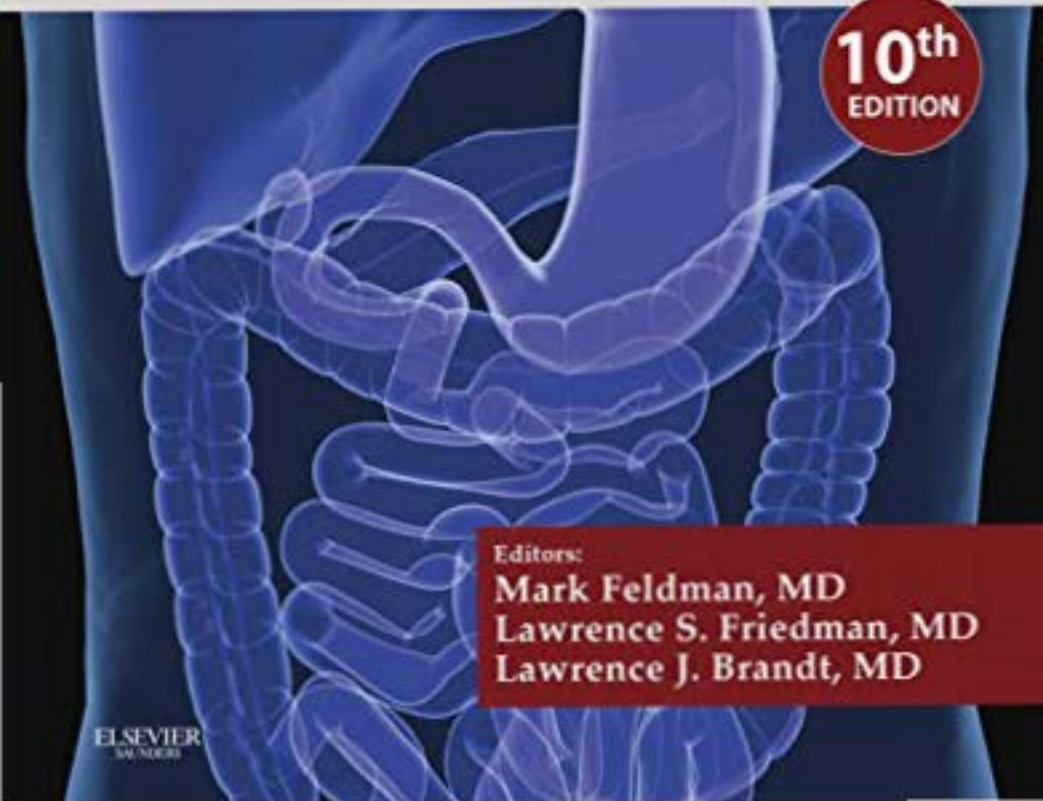
Sleisenger and Fordtran's

GASTROINTESTINAL AND LIVER DISEASE

PATHOPHYSIOLOGY / DIAGNOSIS / MANAGEMENT



10th EDITION

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Sleisenger and Fordtran's Gastrointestinal and Liver Disease

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10th Edition — Volume 1

PATHOPHYSIOLOGY • DIAGNOSIS • MANAGEMENT

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To our wives—Barbara Feldman, Mary Jo Cappuccilli, and Lois Brandt;
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FOREWORD

The tenth edition of *Sleisenger and Fordtran's Gastrointestinal and Liver Disease: Pathophysiology/Diagnosis/Management* continues as the benchmark textbook of gastroenterology and hepatology. It is authoritative, comprehensive, and, although encyclopedic in its coverage, very readable. The editors have done an excellent job ensuring that the organization of chapters is uniform. Thus, chapters have sections on epidemiology, etiology, pathology, pathophysiology, clinical features, diagnosis, differential diagnosis, treatment, and prognosis. This uniform format allows readers to search easily for information under different subheadings to find answers to their questions. As noted in the Preface, the content of the book has changed dramatically in the 42 years since the first edition was published in 1973. Whereas the first edition had 115 chapters and the tenth edition has 132, the additional 17 chapters belie the masterly job the contributors and editors have done in preserving references not only to classic articles but also to the important new advances that have occurred between publications of successive editions. This newer material also includes references that have been updated to include articles published into 2014. As also noted in the Preface, some of the new chapters include up-to-date discussions of enteric microbiota, probiotics and fecal transplantation, and factitious gastrointestinal diseases. An outstanding feature of the textbook is the clarity and detail of the tables and the high quality of the photomicrographs.

The tenth edition of "Sleisenger and Fordtran" will continue to be a premier textbook, as was the case with its predecessors, and will be especially useful to medical residents, gastroenterology fellows, and gastroenterologists. Finally, I can personally attest to the remarkable advances that have been made, as I was author of the chapter on eosinophilic gastroenteritis in the second edition of the textbook, and reading the same chapter in the tenth edition underscores the important advances that have been made in our understanding of the molecular basis as well as the pathophysiology of this and related disorders.

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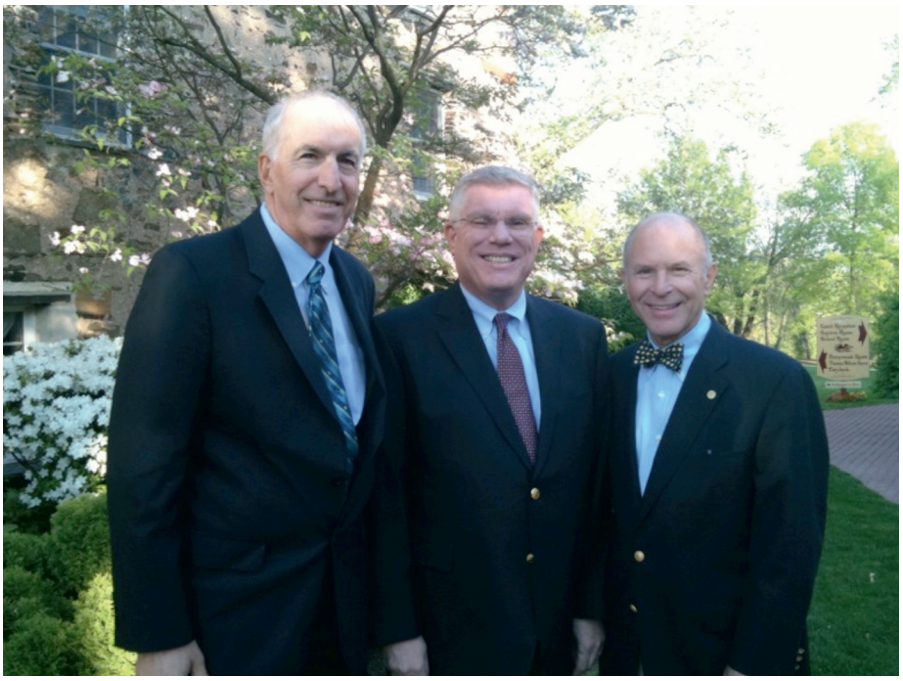

From left: Mark Feldman, MD; Lawrence S. Friedman, MD; Lawrence J. Brandt, MD.

PREFACE

The tenth edition of *Sleisenger and Fordtran's Gastrointestinal and Liver Disease: Pathophysiology/Diagnosis/Management* is among a select group of textbooks that have been valuable to readers over a long time span. Work by its founding editors, Marvin Sleisenger and John S. Fordtran, began more than four decades ago and culminated in the publication of the first edition, *Gastrointestinal Disease*, in 1973. Much has happened in the field of gastroenterology since then, and each edition of the text has methodically incorporated these exciting advances into its pages. Advances have included clearer understanding of the basic mechanisms of health and disease at a cellular, subcellular, genetic, and molecular level; a much clearer comprehension of the pathophysiology of GI and liver diseases; the introduction of numerous diagnostic tests and procedures (many of which displaced now outmoded tests and procedures); combining diagnostic with therapeutic endoscopy; developing many novel pharmaceutical agents and drug classes for conditions that previously had no such treatments; applying laparoscopic surgery in many common GI disorders; and so much more.

Over its 42-year lifespan, the textbook has had six editors: Marvin H. Sleisenger and John S. Fordtran (founding editors), as well as Mark Feldman, Bruce F. Scharschmidt, Lawrence S. Friedman, and Lawrence J. Brandt. These editors have had the good fortune to engage hundreds of superb author-contributors from around the globe who generously shared their knowledge and expertise with readers of the book. The editors also have had the luxury of stalwart support from a highly competent and professional publishing company, Elsevier, throughout the life of the book.

When the first edition of *Gastrointestinal Disease* was published in 1973, it was quite different from this, the tenth edition. The first edition was printed in a single volume of less than 1600 pages, with well over 200 of these pages devoted to a single entity—peptic ulcer disease. There were 115 chapters in the first edition, compared with 132 chapters in the tenth edition. Besides its two founding editors, the first edition had 55 contributors, compared with 217 contributors in the tenth edition. The first edition was written almost entirely by authors based in the United States, whereas authors from 15 countries have contributed to the pages of the tenth edition. The vast majority of chapters in the first edition were written by a single author, whereas most chapters now have two authors. And perhaps most important, there was no coverage of liver diseases in the first edition, or even in the four subsequent editions, until the sixth edition—renamed *Gastrointestinal and Liver Disease: Pathophysiology/Diagnosis/Management*—was published in 1998. In 2007, the British Medical Association awarded the eighth edition of the book its First Prize in the field of gastroenterology.

The first edition was available to readers in print format only, and color was used sparingly. As time went on, the book became available in CD-ROM and then online via a secure website. Enhanced use of color allowed improved depictions of endoscopic images and histopathology. Today the contents of the tenth edition are available on handheld devices such as smartphones, iPads, and Kindles. The online version of the tenth edition also incorporates dozens of video clips that illustrate diagnostic and therapeutic approaches in the field, with narrative descriptions of the procedures. The authors are greatly appreciative of Gregory G. Ginsberg, Christopher J. Gostout, Michael L. Kochman, Ian D. Norton, and the team at Elsevier for allowing our readers access to these valuable educational videos.

Fortunately, with the help of our distinguished contributors, the content of the textbook remains unparalleled. Comparing the contents of the first with the tenth editions, one can appreciate the striking advances in the field. Many conditions that now constitute the core of gastroenterology practice were not even known to exist in 1973. Furthermore, comparing the hepatology section in the sixth edition (1998) with that in the current edition is a striking tribute to the discoveries that have improved the diagnosis and therapy of liver disease, particularly with respect to the panorama of drugs to treat chronic viral hepatitis.

The tenth edition includes three notable chapters not included in earlier editions. An entire chapter, authored by Fergus Shanahan, has been devoted to Enteric Microbiota and another, authored by Christina Surawicz and Lawrence J. Brandt, to Probiotics and Fecal Microbiota Transplantations. These additions reflect our increasing knowledge about the bowel flora and our emerging understanding of the role of intestinal microbiota in the pathogenesis and treatment of a variety of GI (and other) diseases, most notably *Clostridium difficile* colitis. The editors are also delighted to welcome back John S. Fordtran who, along with Marc D. Feldman, has written a scholarly chapter on Factitious Gastrointestinal Disease, a group of disorders that can be most challenging for clinicians to diagnose and treat. Additional changes since the ninth edition are expansions of the chapter on Surgical Treatment of Obesity to include endoscopic treatment, and the chapter on Complications of Gastrointestinal Endoscopy to include preparation for endoscopy; combination of the chapters on Peptic Ulcer Disease and Treatment of Peptic Ulcer Disease into a single chapter; a new chapter on Overview of Cirrhosis; separation of the chapter on Hepatitis B and D into two chapters; and separation of the chapters on Digestion and Absorption of Nutrients and Vitamins into one on Digestion and Absorption of Macronutrients and one on Digestion and Absorption of Micronutrients. We are delighted to welcome many new authors, as well as returning authors, to the tenth edition.

Finally, the editors gratefully acknowledge the capable and spirited roles of Kate Dimock, Suzanne Toppy, Deidre (Dee) Simpson, and Cindy Thoms at Elsevier for facilitating the publication of the tenth edition. Without their support and vision, the editors would have fallen short of the high standards that were set by the founding editors and to which we remain committed.

Mark Feldman, MD
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VIDEO CONTENTS

ABBREVIATION LIST

AASLD	American Association for the Study of Liver Diseases
ACG	American College of Gastroenterology
ACTH	Corticotropin
AFP	Alpha fetoprotein
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotransferase
APACHE	Acute Physiology and Chronic Health Examination
ASGE	American Society for Gastrointestinal Endoscopy
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BICAP	Bipolar electrocoagulation
BMI	Body mass index
CCK	Cholecystokinin
CEA	Carcinoembryonic antigen
CF	Cystic fibrosis
CO_2	Carbon dioxide
COX	Cyclooxygenase
CT	Computed tomography
CTA	Computed tomography angiography/angiogram
DIC	Disseminated intravascular coagulation
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
EGD	Esophagogastroduodenoscopy
EGF	Epidermal growth factor
ERCP	Endoscopic retrograde cholangiopancreatography
EUS	Endoscopic ultrasonography
FDA	U.S. Food and Drug Administration
FNA	Fine-needle aspiration
GERD	Gastroesophageal reflux disease
GGTP	Gamma glutamyl transpeptidase
GI	Gastrointestinal
GIST	GI stromal tumor
H&E	Hematoxylin & eosin
HCG	Human chorionic gonadotropin
H2RA	Histamine-2 receptor antagonist
HAART	Highly active antiretroviral therapy
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HELLP	*H*emolysis, *e*levated *l*iver enzymes, *l*ow *p*latelet count
HEV	Hepatitis E virus
Hgb	Hemoglobin
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPV	Human papillomavirus
HSV	Herpes simplex virus
Hp	*Helicobacter pylori*
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
ICU	Intensive care unit
INR	International normalized ratio
IV	Intravenous
LDH	Lactate dehydrogenase
MELD	Model for End-Stage Liver Disease
MEN	Multiple endocrine neoplasia
MRA	Magnetic resonance angiography/angiogram
MRCP	Magnetic resonance cholangiopancreatography
MRI	Magnetic resonance imaging
NG	Nasogastric
NPO	Nil per os (nothing by mouth)
NSAIDs	Nonsteroidal antiinflammatory drugs
O_2	Oxygen
PBC	Primary biliary cirrhosis
PCR	Polymerase chain reaction
PET	Positron emission tomography
PPI	Proton pump inhibitor
PSC	Primary sclerosing cholangitis
PSS	Progressive systemic sclerosis
PUD	Peptic ulcer disease
RA	Rheumatoid arthritis
RNA	Ribonucleic acid
SBP	Spontaneous bacterial peritonitis
SIBO	Small intestinal bacterial overgrowth
SLE	Systemic lupus erythematosus
SOD	Sphincter of Oddi dysfunction
TB	Tuberculosis
TG	Triglyceride(s)
TNF	Tumor necrosis factor
TNM	Tumor Node Metastasis (staging)
TPN	Total parenteral nutrition
UC	Ulcerative colitis
US	Ultrasonography
USA	United States of America
WBC	White blood cell
WHO	World Health Organization
ZES	Zollinger-Ellison syndrome

CHAPTER

1

Cellular Growth and Neoplasia

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CHAPTER OUTLINE

Neoplasia in the GI tract remains one of the most frequent diseases gastroenterologists encounter. Advances in our understanding of the cellular and molecular basis of GI neoplasia have provided a foundation for the development of novel preventive, diagnostic, and therapeutic approaches. Although some features of carcinogenesis are tissue site–specific, many mechanisms are universal to all sites throughout the GI tract. This chapter reviews mechanisms of normal cell growth and the fundamental cellular and molecular alterations that facilitate malignant transformation. The basic concepts discussed in this chapter provide the framework for discussion of specific GI neoplasms in later chapters.

MECHANISMS OF NORMAL CELL HOMEOSTASIS

Cellular Proliferation

Neoplasia results from the disruption of an intricate network of homeostatic mechanisms regulating cell cycle progression, differentiation, senescence, and programmed cell death. Proliferation occurs as cells traverse the cell cycle (Fig. 1-1). In preparation for cell division, there is a period of biosynthetic activity called the *G_1 phase* that is typically associated with an increase of cell size. This phase is followed by precise duplication of the genome, designated the *S phase.* After an intervening gap period designated the *G_2 phase,* mitosis occurs in the *M phase.*

The commitment to proceed to DNA replication occurs during the G_1 phase at the G_1/S checkpoint or restriction (R) point. Cells may exit this cycle of active proliferation before reaching the R point and enter a quiescent phase, G_0. Cells can subsequently re-enter the cell cycle from the G_0 state (see Fig. 1-1). Another checkpoint exists at the boundary between the G_2 and M phases. The G_2/M checkpoint ensures that mitosis does not proceed prior to the repair of any damaged DNA after genome replication. Impaired function of these checkpoints is frequently observed in cancers.

Regulation of cell cycle progression appears to be achieved principally by cyclins and cyclin-dependent kinase activity at the G_1/S and G_2/M checkpoints. Cyclins A and B are predominantly expressed during the S and G_2 phases, respectively (see Fig. 1-1). In contrast, cyclins D and E are most active during the G_1 phase.[1] Overexpression of cyclin D1 in fibroblasts results in more rapid entry of cells into the S phase. Cyclin D1 is frequently overexpressed in a number of GI and non-GI malignancies.[2]

Each cyclin forms a complex with a cyclin-dependent kinase (CDK) in a cell cycle–dependent fashion. Cyclins function as catalysts for CDK activity (see Fig. 1-1). The cyclin-CDK complexes regulate cell cycle progression through phosphorylation of key target proteins, including the retinoblastoma gene product (pRb) as well as the Rb family members p130 and p107.[3] The final result is progression out of G_1 into the S phase of the cell cycle.

The cell cycle is also regulated by multiple CDK inhibitors; $p21^{CIP1/WAF1}$ and $p27^{KIP1}$ are inhibitors of cyclin E/CDK2. Originally discovered to be part of the complex containing cyclin D1 and CDK4/6, $p21^{CIP1/WAF1}$ is transcriptionally activated by several tumor suppressor genes, most notably *TP53*.[4] Another CDK inhibitor, $p16^{INK4A}$, specifically inhibits CDK4 and CDK6 and is part of a larger family of related inhibitors that includes p14, p15, and p18[5]; $p16^{INK4A}$ is frequently

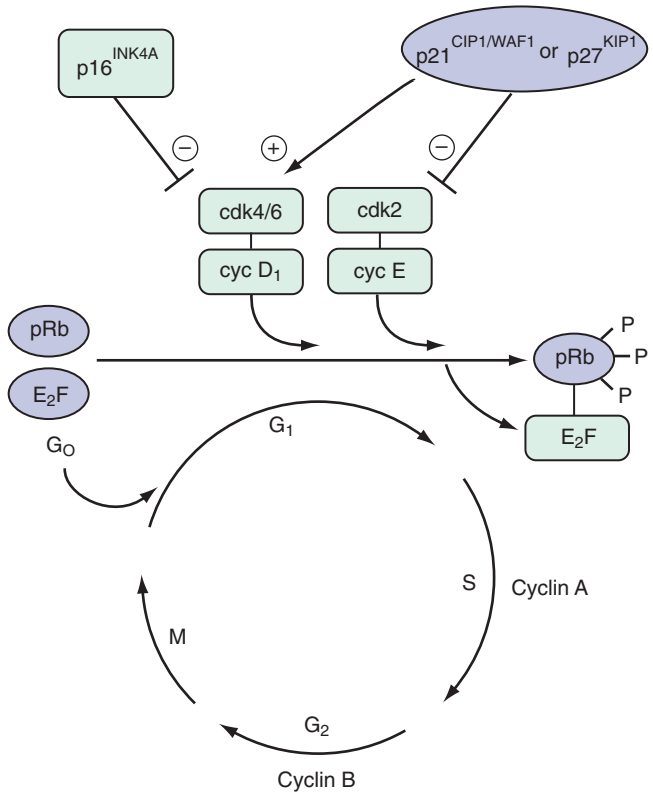

FIGURE 1-1. Regulation of the cell cycle by cyclins (cycs), cyclin-dependent kinases (cdks), and cdk inhibitors. In the normal cell cycle, DNA synthesis (in which chromosomal DNA is duplicated) occurs in the S phase, whereas mitosis (in which nuclei first divide to form a pair of new nuclei, followed by actual cellular division to form a pair of daughter cells) takes place in the M phase. The S and M phases are separated by 2 gap phases, the G_1 phase after mitosis and before DNA synthesis, and the G_2 phase following the S phase. During these gap phases, the cell is synthesizing proteins and metabolites, increasing its mass, and preparing for the S phase and M phase. Cell cycle progression is regulated primarily at 2 points, the G_2/M and G_1/S checkpoints, through the coordinated activities of cyclins and CDKs, which in turn are negatively regulated by CDK inhibitors (INK4 and CIP/KIP families). The mid-G_1 phase is characterized by the interaction between cyclin D_1 and cdk4/6. This complex hyperphosphorylates the retinoblastoma protein (pRb) and its family members (e.g., p130). Another important complex at the G_1/S boundary is that of cdk2 and cyclin E (cyc E). The result is to release transcription factors such as E_2F that are complexed with pRb. In turn, E_2F binds to and activates the promoters of genes important in DNA synthesis.

inactivated in most GI cancers, a finding consistent with its function as a tumor suppressor gene.[6,7] It is known that $p16^{INK4A}$ disrupts the complex of cyclin D1 and CDK4/6, thereby freeing $p21^{CIP1/WAF1}$ and $p27^{KIP1}$ to inhibit the activity of cyclin E/CDK2.[8] In addition, $p16^{INK4A}$ expression results in increased stability of the tumor suppressor p53.[9]

Apoptosis

Apoptosis (programmed cell death) is an important mechanism that counterbalances cell proliferation, and escape from normal apoptotic mechanisms plays a critical role in oncogenesis. Apoptosis is characterized by distinctive features that include chromatin compaction, condensation of cytoplasm, and mild convolution of the nucleus and cytoplasm. These changes are followed by nuclear fragmentation and marked convolution of the cell surface. Eventually, membrane-bound apoptotic bodies that represent the cellular residue are produced and phagocytosed.

Apoptosis may be triggered by internal or external stimuli. Apoptosis routinely occurs during normal development to facilitate tissue patterning. Internal stimuli of apoptosis may include nutrient deprivation, hypoxia, DNA damage, or other stressors. Ultimately, these internal apoptotic signals converge to increase permeability of the mitochondrial membrane and collapse the electrical gradient required for aerobic respiration (Fig. 1-2). Small mitochondria-derived activators of caspases (SMACs) and cytochrome c are released into the cytoplasm. SMACs and the so-called apoptosome complex (cytochrome c, caspase 9, and Apaf1) then activate downstream caspases, such as caspase 3, precipitating cell death. Caspases are intracellular cysteine proteases and are key mediators of programmed cell death in mammalian cells.

The Bcl-2 family of proteins has been shown to modulate the activity of mitochondrial permeability pores. Bax and Bak help form the pore, while Bcl-2, Bcl-xL, and Mcl-1 inhibit pore formation. The stoichiometric ratio between pro-apoptotic and anti-apoptotic members of the Bcl-2 family can determine the balance between cell survival and cell death.[10] In neoplasia, this balance is skewed toward anti-apoptotic factors.

Apoptosis may also be stimulated by external signals. Activation of the TNF receptors, TNFR1 and TNFR2, by TNF cytokines results in activation of caspases. Activation of Fas receptor by the Fas ligand also results in the death-induced signaling complex that activates caspases. In addition to these well-characterized pathways, toxins, chemical signals, and pathogens may trigger apoptosis (see Fig. 1-2).

Senescence

Senescence is the process by which cells permanently lose their ability to divide. Senescence may occur in response to the stress induced by activation of oncogenes, DNA damage, or after a fixed number of cellular divisions (replicative senescence). These processes limit dysregulated or excessive proliferation. However, these mechanisms also contribute to aging and depletion of stem cells.[11] During carcinogenesis, these tumor-suppressive mechanisms are bypassed or lost.

When grown in vitro, most primary cells have a limited replicative potential and eventually undergo replicative senescence.[12] Telomeres are repetitive DNA sequences at the ends of all chromosomes that regulate chromosomal stability. Telomeres shorten with each cell division, and when they have been reduced to a certain critical length, senescence typically occurs through activation of DNA damage signaling. Cancer cells are able to maintain their telomere length despite multiple cell divisions through reactivation of telomerase enzyme activity, which adds additional telomeres to the end of chromosomes.[13] Aberrant DNA damage signaling in cancers may result in chromosomal fusions and aneuploidy when telomeres are exhausted.

