Colitis

A Practical Approach to Colon and Ileum Biopsy Interpretation

Anne Jouret-Mourin
Gavino Faa
Karel Geboes
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Preface

Inflammatory bowel disease is a group of chronic inflammatory disorders primarily involving the digestive tract and particularly the ileocolon. These diseases have been reported from all over the world. They affect children as well as adults. Inflammatory bowel diseases must be differentiated from other conditions such as infectious colitis and microscopic colitis because treatment is different. Because of the widespread use of colonoscopy, the number of biopsies arriving in the laboratories of pathology is increasing. At present, the diagnosis of colitis is therefore a routine task of pathologists and frequently a challenge. In addition the ileum may be involved in a variety of inflammatory conditions, and ileum biopsies combined with colon samples improve the diagnostic yield. Furthermore the occurrence of isolated ileitis represents a diagnostic challenge.

This second edition has been developed in a close collaboration between pathologists and endoscopists because this provides a realistic approach of the needs and daily routine practice. After a chapter with the presentation of the normal histology of the colonic and ileal mucosae, contributions with an update on procedures needed for optimal biopsies, a methodological approacht to the microscopic analysis of ileocolonic biopsies, and the presentation of the basic lesions observed in ileocolitis, illustrated by schematic drawings, the following chapters are dealing with the particular histology of the major types of colitis and the possible differential diagnosis between these lesions. The data presented are based on personal clinical practice and research, on teaching experience, and on a review of the literature. The final chapter is devoted to scoring systems for disease activity in inflammatory bowel disease, an issue which may become a new challenge for pathologists.

We are particularly grateful to Maria Leo for her artistic work in making the drawings and to Sebastien Godecharles for his help in scanning microphotographs.

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Chapter 1
Introduction

Anne Jouret-Mourin, Karel Geboes, and Gavino Faa

Abstract The first colon fibroscope prototypes were developed in 1963 but it was not until American Cystoscope Makers Inc. entered the field in the late 1960s that clinical coloscopy began to flourish. Endoscopy presented some advantages in the ability to see variations in the color of the mucosa, visual resolution of tiny lesions, and the means to obtain tissue diagnosis. In the early 1970s, various studies demonstrated the value of coloscopy with biopsy for the differential diagnosis of inflammatory bowel diseases (IBD) [1, 2]. By the early 1980s, it became clear that the diagnosis and differential diagnosis of IBD, colitis in general, and diarrhea are indications for coloscopy and biopsy as stated in guidelines of the American Society for Gastrointestinal Endoscopy. Over the years, the number of endoscopic biopsies of the colon coming to the pathology laboratory has therefore gradually increased and today they present a daily challenge for pathologists.

Diarrhea (four or more bowel movements per day, liquid stools) lasting more than 4 weeks, abdominal pain, and constipation are common symptoms in adults. The prevalence is approximately 1–5%, making it a major cause of disability [3]. A small number of patients (approximately 1%) need specialized investigations or hospitalization [4]. The etiology is highly variable and includes among others infections, endocrine diseases, chronic inflammatory bowel disorders, food intolerance, and drugs. Patients with chronic diarrhea, with or without the passage of blood, are likely to be fully investigated. Several studies show that coloscopy with biopsy is useful in the investigation of chronic diarrhea without blood loss, yielding a histological diagnosis in 22–31% of patients who had a macroscopically normal

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colon. One study evaluating more than 800 patients found that 122 (15%) had abnormal histopathology. Of those with abnormal biopsies, 2% would have been missed if only a flexible sigmoidoscopy had been performed. Coloscopy is the method of choice in patients older than age 50 years [4–9]. Histological diagnoses include a variety of conditions such as spirochetosis, pseudomelanosis coli, and microscopic colitis. Various forms of colitis can thus be present in the absence of radiological and endoscopic lesions or features of colitis. Ileocoloscopy with biopsy is certainly indicated in patients with chronic diarrhea with blood loss.

Because of the limitations of the patterns of tissue response to a varied range of insults, the precise histological diagnosis of colitis requires a good knowledge of the normal histology of the mucosa, of the different etiological possibilities, and of the microscopic features of different types of colitis and ileocolitis [10–13].

