. However, the stomach can also be transiently colonized by a wide variety of organisms from the oropharynx through swallowing such that in the context in which gastric acidity is decreased, these organisms can be quite substantial in number and theoretically predispose such individuals to aspiration pneumonias. In a similar manner, a few species of bacteria can colonize the upper gastrointestinal tract, most of which are *streptococci* and *staphylococci*. Beginning with the distal small

bowel (ileum), the numbers and complexity of microorganisms increase such that by the time the distal ileum is reached, these can assume the levels of the colon. It has been estimated that the colon contains hundreds of species of bacteria, the majority being anaerobes, which outnumber the aerobes and facultative anaerobes by as much as 1,000:1. Colonization presumably begins early in neonatal life and is presumably established quite rapidly and remains persistent presumptively for the lifetime of the individual. Good prospective studies on the duration of colonization have not been performed but certainly there are data to suggest that it persists over a period of years.

The microbiota of the normal colon establish a complex interaction with the mucus gel either at the luminal interface, within the mucus gel itself or adherent to the epithelial cell via the glycocalyx. A symbiosis exists between the normal microbiota and the host such that it is presumed that the microorganisms assist in establishing the mucus gel layer and do not function in its digestion. For example, certain microorganisms induce the production of epithelial cell mucosal transferases that are important to the generation of mucus gel structure. It has also been suggested that bacteria “graze” on the mucus gel and modify the carbohydrate structure maintaining the integrity of this barrier. Through this and other features, such as the production of factors that inhibit pathogenic invaders, the normal microbiota has been considered a form of colonization resistance against invasion from pathogenic organisms. Such colonization resistance has been shown, for example, in the resistance to *Clostridium difficile*.

Given the persistence of this presumably stable population of microorganisms of the epithelial cell surface, it is not surprising that the organisms are experienced by the immune system of the host as if they were self. As such, the normal host is immunologically tolerant to the organisms present in the lumen. In certain circumstances, this tolerance can be viewed as immunologic ignorance wherein no immune response can be detected against the organism. In certain circumstance, this tolerance is associated with cellular tolerance but evidence of secretory immunity based upon the presence of natural (IgM) or secretory (sIgA) antibodies, which have been shown in many circumstances to coat the organisms. This concept of tolerance to the autochthonous microbiota is extremely important to understanding the pathogenesis of human inflammatory bowel disease. The microorganisms may communicate directly with epithelial cells and cause the secretion of cytokines that promote intestinal epithelial cell barrier function (see below) such as interleukin-10 and TGF- $\beta$ .

5. Innate humoral factors: A number of protein factors are secreted into the lumen of the intestine constitutively that have either anti-microbial or natural anti-microbial activity or help to maintain the epithelial barrier. *Lactoferrin* is found in a wide variety of exocrine secretions, blood and leukocytes and consists of three major forms: apolactoferrin, diferic-lactoferrin, which contains two bound irons, and ferric lactoferrin, which contains one bound iron. Lactoferrin has broad antimicrobial activity against viruses (e.g., rotavirus, RSV, HIV), gram negative organisms (e.g., *Neisseria meningitides*, *H. influenzae*, *Salmonella typhimurium*), gram positive organisms (e.g., *Clostridium sp*), fungi (*Candida sp.*), parasites (e.g., *Giardia lamblia*). Lactoferrin also binds to lactoferrin receptors, which have been identified in the gastrointestinal tract epithelial cell which may regulate epithelial cell growth as well as inhibit epithelial cell tumors. Lactoferrin is upregulated in inflammation, which may play a role not only in antimicrobial pathogenesis but also in antioxidant defense. *Human lysozyme* is a compact polypeptide due to the formation of four disulfide bridges that has a molecular mass of 14.6 kDa. Lysozyme is found broadly in most secretions: stomach (43-106  $\mu\text{g/ml}$ ), saliva (10-200  $\mu\text{g/ml}$ ), tears (1,200-1,300  $\mu\text{g/ml}$ ). Human lysozymes has potent antimicrobial activity through its ability to hydrolyze the peptidoglycans of bacterial cell walls, by activating bacterial autolysins, and are able to aggregate bacteria and block their adherence. It has been shown lysozymes can act either alone or synergistically with other innate luminal factors including lactoferrin and peroxidases. *Peroxidase* derived from polymorphonuclear leukocytes (myeloperoxidase) and eosinophils (eosinophyl peroxidase), are known to be constitutively present and to protect the mucosal surfaces by catalyzing the peroxidation of halides (chloride, bromide, thiocyanate and iodine). Such properties have major anti-microbial activities which can synergize with other innate factors. *Trefoil peptides* consist of approximately 5-6 kDa polypeptides that form a compact structure. This consists of three loops due to the formation of three pairs of intrachain disulfide bonds that

ultimately resemble a cloverleaf structure from which the name is derived. These molecules associate with the mucus gel and perhaps the epithelial cell itself to help maintain barrier function. In mice that are deficient in trefoil peptides, these animals are highly sensitive to disruption of the intestinal epithelial cell barrier and develop severe colitis. Intestinal epithelial cells that are able to synthesize and secrete the *complement components* (C3, C4, and factor B) into the lumen and express on their cell surface decay accelerating factor which protects the epithelial cell from activated complement pathways on the apical cell surface. In diseases such as ulcerative colitis, deposition of complement in association with IgG can be observed on the cell surface of the epithelial cell. The presence of complement in the lumen might provide important antimicrobial activity. Finally, human secretions contain a variety of other small polypeptides including histatins (histatin 1-12), proline-rich proteins, cistatins, secretory leukocyte protease inhibitor (SLPI), and cationic antimicrobial peptides, all of which help synergize with the other innate factors to provide antimicrobial resistance.

*Defensins* are a large family of 30-40 amino acid residue containing peptides that fall into two branches: alpha-defensins and beta-defensins. Humans express six different alpha-defensins and two different beta-defensins. These molecules are expressed by a wide variety of animals and are presumed to represent a primitive defense mechanism. Two of the six human alpha-defensins have been defined in Paneth cells (HD-5 and HD-6). Human beta-defensin-1 (HBD-1) has been shown to be expressed by absorptive epithelial cells. Defensins are complexly folded macrocyclic molecules that have potent antimicrobial activity against gram-positive bacteria (*Neisseria gonorrhoea*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*), fungi (*Candida sp.*), mycobacteria and spirochetes as well as enveloped viruses and protozoa (*Giardia lamblia*). It is hypothesized that the defensins form pores in organisms that allow permeability of other antimicrobial factors. Human defensins have also been shown to have anti-tumor activity. Given their presence in Paneth cells, alpha-defensins have also been called cryptidins.

#### B. Luminal factors (specific extrinsic or immunologic barriers): secretory immunoglobulins)

The major secretory immunoglobulin, IgA, exists in secretions in association with secretory component which is derived from the polymeric Ig receptor. Whereas most IgA in the human serum is monomeric, most IgA in the secretions is polymeric due to dimerization of the IgA through J chain, another product of plasma cells. Most polymeric secretory IgA is derived from local synthesis within the lamina propria. IgM, another polymeric immunoglobulin, is also found in secretions and is also secreted via polymeric Ig receptor albeit at much lower concentrations. However, in IgA-deficient individuals, IgM and IgG may play a compensatory role in increase and relative concentration. IgG can also be considered a secretory immunoglobulin. It is usually present at low concentrations in the secretions of the upper gastrointestinal tract but can be found at relatively high concentrations in the saliva, colon, especially the rectum, and cervical secretions. Recent studies from parasitic infestations have suggested that IgE must also be considered secretory immunoglobulin since high concentrations are found in the lumen in these diseases. Finally, low concentrations of IgD can be detected in milk and saliva.

1. **IgA biology and transport.** A major protective immune mechanism for the intestinal tract is the synthesis and secretion of dimeric IgA. The intestine contains over 70% of the immunoglobulin-producing cells in the body. Although IgG and IgM antibodies are also produced by lamina propria B cells within the normal intestine, the predominant antibody synthesized and secreted is IgA.



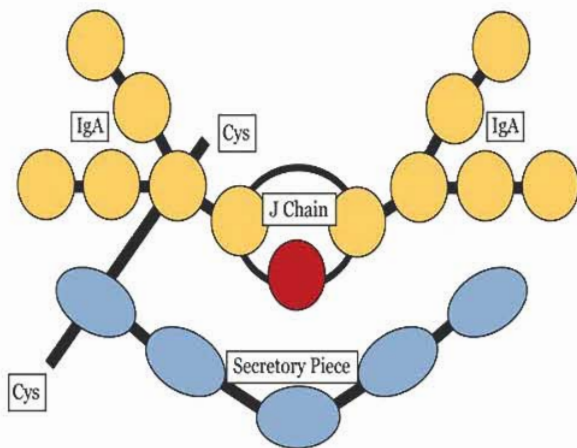


Figure 3: Structure of dimeric IgA. Dimeric IgA consists of two IgA molecules, one J chain and secretory piece that are added during synthesis in plasma cells. (Figure by MIT OCW.)

There are two IgA subclasses: IgA1 and IgA2. In serum, less than 15% of the IgA is IgA2, but in external secretions, as much as 50% of IgA is IgA2. One possible explanation for the preferential production of IgA2 in intestinal secretions is the observation that IgA1 is easily cleaved by proteases produced by bacteria, but IgA2 is more resistant to cleavage and may survive longer in the intestinal lumen. Plasma cells produce IgA in monomeric form or as a dimeric structure in which two IgA monomers are joined by a polypeptide called J chain. J chain is also produced within the plasma cell. J chain produces polymerization of IgA and IgM. By subsequent interaction with another polypeptide called polymeric

immunoglobulin receptor (PIgR), on the basal surface of the epithelial cell, J chain participates in the transport of polymeric IgA and IgM molecules across the intestinal epithelial cell into the lumen.

PIgR, produced by epithelial cells, is a membrane receptor that exhibits selective binding to polymeric immunoglobulins such as IgA and IgM, which contain J chain. PIgR is synthesized in the rough endoplasmic reticulum of the epithelial cell, glycosylated in the Golgi complex, and directed to the basolateral cell surface by a sorting signal in the cytoplasmic tail. After the initial interaction between polymeric IgA and the PIgR, the entire IgA-PIgR complex undergoes endocytosis into the basal side of the epithelial cell. The IgA-PIgR complex is then transported transcellularly in endocytic vesicles, which move to the apical surface of the epithelial cell, where they fuse with the apical membrane and subsequently release a portion of the PIgR, secretory component, as an intact secretory IgA molecule into the intestinal lumen.

The itinerary for transport of secretory IgA by the polymeric Ig receptor (pIgR) has been well worked out. pIgR expression is primarily evident on epithelial cells throughout the gastrointestinal tract and salivary tissues of humans. pIgR expression in humans is regulated by a variety of cytokines, most notably  $\gamma$ -IFN, TNF- $\alpha$  and IL-4. In addition, pIgR is also regulated in other tissues by hormones. The cytoplasmic tail of pIgR contains a basolateral sorting signal that directs pIgR to the basolateral surface. Three amino acids (HIS 656, RH 657 and VAL 660) contain the majority of information for this targeting. From the basolateral surface, pIgR undergoes constitutive transcytosis to the apical cell surface. This transcytosis is markedly increased by binding to secretory IgA at the basolateral surface. Three major steps regulate the transcytosis of the pIgR. The first step involves internalization of pIgR from the basolateral plasma membrane to basolateral early endosomes which are dependent upon two tyrosine-based internalization signals and a serine 726-phosphorylation stimulated internalization signal. Subsequently, pIgR moves from the basolateral early endosomes to apical recycling endosomes wherein phosphorylation of serine 664 inactivates the basolateral signal and thus disables return to the basolateral cell surface. Subsequent exocytosis from the apical recycling endosomes to the apical surface results in activation of phospholipase C, activation of protein kinase C, IP-3-induced release of calcium and further blockade of the basolateral retrieval signal by calmodulin.

At the apical cell surface, proteolytic cleavage in the domain of the polymeric Ig receptor just distal to the transmembrane domain leads to formation of secretory component in association with secretory IgA. Secretory component remains bound to polymeric IgA after its release into the external secretions, although it can also be found by itself in mucosal secretions as a secreted glycoprotein not bound to polymeric immunoglobulins. Secretory component may prevent proteolytic degradation of the secretory IgA molecule and may stabilize the structure of the polymeric IgA complex, protecting the secretory IgA that is secreted into a hostile environment containing numerous proteolytic enzymes, bacteria, and other substances that could otherwise rapidly degrade it.