Signaling Pathways That Regulate Cellular Growth

Cellular proliferation is achieved through transition of cells from G_0 arrest into the active cell cycle (see Fig. 1-1). Although progression through the cell cycle is controlled by the regulatory mechanisms just described, overall proliferation is also modulated by external stimuli. Growth factors that bind to specific transmembrane receptors on the cell surface may be especially important. The cytoplasmic tails of these transmembrane receptor proteins activate intracellular signaling

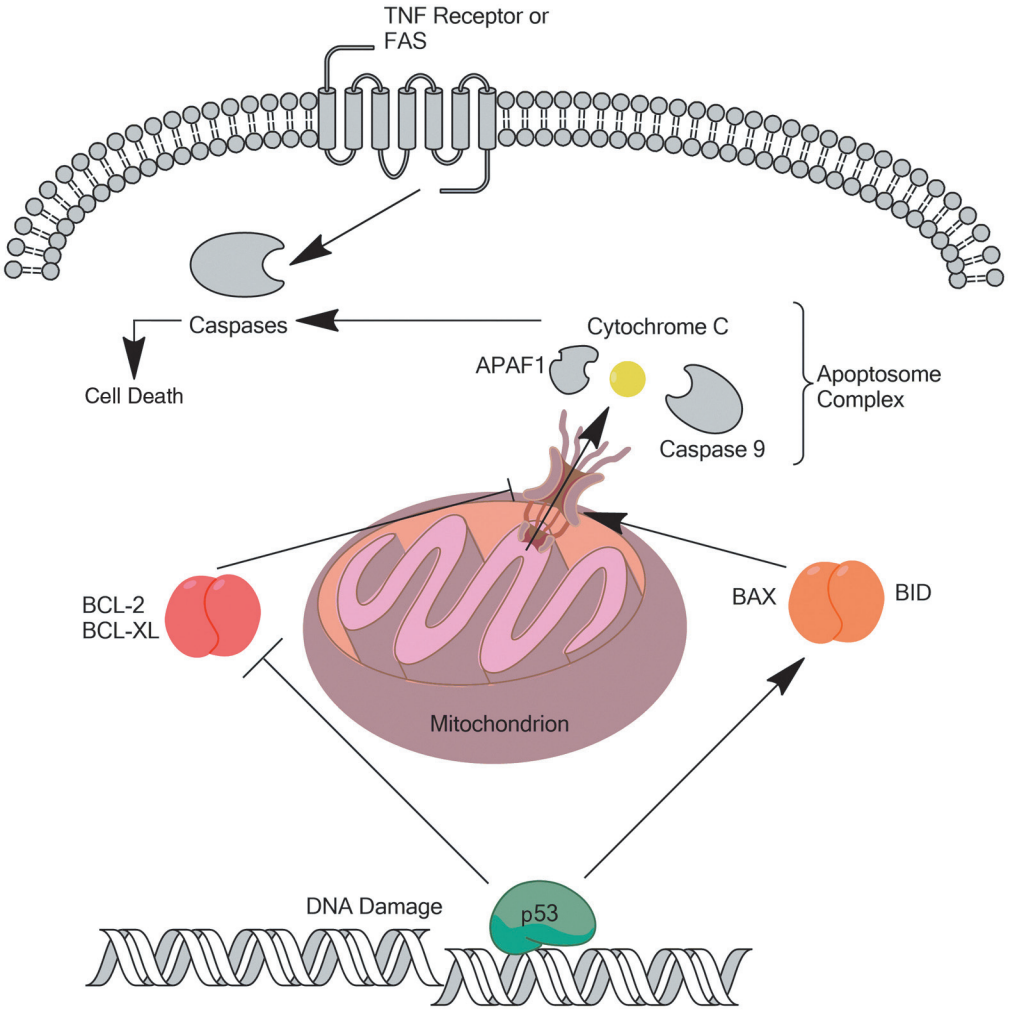

FIGURE 1-2. Apoptosis (programmed cell death) counterbalances cellular proliferation to regulate overall tissue growth. A complex interplay of proapoptotic and antiapoptotic molecules results in downstream activation of caspases that mediate cell death. Some of these signals are initiated through environmental insults that activate the *TP53* tumor suppressor gene, and some are initiated through death receptors, including TNF-R1, TNF-R2, and Fas. In addition, there is an interplay between proapoptotic (Bax, Bak) and antiapoptotic (BCL-2, BCL-XL) molecules. Both pathways converge on the mitochondria, resulting in release of cytochrome c and formation of the apoptosome complex (APAF1, caspase 9, and cytochrome c). This leads to activation of multiple caspases, DNA damage, and ultimately to cell death. BID, bcl-2 interacting domain; TNF-R1, tumor necrosis factor receptor 1; TNF-R2, tumor necrosis factor receptor 2.

cascades after ligand binding. In addition to peptide growth factors, extracellular matrix and cell-cell adhesion molecules (i.e., integrins, cadherins, selectins, proteoglycans) can have a significant impact on cell proliferation. Alterations in cell-matrix or cell-cell interactions are particularly important in contributing to the invasive phenotype of malignant cells.

Interaction of ligands with their receptors at the cell surface induces intracellular signals that alter gene transcription and protein expression. Three important receptor subtypes appear to initiate cellular signaling through ligand-receptor interaction at the cell surface: (1) tyrosine kinases, (2) serine and threonine kinases, and (3) G protein–coupled receptors.

The receptors for many peptide growth factors contain intrinsic tyrosine kinase activity within their intracellular tail. After ligand binding, tyrosine kinase activity is stimulated, leading to phosphorylation of tyrosine residues in target proteins within the cell. Most receptors also autophosphorylate tyrosine residues present in the receptors themselves to magnify signaling and, in some cases, this also causes attenuation of their own activity to effect an intramolecular feedback regulatory mechanism. The receptors for many peptide growth factors, including EGF, belong to this receptor class.

Other receptors on the cell surface possess kinase activity directed toward serine or threonine residues rather than tyrosine. These receptors also phosphorylate a variety of cellular proteins, leading to a cascade of biological responses. Multiple sites of serine and threonine phosphorylation are present on many growth factor receptors, including the tyrosine kinase receptors, suggesting the existence of significant interactions among various receptors present on a single cell.[14] The transforming growth factor (TGF)-β receptor complex is one important example of a serine-threonine kinase-containing transmembrane receptor.

Many receptors are members of the so-called 7-membrane–spanning receptor family. These receptors are coupled to guanine nucleotide binding proteins and designated G proteins. G proteins undergo a conformational change that is

dependent on the presence of guanosine phosphates.[15] Activation of G proteins can trigger a variety of intracellular signals, including stimulation of phospholipase C and the generation of phosphoinositides (most importantly, inositol 1,4,5-triphosphate) and diacylglycerol through hydrolysis of membrane phospholipids, as well as modulation of the second messengers cyclic adenosine monophosphate (cAMP) and guanosine monophosphate (GMP).[16] Somatostatin receptors exemplify a G protein–coupled receptor prevalent in the GI tract.

Binding of growth factors and cytokines to cell surface receptors typically produces alterations in a variety of cellular functions that influence growth. These functions include ion transport, nutrient uptake, and protein synthesis. However, the ligand-receptor interaction must ultimately modify one or more of the homeostatic mechanisms discussed to affect cellular proliferation.

The Wnt pathway is one important example of a signaling pathway that regulates a diverse number of homeostatic mechanisms to control proliferation of intestinal epithelial cells (Fig. 1-3). Evolutionarily conserved among several species, Wnt signaling, as a rule, ultimately results in accumulation of β-catenin in the nucleus, where it binds with the transcription factor Tcf-4 to activate a set of target genes.[17] In normal cells, this signal is initiated by secreted Wnt ligands that bind to cell surface receptors of the Frizzled family. Inhibition of the Wnt signal in mice can be achieved by deletion of Tcf-4 or overexpression of the Wnt inhibitor Dickkopf1, which results in dramatic hypoproliferation of the intestinal epithelium.[18,19] Tissue homeostasis is also maintained by growth-inhibiting signals that counterbalance proliferative signals. TGF-β is a potent growth-inhibiting factor that mediates arrest of the cell cycle at the G_1 phase. TGF-β not only induces transcription of the cell cycle inhibitors $p15^{INK4B}$ and $p21^{CIP1/WAF1}$, it also enhances the inhibitory activity of $p27^{KIP1}$ on the cyclin E/CDK2 complex (see Fig. 1-1).[20] These effects of TGF-β are mediated intracellularly through the Smad family of proteins.

INTESTINAL TUMOR DEVELOPMENT

Multistep Formation

Multiple sequential genetic alterations are required for the transformation of normal intestinal epithelium to neoplasia. This multistep nature of tumorigenesis is most directly illustrated by the changes that accrue in the development of colonic neoplasia (see Chapter 127). The accumulation of genetic and epigenetic alterations parallels the progression from normal

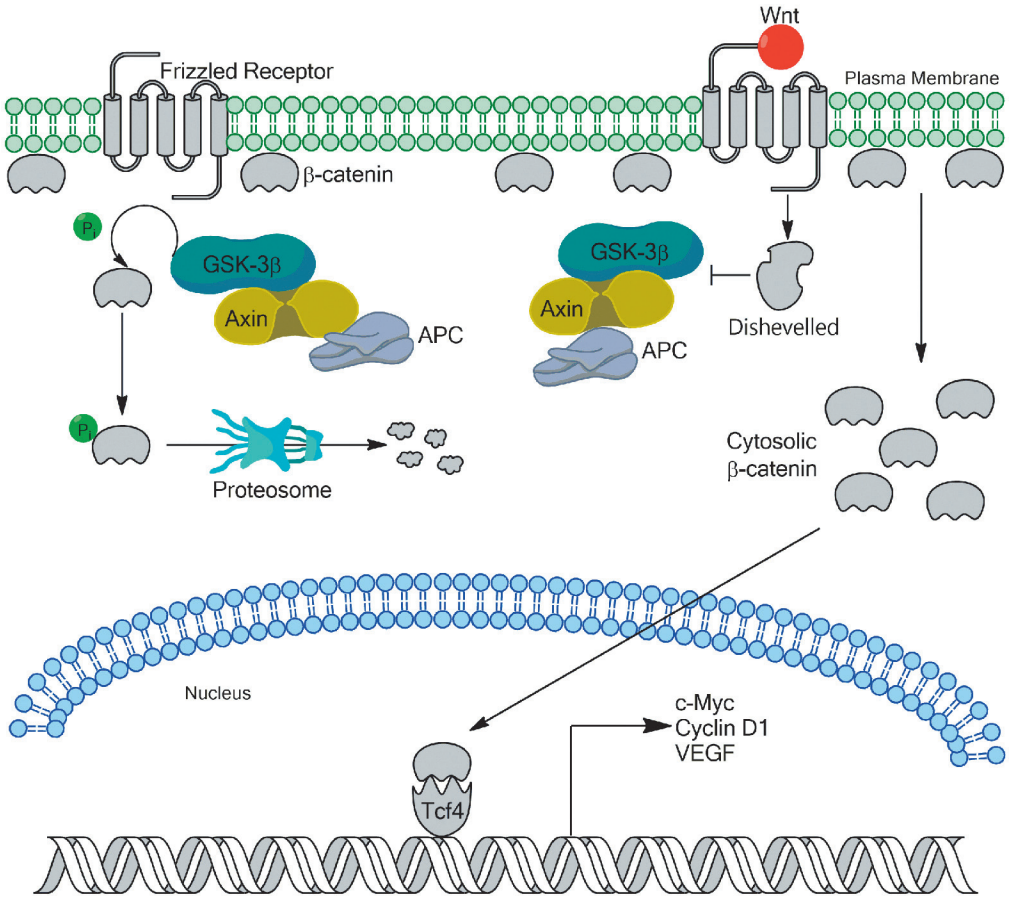

FIGURE 1-3. The Wnt signaling pathway is an important regulator of intestinal epithelial cell proliferation and tumorigenesis. In the absence of a Wnt signal (left top), cytosolic β-catenin forms a cytoplasmic complex with APC, Axin, and glycogen synthase kinase-3β (GSK-3β). This β-catenin destruction complex phosphorylates β-catenin and targets it for degradation via the ubiquitin-mediated proteasomal pathway. In the presence of an active Wnt signal (right top), β-catenin is stabilized, and excess cytoplasmic β-catenin is translocated to the nucleus, where it interacts with the Tcf-4 transcription factor to regulate the expression of many key target genes. APC, adenomatous polyposis coli; P, phosphate group; VEGF, vascular endothelial growth factor.

epithelium through adenomatous polyps to malignant neoplasia. Studies on the molecular pathogenesis of colon cancer have served as a paradigm for the elucidation of genetic alterations in other GI cancers, including gastric and pancreatic cancer.

A genetically unstable environment is necessary for the development of the multiple alterations that ultimately result in cancer. Genomic instability is observed in almost all cancers, regardless of organ site. Instability of the genome may result from several mechanisms. In colon cancer, there are now 3 well-recognized forms of genetic/epigenetic instability that promote carcinogenesis, and they have been termed *chromosomal instability, microsatellite instability,* and *CpG island methylator phenotype* (CIMP).[21,22] Chromosomal instability results in tumor cells that display frequent aneuploidy, large chromosomal deletions, and chromosomal duplications. In contrast, tumors that display microsatellite instability are often diploid or near-diploid on a chromosomal level but harbor frequent alterations in smaller tracts of microsatellite DNA (see later discussion on DNA repair). CIMP-high tumors have excessive gene promoter CpG-island methylation, which results in gene silencing. Thus, there are at least 3 distinct routes to the formation of a colorectal cancer, depending on the nature of the underlying genetic or epigenetic instability (Fig. 1-4). It is important to note that involvement by these pathways is not mutually exclusive.

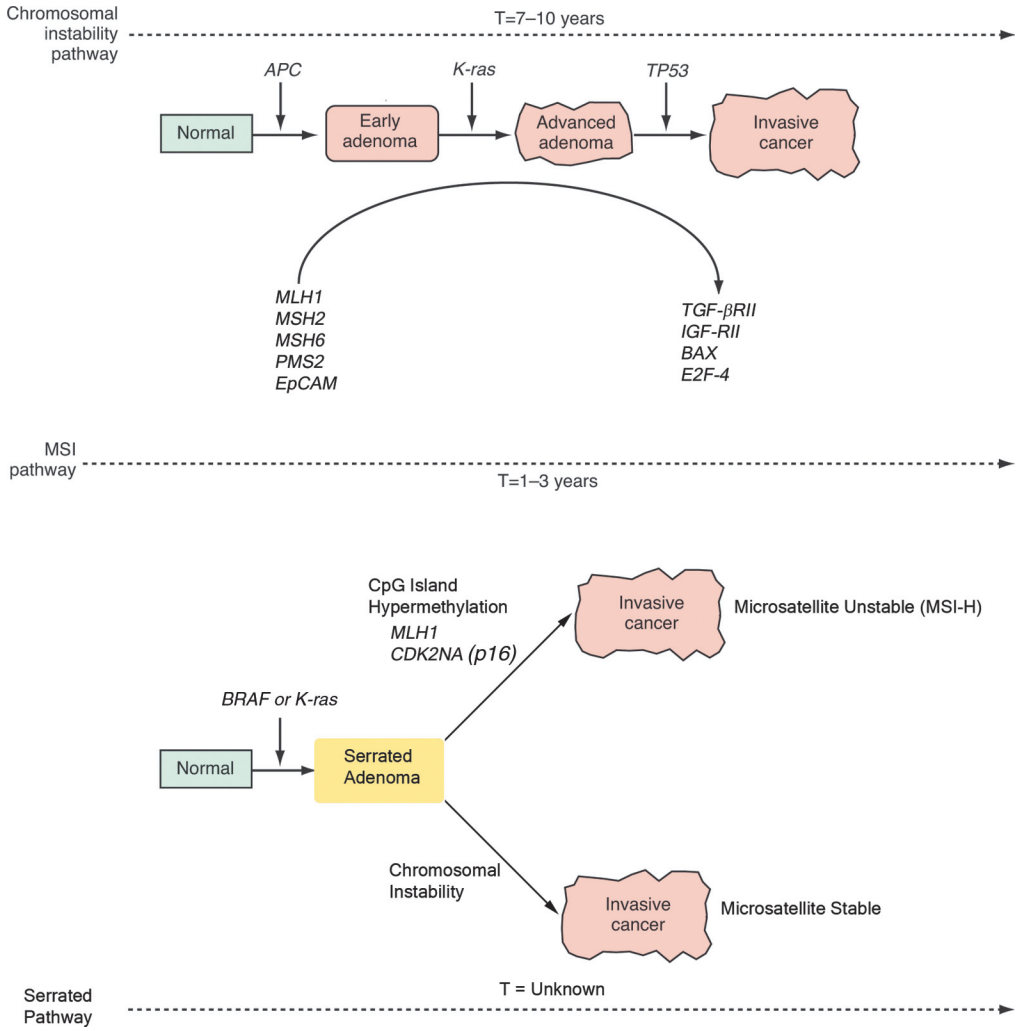

FIGURE 1-4. Multistep models of colorectal cancer based on underlying genetic instability. As shown on the left, there are 3 major pathways: chromosomal instability *(top pathway),* microsatellite instability *(middle pathway),* and serrated *(lower pathway).* The progression from normal colonic epithelium to carcinoma is associated with the acquisition of several genetic and epigenetic alterations. In the chromosomal instability pathway *(top pathway),* these alterations include the concomitant activation of oncogenes (e.g., *K-ras*) through a point mutation and inactivation of tumor suppressor genes (e.g., *APC, TP53*) through a point mutation or deletion. An increasing aggregate number of mutations can be correlated with progression from early benign adenoma to cancer, as reflected by analysis of polyps by size. In the microsatellite instability model *(middle pathway),* mutations in DNA mismatch repair genes create a mutator phenotype in which mutations accumulate in specific target genes (see section on DNA mismatch repair). Tumors develop much more rapidly through this pathway than through the chromosomal instability pathway *(horizontal arrows).* In the serrated pathway *(lower pathway),* the initiating event is hypothesized to be a *BRAF* or *KRAS* activating mutation that results in a serrated adenoma. Serrated adenomas may undergo extensive promoter hypermethylation (CpG island methylator phenotype [CIMP]) to become sporadic microsatellite unstable cancers (MSI-H) through silencing of genes encoding for MLH1 and p16. Alternatively, serrated adenomas can undergo a pathway similar to that of chromosomal instability to become microsatellite stable tumors.

Clonal Expansion

Clonal expansion is essential to tumor development.[23] Whereas germline mutations may lead to altered expression of a gene in all cells in a tissue, subsequent additional somatic mutations generally occur only in a small subpopulation of cells. Clonal expansion of these mutated cells occurs if a specific gene mutation results in a survival advantage for the cells. A second round of clonal expansion occurs when a cell within this population sustains still another genetic alteration that further enhances its growth properties. This iterative process of selection, with accumulating genetic alterations, results in cellular transformation and malignancy. Once frank malignancy has developed, the catalog of mutations harbored may vary between cancer cells. Referred to as *tumor heterogeneity,* this ongoing process may give certain cells selection advantages.[24] Metastasis may be facilitated by the evolution of a subset of tumor cells that acquire the capability of traversing the circulatory system and thriving in a new environment.

Cancer Stem Cells

These observations of tumor heterogeneity have led to the *cancer stem cell hypothesis,* which asserts that there exists a subset of tumor cells that have stem cell–like properties. Cancer stem cells (CSCs) are believed to be the tumor-initiating cells from which clonal expansion occurs. Moreover, it is hypothesized that eradication of these cells is a key therapeutic goal because failure to do so may result in relapse of disease. Within this CSC hypothesis, there are 2 models.[25] The first is a hierarchical model in which CSCs may serve as progenitors of cancer cells with limited reproductive potential. The second stochastic model posits that each cancer cell has the same potential to be a CSC, but this determination is stochastically based on internal factors in addition to external environmental cues. Analysis of putative CSCs demonstrate transcriptional programs and markers shared with normal intestinal stem cells. Markers such as Lgr5 and EphB2 have been used to identify and purify colon CSCs.[26]

NEOPLASIA-ASSOCIATED GENES

The genes that collectively play an important role in oncogenesis generally lead to disruption of the orderly mechanisms of normal cell proliferation. Since normal cell proliferation appears to depend on a wide variety of genes, it is not surprising that alterations in the expression of a diverse set of genes confer part or all of the phenotypic features of transformation. Despite this diversity, all these genes that become altered appear to belong to 1 of 2 distinct groups: (1) oncogenes, which actively confer a growth-promoting property, or (2) tumor suppressor genes, the products of which normally restrain growth or proliferation. An important category within tumor suppressor genes includes DNA repair genes, which prevent accumulation of new mutations. Activation of oncogenes or inactivation of tumor suppressor genes contributes to malignant transformation. Transcriptionally active sites of the genome that do not encode for proteins also play a significant role in regulation of gene expression and carcinogenesis. These noncoding RNAs may harbor oncogenic and tumor suppressive functions as well.

Oncogenes

Typically, oncogenes are genes that encode a normal cellular protein expressed at inappropriately high levels or mutated genes that produce a structurally altered protein that exhibits inappropriately high activity. For example, several genes that encode tyrosine kinase–containing growth factor receptors become oncogenes after a mutation results in unregulated tyrosine kinase activity that is no longer dependent on the presence of the appropriate ligand. The normal cellular genes from which the oncogenes derive are designated *proto-oncogenes.* Most of these genes are widely expressed in many different types of tumor cells.

Several mechanisms can lead to oncogene activation. These include gene transduction or insertion, point mutation, gene rearrangement, and gene amplification. Gene transduction and insertion generally result from retroviral infection. Point mutations result in constitutively active oncogene products. Gene rearrangements can result in oncogenic fusion proteins, and gene amplifications lead to uncontrolled overexpression of a normal gene product.

The proteins encoded by oncogenes comprise at least 4 distinct groups—peptide growth factors that may be secreted into the extracellular milieu, protein kinases, signal-transducing proteins associated with the inner cell membrane surface (membrane-associated G proteins), and transcriptional regulatory proteins located in the nucleus.

Peptide Growth Factor Oncogenes

The transforming effects of enhanced expression of a variety of growth factors have been demonstrated both in vitro and in vivo. Several growth factor–related proteins encoded by oncogenes have now been recognized, including the family of Wnt proteins and Sis, which encodes the β chain of platelet-derived growth factor. Cancer cells may engage in autocrine signaling to promote their growth, or coax the adjacent stroma to hypersecrete such growth-stimulating factors.

Protein Kinase–Related Oncogenes

The largest family of oncogenes encodes proteins with protein kinase activity. These oncogenes encompass the full variety of protein kinases, including receptor/nonreceptor tyrosine kinases and cytoplasmic serine/threonine kinases. Many members of this large oncogene group are expressed by neoplasms of the GI tract, and these include the receptor tyrosine kinases of the EGF receptor family (ERBB1-4) and the Src nonreceptor tyrosine kinase that associates with the inner surface of the plasma membrane.

Signal Transduction–Related Oncogenes (Membrane-Associated G Proteins)

Intermediate steps that effectively translate ligand-receptor binding to an intracellular signal are essential in mediating functional responses of the cell. Mutations in genes that encode key proteins that participate in signal transduction can also lead to cellular transformation.

G proteins regulate signaling of the large family of G protein–coupled receptors (GPCRs) through the exchange of guanosine triphosphate (GTP) with guanosine diphosphate (GDP). Altered *ras* genes, a family of proteins related to the G proteins, are among the most commonly detected oncogenes in GI tract cancers. The *ras* family contains 3 genes: *H-ras, K-ras,* and *N-ras.* Point mutations that result in amino acid substitutions at critical hot spot positions convert the normal gene into an oncogene.

To date, almost all *ras* mutations in GI malignancies occur in the *K-ras* oncogene. The highest mutation frequency is found in tumors of the exocrine pancreas (>90%).[27] *Ras* genes activated through point mutation have been identified in

approximately 50% of colonic cancers as well as a subset of serrated tumors (see Fig. 1-4).[28]

Most oncogenic mutations in *ras* cause biochemical changes that maintain it in the active, GTP-bound state by reducing guanosine triphosphatase (GTPase) activity or by destabilizing the inactive GDP-bound form. However, several *ras* mutants retain significant GTPase activity; therefore, other mechanisms that convert *ras* to a transforming protein may be involved.[29]

A functional consequence of ras activation is phosphorylation of key serine/threonine kinases. One important downstream signaling target of ras is B-raf. In colon cancers without an identifiable *K-ras* mutation, 20% possess an activating *B-raf* mutation,[30] consistent with the concept that activation of an oncogenic pathway can be achieved through an alteration in any of several sequential components of a particular pathway.

Nuclear Oncogenes

Many cellular oncogenes encode proteins that localize to the nucleus. In essence, these nuclear oncogene products are the final mediators of signal transduction pathways that are also affected by cytoplasmic and plasma membrane-bound oncoproteins, because they act as transcription factors that regulate expression of certain genes that enhance cellular proliferation and suppress normal differentiation.

The role of nuclear oncogenes is illustrated by the myc family. The c-Myc protein product is involved in critical cellular functions like proliferation, differentiation, apoptosis, transformation, and transcriptional activation of key genes.[31] Frequently, c-Myc is overexpressed or amplified in many GI cancers. c-Myc has been found to be a transcriptional target of the β-catenin/TCF-4 complex in colorectal cancers (see Fig. 1-3), which may explain the overexpression of c-Myc observed in this cancer type.[32]

Tumor Suppressor Genes

The products of tumor suppressor genes prevent acquisition of the transformed phenotype in vitro and have similar functional properties in vivo. Mutations that disrupt the biological function of these genes are associated with all GI cancers. Germline mutations of this class of gene underlie most of the known inherited cancer syndromes in which a specific gene has been implicated. A number of these genes and their products have been identified and characterized (Table 1-1).

Initial recognition of the existence of tumor suppressor genes was derived from linkage analyses of cancer-prone families. In the GI tract, hereditary colon cancer, gastric cancer, and pancreatic cancer syndromes are the best described and are discussed elsewhere in this text. A number of features are common to GI cancer syndromes with Mendelian patterns of inheritance. Most importantly, the marked increase in risk for a particular tumor is found in the absence of other predisposing environmental factors. In addition, multiple primary tumors often develop within the target tissue, and tumors in these affected members typically arise at a younger age than they do in the general population. Finally, affected individuals are sometimes at risk for tumors outside the GI tract.

These observations led Knudson to hypothesize that tumors in familial cancer syndromes might derive from independent mutations in the 2 alleles of a specific tumor suppressor gene (Fig. 1-5). Specifically, he proposed that the first mutation was present in 1 copy of the gene inherited in the germline and therefore present in all cells in affected family members.[33] A somatic mutation of the remaining normal allele of the tumor suppressor gene that might occur in any cell would then lead to tumor development. The same gene might play a role in the development of the same tumor type in the general population (sporadic cancer), but 2 independent somatic mutations of each of the 2 alleles would be required. However, this combination of events should be uncommon and would explain the lower frequency and later age of diagnosis of similar tumors in the general population. Comings was the first to suggest that the relevant gene in a familial cancer syndrome might encode a tumor-suppressing gene product.[34] Although this 2-hit model has been generally

TABLE 1-1 Mutations Associated with Hereditary Gastrointestinal Cancer Syndromes

Disorder	Gene(s) Mutated
FAP, AFAP	*APC*
Lynch syndrome (HNPCC)	*MSH2, MLH1, MSH6, PMS2, EpCAM*
MUTYH polyposis	*MUTYH*
Peutz-Jeghers syndrome	*LKB1/STK11*
Cowden's disease	*PTEN*
Juvenile polyposis	*SMAD4, BMPR1A*
Hereditary diffuse gastric cancer	*CDH1*
Hereditary pancreatic cancer	*ATM, BRCA1, BRCA2, PALB2, PALLD, CDKN2A, PRSS1, SPINK1, PRSS2, CTRC, CFTR*
MEN1	*Menin*

AFAP, attenuated FAP; APC, adenomatous polyposis coli; FAP, familial adenomatous polyposis; HNPCC, hereditary nonpolyposis colorectal cancer; MEN1, multiple endocrine neoplasia, type 1; MUTYH, mutY homolog.