Diagnosis of inflammatory ileocolic diseases requires close collaboration between pathologists and endoscopists, as well the use of a common language [14]. Therefore, both endoscopic and histological images of the same lesions are necessary. After all, both diagnostic endoscopy and pathology imply an analysis of the "morphology" of lesions. The difference between both is the tool. Endoscopy uses a macroscopic approach, while pathology uses microscopy. The endoscopic description can therefore help the pathologists to formulate a differential diagnosis of one given lesion.

The purpose of the present edition is to review the endoscopic procedures which are needed to take biopsies, to give the different steps to look at a biopsy to reach a diagnosis for colitis, ileocolitis, or ileitis, and to present the most common types of (ileo-)colitis based on both endoscopic and histological features.

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Chapter 2
The Normal Biopsy: Colonic and Ileal Mucosa and Submucosa

Anne Jouret-Mourin, Peter Van Eycken, Maria Leo, and Karel Geboes

Abstract The digestive tract is a hollow tube consisting throughout of three coats or layers. The first layer, the mucosa, is made up of an epithelial lining which borders on the lumen of the bowel and rests upon a basement membrane, the lamina propria and the muscularis mucosae. The mucosa of the colon has a smooth surface and is composed of tubular parallel crypts embedded in a loosely arranged stroma. The mucosa of the ileum is composed of fingerlike villi and displays a number of specific features including the shorter villi and more epithelial goblet cells on the surface than the proximal small intestine and the presence of Peyer's patches. The second coat is the submucosa. The muscularis propria, the third layer, is composed of two layers of smooth muscle separated by a thin layer of connective tissue in which the ganglionated myenteric plexus (Auerbach's) can be observed. The subserosa is composed of loose areolar tissue covered by mesothelium where the tract borders on the body cavity (serosa). Endoscopic biopsies are limited to the mucosa and upper part of the submucosa. A good understanding of the normal histology of the mucosa and submucosa is essential for analysis of endoscopic biopsies of the ileum and the colon.

Keywords Crypt · Mucosa · Architecture · Innominate groove · Intestinal epithelial cell · Goblet cell · Enteroendocrine cell · Paneth cell · Pigmented macrophage · Foamy macrophage · Muciphage · Neutrophil · Cytokine · Fibroblast Collagen · Basement membrane · Lymphocyte · Lamina propria · Eosinophil · Mast cell · Inter-epithelial lymphocyte Peyer's patches · Macrophage · Muscularis mucosae · Submucosa · Adhesion molecule · Integrin · Selectin

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Normal Mucosal Architecture

Colonic Mucosal Architecture

The colonic mucosa is continuously challenged by potentially injurious dietary and microbial luminal factors and acts as a barrier while it is also involved in secretion, terminal digestion, absorption, and transport of nutrients, water, and electrolytes. It contains therefore a combination of epithelial cells and stromal cells with immune competent cells, of which most are illustrated in the drawings (Fig. 2.1).

The mucosa of the colon has a smooth surface and tubular crypts which open into the surface or into innominate grooves or lines. The latter are mucosal areas where several crypts open into one central crypt. They can be seen as delicate, inconstant spiculations on the colonic margin on barium enemas. The crypts are formed in early postnatal life, and the number increases steadily by crypt fission, a process in which new crypts are formed by branching off from existing crypts to accommodate the growth of the organ into adulthood [1]. Crypt fission or branching is therefore not unusual in biopsies from children. The organization of the crypts is responsible for a characteristic normal pattern with roundish pits on the mucosal surface which