IgA is also translocated across the hepatocyte or bile duct epithelium into the bile. IgA in the bile is carried into the duodenum. Important interspecies differences have been observed; the rat and rabbit have IgA and secretory component as major components of their bile, but sheep, dogs, and humans have

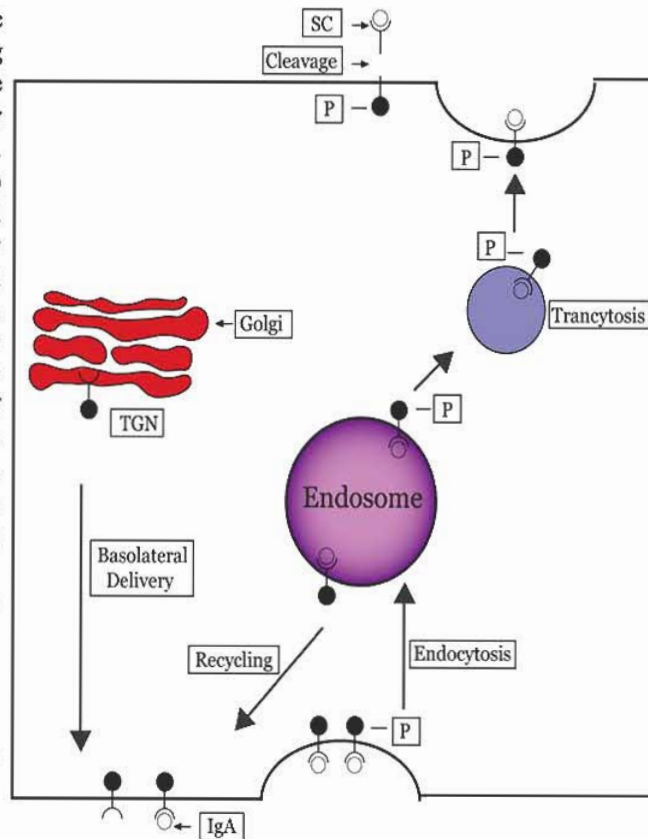


Figure 4: Itinerary for polymeric Ig receptor trafficking across epithelial cell. This trafficking occurs in a basal to apical direction. On the basolateral surface, PiGr picks up the polymeric IgA or IgM and, after endocytosis, transports it through a transcytosis pathway via endosomes to the apical surface whereupon secretory component is cleaved, releasing the polymeric IgA into the lumen. (Figure by MIT OCW.)

much smaller amounts of IgA in their bile. These differences in IgA translocation into bile appear to be related to the presence of PiGr on hepatocytes in rats and rabbits, resulting in highly efficient movement of IgA into the bile, but PiGr is expressed only on biliary epithelium in humans, resulting in less efficient translocation of IgA. The presence of secretory IgA in bile provides passive immunity and protection for the biliary tract and the proximal parts of the small bowel. A second implication of hepatobiliary secretion of IgA is that complexes of IgA and antigen can be transported into bile from the circulation. Hepatic removal of IgA-antigen complexes may protect against harmful absorbed substances, including dietary antigens and bacterial products.

3. **Biologic functions of secretory IgA.** Secretory IgA has been linked with the following functions: inhibition of bacterial adherence (e.g., *H. influenzae*, *E. coli*), mucus trapping for assistance and formation of the mucus gel, virus neutralization (e.g. EBV), and rotavirus (both intracellularly and extracellularly), the neutralization of enzymes and toxins (e.g., *C. difficile* toxin), the inhibition of antigen penetration across the epithelial barrier and ability to interact with an enhanced function of other innate antimicrobial factors including lactoferrin, peroxidases and lysozyme. Secretory IgA cannot activate the classical complement pathway but it has been suggested that it is able to activate the alternate complement pathway when denatured or conformationally altered although this remains controversial. Notably, in tissues, through binding Fc-alpha receptors on monocytes, polymorphonuclear leukocytes, natural killer cells, T cells and B cells, IgA may be able to activate these cell types to stimulate cytotoxicity or cytokine production. Interestingly, a large number of pathogenic bacteria express proteases that are capable of degrading IgG1. It is therefore interesting that the major secretory IgA is of the IgA2 subclass. Such mechanisms of immune evasion by microbes are important to the pathogenesis of a variety of infectious diseases on the mucosal



surfaces.

IgA does not activate complement and does not enhance cell-mediated opsonization or destruction of infectious organisms or antigens. This lack of complement activation sharply contrasts with other immunoglobulins such as IgG, which can also be secreted by B cells in the intestine and which initiate important complement-mediated and cell-mediated protective events within the intestine.

2. IgG biology and transport: Although IgG is at relatively modest concentrations in the mucosal secretions of the upper gastrointestinal tract, it can reach relatively high concentrations in certain secretory sites such as the rectum, cervix and upper airway including the salivary glands. In addition, IgG levels become quite high even in the small intestine and colon in the context of inflammation, including the deposition of IgG at the apical cell surface of epithelial cells in inflammatory bowel disease. Although earlier studies suggested that IgG in the lumen was due to passive diffusion, given the molecular weight of this molecule (150 kDa) the size of the molecule is much greater than would be expected through the paracellular space. With the identification of the neonatal Fc receptor for IgG transport as the molecule responsible for the transcytosis of IgG transepithelially during neonatal life in rodents (rats and mice) across the gastrointestinal epithelial cell surface for the purposes of passive acquisition of immunity from maternal sources, it became evident that a molecular transport system for IgG transport did indeed exist. The neonatal Fc receptor, or FcRn, is a major histocompatibility complex class I-like molecule that, consistent with the structure, is expressed non-covalently with  $\beta_2$ -microglobulin ( $\beta_2$ M). The FcRn was originally cloned from neonatal rodents by Simister and colleagues in 1989, and subsequently from mouse, human, bovine and porcine origin. In all species, the molecule is highly homologous and is associated with  $\beta_2$ M. In rodents, the molecule is primarily expressed on the epithelial cells of the upper gastrointestinal tract for the first two weeks of life whereupon its expression is extinguished. During this early time period, this molecule is responsible for the passive acquisition of immunity as noted. In humans, recent studies have shown clearly that FcRn is expressed in fetal, neonatal and adult tissues at relatively significant levels. This difference between the regulation of these molecules is quite distinct. Recently, published studies in human epithelial cell model systems and transfected epithelial model systems have clearly proven that FcRn is functional in humans as it is in rodents. However, in contrast to the polymeric Ig receptor, which transports from a basal to apical direction, the transport pathway associated with FcRn is bidirectional (apical to basal and basal to apical). These properties might explain, therefore, the ability of FcRn both to deliver IgG cargo into the lumen and to transport IgG cargo, perhaps in conjunction with antigens, back into the basal surface for the purposes of luminal immunosurveillance.

FcRn has a characteristic interaction with its cargo, IgG. It binds IgG at pH 6 due to characteristic histidine residues in the CH2-CH3 domain interface of the IgG molecule, which form dipole interactions with oppositely charged amino acid residues in the FcRn molecule. Acidic pH can be achieved either in the lumen during early life wherein gastric acidification is decreased or, theoretically, after receptor or fluid phase endocytosis into an acidified endosomes wherein the FcRn can bind its cargo, IgG. After binding its cargo, FcRn then transports IgG in a pathway that protects IgG from degradation to the opposite side of the cell wherein the cargo is released at the neutral pH of the interstitium (pH 7.4). Although the itinerary for the bidirectional pathway associated with FcRn is yet to be clearly established, there are clearly motifs present within the cytoplasmic domain of FcRn that regulate this transport including the presence of calmodulin binding motifs, a dilucine motif associated with endocytosis and a critical tryptophan residue. The importance of IgG is further shown by the fact that whereas IgA-deficient individuals do not develop significant compromise of their mucosal surfaces, IgG immunodeficiencies are commonly associated with severe sino-pulmonary and gastrointestinal infections.

3. IgE biology and transport: Recent studies in parasitic infections have suggested that IgE should be considered a secretory immunoglobulin due to the fact that significant levels of this molecule can be achieved in the lumen in these diseases. These levels can be so high that frank crystals (Charcot-Leyden), as are seen in asthma, can be observed in the feces. The molecular basis for this putative transport of IgE lumenally (basal to apical) is unknown. In a similar manner, an apical to basal (abluminally directed) transport pathway for IgE also likely exists as mediated by CD23 in an IL-4-dependent pathway. Delivery of IgG through this mechanism has been linked to intestinal allergy in model systems.

### C. Intrinsic barrier: epithelial cells.

The epithelial cell surface of the intestine is enormous and is estimated to be between 200-400 square meters in size. This enormous cell surface is covered with a one cell thick layer of epithelial cells on the basal lamina and separates the outside from the inside worlds. There are two major types of epithelial cells: those that are associated with the organized gut-associated lymphoid tissue (Peyer's patches), the so-called M cell, and the mucosal epithelial cells that are associated with the non-organized GALT. The latter epithelial cell type consists of the majority of epithelial cells, given the limited localization of Peyer's patches to discrete sections of the gut such as the distal small intestine and can be characterized as either columnar absorptive cells, goblet cells, undifferentiated crypt epithelial cells, Paneth cells, enteroendocrine cells, tuft cells and cup cells.

1. Mucosal epithelial cells: The major mucosal epithelial cell is the mucosal intestinal epithelial cell (IEC) or enterocyte, which is approximately 25 microns in height, 8 microns in width and assumes a columnar shape. The surface of the enterocyte exhibits numerous, tightly-packed microvilli which, as noted, has an integral glycocalyx accompanying a thick, abutting mucus gel layer. Enterocytes have a gasket-like structure which creates the major paracellular barrier against passive transport of macromolecules. This junctional complex has three major parts: the tight junctions (zonula occludens), adhesion junctions (zonula adherens) and desmosomes (macular adherens). These tight junctions, as noted, form a relatively impervious barrier to large macromolecules. The basal membrane of the absorptive enterocyte is attached to the basal lamina, the connective tissue substances, by junctional structures called hemidesmosomes. This thick, basal lamina (20-50 nm) forms a substrate for these cells and helps to stimulate polarity and provides a guidance system for migration enterocytes from the crypt to the villous tip for desquamation. The absorptive enterocyte is, in turn, derived from an undifferentiated epithelial stem cell localized in the mid crypt that represents a continuously renewing cell type. The undifferentiated epithelial stem cell gives rise not only to the absorptive enterocyte but also to *goblet cells*, which are present in the crypt and villi of both the small and large intestine and provide the majority of the mucus gel, the *Paneth cells*, which are restricted to the crypts and are the major cell type containing cryptidins, *tuft cells*, which are characterized caveolated cells with long microvilli, and *enteroendocrine cells*, which are characterized by a narrow apical surface and a wide basal surface and function to secrete a variety of secretory proteins into the luminal microenvironment. Intimately associated with the mucosal epithelial cell at its basal surface above the basal lamina, are a unique group of T cells, the so-called intraepithelial lymphocytes (IEL). It is notable that only two cell types are present above the basal lamina, IELs and IECs, with approximately one IEL for every five IEC. IEL are described in more detail below. Mucosal epithelial cells have three major functions: 1) uptake and transport of nutrients, minerals, water and non-nutrient materials; 2) a physical barrier to entrance of microorganisms, particles and macromolecules; and 3) immunologic function as a non-professional antigen-presenting cell (see below).

2. The M cell: M cells are the major cell type associated with the follicle-associated epithelium within Peyer's patches, the major organized part of the GALT. The Peyer's patch nodule consists of the follicle-associated epithelium (FAE) and its subjacent structures, the follicular area and the parafollicular area. These are described below in consideration of antigen handling and T cell responses. In addition to the M cell, the FAE also contains the columnar absorptive enterocytes, mucous-secreting goblet cells, and an occasional tuft cell.