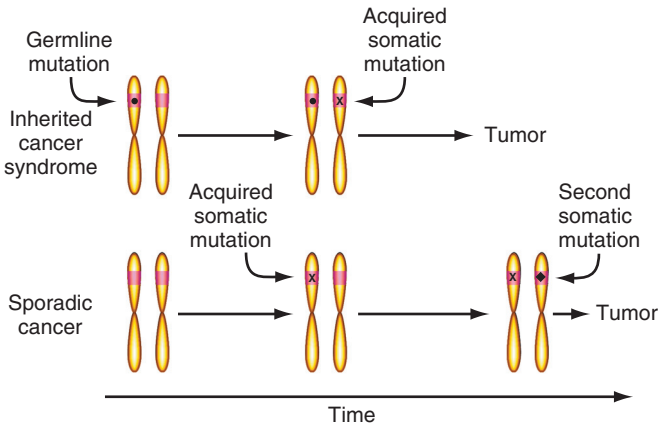

FIGURE 1-5. Knudson's 2-hit hypothesis. In an inherited cancer syndrome, 1 chromosome has an inactive tumor suppressor gene locus because of a germline mutation. The counterpart tumor suppressor gene on the remaining paired chromosome is subsequently inactivated by a somatic mutation, leading to tumor formation. In contrast, in a sporadic cancer, the 2 alleles of the tumor suppressor gene become inactivated through 2 independent somatic mutations, an unlikely event within a single cell.

observed for mendelian cancer syndromes, there are exceptions. Some tumor suppressors may function to increase cancer risk when only 1 allele is mutated. These genes may be so critical that the reduction in gene expression by 1 mutant allele is sufficient to drive tumorigenesis. Also, 1 mutant allele may function in a dominant-negative fashion, blocking the effect of the intact protein encoded by the normal allele.

Tumor Suppressor Gene Inactivation

Some tumor suppressor genes were first cloned through detection of regions of gene deletion in tumor samples from cancer-prone kindreds by DNA screening for markers scattered throughout the genome. These deletions targeted the second wild-type allele and served to pinpoint the chromosomal location of the disease-causing gene present on the other allele. More recently, our knowledge of the genetic variation observed in tumors has greatly increased by next-generation sequencing technologies. By analyzing the genetic changes in tumors in comparison to normal mucosa, we are now aware of the types of genetic changes that occur in cancer cells. *Single nucleotide variants* (SNVs) refer to changes in a single base pair of the genetic code. While many of these mutations are silent, others can result in significant changes in gene expression or function. Missense mutations result in a change in the amino acid encoded by the codon. *Nonsense mutations* refer to the introduction of a premature stop codon. SNVs at splice-acceptor or donor sites may result in exon loss or misexpression of intronic sequences. SNVs in the promoter or untranslated regulatory regions of a gene may dramatically change gene expression. Another type of genetic variation includes insertions or deletions. Small insertion or deletion mutations may result in frameshift mutations within a gene. Larger-scale insertion and deletions are also seen. Each type of variant may result in inactivation of a given gene, and they represent important mechanisms of inactivation of 1 copy of tumor suppressor genes. Another mechanism of tumor suppressor gene inactivation includes promoter hypermethylation. Transcriptional silencing can result from methylation of CpG islands in gene promoters; this has been demonstrated to occur in the genes encoding $p16^{INK4A}$ and E-cadherin.[35] Excess CpG island methylation has been implicated as a cardinal feature in the serrated pathway to colon cancer (see Fig. 1-4).

Tumor suppressor genes do not function identically in every tissue type. Consequently, inactivation of a particular tumor suppressor gene is tumorigenic only in certain tissues. For example, the tumor suppressor genes *RB1* and *VHL* play crucial roles in retinoblastomas and renal cell cancer, respectively, but are rarely mutated in GI malignancies. Three tumor suppressor genes shown to have a critical role in the pathogenesis of GI malignancies, *APC*, *TP53*, and *SMAD4*, are described below.

Adenomatous Polyposis Coli Gene

Genetic linkage analysis revealed markers on chromosome 5q21 that were tightly linked to polyp development in affected members of kindreds with familial adenomatous polyposis (FAP) and Gardner's syndrome.[36] Further work led to identification of the gene responsible for FAP, the adenomatous polyposis coli *(APC)* gene.[37-39] The full spectrum of adenomatous polyposis syndromes attributable to *APC* is discussed in detail in Chapter 126. Somatic mutations in *APC* have also been found in most sporadic colon polyps and cancers.[40,41] Mutations in *APC* are characteristically identified in the earliest adenomas, indicating that *APC* plays a critical role as the gatekeeper in the multistep progression from normal epithelial cell to colon cancer (see Fig. 1-4).

The *APC* gene comprises 15 exons and encodes a predicted protein of 2843 amino acids, or approximately 310 kd. Most germline and somatic *APC* gene mutations result in a premature stop codon and therefore a truncated APC protein product. Mutations occurring in the *APC* amino terminal are associated with a rare variant of FAP, attenuated familial adenomatous polyposis (AFAP).[42] *APC* mutations result in functional changes in key protein-protein interactions. As discussed earlier, *APC* is a negative regulator of the Wnt signaling pathway (see Fig. 1-3). Mutant APC proteins are unable to interact with β-catenin, resulting in uncontrolled activation of the Wnt signaling pathway and the subsequent oncogenic phenotype.

TP53 Gene

Named for a 53-kd-sized gene product, p53 is a nuclear phosphoprotein that plays a key role in cell cycle regulation and apoptosis.[43] The p53 protein was first detected in tumors as the product of a mutated gene that was mapped to chromosome 17p, a region found to exhibit loss of heterozygosity in many tumors. Point mutations in *TP53* have been identified in as many as 50% to 70% of sporadic colon cancers (see Fig. 1-4) but only a small subset of colonic adenomas.[44] Point mutations in *TP53* have also been found in all cancers of the GI tract.[43] Interestingly, aflatoxin appears to induce a mutation in a single hot spot codon (codon 249) of *TP53* in many hepatocellular carcinomas.[45] In addition to the *TP53* point mutations in sporadic cancers, germline *TP53* mutations have been observed in the Li-Fraumeni syndrome, an autosomal dominant familial disorder in which breast carcinoma, soft tissue sarcoma, osteosarcoma, leukemia, brain tumor, and adrenocortical carcinoma can develop in affected persons.[46]

The sequence-specific transcription factor p53 is induced in conditions of cellular stress, such as ionizing radiation, growth factor withdrawal, or cytotoxic therapy (see Fig. 1-2). As a consequence of genotoxic damage, p53 arrests cells at the G_1 phase to facilitate DNA repair, senescence, or trigger apoptosis. Factor p53 mediates some of these responses through induction of the $p21^{CIP1/WAF1}$ inhibitor of the cell cycle or pro-apoptotic genes, including *PUMA*, and c-Myc appears to play a role in this cell fate decision.[47]

SMAD4 Gene

SMAD4 is a tumor suppressor gene located on chromosome 18q and is deleted or mutated in most pancreatic adenocarcinomas and a subset of colon cancers. This gene encodes Smad4, an essential intracellular mediator of the growth inhibitory effects of TGF-β. The Smad4 protein has 2 important domains, the mad homology domains 1 and 2 (MH1 and MH2), which are essential for DNA binding and for oligomerization with other Smad proteins, respectively.[48] Mutant Smad4 blocks TGF-β–induced inhibition of proliferation. Germline mutations in *SMAD4* result in the juvenile polyposis syndrome (see Chapter 126).

DNA Repair Genes

Cellular mechanisms have evolved to preserve the fidelity of DNA. Errors can be introduced into the genome through multiple physiologic and pathologic mechanisms. These errors include spontaneous mismatching of nucleotides during normal DNA replication, oxidative damage of nucleotides, and complete double-strand breaks. Numerous discrete systems exist to repair these types of DNA damage that can arise from a variety of insults, including carcinogens, irradiation, and reactive oxygen species. One type of error that

develops during replication may occur in stretches of microsatellite DNA, which involves regions of mononucleotide (e.g., poly-A) or dinucleotide (e.g., poly-CA) repeats.[49] The DNA mismatch repair system corrects these errors. The enzymes bind mismatched DNA, cut the DNA strand with the mismatched nucleotide, unwind the DNA fragment, fill in the gap with the correct nucleotide, and finally reseal the remaining nick. The family of DNA mismatch repair genes includes *MSH2, MSH3, MSH4, MSH5, MSH6, MLH1, MLH3, PMS1,* and *PMS2.*

MLH1 and *MSH2* are the 2 DNA mismatch repair genes that are most frequently mutated at the germline level in Lynch syndrome, also known as *hereditary nonpolyposis colorectal cancer* (HNPCC).[50,51] Mutations can lead to functional alterations that allow strand slippage during replication. Affected cells are called *replication error* (RER) *positive,* in contrast to the RER-negative phenotype.[52,53] Because microsatellite DNA sequences are primarily affected by this type of genetic instability, the tumor cells are said to display microsatellite instability (MSI). Mechanistically, the absence of DNA repair does not directly cause cancer. Rather, the DNA repair defect creates a milieu that permits accumulation of mutations in a variety of other genes that contain microsatellite DNA sequences, such as the TGF-β type II receptor, IGF type II receptor, BAX, and E2F-4. This MSI pathway represents a novel mechanism for the accumulation of mutations within a tumor (see Fig. 1-4). It is characteristic of all Lynch-related tumors and is observed in approximately 15% of all sporadic colon cancers. Increasing evidence has emerged that these sporadic MSI tumors result from the serrated pathway and *MLH1* promoter hypermethylation (see Fig. 1-4).

Errors can also be introduced when individual nucleotides are damaged by chemical factors; the base excision repair system corrects these types of errors. 8-Oxoguanine residues can result from oxidative DNA damage, and these altered bases will inappropriately pair with adenines, ultimately leading to somatic G:C→T:A mutations if uncorrected. MUTYH is a DNA glycosylase that participates in the repair of these oxidized guanine nucleotides. An autosomal recessive adenomatous polyposis syndrome caused by germline mutations in the *MUTYH* repair gene has been identified.[54,55] Interestingly, G:C→T:A mutations in the *APC* gene were almost universally found in the polyps of patients with germline *MUTYH* mutations, indicating that there are important similarities in the molecular pathogenesis of polyps in the MUTYH and FAP syndromes.

Oncogenic Signaling Pathways

Individual oncogenes or tumor suppressor genes do not necessarily induce cellular transformation directly but typically function as components of larger oncogenic signaling pathways. Some of the pathways that are particularly relevant for GI tumorigenesis include the Wnt and Ras signaling pathways. These are pathways that regulate normal tissue homeostasis but become oncogenic when the signals are transduced in an aberrant or amplified manner. The key features of Wnt signaling are illustrated in Figure 1-3. β-catenin is translocated from the inner plasma membrane to the cytoplasm. There, it forms a macromolecular complex with the APC protein Axin and glycogen synthase kinase-3β (GSK-3β). Phosphorylation of β-catenin by GSK-3β triggers its degradation. In the presence of an active Wnt signal, β-catenin is stabilized, and it enters the nucleus where it interacts with the transcription factor Tcf-4 to up-regulate a number of key target genes, including *c-Myc, cyclin D1,* and *VEGF*. As discussed earlier, Wnt signaling is essential for regulating proliferation of normal intestinal epithelium, and dysregulated Wnt signaling

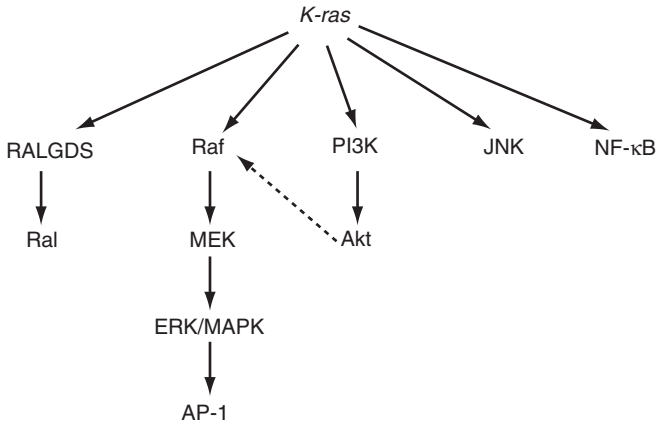

FIGURE 1-6. Diversity of signaling through *K-ras*. Oncogenic *K-ras* can activate multiple signaling pathways. The mechanisms that determine which pathway may be preferentially activated in a given cell type are not fully defined. Crosstalk between these pathways increases the complexity of the signaling networks. These effector pathways can influence cellular biological processes including proliferation, apoptosis, differentiation, and motility.

is an almost universal feature of all colorectal cancers. The latter can result from a mutation in the *APC, Axin,* or *β-catenin* genes, although alterations in the *APC* tumor suppressor gene are the most common. An alteration in just 1 of these components is sufficient to activate the entire pathway. Thus, it is essential to consider individual genetic alterations in the context of the overall signaling pathway in which they function.

Because pathways are typically not linear, additional levels of complexity arise. There is frequent overlap among pathways, and the distinction between pathways can be somewhat arbitrary. For example, mutations in the *K-ras* oncogene result in activation of multiple distinct signaling pathways, including Raf/ERK/MAPK, PI3K/Akt, and NF-κB, all of which play an important role in tumorigenesis (Fig. 1-6). Crosstalk between these effector pathways serves to modulate the cellular responses further. For example, Akt, a target of PI3K, can phosphorylate Raf and thereby regulate signaling through the MAPK pathway.[56] Finally, each of these signaling pathways regulates multiple biological processes related to tumorigenesis,[57] including cell cycle progression, apoptosis, senescence, angiogenesis, and invasion.

Another pathway that plays a particularly important role in GI tumors is the cyclooxygenase-2 (COX-2) pathway. The enzyme COX-2 is a key regulator of prostaglandin synthesis that is induced in inflammation and neoplasia. Although no mutations of COX-2 have been described, overexpression of COX-2 in colonic adenomas and cancers is associated with tumor progression and angiogenesis, primarily through induction of prostaglandin E_2 synthesis. Inhibition of COX-2 with a variety of agents (aspirin, nonsteroidal anti-inflammatory drugs, or COX-2 selective inhibitors) is associated with a reduced risk of colorectal adenomas and cancer.[58]

Noncoding RNAs

Although previously referred to as "junk DNA," a significant portion of the non–protein coding genome remains transcriptionally active. The RNA products, termed *non-coding RNAs* (ncRNAs), consist of a broad category of active RNA molecules including long noncoding RNAs (lncRNAs) and micro

RNAs (miRNAs) that are frequently dysregulated in cancers.[59] Initially processed into small interfering RNAs (siRNAs) by the protein Dicer into 20- to 25-nucleotide sequences, microRNAs play a critical role in transcript silencing.[60] These siRNAs bind to complementary mRNA sequences, and this binding then facilitates the activity of the RNA-induced silencing complex to target the mRNA for cleavage and degradation. LncRNAs may perform diverse functions like gene silencing, splicing, and extension of telomeres.

Epigenetics

Epigenetics refers to changes in the genome that result in change in expression or phenotype without a change in the sequence of the DNA. Often these changes can result from structural alterations of the genome. One major mechanism is promoter CpG-island hypermethylation. The promoters of many genes are enriched with these CG sites ("CpG islands"). Methylation of the cytosine residues in these islands can result in silencing of the downstream gene.

Many cancers exhibit promoter hyperymethylation and silencing of important tumor suppressor genes. In approximately 15% to 20% of colorectal cancers, this process becomes a dominant feature of carcinogenesis. Characterized as CpG island methylator phenotype (CIMP) positive, these tumors have excessive levels of promoter hypermethylation of tumor suppressor genes. Notably, *MLH1* is frequently hypermethylated, resulting in sporadic microsatellite unstable cancers. The mechanisms underlying this promoter hypermethylation remain undefined, but recent studies demonstrate a link between tumor metabolism and global methylation status. Mutations in *IDH1* can induce a CIMP-high phenotype in glioblastomas.[61]

TUMOR METABOLISM

Metabolic cues and nutrient availability play a critical role in cell growth and homeostasis. As previously described, a lack of available nutrients or mitochondrial dysfunction may signal growth arrest or apoptosis. However, tumor cells exhibit abnormal metabolic profiles to facilitate their growth and anabolic needs. Observations in 1924 from Nobel Laureate Otto Heinrich Warburg revealed that tumor cells displayed dramatic increases in aerobic glycolysis and diminished mitochondrial respiration. This hypothesis, known as the *Warburg hypothesis,* has been validated and is a hallmark feature of most malignancies.[62] Many of the genes implicated in GI cancers *(p53, K-Ras, PI3K, mTOR, HIF, Myc)* can in fact regulate metabolic pathways. Moreover, germline mutations in metabolic regulators (e.g., subunits of succinate dehydrogenase [SDH]) that are not classical oncogenes or tumor suppressor genes have been associated with a high risk of tumorigenesis (pheochromocytoma and paraganglioma).[63,64] The selection advantage of increased glycolysis in cancer cells may include greater tolerance to hypoxic environments and shunting of metabolic byproducts to other biosynthetic pathways. These altered metabolic pathways are promising new targets for therapy.

ENVIRONMENTAL AND MICROENVIRONMENTAL INFLUENCES

Fundamentally, cancer is a genetic disorder. Environmental factors play an important role in tumorigenesis, but they ultimately lead to expression of abnormal genes or inappropriate expression of normal genes, the products of which confer the malignant phenotype. Genetic mutation is the common denominator of agents or mechanisms that contribute to the development of neoplasia.

Chemical Carcinogenesis

Metabolic activation by the host is a key determinant of the carcinogenic potential of many compounds. The initial compound, the procarcinogen, is converted by host enzymes to an electrophilic derivative, which then chemically modifies DNA. Mutations result from errors that occur during DNA replication as a result of distorted base pairs. Factors that influence the potency of any chemical carcinogen include the equilibrium between activation of the procarcinogen and deactivation or degradation of the carcinogen.[65] Deactivation typically occurs through a conjugation reaction, usually in the liver.

These principles are exemplified by experimental colonic carcinomas that arise in rodents fed cycasin, a glucosylated compound present in the cycad nut. The glucose residue of cycasin is cleaved in the rat liver by β-glucosidase to form methylazoxymethanol, which is subsequently deformylated by enzymes in the liver and colon to give rise to methyldiazonium, a carcinogen. These same metabolites are formed through hepatic enzymatic modification of the compound dimethylhydrazine and result in colon cancer in the rat.

In humans, regular tobacco use is strongly associated with a higher risk of multiple GI cancers, including pancreatic and colon cancer. Among active smokers with long-term tobacco use, the risk for pancreatic cancer can be elevated 2-fold. Multiple carcinogenic agents including arsenic, benzene, and ethylene oxide have been identified in cigarettes, but the chemicals linked specifically to the development of pancreatic or colon cancer have not yet been defined.

Dietary Factors

Chemical mutagenesis may be especially important in the development of cancers within the GI tract and related organs. The mucosal surfaces from which most primary cancers in the GI tract develop are exposed to a complex mixture of dietary constituents that are potential carcinogens or procarcinogens. The ability of dietary factors to act as mutagens in humans was demonstrated directly in 1995. The frequency of contamination of foodstuffs with aflatoxins, a fungal metabolite, parallels the incidence of hepatocellular carcinoma in various areas of the world.[66] Studies demonstrating that aflatoxins cause mutations in the *TP53* gene in hepatocellular carcinoma have provided a compelling link between genes and the environment.[66]

Nitrates present in many foods appear to be additional dietary constituents that may act as procarcinogens in the GI tract. Diet-derived nitrates can be converted by bacterial action in a hypochlorhydric stomach to nitrites and subsequently to mutagenic nitrosamines.[67] These events may underlie the documented correlation between dietary intake of foods high in nitrates and the incidence of gastric cancer in different populations.

Other dietary factors may modulate the biological potency of dietary procarcinogens. Variations in the relative and absolute amounts of dietary fats may lead to alterations in the composition of the colonic microflora and their metabolic characteristics, resulting in modulation of the production of enzymes that convert dietary constituents into potentially mutagenic compounds. Changes in dietary fiber content

can alter the transit time of luminal contents in the bowel, thereby changing the duration of exposure of the mucosa to potential mutagens. Bile salt content may be an additional luminal factor that can modulate the biological effect of procarcinogens. Deconjugated bile salts may promote carcinogenesis through mucosal injury and enhanced epithelial proliferation.

These mechanisms could explain well-documented correlations between the intake of various dietary constituents and the incidence of colorectal cancer in certain populations. Populations that have a high fiber intake and resulting fast colonic transit times generally exhibit a lower incidence of colorectal cancer than populations with low fiber intake and delayed transit. The incidence of colorectal cancer in Japanese immigrants to the United States who consume a Western diet is much higher than that of native Japanese who consume a traditional Japanese diet.[68]

Microbiome

The human body possesses over 100 trillion microbes. The interaction between these organisms and the host is an area of great interest, particularly for a broad range of autoimmune, metabolic, and neoplastic disorders. The Human Microbiome Project seeks to develop a map for these organisms throughout the body, with the goal of correlating specific bacterial species with disease states. Although the results of this track of investigation are preliminary, evidence is accumulating that the composition of the gut microbiome may affect cancer risk.[69] Altered bacterial populations have the potential to influence metabolic pathways and inflammatory indices in the GI tract.

Viruses also can lead to disruption of normal genes by integration into the host genome in a position that disrupts normal gene sequences (insertional mutagenesis) or through the introduction of aberrant genes present in the virus's own genetic material. Viruses that appear to play a role in oncogenesis in the GI tract through insertional mutagenesis include human papillomavirus in squamous cell cancers of the esophagus and anus, Epstein-Barr virus in gastric lymphoepithelial malignancies, and hepatitis B virus in hepatocellular carcinoma.

Inflammation and Cancer

A number of chronic inflammatory conditions increase the site-specific risk of cancer, such as ulcerative colitis (Chapter 116), chronic gastritis (Chapter 52), chronic pancreatitis (Chapter 59), Barrett's esophagus (Chapter 45), and chronic viral hepatitis (Chapters 79 and 80). In addition to the direct proliferative stimuli, the influences of inflammation on the development of neoplasia are multifaceted and complex. Immune cells may promote remodeling of the vascular network and promote angiogenesis (discussed later). Inflammation may also induce epigenetic changes in cells to favor gene silencing of tumor suppressor genes through DNA damage from cytokine-stimulated production of reactive oxygen species. In addition, cytokines produced by inflammatory cells can lead to activation of nuclear factor (NF)-κB in tumor cells that can serve to inhibit apoptosis and stimulate proliferation.[70] Although chronic inflammation creates a pro-tumorigenic environment, it should be noted that the immune system also plays an important role in tumor suppression through tumor surveillance. Immunosuppressive therapies are associated with an increased risk of malignancy. Maintenance of this tight balance of immunoregulation is critical to prevent the development of a pro-tumorigenic environment.

BIOLOGICAL FEATURES OF TUMOR METASTASIS

The establishment of distant metastases requires multiple processes, many of which involve alterations in interactions between tumor cells and normal host cells. To metastasize, a cell or group of cells must detach from the primary tumor, gain access to the lymphatic or vascular space, adhere to the endothelial surface at a distant site, penetrate the vessel wall to invade the second tissue site, and finally proliferate as a second tumor focus. Angiogenesis is necessary for proliferation of the primary tumor and tumor metastases. Tumor cells must also overcome host immune cell killing. As a result, few circulating tumor cells (<0.01%) successfully initiate metastatic foci. A "survival of the fittest" view of metastasis has been proposed, in which selective competition favors metastasis of a subpopulation of cells from the primary site.[71] Clonal expansion occurs again after formation of a metastatic focus.

Epithelial-Mesenchymal Transition

Modulation of tumor cell interactions with adjacent cells and with the extracellular matrix is an essential step as epithelial tumor cells invade through the basement membrane and ultimately metastasize to distant sites. A similar process occurs during normal embryogenesis, when polarized epithelial cells no longer recognize the boundaries imposed by adjacent epithelial cells or their basement membrane and adopt features of migratory mesenchymal cells. This phenomenon, designated *epithelial-mesenchymal transition* (EMT), has provided insight into understanding tumor progression (Fig. 1-7). E-cadherin is a critical component of adherens junctions that maintain epithelial cell-cell interactions, and loss of E-cadherin is one of the key features of the EMT phenotype.[72] Mutations

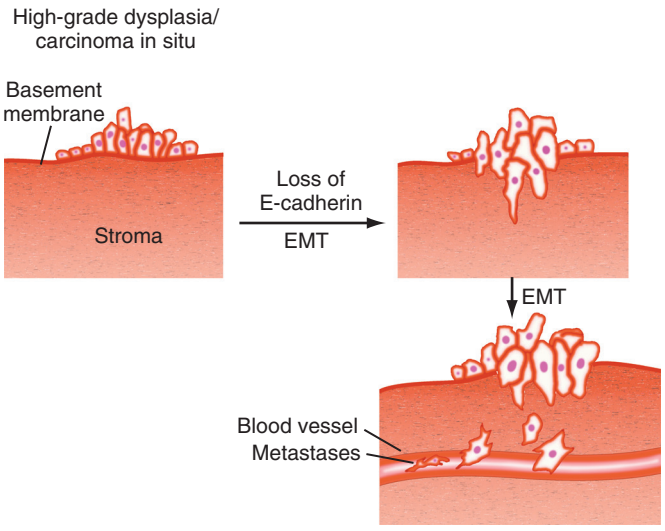

FIGURE 1-7. An epithelial-mesenchymal transition (EMT) provides a model for tumor progression and invasion. Epithelial cells maintain their polarity and boundaries with adjacent cells through many junctional proteins, including E-cadherin. The loss or down-regulation of E-cadherin is a key feature in EMT, wherein epithelial cells can adopt a migratory mesenchymal phenotype. In tumor progression, EMT can occur at multiple levels, including the transition from early carcinoma in situ to invasive cancer, as well as the invasion of a tumor cell into blood and lymphatic vessels.

in E-cadherin are common in many GI cancers, particularly gastric cancer. Germline mutations in E-cadherin are linked to hereditary diffuse gastric cancer.