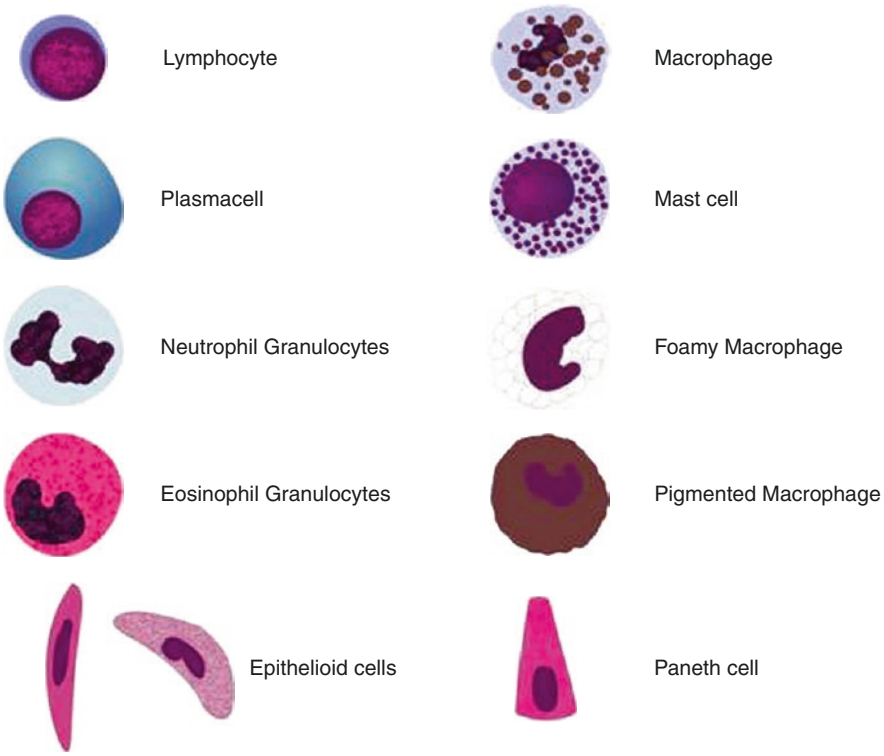


Fig. 2.1 Key to identification of cells in the illustrations

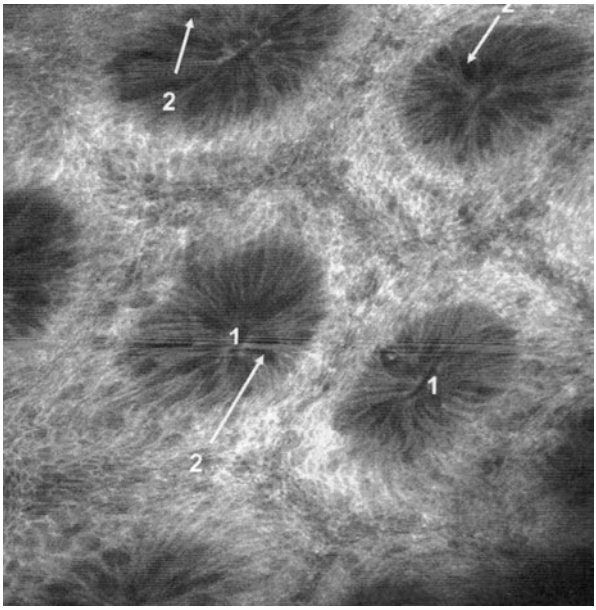


Fig. 2.2 Confocal laser endomicroscopy of the surface of the colon mucosa showing the regularly rounded pits (1) and goblet cells (2)

can be observed during magnifying colonoscopy or confocal laser endomicroscopy (CLE) of the colon (Fig. 2.2). According to the "Kudo classification," the normal appearance is called "pit pattern I" [2]. CLE fluorescein sodium imaging of the normal colon shows a similar surface crypt architecture with ordered and regular crypt orifices covered by a homogeneous epithelial layer with visible "black-hole" goblet cells. Changes in the crypt architecture or pit pattern occur during carcinogenesis but also as a result of chronic inflammation and can be identified with the advanced endoscopic techniques.

The normal surface and crypts are lined by a single layer of low columnar epithelial cells resting on a basement membrane composed of extracellular matrix components including laminins, collagens (predominantly collagen IV), proteoglycans, calcium-binding proteins such as fibulin, and various other structural or adhesive proteins. The membrane supports and separates the epithelium from the underlying connective tissue or lamina propria but also influences the behavior of epithelial cells by controlling their shape, gene expression, adhesion, migration, proliferation, and apoptosis. The normal membrane measures up to 3 or 4 μm [3]. This membrane is thickest in the rectum. The tubular glands or crypts are tightly packed. Variations in the number of crypts per defined area are minimal. Some variations in space between crypts is expected in biopsies of normal patients. The diameter of the crypts and the distance between the crypts are fairly constant. The mean diameter varies between 45 and 105 μm. The inter-glandular distance varies from 4.5 to 36 μm [4]. The crypts have a straight, test tube shape with minimal branching. They run a parallel course from the surface to the muscularis mucosae (Fig. 2.3). The crypts are surrounded by a pericryptal fibroblast sheath composed of fibroblasts and myofibroblasts.