M cells are found through the FAE dome associated with the Peyer's patch nodule. They comprise approximately 50% of the FAE surface in rabbits but only 10% of the surface in humans and mouse. It is believed that M cells are derived from the same stem cell as absorptive enterocytes under the control of unknown factors from lymphocytes, primarily B-lymphocytes. In contrast to absorptive enterocytes, M cells, short, widely spaced, irregular-shaped microvilli in contrast to the large, closely packed villous structure of absorptive intestinal epithelial cells. Due to this microfold structure, these cells have acquired the name, M cells. M cells do have well defined-defined tight junctions and desmosomes like AEC, which, in fact, interact with the IEL. The apical cytosol of the M cell is quite thin, which creates a pocket that allows the influx of closely appended lymphoid cells such that the nucleus is displaced basally. M cells lack a glycocalyx and secretory component and exhibit little enzymatic degradative activity. This is consistent with a role for M cells in mucosal surveillance through uptake of antigens. M cells are quite active in taking up particles and macromolecules and a variety of microbial organisms. Most studies would suggest that the M cell is simply a passive transcytotic conduit for these luminal particulate antigens with little evidence of M cell catabolism for processing for presentation for lymphocytes. However, this latter point is controversial. The selectivity for M cell binding of luminal particulate antigens is unknown but may be related to specific interactions between carbohydrate determinants on the cell surface of the M cell and the cell surface of the particulate to be transported.

## SUBSTANCES and MICROORGANISMS TAKEN UP by M CELLS

### Particles and macromolecules

Native ferritin  
Cationized ferritin  
Horseradish peroxidase  
*Ricinus communis* agglutinins I & II  
Wheat germ agglutinin  
Cholera toxin  
Picibanil (OK-132)  
600 to 750 nm diameter latex particles

### Viruses

Reovirus type 1 and type 3  
Poliovirus type 1  
Human immunodeficiency virus type 1 (HIV-1)  
Mouse mammary tumor virus (MMTV)

### Bacteria

*Vibrio cholerae*  
*Salmonella typhi*  
*Salmonella typhimurium*  
Bacillus Calmette-Guerin (BCG)  
*Mycobacterium paratuberculosis*  
*Brucella abortus*  
*Streptococcus pyogenes*  
*Streptococcus pneumoniae*  
*Campylobacter jejuni*  
*Yersinia enterocolitica*  
*Yersinia pseudotuberculosis*  
*Shigella flexneri*  
*Salmonella enteritidis*  
*Escherichia coli* RDEC-1 strain  
*Escherichia coli* O:124 K:72 strain

### Parasites

Cryptosporidium  
*Eimeria coecicola*

Figure 5: Structures of Peyer's patches. Antigen is taken up across the follicular-associated epithelium, which contains specialized epithelial cells called M cells, whereupon antigens received by professional antigen-presenting cells (dendritic cells [DC] and macrophages) whereupon antigen education of T and B cells occurs. IFR, interfollicular region; HEV, high endothelial venules; SED, subepithelial dome.

### III. INDUCTIVE AND EFFECTOR TISSUES AND CELLS OF THE MUCOSAL IMMUNE SYSTEM

The cellular elements of the gut-associated lymphoid tissue GALT are organized into functional compartments that are anatomically contained within the lamina propria and epithelium. The lamina propria is composed of an organized compartment, including Peyer's patches and follicle-associated epithelium, and a nonorganized compartment that is loosely distributed throughout the lamina propria. Peyer's patches, which are unencapsulated lymphoid nodules, constitute an afferent limb of the GALT that recognizes antigens through the specialized sampling mechanism of the M cell contained within the follicle-associated epithelium. Detection of substances that move across the small bowel epithelium results in the education and dissemination of B and T lymphoblasts to other tissues linked to the MALT, such as the lungs, breast, and genitourinary tract, and the loosely affiliated compartment of the lamina propria. The lamina propria represents an efferent or effector limb of the GALT, which is populated by lymphoid cellular effectors, such as B cells, plasma cells, T cells, and natural killer cells, and by mononuclear and polymorphonuclear phagocytes and mast cells. The immune compartment within the epithelium consists of a resident population



of T cells, the intraepithelial lymphocytes. Evidence from rodent models indicates that dendritic cells found adjacent to intestinal epithelium may also obtain local antigens and migrate to regional lymph nodes (mesenteric lymph nodes) where immune responses are initiated.

#### A. Peyer's Patches

The follicle-associated epithelium contains microfold (M) cells. M cells are derived directly from undifferentiated, immature epithelial stem cells in the crypts that surround the organized lymphoid follicles called Peyer's patches. The evolution of M cells may be influenced by subjacent B cells within the lamina propria. M cells cover the lymphoid follicles in the gastrointestinal tract and provide a site for the selective sampling of intraluminal antigens.

The antigens and microorganisms transported abuminally by the M cell come into contact with lymphocytes, macrophages and DCs, which have migrated into the lymphoid aggregates or Peyer's patches

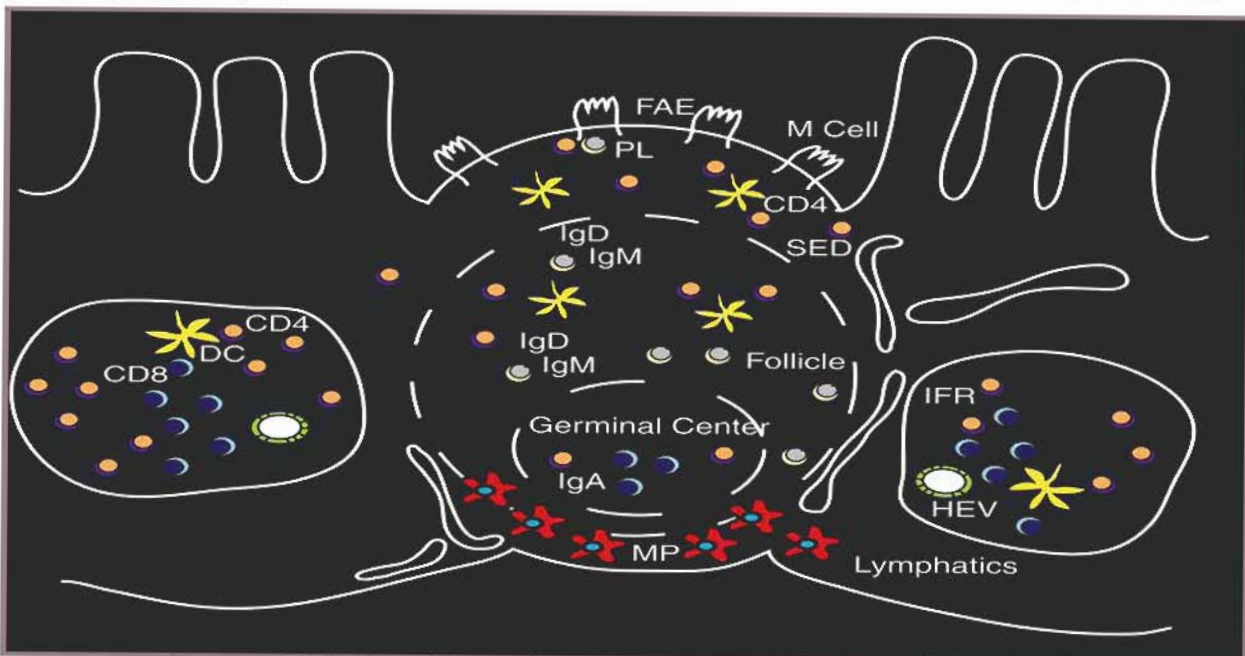


Figure 6: Lymphocyte trafficking in GALT. Naïve T cells enter the Peyer's patches where they receive antigenic education by macrophages and dendritic cells whereupon they leave the Peyer's patch and enter the mesenteric lymph, enter the thoracic duct and migrate to the loosely affiliated GALT (lamina propria and epithelium). (Figure by MIT OCV.)

below the M cells. Some of these mononuclear cells enter an intercellular space or central hollow that indents into the M cell. Naive B and T lymphocytes that have never encountered their cognate antigen express a combination of cell-surface receptors that directs them to emigrate into lymphoid aggregates such as Peyer's patches associated with follicle-associated epithelium. This emigration occurs through interactions between cell-surface receptors on the naive lymphocytes and their counterligands on specialized endothelium, the high endothelial venules present within the organized lymphoid structures. M cells transport selected antigens from the intestinal lumen to facilitate macrophage (and dendritic cell) processing and antigen presentation to the naive lymphocytes in the intestinal lymphoid follicles. A specific mucosal immune response is initiated by these interactions. This interaction may preferentially direct naïve T cells either to a Th2, Th3 or Tr1 phenotype preferentially over a Th1 phenotype. Such an outcome is likely due to the properties of the DCs within the Peyer's patches and play an important role in generating mucosal tolerance. During infection or pathologic conditions such as IBD, a Th1 bias may occur relative to Th3 or Tr1. The observation of lysosomal organelles containing MHC class II components in M cells confirms that, under certain circumstances, M cells may also serve as primary antigen-presenting cells.

Activated lymphocytes from intestinal lymphoid follicles begin a maturational journey in which they leave the intestinal tract and migrate into afferent lymphatics that drain into mesenteric lymph nodes. During

this process, the lymphocytes mature into T and B lymphoblasts enriched in IgA-bearing B cells. The B lymphocytes become surface IgA-bearing lymphoblasts after being promoted to switch their immunoglobulin isotype by regulatory (i.e., "switch") T cells within Peyer's patches. Switch T cells control isotype switching and B-cell clonal expansion by secreting cytokines (e.g., IL-2, IL-4, IL-5, IL-6, IL-10 and TGF- $\beta$ ). Lymphocytes then enter the efferent lymphatics of the mesenteric lymph nodes and pass through the thoracic duct into the peripheral blood. These lymphocytes subsequently reenter the loosely affiliated lamina propria through interactions with flat endothelial cells of the postcapillary venules. After homing to mucosal sites, B lymphoblasts mature into IgA-secreting plasma cells under the control of antigen-activated T lymphocytes that have completed a similar maturational journey. Lymphoblasts that have homed to the gastrointestinal mucosa and have matured into effector cells provide protective immunity within the lamina propria.

Lymphoblasts recirculate or home to the sites of the original antigenic stimulation and to other mucosal secretory sites. After antigenic stimulation in the gastrointestinal tract, IgA lymphoblasts circulate to the mucosal secretory sites of the breast, lung, and eye, where antigen-specific antibodies are secreted. A breast-feeding mother can passively transfer secretory IgA in the breast milk to her nursing child. The breast milk secretory IgA transferred to the infant protects against bacteria or viruses found within the mother's gastrointestinal tract, supporting the importance of the common mucosal immune system. Homing of stimulated lymphoblasts to mucosal secretory sites allows the secretion into lung, breast, and eye fluids of protective antibodies directed against antigens recognized within the gastrointestinal lumen. The intestinal immune system thus has components that allow selective antigen sampling and subsequent induction of immune responses that provide protection for the gastrointestinal tract and other mucosal surfaces.

The selective interaction between lymphocyte-specific proteins and counterligands on endothelial cells in specific organs regulates the distribution of lymphoid effector cells to the intestine and other mucosal secretory sites. Antigenic stimulation and chronic inflammation result in a rapid increase in the number of endothelial venules. The number and adhesiveness of endothelial venules increases because of enhanced differentiation and stimulated proliferation. Cytokines, including IL-1, IFN- $\gamma$ , and tumor necrosis factor- $\alpha$ , increase lymphoblast adherence to endothelial cells, trigger the development of endothelial cell differentiation markers, and enhance the expression of endothelial adhesion molecules. The increased expression of adhesion molecules on endothelial cells stimulates an increase in the influx of antigen-specific, sensitized lymphocytes into areas of chronic inflammation or areas where cell-mediated host defense processes are needed. Endothelial venules are closely associated with dense, lymphocytic infiltrates, particularly if the mononuclear cell-mediated processes are persistent.