The epithelial basement membrane consists of a dense matrix of collagen, glycoproteins, and proteoglycans and normally does not permit passive penetration of cells. The transmigration of tumor cells through the basement membrane likely involves production of key proteolytic activities. Alternatively, the tumor cell may produce factors capable of activating proenzymes present in the extracellular matrix. For example, the tumor may produce urokinase, itself a protease, or plasminogen activator. Having gained access to the interstitial stromal compartment, tumor cells can then enter lymphatic and blood vessels and metastasize.

Angiogenesis and Lymphangiogenesis

Angiogenesis is essential to sustain continued growth of the primary tumor. If new vessels are not developed as the primary tumor expands, cells most distant from available vessels are deprived of an adequate source of nutrition, and central necrosis occurs. Neovascularization is also an important permissive factor in facilitating metastatic dissemination of tumors.[73] A number of protein growth factors produced by malignant tumor cells and stromal cells have been found to be potent stimuli of angiogenesis, including vascular endothelial growth factor (VEGF)-A, basic fibroblast growth factor (bFGF), and TGF-β. VEGF-A is perhaps the most critical factor that is up-regulated in most tumor types, including colorectal cancer. Multiple genetic pathways implicated in GI carcinogenesis modulate *VEGF-A* expression, including Wnt and mutant *ras*.[74] Therapeutic strategies that inhibit VEGF-A are now standard-of-care therapies in metastatic colorectal cancer (see Chapter 127).

Angiogenesis occurs in an ordered series of events. Endothelial cells in the parent vessel are stimulated to degrade the endothelial basement membrane, migrate into the perivascular stroma, and initiate a capillary sprout. The sprout develops into a tubular structure that in turn develops into a capillary network. In vitro models that recapitulate the early events of angiogenesis indicate that this process involves a balance between proteases and protease inhibitors in a manner similar to that during tumor invasion. Indeed, functional parallels between tumor invasion and angiogenesis are evident in their mutual requirement for cellular motility, basement membrane proteolysis, and cell growth.

In addition to angiogenesis, lymphangiogenesis plays an important role in tumor metastasis. Some important clues into the molecular basis of tumor lymphangiogenesis have been obtained. VEGF-C or VEGF-D bind to the VEGF receptor-3 on lymphatic endothelial cells to stimulate formation of new lymphatic vessels.[75] This results in the development of new lymphatic channels within the tumor mass and, consequently, enhanced dissemination of tumor cells to regional lymph nodes.[76] Strategies to inhibit tumor lymphangiogenesis are being actively pursued.

MOLECULAR MEDICINE: CURRENT AND FUTURE APPROACHES IN GASTROINTESTINAL ONCOLOGY

Molecular Diagnostics

Progress in the identification of cancer-associated genes coupled with the inherent power of molecular biological techniques to analyze exquisitely small amounts of DNA and protein are leading to more effective diagnostic markers. The most immediate application is assessment of cancer risk in members of cancer-prone kindreds. Strategies have been developed to identify germline mutations in patients with a variety of inherited GI cancer syndromes, including FAP, Lynch syndrome, and hereditary diffuse gastric cancer (HDGC) (see Table 1-1). Genetic testing is a powerful tool to identify high-risk families and define the cancer risk for individual family members. Application of genetic testing must take into consideration the sensitivity and specificity of the assay as well as issues of patient confidentiality and potential impact on medical insurability. For these reasons, genetic counseling is an essential component of the genetic testing process.

Improved detection of sporadic GI cancers and their precursor lesions has also been the focus of research studies. Small numbers of shed cells obtained from stool or fluid aspiration from cysts can be assessed for the presence of mutations or epigenetic alterations in specific tumor-associated genes (*B-raf, K-ras, APC, TP53,* etc.). MSI testing can be performed on archived colon tumor samples and serves as a useful screening test to identify individuals whose colorectal cancers may have developed as a manifestation of the Lynch syndrome or the serrated pathway to colorectal cancer.[77] Loss of MSH2, MLH1, PMS2, or MSH6 immunohistochemical staining may provide similar information. Studies have demonstrated that the MSI status of a colon tumor is predictive of the response to 5-fluorouracil–based chemotherapy.[78,79] Therapies that target specific signaling pathways are likely to increase as our molecular understanding of GI cancers increases. Antibodies that target EGF receptors and block the EGF receptor signaling pathway have proved therapeutic benefit in colorectal cancer. However, their benefit has been shown only in cancers lacking activating mutations in *K-ras*. Testing for *K-ras* mutations in colorectal cancers is now standard of care before administration of such targeted therapy. In addition, small molecule tyrosine kinase inhibitors of the *c-KIT* oncogene now constitute routine treatment of GI stromal tumors (see Chapter 32).[80] Molecular techniques may also find a role in the staging of disease. For example, capture of small numbers of circulating tumor cells prior to the discovery of metastasis may yield prognostic and therapeutic benefits.[81] Finally, as more tests for genetic markers become available, monitoring for disease recurrence after surgery may become another important application.

Genome-wide Association Studies

Although 11% of individuals with colorectal cancer have 2 close family members with the disease, only a small fraction of those occur within an already defined mendelian cancer syndrome.[82] Moreover, identical twin studies of colorectal cancer only demonstrate a 35% risk in the sibling. Identification of other genetic variants that confer an increased risk of colorectal cancer remains a high priority. Given the development of genotyping and deep-sequencing technologies, many such variants have been discovered. Two underlying hypotheses, which are not mutually exclusive, have driven the search for these variants.

The *common disease–common variant hypothesis* is based on the idea that the heritable risk for illnesses like colorectal cancer is based on the summation of the small effects from genetic variants that are common (minor allelic frequency >5%) in the general population. Thus far, many loci have been identified. However, the small relative risk of each associated common variant has not yielded any more predictive information than family history for diseases like colorectal cancer. Despite this limited clinical applicability, identification of

novel genes not previously associated with the disease raises the possibility of new therapeutic and diagnostic approaches. Another caveat of such studies is that such variants are not necessary causal but merely associated, since other variants may be in linkage disequilibrium with the variant of interest.

The *common disease–rare variant hypothesis* is based on the premise that the genetic risk of diseases such as colorectal cancer are primarily driven by a heterogeneous set of rare or de novo mutations. In most studies, *rare variants* are defined as those with a minor allelic frequency of less than 1% in the general population. Compared to common variants, rare variants are more likely to have larger effect sizes owing to the effect of purifying selection. Recent studies, however, demonstrate a bulk of the rare variants likely occurred over the past 5000 years and were due to population expansion and relatively weaker purifying selection of these variants.[83] Advantages of rare variant studies are that the identified variant is more likely to be directly implicated in disease, given the lack of linkage disequilibrium with other variants. Given the larger effect sizes, these variants may also play a key role in clinical decision making.

Whole Genome Sequencing and Exome Sequencing

Given the decline in DNA sequencing costs, considerable interest exists in incorporating the full genomic profile of tumors and cancers into clinical care, with the goal of identifying tailored therapeutics suitable for each individual. At present, 2 strategies are being actively pursued. The first is whole genome sequencing, where the entire genome of the tumor is detailed. As our understanding of the non–protein coding genome evolves, the expectation is that we may discover novel prognostic and therapeutic strategies based on non–protein coding regions of the genome. Another method is to exclusively focus on the exome, the protein-coding portion of the genome. Although only comprising 1% of the genome, the exome is believed to contain approximately 85% of the mutations associated with disease, and the cost of exome sequencing is a fraction of whole genome sequencing. Multiple efforts, including the National Cancer Institute–sponsored Cancer Genome Atlas Project and International Cancer Genome Consortium, are underway to catalog the variation in a large number of cancers.

KEY REFERENCES

Full references for this chapter can be found on www.expertconsult.com.

2. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012; 487:330-7.
11. Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. Cell 2007; 130:223-33.
22. Weisenberger DJ, Siegmund KD, Campan M, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nat Genet 2006; 38:787-93.
24. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012; 366:883-92.
25. Nguyen LV, Vanner R, Dirks P, et al. Cancer stem cells: An evolving concept. Nat Rev Cancer 2012; 12:133-43.
33. Knudson AG Jr. Mutation and cancer: Statistical study of retinoblastoma. Proc Natl Acad Sci U S A 1971; 68:820-3.
58. Chan AT, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. N Engl J Med 2007; 356:2131-42.
60. Hutvagner G, McLachlan J, Pasquinelli AE, et al. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 2001; 293:834-8.
69. Grivennikov SI, Wang K, Mucida D, et al. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. Nature 2012; 491:254-8.
83. Tennessen JA, Bigham AW, O'Connor TD, et al. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. Science 2012; 337:64-9.

REFERENCES

1. Sherr CJ. The Pezcoller lecture: Cancer cell cycles revisited. Cancer Res 2000; 60:3689-95.
2. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012; 487:330-7.
3. Weinberg RA. The retinoblastoma protein and cell cycle control. Cell 1995; 81:323-30.
4. el-Deiry WS, Tokino T, Velculescu VE, et al. WAF1, a potential mediator of p53 tumor suppression. Cell 1993; 75:817-25.
5. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature 1993; 366:704-7.
6. Liu Q, Yan YX, McClure M, et al. MTS-1 (CDKN2) tumor suppressor gene deletions are a frequent event in esophagus squamous cancer and pancreatic adenocarcinoma cell lines. Oncogene 1995; 10:619-22.
7. Caldas C, Hahn SA, da Costa LT, et al. Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma. Nat Genet 1994; 8:27-32.
8. Muraoka RS, Lenferink AE, Law B, et al. ErbB2/Neu-induced, cyclin D1-dependent transformation is accelerated in p27-haploinsufficient mammary epithelial cells but impaired in p27-null cells. Mol Cell Biol 2002; 22:2204-19.
9. Zhang Y, Xiong Y, Yarbrough, WG. ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. Cell 1998; 92:725-34.
10. Reed JC. Mechanisms of apoptosis. Am J Pathol 2000; 157:1415-30.
11. Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. Cell 2007; 130:223-33.
12. Hayflick L. Mortality and immortality at the cellular level. A review. Biochemistry 1997; 62:1180-90.
13. Cech TR. Beginning to understand the end of the chromosome. Cell 2004; 116:273-9.
14. Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell 2000; 103:211-25.
15. McCormick F. Signalling networks that cause cancer. Trends Cell Biol 1999; 9:M53-6.
16. Parekh DB, Ziegler W, Parker PJ. Multiple pathways control protein kinase C phosphorylation. EMBO J 2000; 19:496-503.
17. Chung DC. The genetic basis of colorectal cancer: Insights into critical pathways of tumorigenesis. Gastroenterology 2000; 119:854-65.
18. Korinek V, Barker N, Moerer P, et al. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. Nat Genet 1998; 19:379-83.
19. Pinto D, Gregorieff A, Begthel H, Clevers H. Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. Genes Dev 2003; 17:1709-13.
20. Siegel PM, Massague J. Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. Nat Rev Cancer 2003; 3:807-21.
21. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. Nature 1998; 396:643-9.
22. Weisenberger DJ, Siegmund KD, Campan M, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nat Genet 2006; 38:787-93.
23. Nowell PC. The clonal evolution of tumor cell populations. Science 1976; 194:23-8.
24. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012; 366:883-92.
25. Nguyen LV, Vanner R, Dirks P, et al. Cancer stem cells: An evolving concept. Nat Rev Cancer 2012; 12:133-43.
26. Merlos-Suarez A, Barriga FM, Jung P, et al. The intestinal stem cell signature identifies colorectal cancer stem cells and predicts disease relapse. Cell Stem Cell 2011; 8:511-24.
27. Sigal I. The ras oncogene: Structure and some function. Nature 1989; 332:485.
28. Bos JL, Fearon ER, Hamilton SR, et al. Prevalence of ras gene mutations in human colorectal cancers. Nature 1987; 327:293-7.
29. Bourne HR, Sanders DA, McCormick F. The GTPase superfamily: A conserved switch for diverse cell functions. Nature 1990; 348:125-32.
30. Rajagopalan H, Bardelli A, Lengauer C, et al. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. Nature 2002; 418:934.
31. Luscher B, Eisenman RN. New light on Myc and Myb. Part I. Myc. Genes Dev 1990; 4:2025-35.
32. He TC, Sparks AB, Rago C, et al. Identification of c-MYC as a target of the APC pathway. Science 1998; 281:1509-12.
33. Knudson AG Jr. Mutation and cancer: Statistical study of retinoblastoma. Proc Natl Acad Sci U S A 1971; 68:820-3.
34. Comings DE. A general theory of carcinogenesis. Proc Natl Acad Sci U S A 1973; 70:3324-8.
35. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 2003; 349:2042-54.
36. Leppert M, Dobbs M, Scambler P, et al. The gene for familial polyposis coli maps to the long arm of chromosome 5. Science 1987; 238:1411-13.
37. Kinzler KW, Nilbert MC, Vogelstein B, et al. Identification of a gene located at chromosome 5q21 that is mutated in colorectal cancers. Science 1991; 251:1366-70.
38. Groden J, Thliveris A, Samowitz W, et al. Identification and characterization of the familial adenomatous polyposis coli gene. Cell 1991; 66:589-600.
39. Nishisho I, Nakamura Y, Miyoshi Y, et al. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science 1991; 253:665-9.
40. Powell SM, Zilz N, Beazer-Barclay Y, et al. APC mutations occur early during colorectal tumorigenesis. Nature 1992; 359:235-7.
41. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990; 61:759-67.
42. Spirio L, Olschwang S, Groden J, et al. Alleles of the APC gene: An attenuated form of familial polyposis. Cell 1993; 75:951-7.
43. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: Clues to cancer etiology and molecular pathogenesis. Cancer Res 1994; 54:4855-78.
44. Nigro JM, Baker SJ, Preisinger AC, et al. Mutations in the p53 gene occur in diverse human tumour types. Nature 1989; 342:705-8.
45. Bressac B, Kew M, Wands J, et al. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. Nature 1991; 350:429-31.
46. Malkin D, Li FP, Strong LC, et al. Germline p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 1990; 250:1233-8.
47. Seoane J, Le HV, Massague J. Myc suppression of the p21(Cip1) Cdk inhibitor influences the outcome of the p53 response to DNA damage. Nature 2002; 419:729-34.
48. Liu F, Pouponnot C, Massague J. Dual role of the Smad4/DPC4 tumor suppressor in TGFbeta-inducible transcriptional complexes. Genes Dev 1997; 11:3157-67.
49. Chung DC, Rustgi AK. DNA mismatch repair and cancer. Gastroenterology 1995; 109:1685-99.

50. Fishel R, Lescoe MK, Rao MR, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 1993; 75:1027-38.
51. Leach FS, Nicolaides NC, Papadopoulos N, et al. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 1993; 75:1215-25.
52. Parsons R, Li GM, Longley MJ, et al. Hypermutability and mismatch repair deficiency in RER+ tumor cells. Cell 1993; 75:1227-36.
53. Aaltonen LA, Peltomaki P, Mecklin JP, et al. Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. Cancer Res 1994; 54:1645-8.
54. Al-Tassan N, Chmiel NH, Maynard J, et al. Inherited variants of MYH associated with somatic G:C→T:A mutations in colorectal tumors. Nat Genet 2002; 30:227-32.
55. Sieber OM, Lipton L, Crabtree M, et al. Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in MYH. N Engl J Med 2003; 348:791-9.
56. Zimmermann S, Moelling K. Phosphorylation and regulation of Raf by Akt (protein kinase B). Science 1999; 286:1741-4.
57. Ulku AS, Der CJ. Ras signaling, deregulation of gene expression and oncogenesis. Cancer Treat Res 2003; 115:189-208.
58. Chan AT, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. N Engl J Med 2007; 356:2131-42.
59. Iorio MV, Croce CM. MicroRNAs in cancer: Small molecules with a huge impact. J Clin Oncol 2009; 27:5848-56.
60. Hutvagner G, McLachlan J, Pasquinelli AE, et al. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 2001; 293:834-8.
61. Turcan S, Rohle D, Goenka A. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. Nature 2012; 483:479-83.
62. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. J Gen Physiol 1927; 8:519-30.
63. Niemann S, Muller U. Mutations in SDHC cause autosomal dominant paraganglioma, type 3. Nat Genet 2000; 26:268-70.
64. Astuti D, Latif F, Dallol A, et al. Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am J Hum Genet 2001; 69:49-54.
65. Miller EC, Miller JA. Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules. Cancer 1981; 47:2327-45.
66. Ozturk M. p53 mutations in nonmalignant human liver: Fingerprints of aflatoxins? Hepatology 1995; 21:600-1.
67. Bortsch H. N-nitroso-compounds and human cancer: Where do we stand? IARC Sci Publ 1991; 103:105.
68. Haenszel W, Kurihara M. Studies of Japanese migrants. I. Mortality from cancer and other diseases among Japanese in the United States. J Natl Cancer Inst 1968; 40:43-68.
69. Grivennikov SI, Wang K, Mucida D, et al. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. Nature 2012; 491:254-8.
70. Lin WW, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest 2007; 117:1175-83.
71. Fidler IJ, Radinsky R. Genetic control of cancer metastasis. J Natl Cancer Inst 1990; 82:166-8.
72. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002; 2:442-54.
73. Liotta LA, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: An imbalance of positive and negative regulation. Cell 1991; 64:327-36.
74. Zhang X, Gaspard JP, Chung DC. Regulation of vascular endothelial growth factor by the Wnt and K-ras pathways in colonic neoplasia. Cancer Res 2001; 61:6050-4.
75. Jeltsch M, Kaipainen A, Joukov V, et al. Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. Science 1997; 276:1423-5.
76. Skobe M, Hawighorst T, Jackson DG, et al. Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nat Med 2001; 7:192-8.
77. Giardiello FM, Brensinger JD, Petersen GM. AGA technical review on hereditary colorectal cancer and genetic testing. Gastroenterology 2001; 121:198-213.
78. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 2003; 349:247-57.
79. Sargent DJ, Marsoni S, Monges G, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol 2010; 28:3219-26.
80. Demetri GD, Benjamin RS, Blanke CD, et al. NCCN Task Force report: Management of patients with gastrointestinal stromal tumor (GIST)—update of the NCCN clinical practice guidelines. J Natl Compr Canc Netw 2007; 5(Suppl 2):S1-29.
81. Liefers GJ, Cleton-Jansen AM, van de Velde CJ, et al. Micrometastases and survival in stage II colorectal cancer. N Engl J Med 1998; 339:223-8.
82. Fletcher RH, Lobb R, Bauer MR, et al. Screening patients with a family history of colorectal cancer. J Gen Intern Med, 2007; 22:508-13.
83. Tennessen JA, Bigham AW, O'Connor TD, et al. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. Science 2012; 337:64-9.

CHAPTER

2

Mucosal Immunology and Inflammation*

IRIS DOTAN AND LLOYD MAYER

CHAPTER OUTLINE

Mucosal immunity refers to immune responses that occur at mucosal sites. The demands upon the mucosal immune system are quite distinct from their systemic counterparts. At mucosal sites, the "outside world" is typically separated from the inner world by a single layer of epithelium. The mucosal immune system exists at a number of sites, including the GI tract, respiratory tract (especially the upper respiratory tract), urogenital tract, mammary glands, eyes, and ears. Regardless of the site, unique lymphoid and other cell populations are required to handle a wide array of environmental stimuli. Together these sites are called *mucosa-associated lymphoid tissue* (MALT).[1-5]

The intestine is the site most often associated with mucosal immunity and is unique in several aspects. Relative to other mucosal sites, the intestine is the least sterile, containing billions to trillions of microorganisms, mainly bacteria. These organisms, along with ingested food, represent an enormous antigenic load that must be tolerated to maintain the status quo in the intestine. This unusual environment and the demands associated with it have resulted in the development of a distinct immune system designated the *gut-associated lymphoid tissue* (GALT).

The specific characteristics and peculiarities of the GALT reflect the unique milieu in which it needs to function. To maintain homeostasis in the intestine, one of the most important tasks of the GALT is to differentiate between potentially harmful antigens (e.g., pathogenic bacteria or toxins) from ones that may benefit the body (e.g., derived from food or commensal bacteria). To achieve homeostasis, unusual cell types, immunoglobulins (Igs), and secreted mediators have to function in a coordinated fashion. In contrast to the systemic immune system, whose focus is to act quickly within seconds of encountering a foreign antigen ("first shoot, then talk"), the GALT is poised to respond but is predominantly tolerant, rejecting harmful antigens but allowing beneficial/harmless ones to persist without evoking immune responses like allergic reactions or inflammation.

The unique ways the GALT performs in its demanding environment are the focus of this chapter, along with the consequences of abnormal GALT function that result in intestinal disease.

*The editors and Dr. Dotan dedicate this chapter to the scientific achievements and the legacy of Lloyd Mayer, MD, a leader in the field of mucosal immunology, a mentor, and a dear colleague and friend.

IMMUNE RESPONSES IN GUT-ASSOCIATED LYMPHOID TISSUE

The hallmark of mucosal immunity, in contrast to systemic immunity, is suppression as exemplified by 2 linked phenomena: controlled/physiologic inflammation and oral tolerance. These 2 processes are mediated by a unique anatomy, distinct resident cell populations, and selective antibody isotypes.

Controlled/Physiologic Inflammation

Billions of activated plasma cells, memory T cells, memory B cells, macrophages, and dendritic cells exist within the lamina propria (LP).[6,7] Given the large surface area of the GI tract and the resident cell populations that inhabit this space, the gut is the largest lymphoid organ in the body. Still, in contrast to activated lymphocytes in the peripheral immune system, significant inflammation is not present in the intestine. This phenomenon has been called *controlled/physiologic inflammation* (Fig. 2-1). Entry of immune cells into the LP and cell activation is antigen driven. Germ-free mice have few immune cells in their LP, but within hours to days following colonization with normal intestinal flora (no pathogens), there is a massive influx and activation of cells.[8-11] Despite the persistence of an

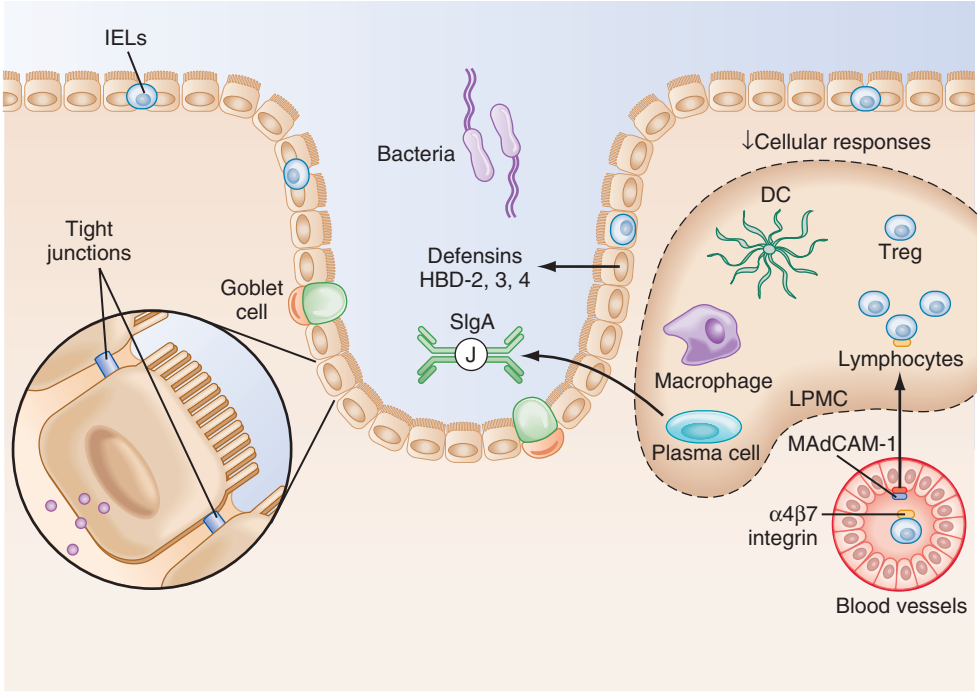

FIGURE 2-1. Mechanisms for damping mucosal immune responses. The intestine uses a number of distinct mechanisms to dampen mucosal immune responses. The major source of antigen in the intestine is the commensal bacterial flora, but both innate and adaptive responses control local responses. Physical barriers like mucins secreted by goblet cells and tight junctions between epithelial cells prevent invasion by luminal flora *(circle inset)*. Defensins like HBD-2, -3, and -4 are thought to maintain sterility of the crypt, whereas secretory immunoglobulin A produced by local plasma cells prevents attachment and invasion by luminal bacteria, thereby reducing antigenic load. Even with antigenic challenge, intestinal lymphocytes, macrophages, and dendritic cells are programmed to not respond as a consequence of decreased expression of pattern recognition receptors (e.g., Toll-like receptors) and a decrease in the ability of lymphocytes to be activated through their antigen receptor. Egress of circulating lymphocytes expressing the integrin α4β7, which recognizes the addressin MAdCAM-1, is also shown. DC, dendritic cell; HBD, human β-defensin; IELs, intraepithelial lymphocytes; LPMC, lamina propria mononuclear cells; MAdCAM, mucosal addressin cell adhesion molecule; SIgA, secretory immunoglobulin A, a dimer with a connecting J chain; Treg, T regulatory cells (formerly known as *suppressor T cells*).

antigen drive (luminal bacteria), the cells fail to develop into aggressive inflammation-producing lymphocytes and macrophages. Bacteria or their products play a role in this persistent state of activation[12] and likely contribute to the controlled inflammatory process as well.

The failure to produce pathology despite the activation state of intestinal lymphocytes is probably the consequence of regulatory mechanisms. The failure of LP lymphocytes (LPLs) to generate "normal" antigen receptor–mediated responses is an important factor in controlled inflammation (i.e., lack of expansion despite the presence of activation). LPLs respond poorly when activated via the T cell receptor (TCR), failing to proliferate, although they can still produce cytokines.[13,14] This is key to the maintenance of controlled inflammation.

Oral Tolerance

The most recognized phenomenon equated with mucosal immunity and associated with suppression is oral tolerance.[15-21] *Oral tolerance* can be defined as the active antigen-specific non-response to antigens administrated orally.[18,22,23]

How does the body regulate the response to the vast amount of antigens introduced via the oral route, specifically those that avoid complete digestion? For example, up to 2% of dietary proteins enter the draining enteric vasculature intact.[23] Non-response to these antigens is achieved by oral tolerance. The mucosal immune system in the intestine is separated from the continuous antigenic bombardment composed of food, intestinal secretions, and microorganisms by a single layer of intestinal epithelial cells (IECs). Its ability to discriminate between harmful and harmless, or even beneficial, antigens and to generate a differential immune response toward each type of antigens is a complex process extensively investigated in animal models and existing in humans.[24,25] Disruption of oral tolerance and of a more local mechanism, mucosal-induced tolerance, may result in food allergies and food intolerances like celiac disease, as well as in inflammatory bowel diseases.

