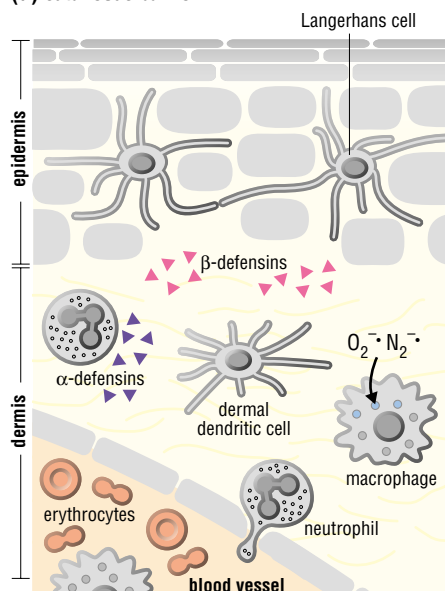


Epithelial Barriers and Defenses against Infection

(a) cutaneous barrier



(b) mucosal barrier

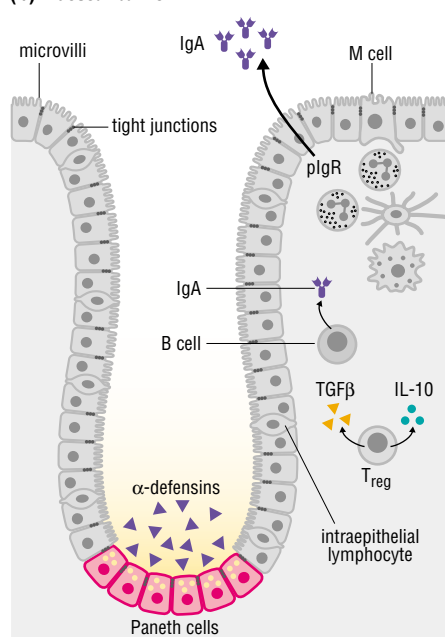


Figure 8-1.1 Epithelial barriers (a)

Squamous epithelium showing keratinized outer layer and dendritic cells in the underlying soft tissue. **(b)** Columnar epithelium showing tight junctions and Paneth cells.

8-3 Epithelia present both a physical and an antimicrobial barrier

Epithelial cells establish barriers at the cutaneous and mucosal interfaces between host and environment. Stratified epithelial cells of the skin and oral cavity secrete glycolipoproteins that maintain a permeability barrier; keratinization of the outermost skin cells reinforces the cutaneous barrier. The simple polarized epithelia of the intestine and airways are sealed by intercellular tight junctions (Figure 8-1.1).

Epithelial cells of the eye, hair follicles, oral, intestinal, respiratory and genitourinary surfaces are coated by a fluid matrix that physically restricts access of organisms to the underlying epithelia. The fluid is constantly replenished and removed by mechanical or ciliary processes that impede the ability of bacteria to adhere. In addition to complex glycosaminoglycans and mucins that bind bacterial outer membrane constituents, the fluid matrix contains antibacterial peptides and proteins that are constitutively secreted and further induced in response to bacterial cell wall components, such as lipopolysaccharide. Antimicrobial peptides, such as **β-defensins** in the lung and skin, **α-defensins** in Paneth cells of the intestinal tract, and antimicrobial proteins such as lysozyme and secretory leukocyte protease inhibitor (SLPI), in fluids bathing the cornea of the eye, are toxic to diverse groups of microbes. Antimicrobial peptides have optimal activities that reflect the distinctive physiology of given sites, such as the acid pH of the stomach, the alkaline pH of the vagina or the salinity of the respiratory tract. Epithelial antimicrobial peptides are complemented by **α-defensins**, which comprise the major constituent of neutrophil specific granules. **Cathelicidins**, another group of antimicrobial defensins produced by activated neutrophils and epithelial cells, reinforce the recruitment of neutrophils and monocytes to inflammatory sites by cross-utilization of FPRL1, the seven-transmembrane formyl peptide receptor-like 1 chemoattractant receptor.

Nonpathogenic **commensals** can cause infections when epithelial barriers are disrupted, particularly in the setting of a further compromised immune system, such as neutropenia. Wounds, burns, intravenous access devices and insect vectors allow commensal organisms on the skin to gain access to the tissues. Cytoablative cancer chemotherapy or irradiation can also compromise epithelial integrity through effects on cycling cells that maintain this dynamic barrier. Cystic fibrosis compromises the volume and salinity of respiratory tract fluids, attenuating the activity of antimicrobial peptides and leading to overgrowth of colonizing bacteria and eventual destruction of lung tissue. Antacids, by neutralizing stomach pH, can allow a normally noninfective bacterial inoculum to reach the lower intestines and cause disease. Antibiotics, by destroying susceptible nonpathogenic commensals, can allow the outgrowth of organisms, such as *Clostridium difficile* in bowel, which secrete toxins that mediate disease.