## B. Non-Organized GALT

1. Lamina Propria Compartment. The efferent, or effector, compartment of the lamina propria consists of T cells, B cells, plasma cells, Natural killer cells, phagocytic cells, and mast cells. Approximately 60% of the lymphoid cells are T cells. CD4<sup>+</sup> and CD8<sup>+</sup> T cells occur in a ratio of about 2:1, which approximates their concentrations in peripheral blood. Virtually all T cells within the lamina propria express the  $\alpha\beta$  T-cell receptor. Closer examination of  $\alpha\beta$  T-cell receptor usage shows some skewing, with an overabundance of certain receptors, suggesting the expansion of some T-cell clones. The CD45 isoform expression confirms that these cells are memory cells, which express the CD45RO isoform, indicating previous encounters with antigens presumably in the Peyer patches. The CD4<sup>+</sup> cells in the lamina propria exert a helper-inducer function for immunoglobulin production, a major function of lamina propria T cells. Consistent with this, in response to antigen, lamina propria lymphocytes (LPL) respond primarily with cytokine production rather than proliferation. The majority of CD8<sup>+</sup> LPLs as well as a significant proportion of CD4<sup>+</sup> LPLs express a unique  $\beta_7$  integrin associated with a novel  $\alpha$  chain,  $\alpha^E$ , which plays a role in intestinal epithelial cell binding. The population of CD8<sup>+</sup> LPLs probably contains precursors, such as TCR- $\alpha\beta^+$ , CD8<sup>+</sup>,  $\alpha^E\beta_7^+$  (HML-1<sup>+</sup>), and CD45RO<sup>+</sup> T cells, in transit to the epithelium.

A set of lymphocytes in the intestine are cytotoxic effector precursor cells that can participate in host mucosal defense mechanisms when needed, without continuously causing damage to the surrounding tissue when not needed. Functional precursor natural killer cells and cytotoxic T lymphocytes can be enriched from isolated lamina propria mononuclear cells. LPLs can be induced to mediate cell-mediated cytotoxicity by incubation with IL-2, interferon, lectins, and monoclonal antibodies directed against the T-cell receptor,

typical of antigen-primed effectors and consistent with their CD45RO phenotype. Although cytotoxic effectors are present, the intestinal LPLs are generally poor mediators of cell-mediated cytotoxicity in a variety of systems, including spontaneous cell-mediated cytotoxicity, antibody-dependent cellular cytotoxicity, and cell-mediated cytotoxicity.

The majority of mucosal T lymphocytes are also CD95L<sup>+</sup> and CD69<sup>+</sup> and exhibit elevated levels of cytoplasmic Ca<sup>2+</sup> consistent with an activated phenotype. Controlled activation of the intestinal immune system may be important in regulating effector cell function. This includes cytotoxic function which may be directed at the lymphocytes themselves for the purposes of downregulating immune responses. In this way, the gut can remain in a state of physiologic inflammation, poised for intervention when necessary but maintaining a general tone of restraint. Humoral extracts from the normal lamina propria, containing humoral substances of unknown origin, are capable of suppressing the activation of peripheral nonintestinal lymphoid populations, which may account for the low proliferative responses to proliferative signals delivered by the T-cell receptor-CD3 complex.

Approximately 40% of the lymphoid cells in the lamina propria are B cells, which are primarily derived from precursors in Peyer patches. These B cells and their progeny plasma cells are predominantly focused on IgA synthesis and focused to a lesser extent on IgM, IgG and IgE synthesis. Lamina propria B cells are induced to terminally differentiate into IgA-secreting cells by IFN- $\gamma$ , IL-4, IL-5, IL-6 and IL-10. IL-4 and IL-5 activate resting B cells and induce the division and growth of activated B cells. IL-6 is critical for the terminal differentiation of IgA plasma cells, resulting in the secretion of large amounts of IgA. The sequence of cytokine-mediated events that regulate B-cell growth, differentiation, and development toward IgA production involve both Th1 (IFN- $\gamma$ ) and Th2 (IL-4, IL-5, IL-6, IL-10) indicating the importance of this antibody to mucosal protection. In diseases such as IBD, lamina propria B cells and plasma cells, which produce IgG, are markedly increased.

### C. Epithelial Compartment

1. Intraepithelial Lymphocytes: The epithelium of the human intestine contains a unique population of lymphoid cells, the intraepithelial lymphocytes (IELs), that reside between intestinal epithelial cells along their basolateral surface. A few IELs are CD4<sup>+</sup> T cells, but most IELs express the CD8  $\alpha\beta$  heterodimer, the CD45RO isoform, and the  $\alpha\beta$  T-cell receptor, indicating that they are memory cells driven by MHC class I or class I-like molecules. Between 5% and 30% of these T cells, especially in the colon, express the  $\gamma\delta$  T-cell receptor. Virtually all of the  $\gamma$  T cells in the human intestine reside within the epithelium, suggesting that they are responsive to specific chemotactic factors or antigens within this location. More than 95% of IELs express the unique  $\alpha^E\beta_7$  integrin, HML-1, which, in view of its role in epithelial cell binding, probably plays an important role in the epithelial cell tropism of these cells.  $\alpha^E\beta_7$  binds E-cadherin, an immunoglobulin supergene family member, on the basolateral surface of the intestinal epithelial cell. In the presence of transforming growth factor- $\beta$  presumably secreted by local intestinal epithelial cells, high levels of this integrin are expressed somewhat reciprocally to the  $\alpha^L\beta_2$  integrin LFA-1 (i.e., CD11a/CD18), a  $\beta_2$  integrin commonly expressed by peripheral blood lymphocytes.

Despite their contiguity to the gut lumen, potential exposure to a variety of antigens, and the expectation that these cells express a diverse, polyclonal array of  $\alpha\beta$  and  $\gamma\delta$  T-cell receptors, IELs within the small and large intestine are oligoclonal and express a small number of  $\alpha\beta$  and  $\gamma\delta$  T-cell receptors based upon an analysis of CDR3 regions. A limited variety of T-cell clones are, in fact, widely disseminated throughout the intestinal epithelium. With a CD45RO phenotype, these cells are memory cells that recognize an extremely limited number of antigens, not the multitude of luminal antigens. The abundant expression of CD8 further indicates that these cells recognize these putative antigens in the context of an MHC class I or class I-like molecule such as CD1 or the MHC class I chain-related gene A gene product. The CD8 expression of IELs suggests that they function biologically as cytolytic effectors as a consequence of antigenic recognition. IELs exhibit a high level of cytolytic activity in a variety of *in vitro* systems, especially after activation, and likely in disease states *in vivo*. However, IELs *in situ* do not express granzyme, perforin or CD94L. This suggests that their major biologic function in health is the secretion of cytokines (e.g., IFN- $\gamma$  and keratinocyte growth factor), which regulate epithelial cell function and possibly responses to luminal antigens.  $\gamma\delta$  IEL, for example, may play a role in regulating responses to orally delivered antigens. Upon activation, IELs may



acquire cytolytic machinery that can contribute to epithelial cell death through apoptosis. Their cytolytic capabilities, large number, and extremely limited T-cell receptor repertoire together indicate that IELs are a regionally specific population of cells involved in immunosurveillance against abnormal epithelial cells based on the recognition of a limited number of proteins not normally expressed on the cell surface of intestinal epithelial cells. IELs may be the first line of defense against deleterious epithelial events. Their numbers are markedly increased in intestinal graft-versus-host disease, gluten-sensitive enteropathy, and protozoal infections of the epithelium, such as those caused by *Cryptosporidium* and *Isospora* species.

Human IELs and the CD8<sup>+</sup> T cells within the lamina propria share many phenotypic characteristics, but their origin is unclear. Studies of murine systems suggest the existence of two IEL populations: thymus-dependent (i.e., selected in the thymus) and thymus-independent (i.e., selected in the intestine) lymphocytes. A feature of thymus-independent mouse IELs is the expression of CD8 as an  $\alpha\alpha$  homodimer rather than the more common  $\alpha\beta$  heterodimer and the expression RAG transcripts. Considering the rarity of this form of CD8 in normal human adult intestine, the extrapolation from the mouse models to humans remains uncertain. Nonetheless, mouse models of rotavirus and reovirus infection indicate that TCR- $\alpha\beta$ <sup>+</sup>, CD8<sup>+</sup>, MHC class I-restricted, virally specific cytotoxic effectors can be found within the epithelium that are probably derived from cytotoxic T-cell precursors in the Peyer's patches. Taken together with other studies showing that most IELs are restricted by classical MHC class I molecules, these studies suggest that, IELs are likely the clonally expanded progeny of MHC class I-restricted cytolytic effector cells.

2. Epithelial cells: Epithelial cells interact with the inflammatory response at several levels. Secretion of electrolytes and water by epithelial cells is an important part of the gastrointestinal response to inflammation. The interaction of inflammation and epithelial cell electrolyte and water secretion has been studied by assessing the effects of individual inflammatory mediators on electrolyte and water secretion or by identifying the mediators that regulate electrolyte and water secretion in specific inflammatory states. Histamine, PGE<sub>2</sub>, 5-HT (serotonin), and LTB<sub>4</sub> are just a few of the inflammatory mediators that induce epithelial cell Cl<sup>-</sup> secretion. Neural mechanisms also affect epithelial cell Cl<sup>-</sup> secretion directly or indirectly, through inflammatory cells. The neurotransmitter acetylcholine induces Cl<sup>-</sup> secretion in epithelial cells directly. Neuropeptides such as substance P, VIP, and NPY induce mast cell activation, resulting in release of histamine and 5-HT and, thus, activate epithelial cell Cl<sup>-</sup> secretion. Cl<sup>-</sup> secretion is accompanied by Na<sup>+</sup> secretion and, consequently, by the passage of water across the epithelium into the intestinal lumen. Diarrhea is the clinical manifestation of the enhanced enterocyte Cl<sup>-</sup> secretion induced by these mediators. These same inflammatory mediators induce the secretion of mucus by goblet cells in the gastrointestinal tract. Diarrhea protects the host from infectious agents and their toxins by speeding their passage through the gastrointestinal tract and out of the organism. Mucus secretion protects the host from infectious agents in the gastrointestinal tract by preventing the binding of the infectious agents and their toxins to epithelial cells.

The interaction of inflammation and epithelial electrolyte and water secretion has also been studied by identifying the inflammatory mediators that regulate electrolyte and water secretion in specific inflammatory conditions. One relatively simple animal model of intestinal inflammation is the sensitization of rats to egg albumin followed by antigenic challenge. In this model, antigenic challenge results in increased Cl<sup>-</sup> secretion and increased paracellular permeability. The increase in paracellular permeability was demonstrated by the increase in the uptake of <sup>51</sup>Cr-EDTA from the intestinal lumen after antigen challenge of previously sensitized rats. The relative contribution of various inflammatory mediators to the increase in Cl<sup>-</sup> secretion seen in this model was tested with a series of blocking agents. Ketanserin (an antagonist of 5-HT), diphenhydramine (an H<sub>1</sub> histaminic antagonist), and piroxicam (a COX inhibitor) inhibited egg albumin-induced Cl<sup>-</sup> secretion by 30%, 42%, and 52%, respectively. The combination of piroxicam and diphenhydramine inhibited the secretory response by 82%. These data suggest that even in this simple model not only does more than one inflammatory mediator contribute to the increase in Cl<sup>-</sup> secretion, but the mediators involved suggest that more than one cell type produces mediators. Histamine and 5-HT are products of mast cell activation. PGE<sub>2</sub>, the prostaglandin most likely involved in Cl<sup>-</sup> secretion, is produced by fibroblasts or by the epithelial cells themselves. In a similar

study, challenge of *Trichinella*-immunized rats with *Trichinella* larvae resulted in increased intestinal fluid secretion. Treatment of the rats with the combination of indomethacin, a COX inhibitor, and diphenhydramine ablated the increase in the fluid secretion induced by exposure to *Trichinella*. In the *Trichinella* model, increased fluid secretion is caused by a combination of prostaglandins and histamine, whereas in the egg albumin model, increased secretion results from a combination of prostaglandins, histamine, and 5-HT.

The epithelium also interacts with the inflammatory response in its function as a barrier. The epithelium separates luminal antigens from lamina propria inflammatory cells, thus preventing activation of the inflammatory cells by luminal agents. The effectiveness of the epithelium as a barrier is influenced by the junctions between the epithelial cells, as noted above. The tighter these junctions, the more effective the epithelium is as a barrier to the passage of noxious agents. The junctions between epithelial cells are fairly loose in the proximal intestine and become progressively tighter in the distal small intestine and colon. Epithelial permeability usually is assessed by measuring the flux of tracer molecules either from the bloodstream into the intestinal lumen or from the intestinal lumen into the bloodstream. One commonly used probe is  $^{51}\text{Cr-EDTA}$ . This compound is placed in the intestinal lumen, the animal is challenged, and then the recovery of  $^{51}\text{Cr-EDTA}$  in the blood is assessed. Epithelial permeability is affected by inflammatory events.  $\text{IFN-}\gamma$ , a cytokine produced during intestinal inflammation, increases paracellular permeability by opening the tight junctions between epithelial cells. Increased paracellular permeability has been described in Crohn's disease and in several animal models of gastrointestinal inflammation.