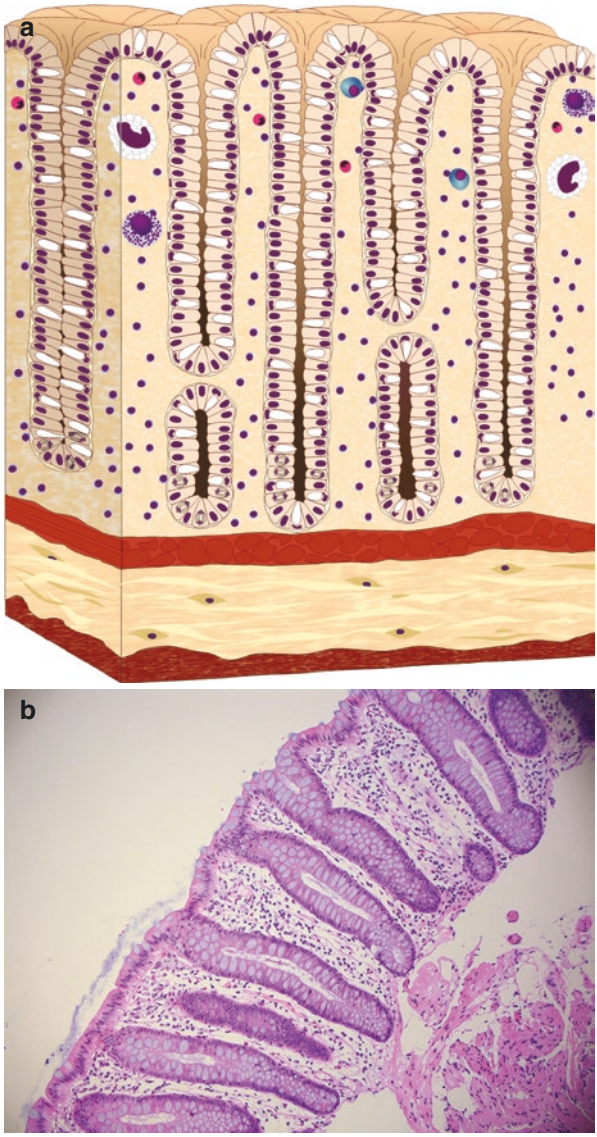
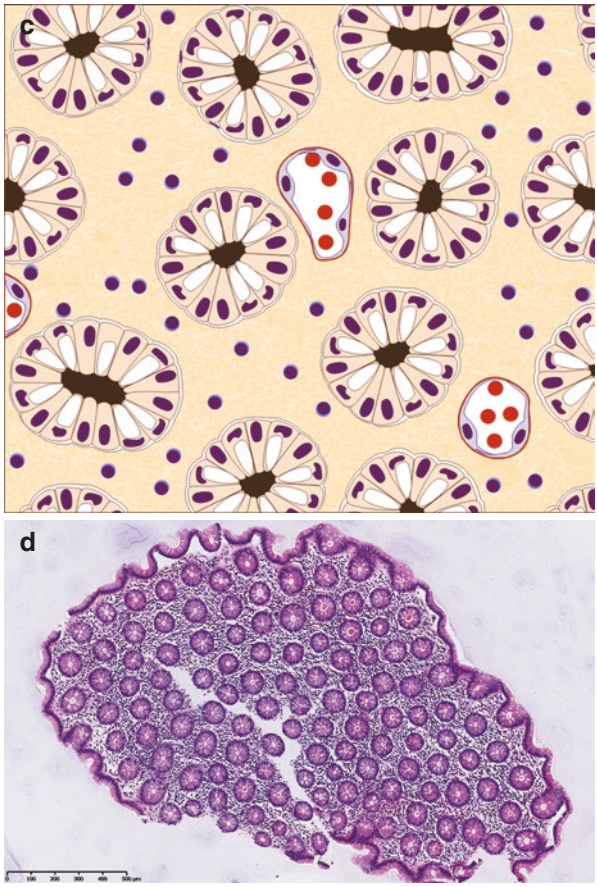