An important difference between oral tolerance against food antigens and mucosal tolerance against microorganisms is that the former has both local (intestinal) and systemic consequences, whereas the latter does not attenuate systemic immune responses.[21] Factors affecting the induction of oral tolerance include the host's age, genetic factors, nature of the antigen, and the tolerogen's form and dose. The state of the intestinal barrier also affects oral tolerance, and when barrier function is reduced, oral tolerance decreases. Part of the explanation for oral tolerance relates to the properties of digestion per se, where large macromolecules are degraded so that potentially immunogenic substances are rendered non-immunogenic.

As just mentioned, oral tolerance is age dependent. Oral tolerance is difficult to achieve in the neonate, probably owing to the rather permeable intestinal barrier that exists in the newborn, as well as the immaturity of the mucosal immune system. Within 3 months of age (in the mouse), oral tolerance

can be induced, and many previous antibody responses to food antigens are suppressed. The limited diet in the newborn may further serve to protect the infant from generating a vigorous response to food antigens. Furthermore, the intestinal flora has been demonstrated to affect the development of oral tolerance. Probiotics (e.g., *Lactobacillus* GG) given to mothers before delivery and during lactation provided protection against development of atopic eczema in their offspring.[26] Continuous exposure to microbial compounds (e.g., lipopolysaccharides) during pregnancy and early infancy was associated with a lower prevalence of atopy and asthma in children.[27,28] The effects of probiotics on oral tolerance are probably mediated through modulation of cytokine responses,[29] the positive effect on intestinal barrier function and restitution of tight junctions,[30,31] suppression of intestinal inflammation via down-regulation of Toll-like receptor (TLR) expression,[32,33] and secretion of metabolites that may inhibit inflammatory cytokine production by mononuclear cells.

A role of genetic factors in oral tolerance has been suggested in murine models, where certain strains develop tolerance more easily than others.[34,35]

The nature and form of the antigen also play a significant role in tolerance induction. Protein antigens are the most tolerogenic, whereas carbohydrates and lipids are much less effective at inducing tolerance.[31] The form of the antigen is also critical; a protein such as ovalbumin (OVA) given in soluble form is quite tolerogenic, whereas aggregation of OVA reduces its potential to induce tolerance. This difference may be associated with an alteration in the sites of antigen sampling.[6] Exposure (prior sensitization) to an antigen through an extraintestinal route also affects the development of tolerance responses.

The dose of antigen administered was also considered critical to the form of oral tolerance generated. In mouse models, high doses of antigen were suggested to be associated with clonal deletion or anergy of T cells.[36,37] In this setting, transfer of T cells from tolerized to non-tolerized animals does not lead to transfer of tolerance. Low doses of antigen, on the other hand, were shown to activate regulatory/suppressor T cells.[38,39] More recent work suggested that high antigen dosing was an effective inducer of $FOXP3^+$ regulatory T cells (Treg cells),[40] but the effect of antigen dose on oral tolerance remains to be redefined. Treg cells of both CD4 and CD8 lineages have a central role in oral tolerance. Th3 cells were the initial regulatory/suppressor cells described as mediators of oral tolerance.[41-43] These cells appear to be activated in the Peyer's patch and secrete transforming growth factor (TGF)-β. This cytokine plays a dual role in mucosal immunity; it is the most potent suppressor of T and B cell responses while promoting the production of IgA (it is the IgA switch factor).[44-47] Production of TGF-β by Th3 cells elicited by low-dose antigen administration helps explain an associated phenomenon of oral tolerance bystander suppression. Oral tolerance is antigen specific, but the effector arm is antigen nonspecific. If an irrelevant antigen is co-administered systemically with the tolerogen, suppression of T and B cell responses to that irrelevant antigen will occur as well (hence, bystander suppression). Secreted TGF-β suppresses the response to the co-administered antigen. Tr1 cells may also participate in bystander suppression and oral tolerance by producing interleukin (IL)-10, another potent immunosuppressive cytokine.[48-50] Evidence for the activation of $CD4^+CD25^+$ Treg cells during oral tolerance also exists.[51-55] Tolerance studies performed in mice depleted of $CD4^+CD25^+$ T cells, coupled with neutralization of TGF-β, demonstrated that $CD4^+CD25^+$ T cells and TGF-β together are involved in the induction of oral tolerance, partly through regulation of the expansion of antigen-specific $CD4^+$ T cells.[56] The ability to identify regulatory $CD4^+CD25^+$ T cell subpopulations was enhanced by the recognition that these cells express the transcription factor Forkhead box P3 (FoxP3). Because not every cell within the $CD4^+CD25^+$ population is a naturally occurring Treg cell, the ability to use FoxP3 as a marker of these Treg cells has been a major breakthrough in our ability to study them.[57-61] Importantly, in mice, absence of $CD4^+$ Treg cell activity results in IBD, whereas its expansion ameliorates murine colitis.[62-66] In IBD patients, the number of Treg cells is generally greater than in controls, and a peripheral-to-intestinal shift has been suggested.[66-72] Whether their failure to protect against IBD is due to an intrinsic defect or microenvironmental effect is still being investigated.[73]

A role for antigen-specific $CD8^+$ T cells in oral tolerance,[74-79] as well as in the regulation of mucosal immune responses, has been suggested by several groups. Specifically, in vitro activation of human $CD8^+$ peripheral blood T cells by normal IECs results in the expansion of $CD8^+CD28^-$ T cells with regulatory activity.[80] Moreover, in the LP of IBD patients, such cells were significantly reduced, supporting a role for these epithelial-induced T regulatory (TrE) cells in the control of intestinal inflammation.[81]

Another important factor affecting tolerance induction is the state of the intestinal barrier. In addition to failure to generate tolerance in the neonate (because intestinal permeability is higher), several other states of barrier dysfunction are associated with aggressive inflammation and a lack of tolerance. In anaphylaxis, increased intestinal permeability allows antigens to pass through paracellular spaces by disrupting tight junctions.[82-84] Treatment of mice with interferon (IFN)-γ can disrupt the mucosal barrier, and these mice fail to develop tolerance in response to OVA feeding. Perhaps even more interesting observations are failure of N-cadherin dominant negative mice to suppress mucosal inflammation (loss of controlled inflammation), possibly because of the enormous antigenic exposure resulting from the leaky barrier in these mice.[85] Increased susceptibility of Nod1- and Nod2-deficient mice to colitis (associated with increased paracellular permeability and decreased E-cadherin) that could be modified using specific commensals and probiotic strains points to an interplay of genetic and microbial factors in intestinal barrier function and controlled or uncontrolled inflammation.[86]

Lastly, oral tolerance may also be influenced by the cell serving as the antigen-presenting cell, as well by as the site of antigen uptake. In mice, orally administered reovirus type III is taken up by M cells expressing reovirus type III–specific receptors (Fig. 2-2).[87] This induces an active IgA response. In contrast, reovirus I infects IECs and induces tolerance. Thus, the route of entry (M cell vs. IEC) of a specific antigen may dictate the type of immune response generated (IgA vs. tolerance). Interestingly, poliovirus, one of the few oral vaccines effective in man, binds to M cells, and this may account for its ability to stimulate active immunity in the gut.[88]

UNUSUAL IMMUNOGLOBULINS OF GUT-ASSOCIATED LYMPHOID TISSUE

The unique antibody, secretory IgA (sIgA), is the hallmark of MALT/GALT immune responses (Fig. 2-3). IgG is the most abundant isotype in the systemic immune system, but IgA is the most abundant antibody in mucosal secretions.[87,89,90] Given the numbers of IgA^+ plasma cells and the extent of the MALT, IgA is the most abundant antibody in the body.

SIgA is a dimeric form of IgA produced in the LP and transported into the lumen through the intestinal epithelium by a specialized pathway (Fig. 2-4). Two IgA molecules (homodimers) are bound together by J chain (produced by plasma cells). Subsequently the homodimer binds to a highly

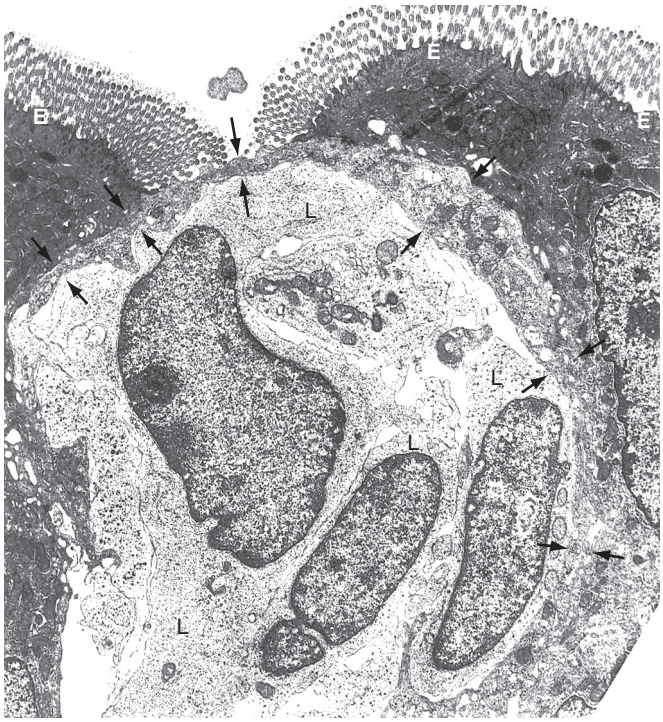

FIGURE 2-2. M cell. Transmission electron micrograph from non-columnar region of a Peyer's patch epithelium shows a cross-sectional view of a microfold (M) cell, as well as associated microvillus-covered intestinal epithelial cells and at least 3 lymphoid cells *(L)*. Note the attenuated cytoplasm of the M cell *(between arrows)* that bridges the surface between microvillus-covered epithelial cells, forming tight junctions with them and producing a barrier between lymphoid cells and the intestinal lumen (×9600). B, B cell; E, intestinal epithelial cell. *(From Owen RL, Jones AL. Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. Gastroenterology 1974; 66:189-203.)*

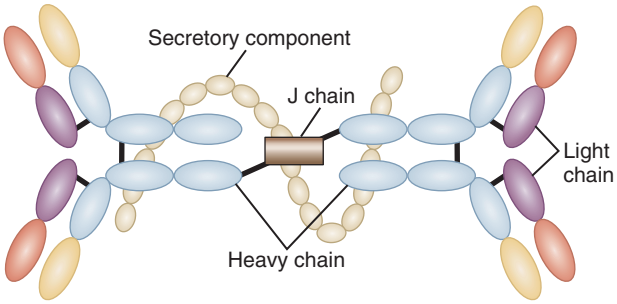

FIGURE 2-3. Secretory immunoglobulin (Ig)A complex. Two IgA molecules are linked by a J chain and stabilized by secretory component (polymeric Ig receptor) to form dimeric secretory IgA.

specialized glycoprotein, secretory component n (also called the *polymeric Ig receptor*), a 55-kd glycoprotein produced by epithelial cells. The polymeric Ig receptor is expressed on the basolateral membrane of the IEC and binds only to dimeric IgA or IgM (also polymerized with J chain). Once bound to the IEC, SIgA is actively transported within vesicles to the apical membrane of the IEC. The vesicle fuses with the apical membrane, and the secretory component/IgA complex is

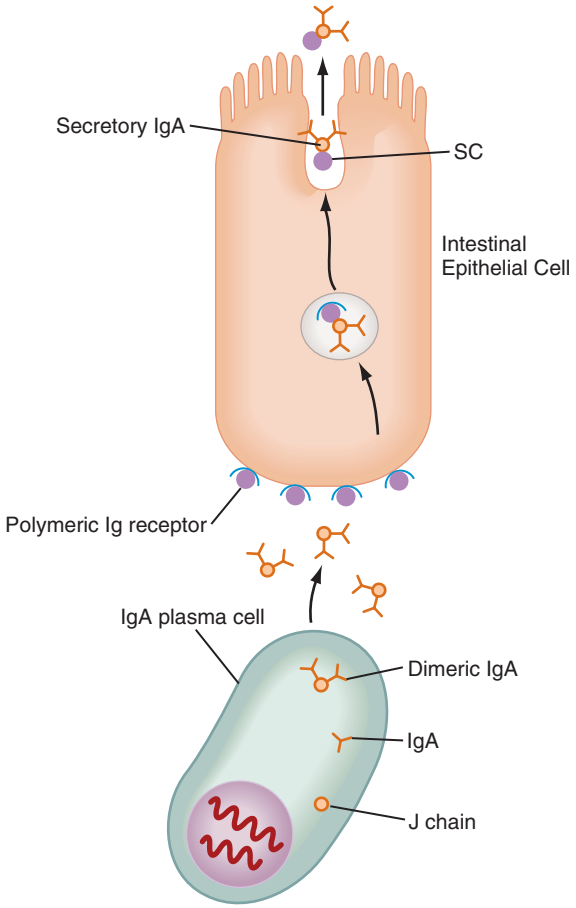

FIGURE 2-4. Assembly and secretion of dimeric immunoglobulin (Ig)A. IgA and J chain produced by IgA-committed plasma cells *(bottom)* dimerize to form polymeric IgA, which covalently binds to membrane-bound polymeric Ig receptor produced by intestinal epithelial cells *(top)*. This complex is internalized, transported to the apical surface of epithelial cell, and secreted into the lumen. SC, secretory component.

released into the intestinal lumen. Within the lumen, secretory component serves its second function, protection of the SIgA dimer from degradation by luminal proteases and gastric acid. SIgA and SIgM are the only antibodies that can bind secretory component and therefore withstand the harsh environment of the GI tract.

In addition to its unique form, SIgA is also unique in that it is anti-inflammatory in nature. It does not bind classical complement components but rather binds luminal antigens like toxins and pathogens, preventing their attachment to the epithelium or promoting their agglutination and subsequent removal of the antigen in the mucus layer overlying the epithelium.[89,91-98] This process reflects "immune exclusion," a process thought to include agglutination, entrapment, and clearance of antigen as the result of a specific interaction with the secreted antibody,[99] as opposed to nonspecific mechanisms of exclusion exerted by the epithelium (e.g., mucus barrier, proteolytic digestion, defensin secretion, etc.). Recently the ability of SIgA to exert specific protective immunity against certain pathogens via more direct mechanisms such as suppression of bacterial virulence was demonstrated,[100] as well as a fragment antigen–binding (Fab)-independent pathway of antibacterial activity mediated mainly via binding to bacterial glycan residues on the free or bound secretory component, or

the SIgA complex.[101,102] M cells in Peyer's patches selectively bind SIgA and SIgA immune complexes.[103,104] Although the M cell receptor for this specific interaction was not clearly identified, it was suggested that SIgA undergoes conformational changes following luminal antigen binding, which contributes to enhanced uptake of the SIgA-pathogen complex, as opposed to the excessively present SIgA.[105] This "retrotransport" of antigens, whether pathogens or allergens, was speculated to be a potential mechanism to dampen local inflammatory responses exerted when the same pathogens or allergens invade the intestinal mucosa uncoated with SIgA.[106]

IgM is another antibody capable of binding secretory component (pIgR). Like IgA, IgM uses J chain produced by plasma cells to form polymers—in the case of IgM, a pentamer. Secretory component binds to the Fc portion of the antibody formed during polymerization. The ability of IgM to bind secretory component may be important in patients with IgA deficiency, where secretory IgM (SIgM) may compensate for the absence of IgA in the lumen.

While SIgA is the major antibody isotype produced in the GALT, IgG has been detected as well.[107,108] The neonatal Fc receptor expressed by IECs (FcR_N) might serve as a bidirectional transporter of IgG[109,110] and may be important in control of neonatal infections and IgG metabolism. In patients with IBD, marked increases in IgG within the LP and lumen have been observed.[111]

Even IgE production may play an important role in intestinal diseases in the GALT. CD23 (low-affinity IgE Fc receptor) has been reported to be expressed by gut epithelial cells, and one model has suggested that it may play a role in facilitated antigen uptake and consequent mast cell degranulation in food allergy. In this setting, IgE transcytosis and mast cell degranulation may be associated with fluid and electrolyte loss into the lumen, an event intimately associated with an allergic reaction in the lung and gut.[112,113]

PHYSIOLOGY OF GUT-ASSOCIATED LYMPHOID TISSUE AND THE INTESTINAL BARRIER

The cells, structures, and mediators separating the intestinal lumen from the LP function as a physical barrier. However, this physical barrier is a biologically active structure that constantly interacts with its ever-changing environment. The intestinal barrier changes not only on a day-to-day basis but also through the years. Many barrier mechanisms are not fully developed at birth, and evidence in animal studies exists to support less restricted antigen transport in neonates compared to adults.

Factors in the upper GI tract influence the antigenic load that reaches the major sites of the GALT in the small and large bowel. Detailed exploration of these factors is beyond the scope of this chapter but include proteolysis, gastric acidity, and peristalsis.

The mucous coat lining the intestinal tract is composed of a mixture of glycoproteins (mucins) heavily glycosylated with *O*-linked oligosaccharides and *N*-glycan chains, linked to a protein backbone. There are at least 21 different mucin genes in the human genome, encoding secreted and membrane bound mucins, each with a distinct carbohydrate and amino acid composition.[114,115] The major colonic mucins are MUC1, MUC2, MUC3A, MUC3B, MUC4, MUC13, and MUC17. MUC2, produced in goblet cells, is a secreted mucin and serves as the primary component of intestinal mucus, while the other mucins listed are membrane bound. The membrane bound mucins participate in processes such as cell signaling, adhesion, growth, and immune modulation. Mucus protects the intestinal wall by several mechanisms. Its stickiness and competitive binding of its glycoprotein receptors decrease the ability of microorganisms to penetrate the intestine.[116-118] It also generates a stream that moves luminal contents away from epithelial cells. Intestinal infection and inflammation are associated with disruption or dysfunction of the mucous barrier involving altered commensal microbes and defective innate and adaptive host immune responses.[119]

Underneath the mucus layer, the physical barrier that prevents penetration of antigens across the intestinal epithelium consists of the epithelial cell per se (the transcellular route) and the tight intercellular spaces (the paracellular route) regulated by tight junction (TJ) complexes (e.g., zona occludens) and the subjunctional space.[120] Of the 2 structures, tight junctions have the greater role in preventing macromolecular diffusion across the epithelium, because these junctions exclude almost all molecules present in the lumen.[121] The barrier formed by the TJ is a dynamic structure that may be modified by various cytokines and growth factors. Some (e.g., IFN-γ, TNF-α, IL-1β, IL-4, IL-6, IL-13) increase intestinal TJ permeability, whereas others (IL-10, IL-17, TGF-β) decrease intestinal TJ permeability,[122] a characteristic that might be crucial for preventing intestinal inflammation like that seen in IBD.[123]

The epithelial cells themselves serve as a physical barrier in several ways: their microvilli are at a distance of about 25 nm from each other and are negatively charged. Thus a negatively charged molecule would be inhibited from passage even if its diameter was well below 25 nm. Despite these barriers, intact antigens may traverse the epithelium by fluid phase endocytosis and enter the circulation.[124]

FUNCTIONAL ANATOMY OF GUT-ASSOCIATED LYMPHOID TISSUE

To accomplish the goals of the mucosal immune system in the intestine (maintenance of homeostasis and clearance of pathogens), several key features have been identified. Compartmentalization of cells into distinct regions and sites despite being millimeters away from each other is a hallmark of the GALT. Cell populations and the immune response in the epithelium, subepithelial region, LP, Peyer's patches, and mesenteric lymph nodes (MLNs) may differ substantially.

The cells residing in these compartments differ not only topographically but also phenotypically and functionally, depending upon the anatomic site within the GALT. Cells with distinct phenotypes and functions are attracted to specific sites within the GALT.

Peyer's Patches and M Cells

The follicle-associated epithelium (FAE) is a specialized epithelium overlying the only organized lymphoid tissue of the GALT: the Peyer's patch. The M (microfold) cells in the FAE, in contrast to the adjacent absorptive epithelium, have few microvilli, a limited mucin overlayer, a thin elongated cytoplasm, and a shape that forms a pocket surrounding subepithelial lymphocytes, macrophages, T cells, B cells, and dendritic cells (DCs) (see Fig. 2-2). M cells are highly specialized for phagocytosis and transcytosis and are capable of taking up large particulate antigens from the lumen and transporting them intact into the subepithelial space.[125-130] They contain few lysosomes, so little or no processing of antigen occurs.[131] M cells are exposed to the lumen, thus having a larger area for contact with luminal contents. The M cell expresses several unique lectin-like molecules that help

promote binding to specific pathogens, the prototype being poliovirus.[132] Antigens that bind to the M cell and get transported to the underlying Peyer's patches generally elicit a positive (secretory IgA) response. Successful oral vaccines bind to the M cell and not to the adjacent epithelium. Thus, M cells appear to be critical for the initial positive aspects of mucosal immunity.[133,134] However, this may be a double-edged sword; certain pathogens or their toxins may exploit M cells and use transcytosis via M cells for penetration of the intestinal mucosa.[135,136]

The M cell is a conduit to the Peyer's patches. Antigens transcytosed across the M cell and into the subepithelial pocket are taken up by macrophages/DCs and carried into the Peyer's patch. Once antigens reach the patch, TGF-β–secreting T cells promote B cell isotype switching to IgA.[137] Importantly, there is a clear relationship between M cells and Peyer's patches. Induction of M cell differentiation has been shown to be dependent upon direct contact between the epithelium and PP lymphocytes.[138] This is mediated, at least in part, by the expression of NOTCH receptors and ligands.[139] In the absence of Peyer's patches there are no M cells. For example, M cells have not been identified in B cell–deficient animals (where there are no Peyer's patches).[140] Even though M cells and Peyer's patches may be involved in oral tolerance,[141-143] Peyer's patch–deficient mice are capable of developing tolerance after oral administration of soluble antigen.[144]

After activation in the Peyer's patch, lymphocytes are induced to express specific integrins (α4β7) that provide a homing signal for mucosal sites (where the ligand is MadCAM-1).[145-147] Lymphocytes then travel to the MLN and subsequently into the main intestinal lymphatic drainage system, the thoracic duct, which eventually empties into the circulation (Fig. 2-5). There, mucosally activated cells with their mucosal "addressins" circulate in the bloodstream to exit in high endothelial venules in various mucosal sites.[148] Those bearing α4β7 molecules exit in the MALT/GALT LP, where they undergo terminal differentiation. Chemokines and their receptors (discussed later) as well as adhesion molecules and ligands may help direct this trafficking pattern.

Intestinal Epithelial Cells

Intestinal epithelium is composed of a single layer of columnar cells. These IECs are derived from the basal crypts and differentiate into absorptive villous or surface epithelium or secretory goblet cells, neuroendocrine cells, and Paneth cells. In addition to their function as a physical barrier in the GALT, IECs contribute to both innate and adaptive immunity in the gut and may play a key role in maintaining intestinal homeostasis.

Antigen Trafficking Across the Epithelium

The ability of intact antigen to cross the lipid bilayer at the surface of the intestinal epithelium (underneath the microvilli) is limited, although invagination of apical membranes regularly occurs, allowing macromolecules to be carried into the cell within membrane-bound compartments.

Binding to the surface of the cell depends on the structure of the antigen and the chemical composition of the microvillous membrane. For instance, bovine serum albumin binds less efficiently to the intestinal epithelial surface than bovine milk protein, and as a consequence is transported less efficiently.[149] Structural alterations in an antigen caused by proteolysis might also affect its binding, because this will change the physicochemical characteristics of the molecule.[150] Several factors influence the transport of antigens from the apical to the basolateral surface of IECs. The rate of vesicular passage to the basolateral membrane depends on the rate of endocytosis, the proportion of vesicles trafficking to the lysosome, and the speed of travel of membrane-bound compartments. Lysosomally derived enzymes determine the rate of breakdown of products contained in membrane compartments. These include proteases like cathepsin B and D (found throughout the length of the intestine, particularly in the mid- and distal third of the small intestine), as well as those that catalyze carbohydrate breakdown, like acid phosphatase and mannosidase. It is the degree to which the organellar contents encounter such enzymes (in the lysosome or in endocytic vesicles) that determines the rate of intracellular destruction of macromolecules.[151] Although cathepsins are capable of catalyzing antigens, they may not completely digest protein and may require further proteolysis by peptidases in the cytoplasm.