Absorptive intestinal epithelial cells can also function as antigen-presenting cells. Epithelial cells of the small intestine constitutively express MHC class II molecules, possibly as a consequence of IEL secretion of  $\text{IFN-}\gamma$ . Colonic epithelial cells do not normally express measurable levels of MHC class II molecules, except in the setting of inflammation, presumably in response to local cytokine production. In vitro studies of intestinal epithelial cell function show that they take up, process, and present soluble antigens to  $\text{CD4}^+$  T cells in the context of MHC class II molecules. Although soluble antigens can be taken up apically and basolaterally, MHC class II molecules primarily segregate basolaterally where antigen presentation to antigen-specific, MHC class II-restricted T cells occurs. There is evidence that under normal conditions intestinal epithelial cells take up, process, and present soluble antigens from the lumen in vivo. The implication of these studies is that intestinal epithelial cells may augment or modify afferent pathways that normally result from antigenic events within Peyer patches. Despite in vitro evidence for functional class II MHC expression, intestinal epithelial cells seem to preferentially engage and stimulate  $\text{CD8}^+$  cells that exhibit suppressor activity—an activity that may contribute to the suppressor tone of the intestine. IELs also express other antigen presenting molecules (CD1d, MICA) and costimulatory molecules (CD86, CEACAM1, CD40).

One consequence of many forms of intestinal injury is breaks in the epithelial barrier. These breaks are seen in infectious disease, in IBD, in celiac disease and in injury caused by radiation and chemotherapeutic agents. There is an orchestrated response to these breaks that begins with rapid migration of remaining epithelial cells from the wound edge to cover the defect. Epithelial cells elongate and then allowing them to cover broad areas of denuded mucosal surface

In the presence of inflammation, epithelial cells express a series of genes that are not expressed in the absence of inflammation. Among these are genes for COX-2, iNOS, and IL-8. An immunohistochemical study of human colon revealed expression of iNOS in epithelial cells from areas of inflammation in ulcerative colitis, Crohn's disease, and diverticulitis, but not in epithelial cells from uninfamed areas of the same surgical resections. Parallel studies of the cellular distribution of iNOS and COX-2 in ulcerative colitis resections demonstrated that these proteins were expressed in exactly the same populations of epithelial cells in areas of inflammation. This colocalization suggests a common regulatory mechanism for the expression of these genes.

Intestinal epithelial cell lines infected with *Salmonella* and other invasive bacteria express IL-8 and COX-2, whereas uninfected cells or cells infected with noninvasive bacteria do not. Intestinal epithelial cell lines in culture were infected with *Salmonella dublin* and then stained for COX-2

expression and for intracellular *Salmonella*. The *Salmonella*-infected cells expressed COX-2, as did a few immediately adjacent epithelial cells. In contrast, cells that were not infected themselves or were not adjacent to infected cells did not express COX-2. The expression of COX-2 in cells adjacent to an infected cell raises the possibility of a paracrine effect mediated by a soluble factor. *Salmonella* infection also induced epithelial cell COX-2 expression in human intestinal xenografts transplanted into severe combined immunodeficiency disease (SCID) mice, which demonstrates that infection from *Salmonella* induces COX-2 expression in normal intestinal epithelial cells as well as in transformed epithelial cell lines. Infection of the colonic epithelial cell line HT-29 with *Salmonella* resulted in the expression of COX-2 and, as a consequence, a 50-fold increase in the production of PGE<sub>2</sub>, a potent stimulus for Cl secretion in epithelial cells. Supernatants from *Salmonella*-infected epithelial cell lines increased Cl secretion by polarized intestinal epithelial cells mounted in an Ussing chamber. The demonstration that the PGE<sub>2</sub> in conditioned media from *Salmonella*-infected epithelial cells in culture can induce Cl secretion in vitro raises the possibility that the diarrhea seen in infection with *Salmonella* in humans may be the result of PGE<sub>2</sub> produced by COX-2 in *Salmonella*-infected epithelial cells. One of the protective responses of the host to infection with enteric pathogens is an increase in electrolyte and water secretion to wash the pathogens out of the gastrointestinal tract. This study raises the possibility that one mechanism for this protective response is the induction of epithelial cell COX-2 expression, which results in increased prostaglandin production and, thus, increased Cl-secretion.

The genes for IL-8, iNOS, and COX-2 each have an NF $\kappa$ B site in their promoters. NF $\kappa$ B is a transcription factor that is important in the regulation of the synthesis of numerous inflammation-related proteins (e.g., TNF- $\alpha$ , IL-1, ICAM-1, E-selectin, IL-8, iNOS, and COX-2.) NF $\kappa$ B is a heterodimer that consists of p50 and p65 subunits. In unstimulated cells, NF $\kappa$ B is bound to I $\kappa$ B, which is found in the cytoplasm. When cells are stimulated, I $\kappa$ B is phosphorylated and degraded and releases NFB. The release of NF $\kappa$ B from I $\kappa$ B allows NF $\kappa$ B to enter the nucleus, where it binds to the promoter regions of target genes. NF $\kappa$ B is activated by proinflammatory cytokines (e.g., TNF- $\alpha$  and IL-1), oxidants, phorbol esters, PAF, and LPS. Some of these agents (e.g., IL-1, TNF- $\alpha$ ) are likely to be present in inflammatory states. The importance of NFB in the response to infection is demonstrated by the finding that mice lacking the p50 subunit of NF $\kappa$ B are unable to clear *Listeria* and other organisms effectively.

Activation of NF $\kappa$ B results in the parallel stimulation of a number of important genes involved in the inflammatory response. It is this parallel stimulation that probably accounts for the coexpression of iNOS and COX-2 in the same population of epithelial cells in ulcerative colitis. The presence of NF $\kappa$ B response elements in the genes for E-selectin, ICAM, IL-8, and TNF- $\alpha$  allows for the coordinated expression of a series of proteins involved in the adhesion of neutrophils to epithelial cells, the migration of neutrophils from the vascular space into gastrointestinal tissue, and the activation of those neutrophils. IL-1, TNF- $\alpha$ , and NF $\kappa$ B are involved in a cycle of activation that results in amplification of the inflammatory response. IL-1 and TNF- $\alpha$  both activate NF $\kappa$ B; in turn, the synthesis of IL-1 and TNF- $\alpha$  is promoted by the binding of NF $\kappa$ B to response elements in their promoters. This positive regulatory cycle amplifies and perpetuates the inflammatory response. Although NF $\kappa$ B has received the most attention, other transcription factors are involved in the regulation of genes associated with inflammation. NF-IL6 sites are presented in the promoters of several of these genes. Preliminary studies suggest that NF-IL6 may be as important as NF $\kappa$ B in the regulation of these genes.

### C. Mast Cells

Mast cells are inflammatory cells with large granules containing preformed mediators of inflammation (e.g., histamine and 5-HT). In response to stimulation, mast cells release these granules and produce newly formed non-granule-associated mediators (e.g., NO, PGD<sub>2</sub>, PAF, and leukotrienes). In the normal gastrointestinal tract, mast cells are found in the lamina propria, submucosa, muscle layers, and on the serosal surface. Increased numbers of mast cells and mast cell activation have been observed in the gastrointestinal mucosa of patients with helminthic infections, ulcerative colitis, Crohn's disease, gastritis, and celiac sprue.



In rodents, the two distinct subpopulations of mast cells are connective-tissue mast cells and mucosal mast cells. The connective-tissue mast cells, such as those found in the rat peritoneum, have granules that contain serotonin, heparin, and large amounts of histamine. They are capable of making PAF, NO, PGE<sub>2</sub>, TNF-, IL-1, IL-3, IL-4, IL-6, IL-10, and IFN-. In contrast, rat mucosal mast cells have granules that contain serotonin and small amounts of histamine. They are capable of making PAF, NO, PGE<sub>2</sub>, leukotrienes, and TNF-. Connective-tissue mast cells and mucosal mast cells arise from a common progenitor cell. There appear to be two subpopulations of mast cells in humans, but the distinctions are not as clear as they are in rodents.

Mast cells can be activated by a variety of factors, but they are associated most commonly with IgE-dependent antigen activation. Antigen-specific IgE binds to receptors on the mast-cell surface through its Fc component. Exposure of the mast cell to an appropriate antigen results in cross-linking of the IgE molecules, which in turn results in activation of the mast cell. IgE-mediated mast cell activation is important as a defense mechanism against intestinal worms and other parasites. Parasite antigens cross-link IgE molecules on intestinal mast cells. Mast-cell activation releases substances that promote intestinal motility and increase electrolyte and water secretion. Histamine, PGD<sub>2</sub>, and peptidyl leukotrienes are released by activated mast cells; all enhance epithelial cell Cl secretion and promote intestinal motility. These physiologic responses allow the infected host to wash the parasites out of the digestive tract. IgE-mediated mast cell activation is also important in allergic disorders. Pollen cross-links IgE molecules on mast cells in the nasal mucosa, resulting in allergic rhinitis. Food antigens activate mast cells in the gastrointestinal tract by similar mechanisms. There would appear to be no evolutionary advantage to being able to mount an allergic response to food antigens, but there would be an evolutionary advantage to being able to mount a response to gut parasites that would clear them from the gastrointestinal tract. It may be that food allergies represent the maladaptation of an inflammatory response designed to deal with intestinal parasites.

In addition to IgE-dependent antigen activation, mast cells also can be activated by the calcium ionophore A23187 and by the complement components C3a and C5a. Some subpopulations can be activated by substance P; activation by substance P is of particular interest in that it is a neurotransmitter that can be released by neural activation in the gastrointestinal tract. Mast-cell activation by substance P would provide a mechanism for induction of intestinal inflammation by neural activation. There are both anatomic and functional interactions between mast cells and the enteric nervous system. In the rat, infection with the intestinal nematode *Nippostrongylus brasiliensis* results in mast cell hyperplasia. Immunohistochemical studies reveal that most of these mast cells are juxtaposed to enteric nerves. There are bidirectional interactions between nerves and mast cells. Neurotransmitters, particularly substance P, cause mast cell degranulation and mast cells, in turn, release VIP, which can act as a neurotransmitter.

Mast-cell activation plays an important role in various allergic reactions. Exposure to an antigen results in B-cell activation and the production of antigen-specific IgE, which binds to specific receptors on mast cells. Reexposure to the sensitizing antigen results in IgE cross-linking and mast cell activation. Earlier in this chapter, we described studies in which sensitized rats were challenged with albumin. The result was increased Cl secretion and increased paracellular permeability. A role for mast-cell activation in this process was demonstrated by the finding that Cl secretion in response to egg albumin could be significantly diminished by coadministration of diphenhydramine, an antagonist of the H<sub>1</sub> histamine receptor. Mast cells are the dominant source of histamine in the gastrointestinal tract.

Additional evidence for a role for mast cells in the mediation of enhanced Cl secretion after antigen exposure comes from studies with the mast-cell-deficient mouse (W-W<sup>v</sup>). Antigen challenge of sensitized W-W<sup>v</sup> mice results in a 70% decrease in Cl secretion compared with antigen challenge of wild-type litter mates. These data suggest that, at least in this model, mast-cell-derived histamine is an important mediator of epithelial cell Cl secretion. The Cl secretion that was seen in the mast-cell-deficient mice (30% of that seen in the wild-type mice) was inhibitable with NSAIDs, suggesting that the prostaglandins that induce Cl secretion are not of mast cell origin.

#### D. Adhesion Molecules and Cell Trafficking

A healthy gastrointestinal tract contains many neutrophils, macrophages, and lymphocytes. Most of these cells are found in the lamina propria, the space between the epithelium and muscularis mucosa. These three cell types arise from the bone marrow, but their life histories are different.