Fig. 2.3 The normal colonic mucosa is composed of surface epithelial cells and tubular glands embedded in a loosely arranged stroma (**a**) schematic view, (**b**) microscopy ×10 of perpendicular sections. (**c**) Schematic view. (**d**) Microscopy ×10 of transverse sections

Fig. 2.3 (continued)



The colonic epithelial cells form a heterogeneous group composed of surface lining cells (absorptive cells and goblet cells), crypt cells, and specialized cells such as enteroendocrine cells (Fig. 2.4). The absorptive cells contain no mucin. The cytoplasm is mildly eosinophilic and nuclei are located basally. They are involved in the formation of a mechanical barrier by the presence of tight junctions in which different proteins are incorporated. Epithelial cells are important for resorption and play a major role in secretion and humoral immunity (secretion of secretory immunoglobulin A = SIgA). Goblet cells contain an ovoid mucoid vacuole. Crypt cells are important for epithelial cell renewal [5]. The crypts contain endocrine cells, precursor cells, and occasional Paneth cells (in the right colon). The endocrine cells, usually situated at the base of the crypts, contain fine eosinophilic granules with secretory proteins. The nuclei are not basal but on the luminal side. Paneth cells are involved in the production of defensins and lysozyme (antimicrobial peptides) and constitute the niche for leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5) stem cells in intestinal crypts. In colon crypts, CD24+ (CD = cluster differentiation) cells residing between Lgr5 stem cells may represent the Paneth cell

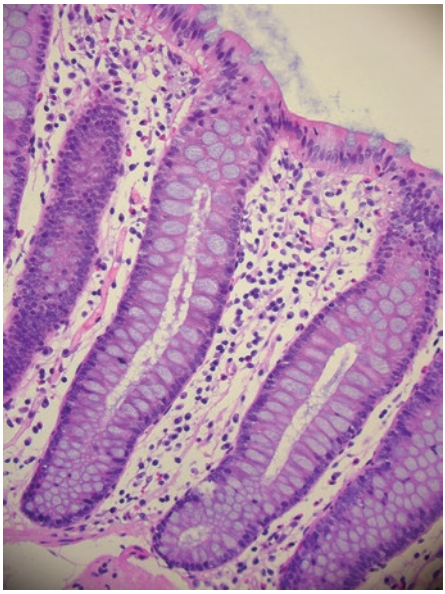


Fig. 2.4 Colonic mucosa showing the parallel crypts and the cells in the lamina propria (×20)

equivalent [6]. Human colonic crypts are lined by a clonal population derived from a multipotential stem cell. Undifferentiated cells at the base of the crypts are precursors of other epithelial cells [7]. These cells can migrate from the crypt base to the surface in 3–8 days, which allows for rapid repair.

Specialized surface epithelial cells such as the follicle-associated epithelial (FAE) cells are well equipped for antigen handling. In the colon they are found in association with mucosal lymphoid aggregates.

Ileal Mucosal Architecture

The mucosal epithelium is divided into the villous and crypt compartments (Fig. 2.5). Each villous surface is covered by a single layer of epithelial cells of various types: the columnar enterocytes and the goblet cells. The columnar enterocyte's function is mainly in terminal digestion of food substances (even more so in the duodenum) and the absorption of nutrients by apical microvilli. They are more abundant in the proximal small intestine. The goblet cells secrete mucins in order to protect the luminal layer. The latter cells are more frequent in the distal small intestine. Scattered endocrine cells are present within the villous epithelium but they are more abundant in the crypts. The crypt epithelium primarily functions in epithelial cell renewal. It's why mitoses are more frequently seen within the crypts. The Paneth cells normally found in the crypt base have a pyramidal shape. They contain and secrete lysozyme, defensins, and other antimicrobial peptides which keep the crypts sterile, and they protect enterocytes and stem cells. Their cytoplasm contains