Recognition of Pathogen-Associated Molecular Patterns by Pattern Recognition Receptors

Classical antigen-presenting cells (APCs) in the systemic immune system possess the innate capacity to recognize components of bacteria and viruses called *pathogen-associated molecular patterns* (PAMPs). Receptors for these PAMPs are expressed on both the cell surface (e.g., TLRs) and inside the cell (e.g., nuclear oligomerization domain [NOD]). Despite the

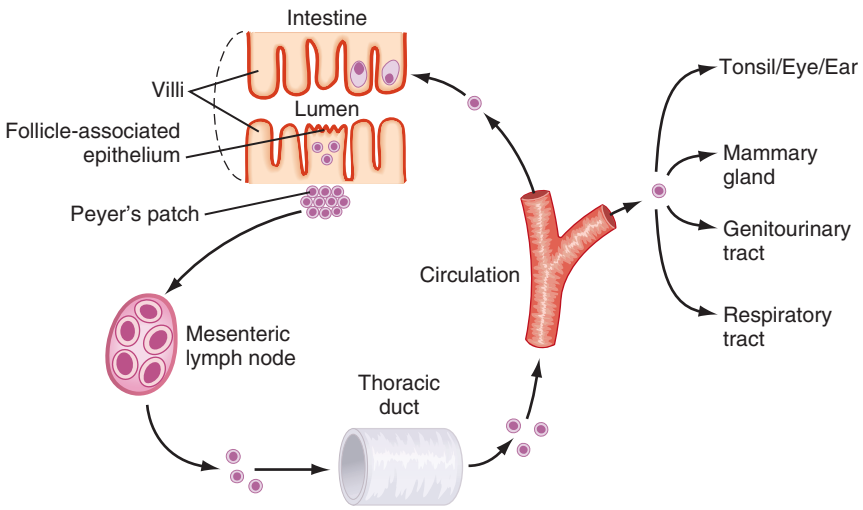

FIGURE 2-5. Mucosal lymphocyte migration. Following antigenic stimulation, T and B lymphocytes migrate from the intestine (Peyer's patch) to the draining mesenteric lymph nodes, where they further differentiate and then reach the systemic circulation via the thoracic duct. Cells bearing appropriate mucosal addressins then selectively home to mucosal surfaces that constitute the common mucosa-associated lymphoid tissue (MALT), including the intestine (gut-associated lymphoid tissue [GALT]).

fact that IECs live adjacent to large numbers of luminal flora, they retain the ability to recognize components of these bacteria. Overall, while pro-inflammatory responses are down-regulated (i.e., in the normal setting, expression of the lipopolysaccharide [LPS] receptor TLR4 is absent), expression of some of these pattern recognition receptors are maintained, such as expression of TLR5, which recognizes bacterial flagellin.[152] This receptor is expressed basolaterally, and it is poised to identify organisms that have invaded the epithelial layer (e.g., *Salmonella* species).[153] Following invasion and engagement of TLR5, the intestinal epithelium is induced to secrete a broad array of cytokines and chemokines that attract inflammatory cells to the local environment to control the spread of infection. In contrast, some bacteria are probiotic and induce anti-inflammatory cytokine production (e.g., IL-10) and increase expression of peroxisome proliferator–activated receptor (PPAR)-γ by IECs.[154,155] Furthermore, other bacterial products (e.g., from *Bacteriodes thetaiotaomicron*) help promote the barrier and IEC differentiation.[156]

Intracellular NOD1 and 2 have been shown to contribute to intestinal inflammation; about 25% of Crohn's disease patients have mutations in the NOD2/CARD15 gene, interfering with their ability to mount an appropriate immune response to bacterial stimuli[157-162] (see Chapter 115). In addition, TLRs that are normally weakly expressed by IECs are expressed at higher levels on IECs from patients with IBD.[163] Expression of different TLRs by IECs, as well as their contribution to innate and adaptive T and B cell responses in both intestinal inflammation and homeostasis, has been demonstrated in several murine models.[164,165] The importance of TLR and NOD2/CARD15 expression and signaling in the intestine has been reviewed.[166-168]

TLR expression by professional APCs is also down-regulated in the LP. This finding, along with others described, contribute to the immunologic non-responsiveness of the GALT.

ANTIGEN PRESENTATION IN THE GUT

Effective immune responses to antigenic proteins require the help of T lymphocytes. This in turn depends on the antigen being presented by APCs that internalize, digest, and couple a small fragment of the antigen to a surface glycoprotein (major histocompatibility complex [MHC] class II or HLA-D in humans) that eventually interacts with a T cell receptor. Several cells in the GALT can act as APCs, including B cells, macrophages, and dendritic cells. The ability of these cells to present antigen depends on the expression of class II MHC on their surface. Class II MHC molecules are also present on the epithelium of the normal small intestine and to a lesser extent colonocytes in both humans[169] and rodents.[170] In vitro studies have demonstrated that isolated enterocytes from rat and human small intestine can present antigens to appropriately primed T cells.[171-173] This raises the possibility that in the intestine, IECs might present peptides to GALT T cells that are localized below the epithelium. Thus, IECs are capable of both antigen processing and presentation in the appropriate context to cells within the LP. Interestingly, bidirectional lymphocyte-epithelial crosstalk exists in the LP, and LP lymphocytes (LPLs) promote mucosal barrier function via Notch-1 signaling and induction of IEC differentiation, polarization, and barrier function.[174,175] Importantly, in IBD, increased expression of MHC class II molecules by IECs has been reported.[176,177] This would be expected to increase the potential of IECs to activate lymphocytes, as indeed reported.[178,179]

Interestingly, drugs used to treat IBD (e.g., 5-aminosalicylate [5-ASA] preparations) may reduce IEC MHC class II expression.[180] In addition to MHC class II expression, IECs (from normal or IBD patients) express a variety of co-stimulatory molecules required for T cell activation (Fig. 2-6). These include intercellular adhesion molecule (ICAM)-1, which binds to leukocyte function associated antigen (LFA)-l on the T cell, B7h, and B7H1. B7-2 (which binds to CD28 and CTLA-4)[181,182] has been shown to be expressed by ulcerative colitis IECs. Interestingly, unique expression of these co-stimulatory molecules by IECs may be involved in the distinct regulation of mucosal responses. Failure to engage CD28 by B7 family members may result in T cell tolerance in naive T cells. This may be less of an issue in the GALT, where cells express the memory phenotype.[183-184] Indeed, phase III trials in patients with moderate to severe Crohn's disease and ulcerative colitis revealed no demonstrable evidence for a therapeutic benefit of CTLA4-Ig (abatacept).[185] There may be several explanations for the clinical result (e.g., CD28-related pathways are of marginal importance in IBD pathogenesis, or use of CTLA4-Ig might have impeded Treg function in addition to preventing effector T cell activation), but the most plausible explanation is the relative lack of dependence on co-stimulation that effector memory T cells, the predominant type of T cell in the gut, exhibit.[186] Small intestinal IECs do not express B7-1 (CD80),[187] so activation of naive T cells by IECs is improbable, aiding in the down-regulation of T cell responses. However, increased expression during intestinal inflammation may serve to augment T cell stimulation.[188]

MHC class I and non-classical class I molecules are also expressed by IECs. Thus, antigen presentation to unique T cell populations is possible and has been reported by several groups.[172,189-195] Specifically, CD1d expressed on human IECs is able to present antigen (in a complex with CEACAM5) to $CD8^+$ T cells.[196-199] CD1d-restricted natural killer T (NKT) cells, effector memory cells that share characteristics of innate and adaptive lymphocytes, are among the earliest responders in immune reactions and affect activation of other immune cell lineages like NK cells, T cells, and B cells. NKT cells have a role in infectious, malignant, and immune-mediated diseases.[200] Other non-classical class I molecules are expressed by IECs. The role of MICA, a stress-induced MHC-related molecule expressed on normal IECs and recognized by the NKG2D-activating receptor on $CD8^+$ T cells, gammadelta T cells, and NK cells, may be of specific importance because it has been reported that Crohn's disease patients had increased numbers of $CD4^+NKG2D^+$ T cells with a Th1 cytokine profile and expressing perforin in the periphery and in the intestinal mucosa.[201] Other non-classical MHC molecules expressed by IECs are being explored (MR-1, TL, Hmt-1, HLA-E, HLA-G), stressing the potential of the intestinal epithelium to serve as a non-classical APC in the gut.[202-205]

In humans, IECs specifically activate $CD8^+$ Treg cells.[172] These regulatory cells may be involved in local tolerance as well as interaction with intra-epithelial lymphocytes ($CD8^+$ T cells). The role of IECs in the regulation of mucosal immunity is best demonstrated in studies with IBD tissues. IECs derived from IBD patients, in contrast to normal IECs, stimulate $CD4^+$ T cells in vitro rather than regulatory $CD8^+$ cells.[178,179,206] Furthermore, oral antigen administration does not result in tolerance in IBD patients, but rather results in active immunity.[25]

INTESTINAL MONONUCLEAR CELLS

Intraepithelial Lymphocytes

Juxtaposed to IECs reside 2 unusual lymphocyte populations, each very different from the other. These include intraepithelial lymphocytes (IELs) and LPLs (discussed later). The clear

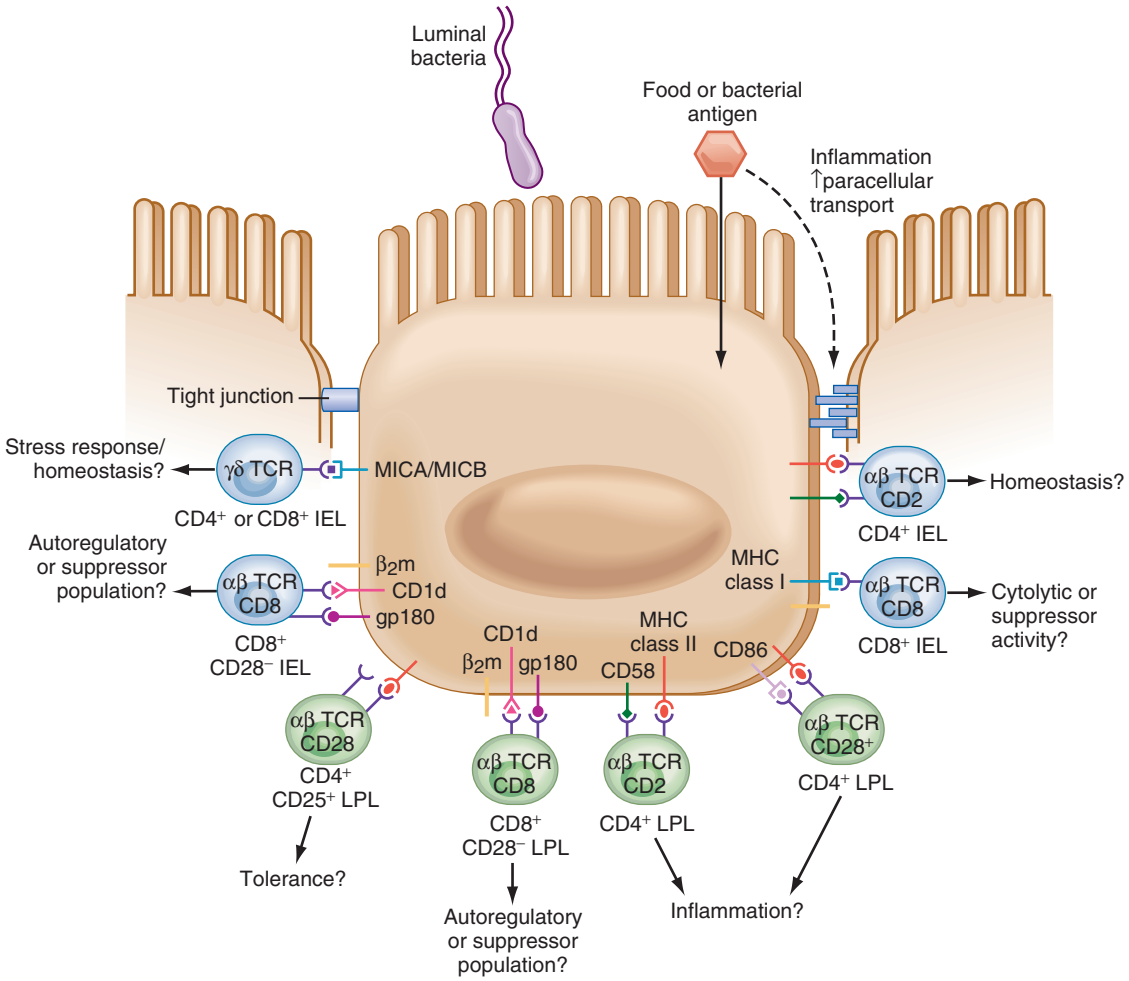

FIGURE 2-6. A normal intestinal epithelial cell (IEC). The IEC is shown to express classic MHC molecules (classes I and II) that have the potential to present conventional antigen to local T cell populations and a broad array of nonclassic class I molecules (e.g., CD1d, MICA/MICB, and β2m [shown in the figure] and MR-1, ULBP, HLA-E, and FcRn [not shown]), which have the potential to present unconventional antigens to unique T cell populations. In addition, alternate pathways of activation appear to be functional in the intestine (e.g., activation via a CD58-CD2 interaction), and classic co-stimulatory molecules are not expressed on IECs, although CD86 may be induced in patients with UC. Other members of the B7 family are expressed (B7h and B7H-1) and may play a role in local T cell activation. β2 Microglobin (β2m) associates with MHC class I, CD1d, HLA-E, HLA-G, and FcRn. β2m, β2 microglobulin; gp180, membrane glycoprotein 180 (a CD8 ligand); IEL, intraepithelial lymphocyte; LPL, lamina propria lymphocyte; MHC, major histocompatibility complex; MICA/MICB, MHC class I-related chains A and B; TCR, T cell receptor.

compartmentalization of these 2 distinct cell populations correlates with their ability to respond to distinct microenvironmental cues.

IELs form one of the main branches of the intestinal immune system, balancing protective immunity with support of epithelial barrier integrity. In the small intestine, IELs are more than 98% T cells and are mostly $CD8^+$,[207-214] including $CD8^+\alpha\alpha$ T cells, as well as $CD4^+CD8^+$ double-positive, and $CD4^-CD8^-$ double-negative cells. Greater numbers of these cells also express the γδ TCR, in contrast to the αβ TCR expressed by T cells in systemic immune system.[215] Roughly half of murine small bowel IELs express the γδ TCR,[216] while both the murine and human large intestine contain primarily αβ $CD4^+$ or $CD8^+$ T cells similar to those found in the systemic immune system.

Based on their phenotype, IELs have been classified into 2 subsets, a and b, where type a includes TCRαβ T cells selected in the thymus, with conventional MHC class I and II, and type b includes TCRαβ $CD8^+\alpha\alpha$, TCRγδ double-positive, and TCRγδ double-negative cells. Both subpopulations have been shown to be cytolytic, killing via granzyme or by engagement of Fas. They also secrete Th1 cytokines. However, antigen-specific type a IELs can transfer protection against a variety of pathogenic organisms, whereas type b IELs are unable to transfer immunologic protection and do not possess immunologic memory. This is possibly due to their activation by IECs in situ by non-classical MHC molecules rather than by the polymorphic MHC-expressed molecules on professional APCs that activate type a IELs.[216] IELs express a variety of activation markers and are $CD45RO^+$ (memory cells). IELs also express the GALT-specific integrin αEβ7.[217,218] It is induced by TGF-β, and its ligand on IECs is E-cadherin, which is involved in cell signaling and cytoskeletal rearrangement.[218] When isolated, IELs are difficult to activate through their TCR and barely proliferate even in response to potent stimuli.[213] They may be activated by alternative pathways (e.g., via CD2).

Type a IELs secrete cytokines that are different from the ones secreted by their peripheral blood counterparts (e.g.,

IL-7).[212,219-221] A broad spectrum of cytokines are produced by IELs, including IFN-γ, TNF-α, IL-2, IL-4, IL-6, IL-10, TGF-β, keratinocyte growth factor (KGF), and IL-17, with important effects on intestinal barrier function and local immune responses.[222]

Functionally, it has been suggested that IELs potentially kill epithelial cells that have undergone some form of stress such as infection, transformation, or invasion by other cells.[214-216,223] Alternatively, it has been proposed that IELs are active in suppressing local immunity, although the evidence that they actually function in luminal antigen recognition is weak. IELs do not travel in and out of the epithelium. Rather, the epithelial cells grow over the IELs as they move from the crypt to the surface. Thus, IELs likely serve as sentinels for epithelial integrity.

Lamina Propria Mononuclear Cells

The LP is the major effector site in the GALT. It has been suggested that the LP may be an inductive site as well, because antigen presentation by professional and non-professional APCs may occur in the LP itself. The LP is also considered a graveyard for activated lymphocytes. LP lymphocytes (LPLs) are more prone to undergo apoptosis than their peripheral counterparts. This may be a regulatory mechanism limiting the potentially inflammatory effects of activated lymphocytes. In inflammatory bowel diseases like Crohn's disease, a major reported defect is the resistance of IBD LPL to undergo apoptosis when activated inappropriately (see later).

Clearly the GALT operates under a distinct set of rules compared to the systemic immune system. This is reflected not only in its functional anatomy (no organized structure) but also in its responses and regulation. As already alluded to, highly specialized cells mediate these effects, some detected only in the GALT.

Lamina propria mononuclear cells (LPMCs) are a heterogeneous group of cells[224,225] (see Fig. 2-1). The most prevalent cell type is the IgA^+ plasma cell, but there are more than 50% T cells and B cells (together comprising the LPL population), macrophages, and dendritic cells (DCs). In contrast to IELs, LPLs express the mucosal addressin α4β7. Similar to IELs, they express an activated memory phenotype and do not proliferate in response to engagement of the TCR. Alternate pathways of LPL activation are mainly via CD2 and CD28.[215,226,227]

Down-regulating the ability of these cells to respond to stimulation via the TCR (i.e., to antigen) may be another mechanism involved in dampening immune responses to normal luminal contents, along with the increased tendency for LPLs to undergo apoptosis if activated inappropriately. The mechanism underlying this latter phenomenon possibly relates to engagement of the death receptor Fas and its ligand on activated LPLs, and by the imbalance between the intracellular anti- and pro-apoptotic factors, Bcl2 and Bax. Defects in this pro-apoptotic balance have been reported in Crohn's disease.[228,229]

The observations described thus far all contribute to the normal scenario within the LP, called *controlled/physiologic inflammation*. This state of inflammation is the norm in the gut, whereas it would be considered indicative of disease in any other organ. When regulatory mechanisms go awry—an increase in cell recruitment coupled with a decrease in apoptosis—the result is *uncontrolled inflammation*, such as what is observed in patients with IBD.

T Cell Differentiation

As already described, within the LP there is an organized lymphoid structure, the Peyer's patch (see Fig. 2-5). There, B and T lymphocytes interact with antigen sampled via M cells in the follicle-associated epithelium (FAE). Activation and maturation of T lymphocytes from naive Th0 cells to distinct biased subpopulations is strongly influenced by the microenvironment. Specifically, contact with DCs, professional APCs within the GALT and their secreted mediators, will skew T lymphocytes to one of several effector cells. IL-2–, IFN-γ–, and TNF-α–secreting Th1 cells develop when DCs secrete the IL-12/p35-40 heterodimer.[230] This induces activation and phosphorylation of the transcription factor STAT-4 (signal transducer and activator of transcription factor 4).[231] STAT-4 in turn induces IFN-γ expression and production. IFN-γ induces activation of STAT-1 and consequently of T box expressed in T cells (T-bet), which is the master transcription factor that induces Th1 cytokine as well as IL-12 receptor β2 production, while simultaneously suppressing Th2 cytokine production. Thus, a cycle promoting Th1 and suppressing Th2 responses is created. Overactivation of T-bet is possibly an essential step for Th1-mediated mucosal diseases, such as those seen in some patients with Crohn's disease.[231] Another Th1-promoting cytokine is IL-18, mediating its effects by augmenting IL-12Rβ2 chain expression on T cells and AP-1(c-fos/c-jun)-dependent transactivation of the IFN-γ promoter. It also activates nuclear factor κB (NF-κB) in T cells.[230]

In contrast, when IL-4 is secreted, Th2 cytokine production (IL-4, IL-5, IL-6, IL-9, IL-10, IL-13) occurs by activation of STAT-6 followed by activation of the transcription factor GATA-3. GATA-3 is capable of promoting the expression of several Th2 cytokines, including IL-4, IL-5, and IL-13.[232] In addition to IL-4, IL-13 also plays an important role in Th2 development and IgE synthesis in an IL-4–independent fashion. These cytokines appear to play a role in the development of food allergies (see Chapter 10). IL-5 induces B cells expressing surface IgA to differentiate into IgA-producing plasma cells. IL-6 causes a marked increase in IgA secretion, with little effect on either IgM or IgG synthesis.[233] Thus, in the normal state in the GALT, a Th2 bias might exist.

Recently, additional T helper populations were identified. Among those, the Th17 population seems to be most important, with specific relevance to intestinal inflammation. Importantly, part of the Th1 data previously reported should be reevaluated because it may be related to Th17 cells. The reason is that the Th1-polarizing cytokine IL-12, composed of the p40 and p35 subunits, has similarities with the Th17-polarizing cytokine IL-23, composed of p40 and the unique p19 subunit. Thus, antibodies targeting the common p40 subunit shared by both IL-12 and IL-23 may fail to differentiate between Th1 and Th17 inflammatory pathways. The possibility that some of the inflammatory activity previously attributed to an IL-12–driven Th1 pathway might actually be an IL-23–driven Th17 pathway was supported by studies showing that intestinal inflammation was still possible when IL-12 was inhibited, and that inhibition of IL-23 rather than IL12 ameliorated inflammation.[234-238] Thus, in Crohn's disease, where increased expression of both IL-12 and IL-23 exists, inhibition of both Th1 and Th17 may be a reasonable therapeutic option. Accordingly, inhibition of the common p40 subunit of IL-12 and IL-23 was beneficial in clinical studies in Crohn's disease patients.[239,240] Th17 cells express retinoid-related orphan receptor-χt (RORχt), which is the master transcription factor for these cells. In addition to RORχt, human Th17 cells express IL-23R, CCR6, and CD161, whereas they lack CXCR3, a chemokine receptor characteristic of Th1 cells.[241-244] The main effector cytokines secreted by Th17 cells are IL-17A, IL-17F, IL-21, IL-22, IL-26, TNF-α, and the chemokine CCL20. Th17 cells differentiate under the influence of IL-1β, IL-6, IL-21, IL-23, and TGF-β.[243] In humans, not all Th17 cells produce IL-22, and a Th22 subset of CD4 helper T cells that produces

IL-22 but not IL-17 has been identified.[244] While IL-17 promotes recruitment and activation of neutrophils, IL-22 promotes mucosal healing through epithelial proliferation and increased mucus production.[245] A role for IL-17/IL-22 imbalance in the pathogenesis of ulcerative colitis has recently been suggested.[246]

The biology of T cell lineages in the LP is complex. Part of this complexity is related to the plasticity of these cell populations. Under specific circumstances, Th17 cells may become Th1 cells. Moreover, regulatory Foxp3$^+$ cells expressing Th17 cytokines and having potent suppressor activity in vitro were recently identified in humans.[247] This suggests that a certain degree of plasticity in vivo exists in all known T cell subsets, reflected in their capacity to produce cytokines depending on the specific microenvironment. The complexity of T cell, specifically Th17 cell, biology in the intestinal LP may be one reason for the failure of anti-IL-17A monoclonal antibody therapy in active Crohn's disease.[248,249] Other Th17 cytokines remained uninhibited, thus potentially contributing to the lack of a therapeutic effect of such a strategy. Addressing the complexity of the LP milieu with its vast amounts of mediators and effectors, including the microbiota, may assist in better design of future therapeutic strategies, as well as our attempts to modify intestinal inflammation, such as the one resulting in IBD.

Innate Lymphoid Cells

Innate lymphoid cells (ILCs) produce Th cell–associated cytokines but do not express cell-surface markers that are associated with other immune cell lineages. Moreover, ILCs are lineage marker negative and do not express a T cell receptor. Thus, their immune response is not antigen specific. ILCs are effectors of innate immunity and regulators of tissue modeling. These recently identified cells have several subpopulations with distinct cytokine expression patterns that resemble the helper T cell subsets Th1, Th2, and Th17.

Group I ILCs include ILC1 cells and NK cells. ILC1 cells express the transcription factor T-bet and respond to IL-12 by producing IFN-γ. They differ from NK cells in that they do not express the NK cell markers CD16 and CD94 and lack perforin and granzyme B. ILC1 may develop from the RORχt ILC3 cells. Thus, it is still unclear whether they are a distinct group or a stage in the differentiation of ILC3 or NK cells.[250] ILC1 cells are increased in the inflamed intestine of Crohn's disease patients, suggesting a role for ILC1 cells in the pathogenesis of intestinal inflammation.

Group 2 ILCs include ILC2 cells (also termed *natural helper cells*, *nuocytes*, and *innate helper* 2). Their transcription factors are retinoic acid receptor-related orphan receptor-α (RORα) and GATA3, and they have key roles in anthelminthic responses and allergic lung inflammation.

Group 3 ILCs include ILC3 and lymphoid tissue inducer (LTi) cells. This group expresses the NK cell-activating receptor NKp46, depends on the transcription factor RORχt, and lacks cytotoxic effectors like perforin and granzymes. Group 3 ILCs express IL-22 but not IFN-γ or TNF. ILCs were recently identified in humans. Their potential contribution to mucosal homeostasis and intestinal inflammation is still unclear and under intensive research.[251]

Dendritic Cells

DCs play an important role in tolerance and immunity in the gut. DCs continuously migrate within lymphoid tissues and present self-antigens (likely from dying apoptotic cells to maintain self-tolerance) as well as non-self antigens.[252] Within the LP of the distal small intestine, they express the chemokine receptor CX3CR1 and form transepithelial dendrites that allow direct sampling of luminal antigen.[253] It has been suggested that IECs expressing CCL25 (the ligand for CCR9 and CCR10) attract DCs to the small bowel, while CCL28 (the ligand for CCR3 and CCR10) attracts them to the colon.[254-256]

DCs process internalized antigens more slowly than macrophages,[178,257] and this probably contributes to local tolerance.[179,180,258,259] Tolerance induction by DCs is associated with their degree of maturation at the time of antigen presentation to T cells (immature DCs activate Tregs), down-regulation of co-stimulatory molecules CD80 and CD86, production of the suppressive cytokines IL-10, TGF-β and IFN-α, and interaction with the co-stimulatory molecule CD200.[181-183,260-262] Recent reports demonstrate that murine CD103$^+$ DCs were able to perform all stages of antigen processing, including uptake, transportation, and presentation of bacterial antigens.[263]

Intense recent research also showed that LP-resident CD103$^+$ DCs share the burden of immunosurveillance with CX3CR1$^+$ macrophages, and that impaired function of these subpopulations may contribute to the development of IBD.[264]

GUT-ASSOCIATED LYMPHOID TISSUE–RELEVANT CHEMOKINES

Many of the chemokines secreted in the GALT are produced by IECs, one more piece of evidence for their active participation in regulating intestinal immune responses. This is especially true in inflammatory bowel diseases, where the secretion of both IEC-derived chemokines and cytokines are increased, mainly owing to enhanced bacterial translocation and IFN-γ production, contributing to the augmentation of mucosal inflammation. Of the chemokines secreted, those secreted by IECs have the capacity to attract inflammatory cells like lymphocytes, macrophages, and DCs.