Neutrophils differentiate in the bone marrow before entering the peripheral blood. They leave the peripheral circulation and enter the gastrointestinal tissues by binding to adhesion molecules expressed on the endothelium of postcapillary venules. They stay in the gastrointestinal tract only one or two days before passing between epithelial cells into the intestinal lumen, where they die and are expelled in the stool. Although neutrophils differentiate in the bone marrow, they are primed and activated in the lamina propria. Priming enhances the neutrophils' ability to produce reactive oxygen species, and activation induces production of the reactive oxygen species. Neutrophil priming and activation are mediated by interaction with particulate stimuli (e.g., bacteria) or by stimulation with soluble factors, such as cytokines, inflammatory mediators, and bacterial products (e.g., endotoxin). Neutrophils are incapable of proliferation; the increase in the number of neutrophils in the lamina propria in inflammatory states reflects increased trafficking out of the bloodstream and into the gastrointestinal tissues.

Gastrointestinal macrophages are derived from circulating monocytes produced in the bone marrow. The large increase in the macrophage numbers seen in clinically apparent inflammation reflects increased migration of monocytes out of the bloodstream and into the lamina propria rather than proliferation of the resident macrophages. Monocytes enter the circulation and, like neutrophils, bind to adhesion molecules expressed on endothelial cells in the postcapillary venules of the intestine. After binding to these adhesion molecules, monocytes pass between endothelial cells and enter the gastrointestinal tissue.

After entering the lamina propria, the monocyte begins to differentiate into a mature macrophage. As the monocyte differentiates, it can acquire capacities for phagocytosis, proliferation, and bacterial killing. Macrophage differentiation is controlled by cytokines and other soluble factors present in the lamina propria; different combinations of cytokines and mediators result in macrophages with different phenotypes. Macrophage phenotypes are characterized by their surface receptors; these surface receptors determine the stimuli to which the macrophage can respond. No detailed surveys exist of the life span of macrophages in the gastrointestinal tract, but as a group their stay is far longer than that of neutrophils.

For the most part, neutrophil and monocyte trafficking in the gastrointestinal tract is similar to that in other organ systems. The mechanisms of leukocyte trafficking in various organs are qualitatively similar, but the number of leukocytes passing through the gastrointestinal tract greatly exceeds that in other organs. The gastrointestinal tract has a large surface area; as a result, even the modest degree of inflammation seen in the normal small intestine and colon represents the trafficking of a substantial number of monocytes and neutrophils. In diffuse inflammatory diseases of the gastrointestinal tract, such as ulcerative colitis, the trafficking of leukocytes through the inflamed mucosa expands to the point that most leukocytes produced in the bone marrow travel through the gastrointestinal mucosa into the lumen.

The migration of neutrophils and monocytes from the peripheral circulation into the lamina propria of the gastrointestinal tract is mediated by the expression of adhesion molecules on vascular endothelial cells and on the leukocytes themselves. Adhesion molecules play an important role in many biologic processes. They are involved not only in interactions between inflammatory cells and vascular endothelium but also in interactions between different inflammatory cell types, between inflammatory and noninflammatory cells, and in the binding of cells to the extracellular matrix. The adhesion molecules that participate in the binding of inflammatory cells to vascular endothelium fall into three groups: selectins,  $\beta 2$  integrins, and the immunoglobulin superfamily of adhesion molecules.

As inflammation is initiated, leukocytes and endothelial cells express selectins. The three members of the selectin family are L-selectin (leukocyte adhesion molecule-1 or LAM-1), E-selectin (endothelial leukocyte adhesion molecule-1 or ELAM-1) and P-selectin. The natural ligands for all three selectins are sialylated Lewis X oligosaccharides, which are found on almost all cell types. Among the molecules with sialylated Lewis X moieties are the selectins themselves, so that L-selectin on neutrophils can bind to E-selectin or P-selectin on endothelial cells.

L-selectin is expressed on lymphocytes, monocytes, and neutrophils; it mediates their adherence to endothelial cells. L-selectin expression is stimulated by inflammatory cytokines (e.g., interleukin-1 [IL-

1] and tumor necrosis factor- $\alpha$  [TNF- $\alpha$ ]), mediators of inflammation (i.e., leukotriene B<sub>4</sub> [LTB<sub>4</sub>]), and lipopolysaccharide (LPS). E-selectin is found only on stimulated endothelial cells, where it promotes leukocyte adherence. It is expressed in response to cytokine stimulation [e.g., IL-1, TNF- $\alpha$  and interferon- $\gamma$  (IFN- $\gamma$ )]. P-selectin is expressed on platelets and is involved in thrombosis. It is also expressed on endothelial cells and is involved in leukocyte adhesion. In contrast to E-selectin, which is only expressed in response to stimulation, P-selectin is constitutively expressed on endothelial cells, at least in some organs. Proinflammatory cytokines, histamine, and LPS induce P-selectin expression, but different organs have different levels of sensitivity. LPS induces endothelial expression of P-selectin and E-selectin, whereas histamine induces P-selectin but not E-selectin.

Selectin bonds are responsible for leukocyte rolling. The selectin bonds that form between leukocytes and endothelial cells are weak; the weakness of these bonds allows leukocytes to roll along the surface of the endothelium by making and breaking selectin-mediated bonds. Rolling reduces leukocyte velocity before the formation of stronger adhesion bonds that fully immobilize leukocytes on the surface of the endothelium. These stronger bonds are formed between  $\beta$ 2-integrins expressed on the surface of the leukocytes and intercellular adhesive molecules 1 and 2 (ICAM-1 and ICAM-2), members of the immunoglobulin superfamily of adhesion molecules, which are expressed on endothelial cells.

The integrins form a large group of adhesion molecules. Each integrin is a heterodimer that consists of noncovalently associated  $\alpha$ - and  $\beta$ -subunits. Integrins are divided into subfamilies based on common  $\beta$ -subunits; the  $\beta$ 1- and  $\beta$ 2-subfamilies are the most important in inflammation.  $\beta$ 1-integrins are involved in lymphocyte trafficking.  $\beta$ 2-integrins are involved in the adhesion of monocytes and neutrophils to endothelial cells. One  $\beta$ 2 integrin, CD11a/CD18 (LFA-1), binds to both ICAM-1 and ICAM-2 on endothelial cells. Another  $\beta$ 2 integrin, CD11b/CD18 (MAC-1), binds only to ICAM-1. CD11a/CD18 is expressed on neutrophils in the basal state, and its expression is not enhanced by cytokines or inflammatory mediators. In contrast, the expression of CD11b/CD18 on leukocytes is induced by products of bacteria [e.g., formylmethionylleucylphenylalanine (FMLP)] and inflammatory cells [e.g., TNF-, IL-1, IL-8, LTB<sub>4</sub>, and platelet-activating factor (PAF)].

The third group of molecules important in the binding of leukocytes to endothelial cells is the immunoglobulin superfamily of adhesion molecules. The most prominent members of this family, ICAM-1 and ICAM-2, are expressed on endothelial cells in the basal state; ICAM-2 is expressed at higher levels than ICAM-1. The expression of ICAM-2 is not increased by cytokine stimulation, whereas the expression of ICAM-1 is enhanced by IL-1, TNF- $\alpha$ , and IFN- $\gamma$ . Thus, the relative importance of ICAM-1 increases in inflammation. ICAM-1 binds both CD11a/CD18 and CD11b/CD18, whereas ICAM-2 binds only CD11a/CD18.

Enhanced expression of adhesion molecules occurs in human diseases marked by gastrointestinal inflammation and in animal models of inflammation. In inflamed colonic mucosa, the expression of ICAM-1, E-selectin, and P-selectin is upregulated in the vascular endothelium. Quantitative immunohistochemistry reveals increased numbers of E-selectin-positive vessels in inflamed areas of a colon affected by ulcerative colitis compared with the normal colon.

The role of adhesion molecules in the pathogenesis of intestinal inflammation was carefully addressed in a study of indomethacin-induced enteritis in the rat. Indomethacin treatment results in mucosal ulceration and granulocyte infiltration in the rat intestine and a corresponding inflammatory response in the mesentery characterized by an increase in the number of adherent and extravascular leukocytes and a reduction in leukocyte rolling velocity. The role of adhesion molecules was assessed by the coadministration of indomethacin and monoclonal antibodies directed against P-selectin, E-selectin, or CD11b/CD18. The indomethacin-induced leukocyte-epithelial cell adhesion in mesenteric venules was reduced with coadministration of monoclonal antibodies against CD11b/CD18 or E-selectin but not by the monoclonal antibody against P-selectin. This study suggests that both CD11b/CD18 (expressed on neutrophils) and E-selectin (expressed on endothelial cells) contribute to neutrophil accumulation in this model.

After neutrophils attach firmly to the endothelium, they migrate between the endothelial cells into the extravascular space. On neutrophils, L-selectin (LAM-1) and CD11b/CD18 are expressed

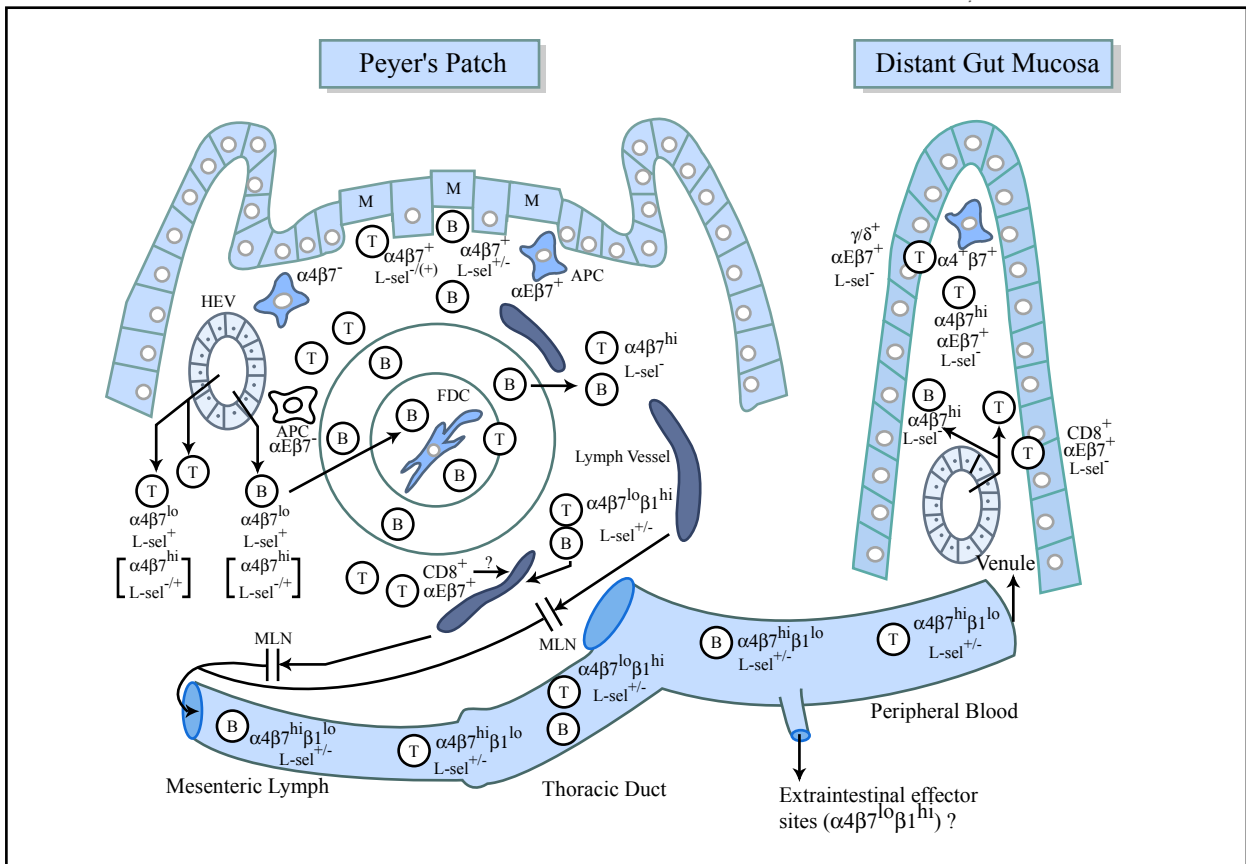
consecutively; that is, the expression of CD11b/CD18 increases as LAM-1 is shed. Immunohistochemistry of inflamed tissue demonstrates that some intravascular neutrophils are LAM-1 positive and others are CD11b/CD18 positive, but extravascular neutrophils are all CD11b/CD18 positive and LAM-1 negative, suggesting that both LAM-1 and CD11b/CD18 are important in the adhesion of neutrophils to endothelial cells but only CD11b/CD18 is important in the subsequent neutrophil migration into the extravascular space.