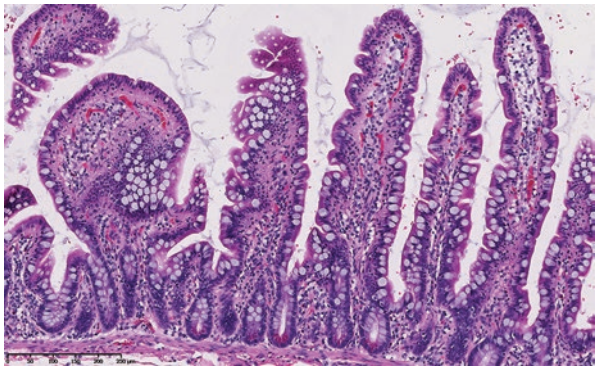


Fig. 2.5 The normal ileal mucosa is composed of the villous and the crypts compartments (×10)

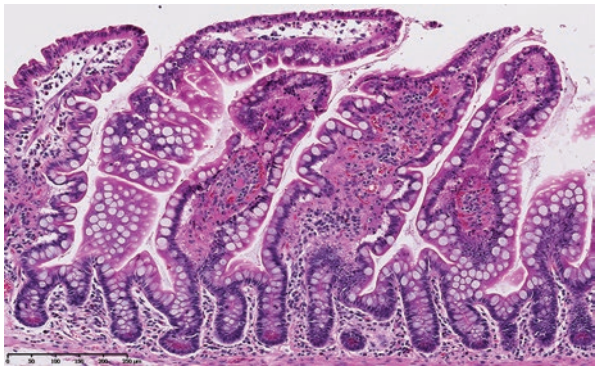


Fig. 2.6 Paneth's cells are normally found in the crypt base. Their cytoplasm contains eosinophilic granules

supranuclear, eosinophilic granules (Fig. 2.6). These four major epithelial cell types arise from stem cells which have been identified by the Wnt target gene leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5) [8]. Scattered intraepithelial lymphocytes (one lymphocyte for five epithelial cells) are seen between the epithelial cells just above the basement membrane. Crypts also contain intraepithelial lymphocytes but neutrophils and plasma cells are normally absent.

The distinctive mucosal characteristics of the terminal ileum include an increased proportion of goblet cells within the ileal epithelium, relatively shorter villi compared with jejunal villi, and the presence of specialized clusters of lymphoid aggregates (Peyer's patches) most prominently located in the mucosa. This organized lymphoid tissue can extend into the submucosa and is concentrated in the terminal 10–15 cm ileum where it forms a lymphoid ring. Peyer's patches are a major component of the gut-associated lymphoid tissue (GALT) acting as immune-inductive sites by supplying the lamina propria with immunocompetent surface IgA-positive B cells that become functional secretory plasma cells.

In children, these can be grossly seen near the ileocecal junction. Hyperplastic Peyer's patches can cause intussusception occurring in the ileocecal region during childhood [9]. Structurally, four distinct compartments can be distinguished in Peyer's patches: the follicle, the dome, the interfollicular region, and the

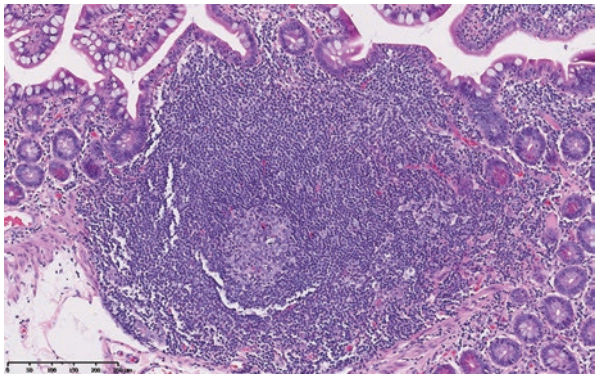


Fig. 2.7 Peyer's patches are composed of four distinct compartments: follicle, dôme, interfollicular region, and follicle-associated epithelium (×10)

follicle-associated epithelium [10] (Fig. 2.7). Lymphoid follicles contain a germinal center populated by IgA-positive B cells with occasional CD4-positive T cells and macrophages, surrounded by a mantle zone which contains small IgD- and IgM-positive B cells. The dome is the area between the follicle and the surface epithelium. It includes B cells, macrophages, and some plasma cells.