The chemokine CCL5 (regulated on activation, normal T cell expressed and secreted [RANTES]) is secreted predominantly by macrophages but can also be produced by human IECs.[265] RANTES may have a role in innate as well as adaptive mucosal immunity,[266] and increased RANTES expression has been demonstrated in the mucosa of patients with ulcerative colitis.[267-270] Bacterial induction of RANTES in the epithelium of inflammasome-deficient mice led to exacerbation of colitis, creating an autoinflammatory circuit.[271]

The CXC chemokines—monokine induced by interferon-γ (MIG, CXCL9); interferon-γ-inducible protein 10 (IP-10, CXCL10), a chemokine that appears to promote Th1 responses and therefore may be relevant in Crohn's disease; and IFN-γ-inducible T cell α-chemoattractant (ITAC, CXCL11)—are constitutively expressed by lymphocytes, endothelial cells, and human colonic IECs.[272-273] Their expression and polarized basolateral secretion increase after IFN-γ stimulation. CXC chemokines attract Th1 cells expressing high levels of CXCR3.[274] They also contribute to NK T cell chemotaxis and increased cytolytic responses[275] and activate subsets of DCs.[276] By attracting CD4$^+$ Th1 cells that produce IFN-γ, up-regulation of expression and secretion of CXC chemokines occurs as IECs express IFN-γ receptors. This appears to contribute to a positive feedback loop that may be relevant in inflammatory states, specifically IBD and celiac disease. Importantly, blockade of the CXCR3-CXCL10 axis has been shown to be beneficial in ameliorating murine colitis,[277] as well as in a phase II study in patients with ulcerative colitis.[278]

In contrast to the inflammation-related CXCR3 receptor, a tissue-specific chemokine receptor, CCR9, is constitutively expressed on small bowel IELs and LPLs.[279-281] Its ligand, the chemokine thymus-expressed chemokine (TECK, CCL25) is

differentially expressed in the jejunal and ileal epithelium, where decreasing levels of expression from the crypt up to the villous have been reported.[282] In murine models it was shown that CCL25/CCR9 is associated with selective localization of mesenteric lymph node–activated CD8αβ$^+$ lymphocytes, co-expressing αEβ7 to the small intestine.[283] CCL25 expression by IECs has been shown to be increased in the inflamed small bowel of patients with Crohn's disease, with increased CCR9 expression by peripheral blood lymphocytes and decreased expression by LPLs,[280] supporting its role in the specific attraction of peripheral lymphocytes to the small bowel in Crohn's disease. This chemokine-receptor pair has also been used as a target for therapeutic intervention in Crohn's disease using a specific orally administered CCR9 antagonist, with positive results specifically in the maintenance phase.[284]

Fractalkine (CX3CL1) is a unique chemokine expressed by IECs that combines the properties of chemokines and adhesion molecules. It attracts NK cells, monocytes, CD8$^+$ T lymphocytes, and to a lesser extent CD4$^+$ T lymphocytes, which express the specific receptor CX3CR1.[285] Its expression is increased in Crohn's disease, specifically in the basolateral aspect of IECs.[286,287] It was suggested that polymorphism of the receptor CX3CR1 influences Crohn's disease phenotype and localization, because it was associated with more stenosis and ileocolonic disease location.[287]

Mucosa-associated epithelial chemokine (MEC, CCL28) may also have a role in intestinal immunity. This chemokine and its receptors CCR3 and CCR10 are expressed by colonic IECs. CD4$^+$ memory lymphocytes and eosinophils are attracted by this chemokine in vitro, although its function in vivo has not yet been demonstrated.[288]

Macrophage-derived chemokine (MDC, CCL22) is constitutively expressed and secreted by colonic IECs. It is unique in that it attracts CCR4$^+$ Th2 cytokine-producing lymphocytes. Polarized basolateral secretion of MDC/CCL22 from stimulated colonic IEC lines has been reported.[289] The specific recruitment of lymphocytes that preferentially secrete anti-inflammatory cytokines supports a role for the intestinal epithelium in orchestrating normal mucosal homeostasis, and adds to the accumulating evidence that these cells possess the ability to regulate mucosal immune responses.

The chemokine macrophage inflammatory protein-3α (MIP3α, CCL20) is unique in its ability to specifically attract immature DCs as well as memory CD4$^+$ T lymphocytes.[290-292] CCL20 is also expressed and produced by human small intestinal ECs (mainly in the follicle-associated epithelium) and by colonic IECs and has been suggested to be the mediator of lymphocyte adhesion to the α4β7 ligand MAdCAM-1.[290] MIP3α expression and secretion is increased in colonic IECs derived from IBD patients.[293] Its stimulated secretion is polarized to the basolateral compartment, supporting its ability to attract immune cells into the LP. Mucosal memory T cells, as well as IECs, express CCR6, the cognate receptor for MIP3α. The interesting observation that CCR6 as well as CCR9 are co-expressed in T cells expressing the α4β7 integrin, characteristic of mucosal lymphocytes, may suggest that in inflammatory states and to some extent in the normal state, MIP-3α and TECK (CCL20 and CCL25, respectively) expression by IECs attract CCR9$^+$ or CCR6$^+$ lymphocytes. These are activated in mesenteric lymph nodes, enter the peripheral blood, and then are recruited to the intestinal mucosa, where they undergo either activation-induced apoptosis (if they are aberrantly activated) or terminal differentiation. Interestingly, NKG2D$^+$ CD4 T cells from patients with Crohn's disease expressed CCR6, rendering them potentially more responsive to CCL20, as well as to IL23, thus potentially contributing to further intestinal inflammation.[294]

CXCL12 (stromal cell derived factor-1) and its main receptor CXCR4 are expressed by IECs in the normal intestinal mucosa,[295-297] where they have a role in IEC migration, barrier maturation, and restitution.[298] Up-regulation of CXCL12 in IBD IECs was recently reported, as was CXCR4 expression by IECs, peripheral blood, and LP mononuclear cells.[299] Moreover, CXCL12 was able to chemoattract Th1-biased memory CD45RO$^+$ peripheral blood and LP T cells,[300] and CXCR4-mediated IgG plasma cell infiltration of the mucosa of ulcerative colitis patients was recently demonstrated,[301] suggesting that CXCL12-CXCR4 interactions contribute to mucosal deregulation, specifically of memory CD45RO$^+$ LP T cells and plasma cells. CXCR4 antagonists were evaluated as a therapeutic modality in animal colitis models and human disease, with preliminary beneficial effects.[302,303] The potential role of the newly reported CXCL12 receptor CXCR7 in IBD is still unclear.[300]

KEY REFERENCES

Full references for this chapter can be found on www.expertconsult.com.

21. Pabst O, Mowat AM. Oral tolerance to food proteins. Mucosal Immunol 2012; 5:232-9.
25. Kraus TA, Toy L, Chan L, et al. Failure to induce oral tolerance to a soluble protein in patients with inflammatory bowel disease. Gastroenterology 2004; 26:1771-8.
51. Sakaguchi S, Toda M, Asano M, et al. T cell-mediated maintenance of natural self-tolerance: Its breakdown as a possible cause of various autoimmune diseases. J Autoimmun 1996; 9:211-20.
59. Murai M, Krause P, Cheroutre H, Kronenberg M. Regulatory T-cell stability and plasticity in mucosal and systemic immune systems. Mucosal Immunol 2010; 3:443-9.
80. Allez M, Brimnes J, Dotan I, Mayer L. Expansion of CD8$^+$ T cells with regulatory function after interaction with intestinal epithelial cells. Gastroenterology 2002; 123:1516-26.
126. Neutra MR. Current concepts in mucosal immunity. V. Role of M cells in transepithelial transport of antigens and pathogens to the mucosal immune system. Am J Physiol 1998; 274:G785-91.
157. Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001; 411:599-603.
158. Ogura Y, Bonen DK, Inohara N, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 2001; 411:603-6.
202. Perera L, Shao L, Patel A, et al. Expression of nonclassical class I molecules by intestinal epithelial cells. Inflamm Bowel Dis 2007; 13:298-307.
230. Neurath MF, Finotto S, Glimcher LH. The role of Th1/Th2 polarization in mucosal immunity. Nat Med 2002; 8:567-73.
231. Neurath MF, Weigmann B, Finotto S, et al. The transcription factor T-bet regulates mucosal T cell activation in experimental colitis and Crohn's disease. J Exp Med 2002; 195:1129-43.
239. Mannon PJ, Fuss IJ, Mayer L, et al. Anti-interleukin-12 antibody for active Crohn's disease. N Engl J Med 2004; 351:2069-79.
247. Hovhannisyan Z, Treatman J, Littman DR, Mayer L. Characterization of IL-17-producing regulatory T cells in inflamed intestinal mucosa from patients with

inflammatory bowel diseases. Gastroenterology 2011; 140:957-65.
261. Niess JH, Reinecker HC. Lamina propria dendritic cells in the physiology and pathology of the gastrointestinal tract. Curr Opin Gastroenterol 2005; 21:687-91.
263. Farache J, Koren I, Milo I, et al. Luminal bacteria recruit $CD103^+$ dendritic cells into the intestinal epithelium to sample bacterial antigens for presentation. Immunity 2013; 38:581-95.

REFERENCES

1. Brandtzaeg P, Farstad IN, Haraldsen G, et al. Cellular and molecular mechanisms for induction of mucosal immunity. Dev Biol Stand 1998; 92:93-108.
2. Mowat AM, Viney JL. The anatomical basis of intestinal immunity. Immunol Rev 1997; 156:145-66.
3. Moldoveanu Z, Clements ML, Prince SJ, et al. Human immune responses to influenza virus vaccines administered by systemic or mucosal routes. Vaccine 1995; 13:1006-12.
4. Bloom PD, Boedeker EC. Mucosal immune responses to intestinal bacterial pathogens. Semin Gastrointest Dis 1996; 7:151-66.
5. Brandtzaeg P. The human intestinal immune system: Basic cellular and humoral mechanisms. Baillieres Clin Rheumatol 1996; 10:1-24.
6. Mayer L, Sperber K, Chan L, et al. Oral tolerance to protein antigens. Allergy 2001; 56(Suppl 67):12-5.
7. Mayer L. Mucosal immunity and gastrointestinal antigen processing. J Pediatr Gastroenterol Nutr 2000; 30(Suppl): S4-12.
8. Anderson JC. The response of gut-associated lymphoid tissue in gnotobiotic piglets to the presence of bacterial antigen in the alimentary tract. J Anat 1977; 124:555-62.
9. Ishikawa K, Satoh Y, Tanaka H, et al. Influence of conventionalization on small-intestinal mucosa of germ-free Wistar rats: Quantitative light microscopic observations. Acta Anat (Basel) 1986; 127:296-302.
10. Cebra JJ, Periwal SB, Lee G, et al. Development and maintenance of the gut-associated lymphoid tissue (GALT): The roles of enteric bacteria and viruses. Dev Immunol 1998; 6:13-8.
11. Rothkotter HJ, Ulbrich H, Pabst R. The postnatal development of gut lamina propria lymphocytes: Number, proliferation, and T and B cell subsets in conventional and germ-free pigs. Pediatr Res 1991; 29:237-42.
12. Saparov A, Kraus LA, Cong Y, et al. Memory/effector T cells in TCR transgenic mice develop via recognition of enteric antigens by a second, endogenous TCR. Int Immunol 1999; 11:1253-64.
13. Qiao L, Schurmann G, Betzler M, et al. Activation and signaling status of human lamina propria T lymphocytes. Gastroenterology 1991; 101:1529-36.
14. De Maria R, Fais S, Silvestri M, et al. Continuous in vivo activation and transient hyporesponsiveness to TcR/CD3 triggering of human gut lamina propria lymphocytes. Eur J Immunol 1993; 23:3104-8.
15. Xiao BG, Link H. Mucosal tolerance: A two-edged sword to prevent and treat autoimmune diseases. Clin Immunol Immunopathol 1997; 85:119-28.
16. Whitacre CC, Gienapp IE, Meyer A, et al. Treatment of autoimmune disease by oral tolerance to autoantigens. Clin Immunol Immunopathol 1996; 80:S31-9.
17. Weiner HL, Mayer LF. Oral tolerance: Mechanisms and applications. Introduction. Ann N Y Acad Sci 1996; 778:xiii-xviii.
18. Titus RG, Chiller JM. Orally induced tolerance. Definition at the cellular level. Int Arch Allergy Appl Immunol 1981; 65:323-38.
19. Strober W, Kelsall B, Marth T. Oral tolerance. J Clin Immunol 1998; 18:1-30.
20. MacDonald TT. T cell immunity to oral allergens. Curr Opin Immunol 1998; 10:620-7.
21. Pabst O, Mowat AM. Oral tolerance to food proteins. Mucosal Immunol 2012; 5:232-9.
22. Challacombe SJ, Tomsai TB. Systemic tolerance and secretory immunity after oral imunization. J Exp Med 1980; 152:1459-72.
23. Mattingly JA, Waksman BH. Immunologic suppression after oral administration of antigen. I. Specific suppressor cells formed rat Peyer's patches after oral administration. J Immunol 1978; 121:1878-83.
24. Kapp,K, Maul J, Hostmann A, et al. Modulation of systemic antigen-specific immune responses by oral antigen in humans. Eur J Immunol 2010; 40:3128-37.
25. Kraus TA, Toy L, Chan L, et al. Failure to induce oral tolerance to a soluble protein in patients with inflammatory bowel disease. Gastroenterology 2004; 26:1771-8.
26. Kalliomaki M, Salminen S, Poussa T, et al. Probiotics and prevention of atopic disease: 4-year follow-up of a randomized placebo-controlled trial. Lancet 2003; 361:1869-71.
27. Riedler J, Barunfahrländer C, Eder W, et al. Exposure to farming early life and its development of asthma and allergy: A cross-sectional survey. Lancet 2001; 358:1129-33.
28. Sordillo JE, Hoffmann EB, Celedon JC, et al. Multiple microbial exposures in the home may protect against asthma or allergy in childhood. Clin Exp Allergy 2010; 40:902-10.
29. Pohjavuori E, Viljanen M, Korpela R, et al. *Lactobacillus* GG effect in increasing IFN-gamma production in infants with cow's milk allergy. J Allergy Clin Immunol 2004; 114:131-6.
30. Otte JM, Cario E, Podolsky DK. Mechanisms of cross hyporesponsiveness to Toll-like receptor bacterial ligands in intestinal epithelial cells. Gastroenterology 2004; 126:1054-70.
31. Rosenfeldt V, Benfeldt E, Valerius NH, et al. Effect of probiotics on gastrointestinal symptoms and small intestinal permeability in children with atopic dermatitis. J Pediatr 2004; 145:612-6.
32. Bullock NR, Booth JC, Gibson GR. Comparative composition of bacteria in the human intestinal microflora during remission and active ulcerative colitis. Curr Issues Intest Microbiol 2004; 5:59-64.
33. Gosselink MP, Schouten WR, van Lieshout LM, et al. Delay of the first onset of pouchitis by oral intake of the probiotic strain *Lactobacillus rhamnosus* GG. Dis Colon Rectum 2004; 47:876-84.
34. Lamont AG, Mowat AM, Browning MJ, Parrott DM. Genetic control of oral tolerance to ovalbumin in mice. Immunology 1988; 63:737-9.
35. Gomes-Santos AC, Moreira TG, Castro-Junior AB, et al. New insights into the immunological changes in IL-10-deficient mice during the course of spontaneous inflammation in the gut mucosa. Clin Dev Immunol 2012; 2012:560817.
36. Whitacre CC, Gienapp IE, Orosz CG, et al. Oral tolerance in experimental autoimmune encephalomyelitis. III. Evidence for clonal anergy. J Immunol 1991; 147:2155-63.
37. Chen Y, Inobe J, Marks R, et al. Peripheral deletion of antigen-reactive T cells in oral tolerance. Nature 1995; 376:177-80.
38. Friedman A, Weiner HL. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. Proc Natl Acad Sci U S A 1994; 91:6688-92.
39. Hafler DA, Kent SC, Pietrusewicz MJ, et al. Oral administration of myelin induces antigen-specific TGF-beta 1 secreting T cells in patients with multiple sclerosis. Ann N Y Acad Sci 1997; 835:120-31.
40. Siewert C, Lauer U, Cording S, et al. Experience-driven development: Effector/memory-like alphaE^{+}Foxp3^{+} regulatory T cells originate from both naive T cells and

naturally occurring naive-like regulatory T cells. J Immunol 2008; 180:146-55.
41. Fukaura HS, Kent C, Pietrusewicz MJ, et al. Induction of circulating myelin basic protein and proteolipid protein-specific transforming growth factor-beta1-secreting Th3 T cells by oral administration of myelin in multiple sclerosis patients. J Clin Invest 1996; 98:70-7.
42. Inobe J, Slavin AJ, Komagata Y, et al. IL-4 is a differentiation factor for transforming growth factor-beta secreting Th3 cells and oral administration of IL-4 enhances oral tolerance in experimental allergic encephalomyelitis. Eur J Immunol 1998; 28:2780-90.
43. Chen Y, Inobe J, Kuchroo VK, et al. Oral tolerance in myelin basic protein T-cell receptor transgenic mice: Suppression of autoimmune encephalomyelitis and dose-dependent induction of regulatory cells. Proc Natl Acad Sci U S A 1996; 93:388-91.
44. Strobel SS. Neonatal oral tolerance. Ann N Y Acad Sci 1996; 778:88-102.
45. Kunimoto DY, Ritzel M, Tsang M. The roles of IL-4, TGF-beta and LPS in IgA switching. Eur Cytokine Netw 1992; 3:407-15.
46. Kim PH, Kagnoff MF. Transforming growth factor beta 1 increases IgA isotype switching at the clonal level. J Immunol 1990; 145:3773-8.
47. Coffman RL, Lebman DA, Shrader B. Transforming growth factor beta specifically enhances IgA production by lipopolysaccharide-stimulated murine B lymphocytes. J Exp Med 1989; 170:1039-44.
48. Groux H, O'Garra A, Bigler M, et al. A $CD4^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 1997; 389:737-42.
49. Levings MK, Roncarolo MG. T-regulatory 1 cells: A novel subset of CD4 T cells with immunoregulatory properties. J Allergy Clin Immunol 2000; 106:S109-12.
50. Roncarolo MG, Bacchetta R, Bordignon C, et al. Type 1 T regulatory cells. Immunol Rev 2001; 182:68-79.
51. Sakaguchi S, Toda M, Asano M, et al. T cell-mediated maintenance of natural self-tolerance: Its breakdown as a possible cause of various autoimmune diseases. J Autoimmun 1996; 9:211-20.
52. Sakaguchi S, Sakaguchi N, Shimizu J, et al. Immunologic tolerance maintained by $CD25^+$ $CD4^+$ regulatory T cells: Their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. Immunol Rev 2001; 182:18-32.
53. Shevach EM, Thornton A, Suri-Payer E. T lymphocyte-mediated control of autoimmunity. Novartis Found Symp 1998; 215:200-11.
54. Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. J Exp Med 2001; 194:629-44.
55. Zhang X, Izikson L, Liu L, Weiner HL. Activation of CD25(+)CD4(+) regulatory T cells by oral antigen administration. J Immunol 2001; 167:4245-53.
56. Chung Y, Lee SH, Kim DH, et al. Complementary role of $CD4^+CD25^+$ regulatory T cells and TGF-beta in oral tolerance. J Leukoc Biol 2005; 77:906-13.
57. Bluestone JA, Abbas AK. Natural versus adoptive regulatory T cells. Nat Rev Immunol 2003; 3:253-7.
58. Hai S, Namura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003; 299:1057-61.
59. Murai M, Krause P, Cheroutre H, Kronenberg M. Regulatory T-cell stability and plasticity in mucosal and systemic immune systems. Mucosal Immunol 2010; 3:443-9.
60. Yuan X, Malek TR. Cellular and molecular determinants for the development of natural and induced regulatory T cells. Human Immunol 2012; 73:773-82.
61. Gravano DM, Vignali DA. The battle against immunopathology: Infectious tolerance mediated by regulatory T cells. Cell Mol Life Sci 2012; 69:1997-2008.
62. Veltkamp C, Ruhwald R, Giesem T, et al. $CD4^+CD25^+$ cell depletion from the normal $CD4^+$ T cell pool prevents tolerance toward the intestinal flora and leads to chronic colitis in immunodeficient mice. Inflamm Bowel Dis 2006; 12:437-46.
63. Morrissey PJ, Charrier K, Braddy S, et al. $CD4^+$ T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodefficient mice. Disease development is prevented by cotransfer of purified $CD4^+$ T cells. J Exp Med 1993; 178:237-44.
64. Powrie F, Leach MW, Mauze S, et al. Phenotypically distinct subsets of $CD4^+$ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. Int Immunol 1993; 5:1461-7.
65. Collins CB, Aherne CM, McNamee EN, et al. Flt3 ligand expands $CD103^+$ dendritic cells and $FoxP3^+$ T regulatory cells, and attenuates Crohn's-like murine ileitis. Gut 2012; 61:1154-62.
66. Wang Y, Liu XP, Zhao ZB, et al. Expression of $CD4^+$ forkhead box P3 $(FOXP3)^+$ regulatory T cells in inflammatory bowel disease. Dig Dis Sci 2011; 12:286-94.
67. Holmén N, Lundgren A, Lundin S, et al. Functional $CD4^+CD25^{high}$ regulatory T cells are enriched in the colonic mucosa of patients with active ulcerative colitis and increase with disease activity. Inflamm Bowel Dis 2006; 12:447-56.
68. Hölttä V, Klemetti P, Sipponen T, et al. IL-23/IL-17 immunity as a hallmark of Crohn's disease. Inflamm Bowel Dis 2008; 14:1175-84.
69. Makita S, Kanai T, Oshima S, et al. $CD4^+$CD25 bright T cells in human intestinal lamina propria as regulatory cells. J Immunol 2004; 173:3119-30.
70. Maul J, Loddenkemper C, Mundt P, et al. Peripheral and intestinal regulatory $CD4^+$ CD25(high) T cells in inflammatory bowel disease. Gastroenterology 2005; 128:1868-78.
71. Saruta M, Yu QT, Fleshner PR, et al. Characterization of $FOXP3^+CD4^+$ regulatory T cells in Crohn's disease. Clin Immunol 2007; 125:281-90.
72. Takahashi M, Nakamura K, Honda K, et al. An inverse correlation of human peripheral blood regulatory T cell frequency with the disease activity of ulcerative colitis. Dig Dis Sci 2006; 51:677-86.
73. Mayne CG, Williams CB. Induced and natural regulatory T cells in the development of inflammatory bowel disease. Inflamm Bowel Dis 2013; 19:1772-88.
74. Mowat AM, Lamont AG, Strobel S, Mackenzie S. The role of antigen processing and suppressor T cells in immune responses to dietary proteins in mice. Adv Exp Med Biol 1987; 216A:709-20.
75. Mowat AM, Lamont AG, Parrott DM. Suppressor T cells, antigen-presenting cells and the role of I-J restriction in oral tolerance to ovalbumin. Immunology 1988; 64:141-5.
76. Mowat AM. Depletion of suppressor T cells by 2'-deoxyguanosine abrogates tolerance in mice fed ovalbumin and permits the induction of intestinal delayed-type hypersensitivity. Immunology 1986; 58:179-84.
77. Mowat AM. The role of antigen recognition and suppressor cells in mice with oral tolerance to ovalbumin. Immunology 1985; 56:253-60.