The final step in leukocyte trafficking in intestinal inflammation is the passage of leukocytes between epithelial cells and out into the lumen. Leukocytes that have passed into the lumen can be found in the stool; indeed, the presence of fecal leukocytes signifies gastrointestinal inflammation. Adhesion molecules, including ICAM-1, are expressed on epithelial cells in the presence of inflammation, and neutrophil chemotactic factors have been identified in the colonic lumen.

Adhesion molecules are possible therapeutic targets in gastrointestinal inflammation. Antibodies to adhesion molecules, both neutrophil adhesion molecules and endothelial adhesion molecules, have been used to block leukocyte trafficking. Monoclonal antibodies directed against ICAM-1 prevent the passage of neutrophils between endothelial cells. A set of experiments using antibodies against different components of the  $\beta$ 2-integrins defined their role in the migration of neutrophils between endothelial cells. Antibodies against CD18 inhibited neutrophil migration, and antibodies against CD11a or CD11b also inhibited migration. Therefore, the binding of both CD11a/CD18 and CD11b/CD18 to ICAM-1 appears to be important in the migration of neutrophils between endothelial cells. In contrast, antibodies to L-selectin did not inhibit neutrophil migration, which is consistent with the finding that extravascular neutrophils are CD11b/CD18 positive but L-selectin negative. Protein synthesis can be blocked by administering the appropriate antisense mRNA, which will bind to the sense mRNA and prevent translation. Antisense mRNA for ICAM-1 was given intravenously to patients with Crohn's disease in an attempt to improve clinical status by diminishing leukocyte trafficking. Preliminary results in a small group of patients revealed a better clinical outcome in the patients who are receiving ICAM antisense mRNA than in those receiving placebo.

Lymphocyte trafficking shares some similarity with neutrophil and monocyte trafficking but there are also substantial differences. Lymphocyte trafficking is mediated by the expression of receptors and counter receptors on lymphocytes and endothelial cells. The interaction of lymphocytes with endothelial cells (like the interaction of leukocytes with endothelial cells) involves three families of cell-surface proteins: integrins, selectins and immunoglobulin-like adhesion receptors. Lymphocytes destined for the intestine display some integrins such as CD11a/CD18 which are also expressed on leukocytes but they also express lymphocyte-specific integrins including  $\alpha$ 4 $\beta$ 7, an integrin expressed in CD4 and CD8 gut trophic lymphocytes, and  $\alpha$ <sup>E</sup> $\beta$ 7, which is expressed on almost all intraepithelial lymphocytes and 40% of lamina propria lymphocytes.





(Image by MIT OCW.)

Figure 7: Molecules involved in the trafficking of lymphocytes through endothelial cells. Molecules expressed on the cell surface of lymphocytes and endothelial cells are shown on the left. The functional location of these molecules during the process of lymphocyte homing is shown on the right.

The  $\alpha 4 \beta 7$  integrin binds to MAdCAM-1, a member of the immuno-globulin superfamily, which is selectively expressed on the high endothelial venules of mucosal lymphoid organs. Thus the migration of selected lymphocyte populations to the intestine is directed by the selective expression of  $\alpha 4 \beta 7$  on these lymphocytes and the selective expression of MAdCAM1 on certain endothelial populations. The  $\alpha 4 \beta 7$  integrin binds to E cadherin on the basolateral surface of endothelial cells. This interaction directs the migration of intraepithelial lymphocytes to the epithelium after they leave the vascular space.

Selectins play a role in the interaction of lymphocytes with the endothelium. By nature of the lectin-binding domain, all selectins bind fucosylated lactosamine structures related to the sialyl Lewis<sup>x</sup> (sLe<sup>x</sup>) blood group antigens. The sLe<sup>x</sup> blood group antigens decorate the molecules such as MAdCAM-1, making it a counter

ligand for L-selectin-bearing lymphocytes. E-selectin plays a role in the adhesion of a subpopulation of memory lymphocytes, neutrophils, and monocytes, and the concentration of E-selectins is increased in inflammatory lesions of the intestine.

Regulated expression of these molecules controls the migration of naive lymphocytes

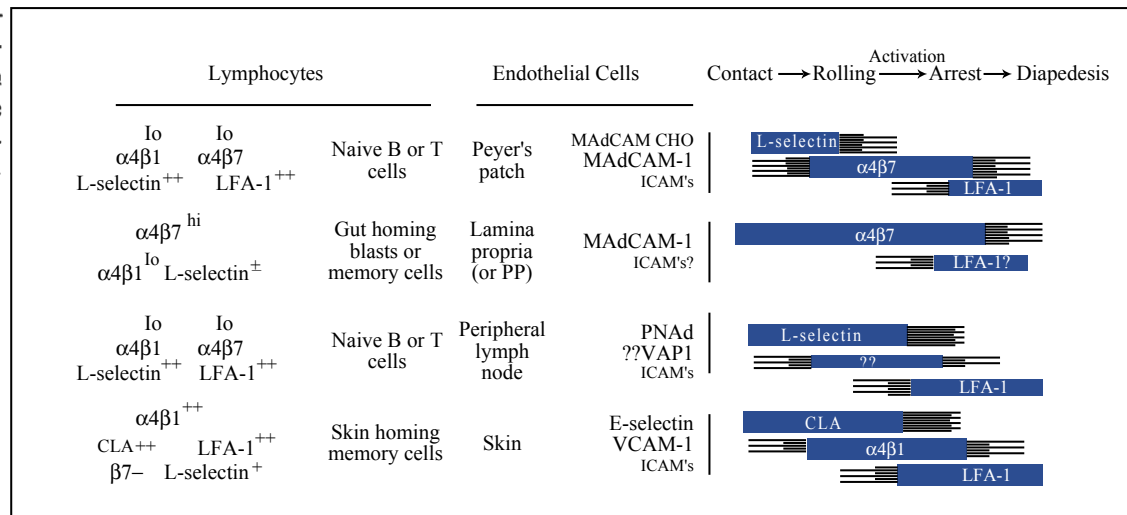


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Figure 8: Mechanisms of tolerance. Low dose tolerance occurs when low concentrations of antigens enter Peyer's patch and lead to the development of regulatory cells that secrete either IL-10, TGF $\beta$  or IL-4. High dose tolerance occurs when high concentrations of antigens enter Peyer's patch leading to the deletion and/or anergy of antigen-specific T cells.

into Peyer patches through high endothelial venules and the subsequent homing of activated memory lymphocytes into the effector compartments of the loosely affiliated lamina propria. Naive T lymphocytes that migrate into Peyer patches in search of an antigen encounter are CD3<sup>+</sup>, CD45RA<sup>+</sup>, L-selectin<sup>+</sup>, and  $\alpha 4\beta 7^{L o}$ ; however, antigen-activated T lymphocytes that migrate into lamina propria are CD3<sup>+</sup>, CD45RO<sup>+</sup>, L-selectin<sup>-</sup> and  $\alpha 4\beta 7^{H i}$ . Similarly, naive B cells with a phenotype of surface IgD<sup>+</sup>, CD20<sup>+</sup>, L-selectin<sup>+</sup>, and  $\alpha 4\beta 7^{L o}$ , and memory B cells with a phenotype of sIgD<sup>-</sup> CD20<sup>+</sup>, L-selectin<sup>-</sup> and  $\alpha 4\beta 7^{H i}$  are preferentially observed in Peyer patches and lamina propria, respectively.

#### IV. FUNCTIONAL CHARACTERISTICS OF MUCOSAL CELLS AND TISSUES

##### A. Oral Tolerance

Because the gastrointestinal tract contains many antigens, including dietary proteins, that could lead to cross-reactivity and activation of self-reactive T cells, an important function of the gastrointestinal immune system is the generation of a state of tolerance to mucosal antigens. The gastrointestinal tract exhibits a fascinating example of specific tolerance to orally ingested antigens, called oral tolerance.

The oral administration of antigens can lead to systemic antigen-specific unresponsiveness, which results in the lack of specific T-cell and B-cell responsiveness to those antigens. Concurrently, local specific secretory immunity can develop, resulting in lymphoblasts capable of IgA production. This dichotomy between mucosal and systemic compartments appears to reflect a solution to the need for excluding the specific antigen during future encounters and avoiding inappropriate systemic responsiveness. The ability of antigens in the gastrointestinal tract to induce a state of functional anergy is precise. The state of unresponsiveness or anergy holds only for the specific antigen that was presented orally to the gastrointestinal tract and depends on the nature of the antigen. It is not surprising that mechanisms to protect against mounting adverse immunologic reactions are present within the gastrointestinal tract. If this were not the case, numerous bacterial and viral antigens and food components could lead to frequent cross-reactive immunologic stimulatory events and result in intestinal autoimmune disorders, and many food substances could give rise to diverse and uncontrollable food-induced allergic reactions.

In model systems, the induction of oral tolerance depends on the type of antigen, the amount of antigen, the frequency of antigen sensitization, the type and genetic background of animal being studied, the age of the animal and the particular immune response being evaluated. Two mechanisms of tolerance have been hypothesized: T-cell suppression and clonal anergy. Low doses of antigen application to the intestine appears to induce the development, within Peyer patches and mesenteric lymph nodes, of CD4<sup>+</sup> T cells capable of secreting TGF- $\beta$  (Th3) and of CD4<sup>+</sup> T cells with a Th2 cytokine profile, capable of secreting IL-4 and IL-10. These cell types migrate to a variety of lymphoid and nonlymphoid tissues, where they inhibit the generation of antigen-specific effector cells. High doses of a toleragen appear to induce the clonal anergy or apoptosis of antigen-specific Th1 cells. The high-dose administration of antigen may lead to systemic leakage of the antigen, causing inappropriate antigen presentation in the periphery by antigen-presenting cells that lack the necessary costimulatory second signal.

The successful induction of oral tolerance may help prevent the initiation of autoimmune diseases. Mice prone to the development of autoimmune disease exhibit defects in the induction of oral tolerance. There are complex regulatory processes at work in the mucosal immune system that allow the development of a local protective mucosal immune response against pathogenic organisms and prevent the development of adverse systemic autoimmune reactions to the same antigens. Although oral tolerance creates an impediment to vaccination, certain mucosal adjuvants such as cholera toxin can reverse oral tolerance.

Oral tolerance is used to advantage in treating several autoimmune diseases. In murine models, feeding myelin basic protein, collagen type II, S antigen (i.e., retinal autoantigen), porcine insulin, and class II MHC peptides suppresses experimental allergic encephalitis, collagen- or adjuvant-induced arthritis, experimental allergic uveitis, diabetes mellitus, and organ transplant rejection, respectively. This approach is being tested in the treatment of systemic human diseases and inflammatory conditions of the intestine itself.