8-4 Innate mechanisms maintain commensals at epithelial barriers

Deficiencies in adaptive immunity, such as those consequent on the deletion of RAG genes, do not affect the ability of animals to restrict commensal organisms to epithelial barriers. In contrast, failure to maintain barriers against normal microbial flora can be demonstrated in certain genetic deficiencies associated with innate immunity (Figure 8-1.2). Animals with these genetic deficiencies die early in life with multiple intestinal, hepatic, cutaneous and lung microabscesses filled with commensal flora. The phenotype of these animals contrasts with the chronic intestinal inflammation that occurs later in life in mice deficient in various genes that contribute to regulatory modulation of the adaptive immune response to proinflammatory molecules induced by bacteria within the bowel. Mice which develop chronic intestinal inflammation provide instructive models for human inflammatory bowel diseases such as Crohn's disease and ulcerative colitis.

Definitions

commensals: the normal bacterial flora colonizing healthy skin and mucosal surfaces.

defensins: family of cationic antimicrobial peptides with 3-4 intramolecular cysteine disulfide bonds; found in mammals, insects and plants.

cathelicidins: family of cationic antimicrobial peptides generated in pre-pro forms that require processing to generate the active peptide; contain amino-terminal cathelin-like domain and carboxy-terminal antimicrobial domain.

FPRL1: formyl peptide receptor-like 1; chemotactic receptor on neutrophils and monocytes mediating responses to bacterial formyl peptides.

intraepithelial lymphocyte (IEL): lymphocytes positioned at the base of polarized epithelial barriers exposed to the external environment; large numbers occur in the intestine, IEL, and are comprised of both thymus-dependent and independent populations of $\alpha\beta$ and $\gamma\delta$ T cells.

polymeric Ig receptor: receptor mediating transcytosis of immunoglobulin A across epithelial cells to mucosal surfaces.

8-5 Specialized cell types in epithelia initiate and execute adaptive immune responses

Antigen sampling from epithelia occurs by the close association of an interdigitating dendritic cell network in the stratified epithelia of the skin or within the simple columnar epithelia of the terminal bronchioles of the lung, or through specialized lymphoid organs that associate with distinct tissues, such as the tonsils or intestinal MALT. M cells comprise a unique population of cells in intestinal and respiratory epithelia that promote the transport of luminal antigens to abluminal dendritic cells and macrophages (see Figure 8-1.1). In each case, however, dendritic cells represent the sentinel network that escorts bacterially-derived antigens to the adaptive immune system in the draining of specialized lymphoid organs. Epithelial β -defensins promote chemotaxis of immature dendritic cells and memory T cells by cross-utilization of CCR6, the receptor for CCL20 (MIP-3 α /LARC), thus serving to link innate with adaptive immunity.

The mechanisms that maintain the integrity of the epithelial barriers are not known for certain, but $\gamma\delta$ T cells are believed to play a part. Their localization to epithelial basement membranes in both mouse and man is suggestive, and some loss of intestinal epithelial integrity can be demonstrated in $\gamma\delta$ T cell-deficient mice. Gene expression profiling of $\gamma\delta$ intraepithelial lymphocytes, or IELs, reveals a constitutively active array of immune defense genes, including cryptdin and granzymes A and B, as well as transcripts for inhibitory receptors, including CTLA-4, PD-1 and various NK inhibitory receptors. In humans, intestinal V δ 1 T cells express NKG2D and are activated by the stress-induced nonpolymorphic MHC class I molecules, MIC-A and MIC-B.

IgA is the most prevalent immunoglobulin in mucosal secretions and is believed to contribute to epithelial defense by binding to antigens, including bacteria and toxins, thus preventing their uptake. Epithelial cells in the intestine and hepatocytes of the liver mediate transfer of dimeric serum IgA to the intestinal lumen via the **polymeric Ig receptor**. After engagement at the basolateral surface by IgA, the polymeric Ig-IgA complex undergoes transcytosis to the apical membrane by a process dependent on the src kinase p62^{yes}. Proteolytic cleavage of the ectodomain of the polymeric Ig receptor, termed secretory component, releases the complex into the lumen. Secretory component is believed to protect luminal IgA from degradation by bacterial proteases. Recent studies indicate that at least some IgA can be produced in mice deficient in IgM or IgD, and can participate in intestinal responses to commensal flora. IgA deficiency is a common immunodeficiency in humans. Although some individuals suffer from recurrent sinus, pulmonary and/or intestinal infections, the majority remain asymptomatic, probably because IgM, which also binds the polymeric Ig receptor, can compensate for IgA in certain cases, for reasons incompletely understood.