78. Allez M, Mayer L. Regulatory T cells: peace keepers in the gut. Inflamm Bowel Dis 2004; 10:666-76.
79. Arnaboldi PM, Roth-Walter F, Mayer L. Suppression of Th1 and Th17, but not Th2, responses in a CD8(+) T cell-mediated model of oral tolerance. Mucosal Immunol 2009; 2:427-38.
80. Allez M, Brimnes J, Dotan I, Mayer L. Expansion of $CD8^+$ T cells with regulatory function after interaction with intestinal epithelial cells. Gastroenterology 2002; 123:1516-26.
81. Brimnes J, Allez M, Dotan I, et al. Defects in $CD8^+$ regulatory T cells in the lamina propria of patients with inflammatory bowel disease. Immunol 2005; 174:5814-22.
82. Brandt EB, Strait RT, Hershko D, et al. Mast cells are required for experimental oral allergen-induced diarrhea. J Clin Invest 2003; 112:1666-77.
83. Li XM, Schofield BH, Huang CK, et al. A murine model of IgE-mediated cow's milk hypersensitivity. J Allergy Clin Immunol 1999; 103:206-14.
84. Berin MC, Kiliaan AJ, Yang PC, et al. The influence of mast cells on pathways of transepithelial antigen transport in rat intestine. J Immunol 1998; 161:2561-6.
85. Hermiston ML, Gordon JI. Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin. Science 1995; 270:1203.
86. Natividad JM, Petit V, Huang X, et al. Commensal and probiotic bacteria influence intestinal barrier function and susceptibility to colitis in Nod1-/-; Nod2-/- mice. Inflamm Bowel Dis 2012; 18:1434-46.
87. Kaetzel CS, Robinson JK, Chintalacharuvu KR, et al. The polymeric immunoglobulin receptor (secretory component) mediates transport of immune complexes across epithelial cells: A local defense function for IgA. Proc Natl Acad Sci U S A 1991; 88:8796-800.
88. Siciński P, Rowiński J, Warchoł JB, et al. Poliovirus type 1 enters the human host through intestinal M cells. Gastroenterology 1990; 98:56-8.
89. Mestecky J, McGhee JR, Elson CO. Intestinal IgA system. Immunol Allergy Clin North Am 1988; 8:349-68.
90. Goldblum RM, Ahlstedt S, Carlson B, et al. Antibody-forming cells in human colostrum after oral immunization. Nature 1975; 257:797-8.
91. Challacombe SJ, Rahman D, O'Hagan DT. Salivary, gut, vaginal and nasal antibody responses after oral immunization with biodegradable microparticles. Vaccine 1997;15:169-75.
92. Apter FM, Lencer WI, Finkelstein RA, et al. Monoclonal immunoglobulin A antibodies directed against cholera toxin prevent the toxin-induced chloride secretory response and block toxin binding to intestinal epithelial cells in vitro. Infect Immun 1993; 61:5271-8.
93. Helander A, Miller CL, Myers KS, et al. Protective immunoglobulin A and G antibodies bind to overlapping intersubunit epitopes in the head domain of type 1 reovirus adhesion sigma1. J Virol 2004; 78:10695-705.
94. Hutchings AB, Helander A, Silvery KJ, et al. Secretory immunoglobulin A antibodies against the sigma1 outer capsid protein of reovirus type 1 Lang prevent infection of mouse Peyer's patches. J Virol 2004; 78:947-57.
95. Mantis NJ, McGuinness CR, Sonuyi O, et al. Immunoglobulin A antibodies against ricin A and B subunits protect epithelial cells from ricin intoxication. Infect Immun 2006; 74:3455-62.
96. Stubbe H, Berdoz J, Kraehenbuhl JP, Corthésy B. Polymeric IgA is superior to monomeric IgA and IgG carrying the same variable domain in preventing *Clostridium difficile* toxin A damaging of T84 monolayers. J Immunol 2000; 164:1952-60.
97. Uren TK, Wijburg OL, Simmons C, et al. Vaccine-induced protection against gastrointestinal bacterial infections in the absence of secretory antibodies. Eur J Immunol 2005; 35:180-8.
98. Lycke N, Erlandsson L, Ekman L, et al. Lack of J chain inhibits the transport of gut IgA and abrogates the development of intestinal antitoxic protection. J Immunol 1999; 163:913-9.
99. Mantis NJ, Roll N, Corthesy B. Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. Mucosal Immunol 2011; 4:603-11.
100. Forbes SJ, Bumpus T, McCarthy EA, et al. Transient suppression of *Shigella flexneri* type 3 secretion by a protective O-antigen-specific monoclonal IgA. MBio 2011; 2:e00042-11.
101. Lu L, Lamm ME, Li H, et al. The human polymeric immunoglobulin receptor binds to *Streptococcus pneumoniae* via domains 3 and 4. J Biol Chem 2003; 278:48178-87.
102. Murthy AK, Chaganty BK, Troutman T, et al. Mannose-containing oligosaccharides of non-specific human secretory immunoglobulin A mediate inhibition of *Vibrio cholera* biofilm formation. PLoS One 2011; 6:e16847.
103. Mantis NJ, Cheung MC, Chintalacharuvu KR, et al. Selective adherence of IgA to murine Peyer's patch M cells: Evidence for a novel IgA receptor. J Immunol 2002; 169:1844-51.
104. Weltzin R. Binding and transepithelial transport of immunoglobulins by intestinal M cells: Demonstration using monoclonal IgA antibodies against enteric viral proteins. J Cell Biol 1989; 108:1673-85.
105. Duc M, Johansen FE, Corthésy B. Antigen binding to secretory immunoglobulin A results in decreased sensitivity to intestinal proteases and increased binding to cellular Fc receptors. J Biol Chem 2010; 285:953-60.
106. Boullier S, Tanguy M, Kadaoui KA, et al. Secretory IgA-mediated neutralization of *Shigella flexneri* prevents intestinal tissue destruction by down-regulating inflammatory circuits. J Immunol 2009; 183:5879-85.
107. Raux M, Finkielsztejn L, Salmon-Ceron D, et al. IgG subclass distribution in serum and various mucosal fluids of HIV type 1-infected subjects. AIDS Res Hum Retroviruses 2000; 16:583-94.
108. Schneider T, Zippel T, Schmidt W, et al. Increased immunoglobulin G production by short term cultured duodenal biopsy samples from HIV infected patients. Gut 1998; 42:357-61.
109. Israel EJ, Taylor S, Wu Z, et al. Expression of the neonatal Fc receptor, FcRn, on human intestinal epithelial cells. Immunology 1997; 92:69-74.
110. Dickinson BL, Badizadegan K, Wu Z, et al. Bidirectional FcRn-dependent IgG transport in a polarized human intestinal epithelial cell line. J Clin Invest 1999; 104:903-11.
111. MacDermott RP, Nash GS, Bertovich MJ, et al. Alterations of IgM, IgG, and IgA synthesis and secretion by peripheral blood and intestinal mononuclear cells from patients with ulcerative colitis and Crohn's disease. Gastroenterology 1981; 81:844-52.
112. Berin MC, Kiliaan AJ, Yang PC, et al. Rapid transepithelial antigen transport in rat jejunum: Impact of sensitization and the hypersensitivity reaction. Gastroenterology 1997; 113:856-64.
113. Berin MC, Yang PC, Ciok L, et al. Role for IL-4 in macromolecular transport across human intestinal epithelium. Am J Physiol 1999; 276:C1046-52.
114. Senapati S, Das S, Batra SK. Mucin-interacting proteins: From function to therapeutics. Trends Biochem Sci 2010; 35:236-45.

115. Podolsky DK. Oligosaccharide structures of isolated human colonic mucin species, J Biol Chem 1985; 260:15510-5.
116. Leitch GJ, Dickey AE, Udezulu IA, et al. *Entamoeba histolytica* trophozoites in the lumen and mucus blanket of rat colons studied in vivo. Infect Immun 1985; 47:68-73.
117. Moncada DM, Kammanadiminti SJ, Chadee K. Mucin and Toll-like receptors in host defense against intestinal parasites. Trends Parasitol 2003; 19:305-11.
118. McAuley JL, Linden SK, Png CW, et al. MUC1 cell surface mucin is a critical element of the mucosal barrier to infection. J Clin Invest 2007; 117:2313-24.
119. Boltin D, Perets TT, Vilkin A, Niv Y. Mucin function in inflammatory bowel disease: An update. J Clin Gastroenterol 2013; 47:106-11.
120. Madara JL. Loosening tight junctions. Lessons from the intestine. J Clin Invest 1989; 83:1089-94.
121. Tsukita S, Furuse M, Itoh M. Multifunctional strands in tight junctions. Nat Rev Mol Sci Biol 2001; 2:285-93.
122. Suzuki T. Regulation of intestinal epithelial permeability by tight junctions. Cell Mol Life Sci 2013; 70:631-59.
123. Teshima CW, Dieleman LA, Meddings JB. Abnormal intestinal permeability in Crohn's disease pathogenesis. Ann N Y Acad Sci 2012; 1258:159-65.
124. Warshaw AL, Walker WA, Isselbacher KJ, et al. Protein uptake by the intestine: Evidence for absorption of intact macromolecules. Gastroenterology 1974; 66:987-92.
125. Bhalla DK, Owen RL. Migration of B and T lymphocytes to M cells in Peyer's patch follicle epithelium: An autoradiographic and immunocytochemical study in mice. Cell Immunol 1983; 81:105-7.
126. Neutra MR. Current concepts in mucosal immunity. V. Role of M cells in transepithelial transport of antigens and pathogens to the mucosal immune system. Am J Physiol 1998; 274:G785-91.
127. Owen RL. Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: An ultrastructural study. Gastroenterology 1977; 72:440-51.
128. Mabbott NA, Baillie JC, Hume DA, Freeman TC. Meta-analysis of co-expressed gene signatures in mouse leukocyte populations. Immunobiology 2010; 215:724-36.
129. Bradford BM, Sester D, Hume DA, Mabbott NA. Defining the anatomical localisation of subsets of the murine mononuclear phagocyte system using integrin alpha X (ITGAX) and colony stimulating factor 1 receptor (CSF1-R) expression fails to discriminate dendritic cells from macrophages. Immunobiology 2011; 216:1228-37.
130. Hume DA, Mabbott N, Raza S, Freeman TC. Can DCs be distinguished from macrophages by molecular signatures. Nat Immunol 2013; 14:876.
131. Allan CH, Mendrick DL, Trier JS. Rat intestinal M cells contain acidic endosomal-lysosomal compartments and express class II major histocompatibility complex determinants. Gastroenterology 1993; 104:698-708.
132. Sicinski P, Rowinski J, Warchol JB, et al. Poliovirus type 1 enters the human host through intestinal M cells. Gastroenterology 1990; 98:56-8.
133. Wang J, Gusti V, Saraswati A, Lo DD. Convergent and divergent development among M cell lineages in mouse mucosal epithelium. J Immunol 2011; 187:5277-85.
134. Hase K, Kawano K, Nochi T, et al. Uptake through glycoprotein 2 of FimHþ bacteria by M cells initiates mucosal immune responses. Nature 2009; 462:226-31.
135. Borghesi C, Nicoletti C. Rapid appearance of M cells after microbial challenge is restricted at the periphery of the follicle-associated epithelium of Peyer's patch. Lab Invest 1999; 79:1393-401.
136. Meynell HM, Thomas NW, James PS, et al. Up-regulation of microsphere transport across the follicle-associated epithelium of Peyer's patch by exposure to *Streptococcus pneumoniae* R36a. FASEB J 1999; 13:611-9.
137. Frossard CP, Hauser C, Eigenmann PA. Antigen-specific secretory IgA antibodies in the gut are decreased in a mouse model of food allergy. J Allergy Clin Immunol 2004; 114:377-82.
138. Kerneis S, Bogdanova A, Kraehenbuhl JP, Pringault E. Conversion by Peyer's patch lymphocytes of human enterocytes into M cells that transport bacteria. Science 1997; 277:949-52.
139. Hsieh EH, Lo DD. Jagged1 and notch1 help edit M cell patterning in Peyer's patch follicle epithelium. Dev Comp Immunol 2012; 27:306-12.
140. Gonnella PA, Waldner HP, Weiner HL. B cell-deficient (mu MT) mice have alterations in the cytokine microenvironment of the gut-associated lymphoid tissue (GALT) and a defect in the low dose mechanism of oral tolerance. J Immunol 2001; 166:4456-64.
141. Fujihashi K, Dohi T, Rennert PD, et al. Peyer's patches are required for oral tolerance to proteins. Proc Natl Acad Sci U S A 2001; 98:3310-5.
142. Spahn TW, Fontana A, Faria M, et al. Induction of oral tolerance to cellular immune responses in the absence of Peyer's patches. Eur J Immunol 2001; 31:1278-87.
143. Spahn TW, Weiner HL, Rennert PD, et al. Mesenteric lymph nodes are critical for the induction of high-dose oral tolerance in the absence of Peyer's patches. Eur J Immunol 2002; 32:1109-13.
144. Kunkel D, Kirchhoff D, Nishikawa S, et al. Visualization of peptide presentation following oral application of antigen in normal and Peyer's patches-deficient mice. Eur J Immunol 2003; 33:1292-301.
145. Butcher EC. Leukocyte endothelial cell recognition: Three or more steps to specificity and diversity. Cell 1991; 67:1-4.
146. Chin YH, Sackstein R, Cai JP. Lymphocyte-homing receptors and preferential migration pathways. Proc Soc Exp Biol Med 1991; 196:374-80.
147. Picker LJ, Butcher EC. Physiological and molecular mechanisms of lymphocyte homing. Ann Rev Immunol 1992; 10:561-91.
148. Hart AL, Ng SC, Mann E, et al. Homing of immune cells: Role in homeostasis and intestinal inflammation. Inflamm Bowel Dis 2010; 16:1969-77.
149. Stern M, Walker WA. Food proteins and gut mucosal barrier I. Binding and uptake of cow's milk proteins by adult rat jejunum in vitro. Am J Physiol 1984; 246:G556-62.
150. Stern M. Food proteins and maturation of small intestinal microvillus membranes (MVM). II. Binding of gliadin hydrolysate fractions and of the gliadin peptide B3142. J Pediatr Gastroenterol Nutr 1988; 7:122-7.
151. Dinsdale D, Healy PJ. Enzymes involved in protein transmission by the intestine of the newborn lamb. Histochem J 1982; 14:811-21.
152. Begue B, Dumant C, Bambou JC, et al. Microbial induction of CARD15 expression in intestinal epithelial cells via toll-like receptor 5 triggers an antibacterial response loop. J Cell Physiol 2006; 209:241-52.
153. Maaser C, Eckmann L, Paesold G, et al. Ubiquitous production of macrophage migration inhibitory factor by human gastric and intestinal epithelium. Gastroenterology 2002; 122:667-80.
154. Zanello G, Berri M, Dupont J, et al. *Saccharomyces cerevisiae* modulates immune gene expressions and inhibits ETEC-mediated ERK1/2 and p38 signaling pathways in intestinal epithelial cells. PLoS One 2011; 6:e18573.

155. Voltan S, Martines D, Elli M, et al. *Lactobacillus crispatus* M247-derived H(2)O(2) acts as a signal transducing molecule activating peroxisome proliferator activated receptor-gamma in the intestinal mucosa. Gastroenterology 2008; 135:1216-27.
156. Resta-Lenert S, Barrett KE. Probiotics and commensals reverse TNF-alpha- and IFN-gamma-induced dysfunction in human intestinal epithelial cells. Gastroenterology 2006; 130:731-46.
157. Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001; 411:599-603.
158. Ogura Y, Bonen DK, Inohara N, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 2001; 411:603-6.
159. Ogura Y, Lala S, Xin W, et al. Expression of NOD2 in Paneth cells: a possible link to Crohn's ileitis. Gut 2003; 52:1591-7.
160. Inohara N, Ogura Y, Fontalba A, et al. Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. J Biol Chem 2003; 278:5509-12.
161. Van Heel DA, Ghosh S, Hunt KA, et al. Synergy between TLR9 and NOD2 innate immune responses is lost in genetic Crohn's disease. Gut 2005; 54:1553-7.
162. Richmond AL, Kabi A, Homer CR, et al. The nucleotide synthesis enzyme CAD inhibits NOD2 antibacterial function in human intestinal epithelial cells. Gastroenterology 2012; 142:1483-92.
163. Cario E, Podolsky DK. Differential expression in intestinal epithelial cell expression of Toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun 2000; 68:7010-7.
164. Shang L, Fukata M, Thirunarayanan N, et al. Toll-like receptor signaling in small intestinal epithelium promotes B-cell recruitment and IgA production in lamina propria. Gastroenterology 2008;135:529-38.
165. Lee J, Mo JH, Katakura K, et al. Maintenance of colonic homeostasis by distinctive apical TLR9 signaling in intestinal epithelial cells. Nat Cell Biol 2006; 8:1327-36.
166. Cario E. Bacterial interactions with cells of the intestinal mucosa: Toll-like receptors and NOD2. Gut 2005; 54:1182-93.
167. Santaolalla R, Abreu MT. Innate immunity in the small intestine. Curr Opin Gastroenterol 2012; 28:124-9.
168. Madsen KL. Interactions between microbes and the gut epithelium. J Clin Gastroenterol 2011; 45(Suppl):S111-4.
169. Wiman K, Curman B, Forsum U, et al. Occurrence of Ia antigens on tissues on non-lymphoid origin. Nature 1978; 276:711-3.
170. Kirby WN, Parr EL. The occurrence and distribution of H-2 antigens on mouse intestinal epithelial cells. J Histochem Cytochem 1979; 27:746-50.
171. Bland PW, Warren LJ. Antigen presentation by epithelial cells of the rat small intestine. II. Selective induction of suppressor T cells. Immunology 1986; 58:9-14.
172. Mayer L, Shlien R. Evidence for function of Ia molecules on gut epithelial cells in man. J Exp Med 1987; 166:1471-83.
173. Kaiserlian D, Vidal K, Revillard JP. Murine enterocytes can present soluble antigen to specific class II-restricted $CD4^+$ T cells. Eur J Immunol 1989; 19:1513-6.
174. Dahan S, Rabinowitz KM, Martin AP, et al. Notch-1 signaling regulates intestinal epithelial barrier function, through interaction with $CD4^+$ T cells, in mice and humans. Gastroenterology 2011; 140:550-9.
175. Dahan S, Roth-Walter F, Martin AP, et al. Lymphoepithelial interactions: A new paradigm. Ann N Y Acad Sci 2009; 1165:323-6.
176. Selby WS, Janossy G, Mason DY, Jewell DP. Expression of HLA-DR antigens by colonic epithelium in inflammatory bowel disease. Clin Exp Immunol 1983; 53:614-8.
177. Salomon P, Pizzimenti A, Panja A, et al. The expression and regulation of class II antigens in normal and inflammatory bowel disease peripheral blood monocytes and intestinal epithelium. Autoimmunity 1991; 9:141-9.
178. Mayer L, Eisenhardt D, Salomon P, et al. Expression of class II molecules on intestinal epithelial cells in humans. Differences between normal and inflammatory bowel disease. Gastroenterology 1991; 100:3-12.
179. Dotan I, Allez M, Nakazawa A, et al. Intestinal epithelial cells from inflammatory bowel disease patients preferentially stimulate $CD4^+$ T cells to proliferate and secrete interferon-gamma. Am J Physiol Gastrointest Liver Physiol 2007; 292:G1630-40.
180. Crotty B, Hoang P, Dalton HR, Jewell DP. Salicylates used in inflammatory bowel disease and colchicine impair interferon-gamma induced HLA-DR expression. Gut 1992; 33:59-64.
181. Linsley PS, Brady W, Grosmaire L, et al. Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. J Exp Med 1991; 173:721-30.
182. Azuma M, Ito D, Yagita H, et al. B70 antigen is a second ligand for CTLA-4 and CD28. Nature 1993; 366:76-9.
183. Schwartz RH. A cell culture model for T lymphocyte clonal anergy. Science 1990; 248:1349-56.
184. Harding FA, Mcarthur JG, Gross JA, et al. CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. Nature 1992; 356:607-9.
185. Sandborn WJ, Colombel JF, Sands BE, et al. Abatacept for Crohn's disease and ulcerative colitis. Gastroenterology 2012; 143:62-9.
186. Mayer L, Kaser A, Blumberg RS. Dead on Arrival: Understanding the failure of CTLA4-immunoglobulin therapy in inflammatory bowel disease. Gastroenterology 2012; 143:13-7.
187. Sanderson IR, Ouellette AJ, Carter EA, et al. Differential regulation of B7 mRNA in enterocytes and lymphoid cells. Immunology 1993; 79:434-8.
188. Nakazawa A, Dotan I, Brimnes J, et al. The expression and function of costimulatory molecules B7H and B7-H1 on colonic epithelial cells. Gastroenterology 2004; 126:1347-57.
189. Bland PW. Antigen presentation by gut epithelial cells: secretion by rat enterocytes of a factor with IL-1-like activity. Adv Exp Med Biol 1987; 219-25.
190. Bland PW, Kambarage DM. Antigen handling by the epithelium and lamina propria macrophages. Gastroenterol Clin North Am 1991; 20:577-96.
191. Hershberg RM, Framson PE, Cho DH, et al. Intestinal epithelial cells use two distinct pathways for HLA class II antigen processing. J Clin Invest 1997; 100:204-15.
192. Hershberg RM, Cho DH, Youakim A, et al. Highly polarized HLA class II antigen processing and presentation by human intestinal epithelial cells. J Clin Invest 1998; 102:792-803.
193. Hershberg RM, Mayer LF. Antigen processing and presentation by intestinal epithelial cells—polarity and complexity. Immunol Today 2000; 1:123-8.
194. Mayer L. The role of the epithelium in mucosal immunity. Res Immunol 1997; 98:498-504.
195. Kaiserlian D, Vidal K, Revillard JP. Murine enterocytes can present soluble antigen to specific class II-restricted $CD4^+$ T cells. Eur J Immunol 1989; 19:1513-6.
196. Yio XY, Mayer L. Characterization of a 180 kDa intestinal epithelial cell membrane glycoprotein, gp180: A candidate

molecule mediating T cell:epithelial cell interactions. J Biol Chem 1997; 272:12786-92.
197. Panja A, Blumberg RS, Balk SP, et al. CD1d is involved in T cell:epithelial cell interactions. J Exp Med 1993; 178:1115-20.
198. Hershberg R, Eghtesady P, Sydora B, et al. Expression of the thymus leukemia antigen in mouse intestinal epithelium. Proc Natl Acad Sci U S A 1990; 87:9727-31.
199. Blumberg RS, Terhorst C, Bleicher P, et al. Expression of a nonpolymorphic MHC class I-like molecule, CD1d, by human intestinal epithelial cells. J Immunol 1991; 147:2518-24.
200. Zeissig S, Blumberg RS. Primary immunodeficiency associated with defects in CD1 and CD1-restricted T cells. Ann N Y Acad Sci 2012; 1250:14-24.
201. Allez M, Tieng V, Nakazawa A, et al. $CD4^+NKG2D^+$ T cells in Crohn's disease mediate inflammatory and cytotoxic responses through MICA interactions. Gastroenterology 2007; 132:2346-58.
202. Perera L, Shao L, Patel A, et al. Expression of nonclassical class I molecules by intestinal epithelial cells. Inflamm Bowel Dis 2007; 13:298-307.
203. Amiot M, Bernard A, Raynal B, et al. Heterogeneity of the first cluster of differentiation: Characterization and epitopic mapping of three CD1 molecules on normal human thymus cells. J Immunol 1986; 136:1752-8.
204. Shawar SM, Cook RG, Rodgers JR, et al. Specialized functions of MHC class I molecules. I. An N-formyl peptide receptor is required for construction of the class I antigen Mta. J Exp Med 1990; 171:897-912.
205. Vitetta ES, Poulik MD, Klein J, Uhr JW. Beta-2 -microglobulin is selectively associated with H-2 and TL alloantigens on murine lymphoid cells. J Exp Med 1976; 144:179-92.
206. Mayer L, Eisenhardt D. Lack of induction of suppressor T cells by intestinal epithelial cells from patients with inflammatory bowel disease. J Clin Invest 1990; 86:1255-60.
207. Viney JL, Kilshaw PJ, MacDonald TT. Cytotoxic alpha/$beta^+$ and gamma/$delta^+$ T cells in murine intestinal epithelium. Eur J Immunol 1990; 20:1623-6.
208. Guy-Grand D, Cerf-Bensussan N, Malissen B, et al. Two gut intraepithelial $CD8^+$ lymphocyte populations with different T cell receptors: A role for the gut epithelium in T cell differentiation. J Exp Med 1991; 173:471-81.
209. Croitoru K, Ernst PB. Leukocytes in the intestinal epithelium: An unusual immunological compartment revisited. Reg Immunol 1992; 4:63-9.
210. Guy-Grand D, Vassalli P. Gut intraepithelial T lymphocytes. Curr Opin Immunol 1993; 5:247-52.
211. Cerf-Bensussan N, Guy-Grand D, Griscelli C. Intraepithelial lymphocytes of human gut: isolation, characterisation and study of natural killer activity. Gut 1985; 26:81-8.
212. Ebert EC. Proliferative responses of human intraepithelial lymphocytes to various T-cell stimuli. Gastroenterology 1989; 97:1372-81.
213. Ebert EC, Roberts AI, Brolin RE, Raska K. Examination of the low proliferative capacity of human jejunal intraepithelial lymphocytes. Clin Exp Immunol 1986; 65:148-57.
214. Roberts AI, O'Connell SM, Biancone L, et al. Spontaneous cytotoxicity of intestinal intraepithelial lymphocytes: Clues to the mechanism. Clin Exp Immunol 1993; 94:527-32.
215. Guy-Grand D, Malassis-Seris M, Briottet C, Vassali P. Cytotoxic differentiation of mouse gut thymus dependent and independent intraepithelial T lymphocytes is induced locally. Correlation between functional assays, presence of perforin and granzyme transcripts, and cytoplasmic granules. J Exp Med 1991; 173:1549-52.
216. Hayday A, Theodoridis E, Ramsburg E, Shires J. Intraepithelial lymphocytes: Exploring the Third Way in immunology. Nat Immunol 2001; 2:997-1003.
217. Higgins JM, Mandlebrot DA, Shaw SK, et al. Direct and regulated interaction of integrin alphaEbeta7 with E-cadherin. J Cell Biol 1998; 140:197-210.
218. Austrup F, Rebstock S, Kilshaw PJ, Hamann A. Transforming growth factor-beta 1-induced expression of the mucosa-related integrin alpha E on lymphocytes is not associated with mucosa-specific homing. Eur J Immunol 1995; 25:1487-91.
219. Targan SR, Deem RL, Liu M, et al. Definition of a lamina propria T cell responsive state. Enhanced cytokine responsiveness of T cells stimulated through the CD2 pathway. J Immunol 1995; 154:664-75.
220. Abreu-Martin MT, Targan SR. Regulation of immune responses of the intestinal mucosa. Crit Rev Immunol 1996; 16:277-309.
221. Pirzer UC, Schurmann G, Post S, et al. Differential responsiveness to CD3-Ti vs. CD2-dependent activation of human intestinal T lymphocytes. Eur J Immunol 1990; 20:2339-42.
222. Qiu Y, Yang H. Effects of intraepithelial lymphocyte-derived cytokines on intestinal mucosal barrier function. J Interferon Cytokine Res 2013; May 21. [Epub ahead of print]
223. Mosley RL, Wang J, Hamad M, Klein JR. Functional heterogeneity of murine intestinal intraepithelial lymphocytes: Studies using TCR-alpha $beta^+$ IEL lines and fresh IEL isolates reveal multiple cytotoxic subsets differentiated by CD5, CD8 alpha/alpha, and CD8 alpha/beta expression. Dev Comp Immunol 1994; 18:155-64.
224. James SP, Zeitz M, Kagnoff M, et al. Intestinal lymphocyte populations and mechanisms of cell-mediated immunity. Immunol Allergy Clin North Am 1988; 8:369-91.
225. Zeitz M, Greene WC, Peffer NJ, James SP. Lymphocyte isolated from the intestinal lamina propria of normal nonhuman primates have increased expression of genes associated with T-cell activation. Gastroenterology 1988; 94:647-55.
226. Qiao L, Braunstein J, Golling M, et al. Differential regulation of human T cell responsiveness by mucosal versus blood monocytes. Eur J Immunol 1996; 26:922-7.
227. Boirivant M, Fuss I, Fiocchi C, et al. Hypoproliferative human lamina propria T cells retain the capacity to secrete lymphokines when stimulated via CD2/CD28 pathways. Proc Assoc Am Physicians 1996; 108:55-67.
228. Boirivant M, Pica R, DeMaria R, et al. Stimulated human lamina propria T cells manifest enhanced Fas-mediated apoptosis. J Clin Invest 1996; 98:2616-22.
229. De Maria R, Boirivant M, Cifone MG, et al. Functional expression of Fas and Fas ligand on human gut lamina propria T lymphocytes. A potential role for the acidic sphingomyelinase pathway in normal immunoregulation. J Clin Invest 1996; 97:316-22.
230. Neurath MF, Finotto S, Glimcher LH. The role of Th1/Th2 polarization in mucosal immunity. Nat Med 2002; 8:567-73.
231. Neurath MF, Weigmann B, Finotto S, et al. The transcription factor T-bet regulates mucosal T cell activation in experimental colitis and Crohn's disease. J Exp Med 2002; 195:1129-43.
232. Nakayama T, Yamashita M. Initiation and maintenance of Th2 cell identity. Curr Opin Immunol 2008; 20:265-71.
233. McGhee JR, Kyono H, Kubota M, et al. Mucosal Th1-versus Th2-type responses for antibody- or cell-mediated immunity to simian immunodeficiency virus in rhesus macaques. J Infect Dis 1999; 179(Suppl 3):S480-4.