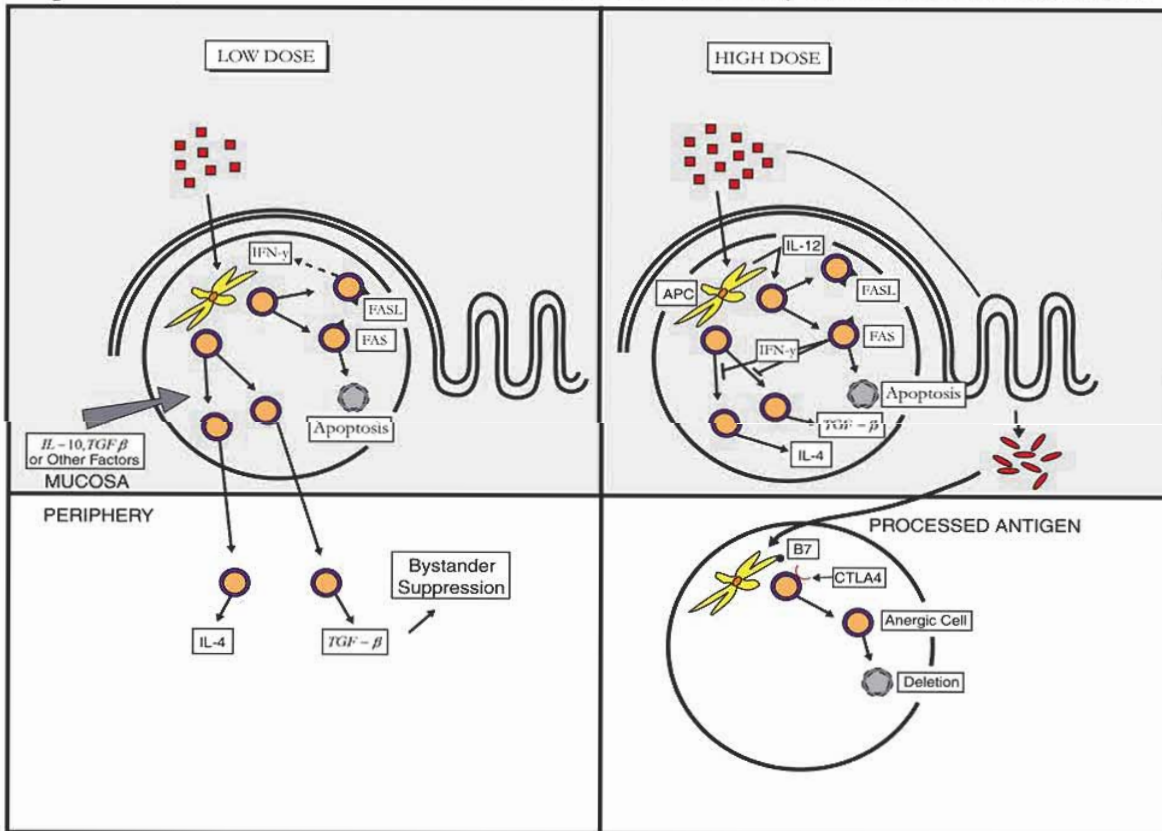


Figure by MIT OCW.

### B. Inflammatory Bowel Disease

Inflammatory Bowel Disease (IBD) is a chronic relapsing and remitting inflammatory condition of the gastrointestinal tract that is phenotypically manifest as one of two usually distinct, but sometimes overlapping clinical entities, ulcerative colitis (UC) and Crohn's disease (CD). UC affects only the colon and is a superficial ulcerative disease, whereas CD is a transmural granulomatous condition that affects any part of a GI tract and has a predilection for the terminal ileum, ascending colon, and rectum. Both forms of IBD are associated with prominent extra-intestinal manifestations and a significant incidence of GI cancer; in addition, both begin relatively early in life and then persist for long periods, leading to measurably decreased quality of life indices and a greater than two-fold increase in mortality rate. By and large, IBD is a disease of "urbanized" areas such as the US and Europe, where it occurs at an incidence of 6 to 12 and 5 to 7 per 100,000 for UC and CD, respectively. This translates to 45,000 new cases per year and 1,000,000 affected individuals in the US alone, which places a monetary burden on the US health care system of approximately 1.8 billion dollars per year (1990 estimate). Clearly, IBD is a major gastroin-

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Figure 2 in Blumberg, Richard S., and Warren Strober.  
"Prospects for Research in Inflammatory Bowel Disease."  
*J Am Med Assoc* 285 (2001): 643-7.

Figure 9: Pathogenesis of inflammatory bowel disease. IBD is initiated by the promiscuous leakage of antigens across the epithelial barrier, which leads to the promiscuous activation of aggressor T cells relative to regulatory T cells.

testinal disease that is an enormous problem to its victims and to society as a whole.

The cause of the two forms of IBD is slowly emerging from a half century of intense investigation. The results of these efforts can be generalized by saying that both UC and CD result from interrelated genetic and environmental factors that, in all likelihood, are channeled through an abnormality in mucosal immune function. Genetic factors were initially apparent from the observation that there is an increased occurrence of either UC or CD in families of patients with both forms of IBD, particularly in first degree relatives who are genetically most related to the patient. This accorded with later studies showing that monozygotic twins have a high concurrence rate (approximately 60%). Most recently, these classical genetic studies have been



augmented by genome-wide scans of humans with IBD which have disclosed disease loci on chromosome 16 (IBD1) and 12 and potential loci on chromosomes 1, 3, 6, and 7. These newer findings are consistent with the notion that IBD is a polygenic disease and explains the fact that familial inheritance patterns are non-Mendelian. Environmental factors are apparent from the aforementioned association of IBD with urbanization. This epidemiologic pattern suggests that IBD is more common in a relatively "clean" environment, and is not due to a mucosal pathogen. Indeed, repeated attempts to prove that IBD is due to a mucosal infection have consistently proven negative.

The concept that the proximal cause of IBD is immunologic in nature was first hinted at by the observation that IBD is characterized by massive cellular infiltration of the intestine and is associated with various abnormalities of the immune system including inappropriate production of antibodies and certain types of T cell dysfunction. More recently, these findings have been clarified by studies of patient lamina propria (LP) cells which show that in CD, cells overproduce cytokines indicative of a typical T helper 1 (Th1) T cell response, namely increased production of IL-12 by LP macrophages and increased production of IFN- $\gamma$  by LP T cells. In addition, LP T cells from patients with UC manifest a cytokine profile that is compatible with a Th2 T cell response; thus, in this case, while the cells do not overproduce the major Th2 cytokine IL-4, they do produce increased amounts of another Th2 cytokine, IL-5. This cytokine production pattern accords with the fact that UC (but not CD) is associated with autoantibodies that in general require Th2 T cell helper responses.

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Figure 1 in Blumberg, Richard S., and Warren Strober. "Prospects for Research in Inflammatory Bowel Disease." *J Am Med Assoc* 285 (2001): 643-7.

Figure 10: Mechanisms by which effector and regulatory cells are generated. In the Peyer's patch it is presumed that effector and regulatory cells are generated through the interactions of antigen with special subsets of dendritic cells. In the normal state, it is assumed that regulatory cells are educated in excess to effector cells secreting either Th1 or Th2 cytokines. In IBD, it is assumed that effector cells are generated in excess over regulatory cells either through excess effector cell generation or inadequate regulatory cell generation.

Taken together, the above data provided strong circumstantial evidence that the two major forms of IBD are due to dysregulated or excessive Th1 (CD) or Th2 (UC) T cell responses. As to the all-important question regarding the nature of the factors that induce these abnormal responses, there is considerable evidence that IBD patients have inappropriate T cell responses to antigenic components of their own intestinal microflora, either because of dysfunction in the primary or secondary mechanisms that normally drive/regulate such responses or because of some dysfunction in the intestinal epithelial cell barrier which leads to inappropriate penetration of and responses to specific microbial antigens. In effect, this means that patients with IBD have a disturbance in "oral tolerance," the normal mucosal immune system mechanism that

ensures the downregulation of responses to harmless constituents in the microflora or the food stream, while allowing robust effector cell responses to mucosal pathogens.

A major advance in the study of IBD and one that provides important evidence that immune factors are in fact the cause of IBD rather than just an accompaniment of the disease, has been the discovery and subsequent analysis of a number of mouse models of mucosal inflammation that resemble IBD. A large number of quite distinct underlying genetic abnormalities can lead to mucosal inflammation in the murine models. Nevertheless, in all of the models, the inflammation is either associated with a Th1 response and resembles Crohn's disease or a Th2 response and resembles UC. Thus, multiple abnormalities appear to be funneled into one of the two "final common pathways" of inflammation associated with the human disease. By analogy, it is likely that the underlying defects in CD and UC are also due to multiple underlying defects that are ultimately expressed as Th1 or Th2 T-cell-induced inflammations.

Studies of murine models of intestinal inflammations resembling human IBD also teach that the normal bacterial flora plays an essential role in the cause of the experimental inflammation. This is seen first in the fact that no inflammation occurs in any of the models when the mice are in a germ-free environment and treatment of mice with certain models of inflammation with antibiotics reduce the intestinal inflammation. In addition, it is seen in the fact that manipulation of bacterial flora can exacerbate or ameliorate disease. These observations provide strong support for the theory alluded to above which holds that abnormal responses to antigens in the normal bacterial flora, i.e., breaks in oral tolerance, are the driving force for the abnormal immunologic response and buttress the idea that the normal flora contains antigens that induce or initiate the immune responses that cause human IBD.

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Figure 11: Amplification of IBD. The cytokine milieu causes increased adhesion and recruitment of leukocytes.

The insights gleaned from studies of murine models of inflammation are leading to or providing a rationale for bold new therapies for human IBD based upon a pathophysiological sequence of events described in the attached figures, in this scenario a *leaky epithelial barrier* (above) in association with in association with abnormal *antigen recognition and immunoregulation* at the mucosal surface leads to

*activation of vascular endothelium and adhesion and recruitment of leukocytes* resulting in chronic inflammation. The operative approach here is to take advantage of our emerging knowledge of the cascade of events that are known to underlie normal and pathologic mucosal immune responses, particularly the events comprising the Th1 and Th2 final common pathways discussed above, to devise rational treatment approaches to IBD. One immunologically-related approach already in play in the treatment of Crohn's disease is the administration of anti-TNF antibody or soluble TNF receptor, both agents that block the action of TNF. The use of these agents conforms to the paradigm set up above that holds that blockade of the Th1 T cell pathway can be a successful means of treating forms of IBD associated with this pathway. Along similar lines, clinical studies are now underway which will test the use of anti-IL-12 in the treatment of CD. This new antibody-based treatment is a direct outgrowth of the observation that anti-IL-12 administration is uniformly effective in reversing disease in all murine models characterized by Th1 T cell-induced inflammation, as might be predicted by the fact that IL-12 is the master cytokine of the Th1 response. Further down the road, but already in development, are treatments that utilize the administration of cytokines such as TGF- $\beta$  and IL-10, which are the counter-regulatory (suppressor) cytokines of the normal mucosal immune response and the cytokines that help maintain oral tolerance. An example of this approach is the successful treatment of one murine model of mucosal inflammation by the administration of intranasal DNA encoding TGF- $\beta$ , which has the effect of inducing intestinal T cells producing TGF- $\beta$ . This result is also important because it suggests that the use of cytokines or anti-cytokines in IBD treatment will inevitably involve the use of gene therapy technique as a means of delivering highly targeted and persisting therapeutic agents.

The therapeutic strategies outlined above all fall within the category of treatments that are directed towards already defined components of mucosal immune function in mucosal inflammation. The more distant challenge of the coming decades will be to identify and address the basic abnormalities of IBD that are akin to the individual genetic defects present in the mouse models and/or the individual disease genes in humans with IBD. First and foremost in this endeavor will be the further definition of the genetic basis of the IBD so as to identify not just genetic loci but actual genes associated with the disease. Initially, this will allow identification of genetic markers that can be used in disease diagnosis and prediction of disease course. Later, it will produce new targets for gene therapy which in this case could lead to permanent reconstitution and cure.

A second category of basic abnormalities relates to the role of environment in the causation of IBD. The highest priority goal here is the acquisition of a more complete understanding of those features of the microflora that initiate and perpetuate disease or alternatively, prevent disease in the general population. One working hypothesis that requires exploration in this regard is that while certain microbial elements elicit detrimental or disease causing immune responses, others evoke protective responses that prevent disease. Data derived from exploring this idea, may enable one to define specific microbial substances in specific patients that are the cause and cure of their IBD.

Finally, future studies of the microflora must move in lockstep with additional studies of a third category of basic abnormalities, those relating to the mucosal immune system itself. The goal here will be to acquire more definitive knowledge of how the mucosal immune system deals with mucosal antigens so as to mount appropriately strong responses to potential pathogens on the one hand and appropriately weak or tolerogenic responses to harmless antigens in the gut. This will require a far more comprehensive understanding than now available of how antigens are processed in the mucosal immune system and once processed, how it elicits the array of T cells that determine the outcome of immune responses. In this context, it should not be forgotten that mucosal immune cells are unique in their juxtaposition to a large population of non-lymphoid cells, namely epithelial cells; thus it will be necessary to explore further the properties of epithelial cells in relation to how the latter participate and influence immune responses, as well as how they directly contribute to or inhibit IBD.

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