After opsonization with IgA or IgM, organisms are endocytosed by Fc α / μ R expressed on mature B cells, macrophages and dendritic cells in mice and humans. The Fc α / μ R gene is located with other FcRs on mouse chromosome 1 and the syntenic region on human chromosome 1q.32.3. Expression on APC suggests a role for the receptor in antigen priming for appropriately opsonized antigens. A second IgA receptor, Fc α RI (CD89), is an Ig superfamily member expressed on human monocytes, neutrophils and eosinophils in association with the adapter protein, Fc γ R. Fc α RI is a low-affinity receptor for complexed serum IgA, but cannot bind secretory IgA which has a blocked receptor binding site. Activation of Fc α RI on phagocytic cells triggers phagocytosis, the respiratory burst and cytokine production. A murine homolog for CD89 remains undiscovered.

Genetic deficiencies affecting responses to commensal bacterial flora

Primary deficiency	Examples	Mouse phenotype	Human disease
PMN defects	P x E selectins C/EBP ϵ	skin, mucocutaneous, conjunctival abscesses	human specific granule deficiency
	Phox x NOS2	abdominal abscesses	CGD (phox)
intestinal mucosa	SMAD3	abdominal abscesses	
	matrilysin	Paneth cell α -defensins absent with exuberant bacterial colonization	
	IL-10 N-cadherin	inflammatory bowel disease	
	IL-2, IL-2R α IL-2R β , TCR α	inflammatory bowel disease	
	NOD2		Crohn's Disease

Figure 8-1.2 Table of representative genetic deficiencies causing abnormal responses to commensal bacterial flora C/EBP ϵ : myeloid-specific CCAAT/enhancer binding transcription factor family; mutation in mice and humans leads to loss of PMN specific granules that contain α -defensins; Phox: phagocyte oxidase; disrupted gp91 used in this example; NOS2: inducible nitric oxide synthase; SMAD3: adapter for TGF- β family serine/threonine receptor kinases; homologs of *C. elegans* Sma and *Drosophila* Mad proteins; Matrilysin: MMP-7, a Paneth cell metalloproteinase required to process pro- α -defensins to active α -defensin; NOD2: Apaf-1/Ced-4 superfamily of apoptosis regulators; leucine-rich domain and NF κ B-activating domains related to plant antimicrobial resistance proteins; mutated in some patients with Crohn's disease.

References

- Fahrer, A.M. *et al.*: **Attributes of $\gamma\delta$ intraepithelial lymphocytes as suggested by their transcriptional profile.** *Proc. Natl. Acad. Sci. USA* 2001, **98**:10261-10266.
- Frenette, P.S. *et al.*: **Susceptibility to infection and altered hematopoiesis in mice deficient in both P- and E-selectins.** *Cell* 1996, **84**:563-574.
- Hooper, L.V. and Gordon, J.I.: **Commensal host-bacterial relationships in the gut.** *Science* 2001, **292**:1115-1118.
- Macpherson, A.J. *et al.*: **A primitive T cell-independent**

mechanism of intestinal mucosal IgA responses to commensal bacteria. *Science* 2000, **288**:2222-2226.

Mellman, I. and Steinman, R.M.: **Dendritic cells: specialized and regulated antigen processing machines.** *Cell* 2001, **106**:255-258.

Shibuya, A. *et al.*: **Fc α / μ receptor mediates endocytosis of IgM-coated microbes.** *Nature Immunol.* 2000, **1**:441-446.

Shiloh, M.U. *et al.*: **Phenotype of mice and macrophages deficient in both phagocyte oxidase and inducible nitric oxide synthase.** *Immunity* 1999, **10**:29-38.

van Egmond, M. *et al.*: **IgA and the IgA Fc receptor.** *Trends Immunol.* 2001, **22**:205-211.

Wilson, C. L. *et al.*: **Regulation of intestinal α -defensin activation by metalloproteinase matrilysin in innate host defense.** *Science* 1999, **286**:113-117.

Yang, D. *et al.*: **β -defensins: linking innate and adaptive immunity through dendritic and T cell CCR6.** *Science* 1999, **286**:525-528.