The follicle-associated epithelium overlying lymphoid aggregates has fewer goblet cells and contains specialized low cuboidal and flattened enterocytes, the so-called M-cells (M is derived from microfold because the cells have small folds on the surface or from membrane because they are very thin). These M-cells play a key role in immunity facilitating interaction between luminal antigens and immunocompetent lymphoid cells present in the mucosa. The abundant lymphoid population between follicles is the fourth component of Peyer's patches and corresponds to the T-cell-rich interfollicular zone.

Granular brown-black pigment is commonly seen in the deep portions of Peyer's patches or in the lamina propria of the ileum in adults. Accumulating within the macrophages, the pigment has no known clinicopathological significance. The origin is probably atmospheric dust or from dietary sources [11].

The mucosal ileocolic transition demonstrates a gradual loss of villi occurring at variable lengths along the short intracecal ileal segment. The ileal mucosa blends rather imperceptively with the mucosa of the large bowel.

Lamina Propria Cells of the Intestinal Mucosa

The lamina propria cells include lymphocytes, cells of the monocyte/macrophage lineage, eosinophils, mast cells, connective tissue cells, vascular structures and nerve endings (in the small intestine), and smooth muscle cells in the muscularis mucosae.

In animals raised in germ-free environments, very few leukocytes are found in the lamina propria. The number increases rapidly following conventionalization forming the normal immune system in the digestive tract. These cells are usually situated in the upper part of the lamina propria. In the normal human rectal

mucosa, the number of nuclei in the lamina propria for a well-defined area is fairly constant. Relative to the left colon and rectum, the right colon contains greater numbers of inflammatory cells in the lamina propria as well as the ileal mucosa.

The lymphocytes are a heterogeneous and dynamic population. Functionally they are grouped in an inductor (of which Peyer's patches and the well-organized mucosa-associated lymphoid tissue in general are the major examples) and an effector immune system. Architecturally different compartments can be distinguished: the inter- or intraepithelial lymphocytes (IEL), the lamina propria lymphocytes (LPL), and the lymphocytes organized in follicles in the mucosa in association with epithelial lymphocytes (lymphoepithelial or lymphoglandular complexes) or not. The inter-epithelial lymphocytes are mainly present in between the surface lining cells (Fig. 2.8). The normal number is estimated at four to five per 100 surface epithelial cells. They tend to be more numerous on the right side as compared with the left side, and one should not count intraepithelial lymphocytes overlying a lymphoid aggregate (where they are normally present in large numbers). They are mainly T lymphocytes expressing the CD3/CD8 suppressor, cytotoxic phenotype (CD = cluster differentiation). The lamina propria lymphocytes are B (15–40%) and T cells (40–90%) and a limited number of natural killer cells. B cells are mainly present as plasma cells with a predominance of IgA over IgM and IgG (7/2/2) (Ig = immune globulin) in the rectum and 90%/6%/4% in the large intestine. The majority of the T cells are CD4+ helper cells (65%).

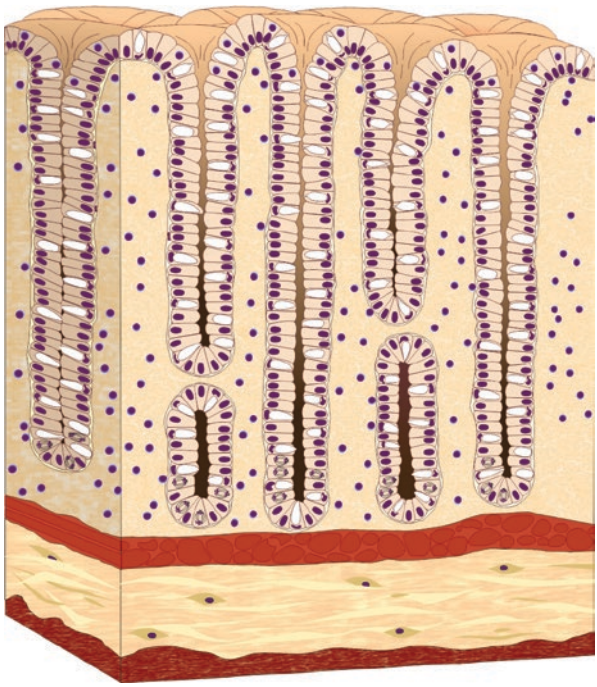


Fig. 2.8 Normal distribution of intraepithelial lymphocytes

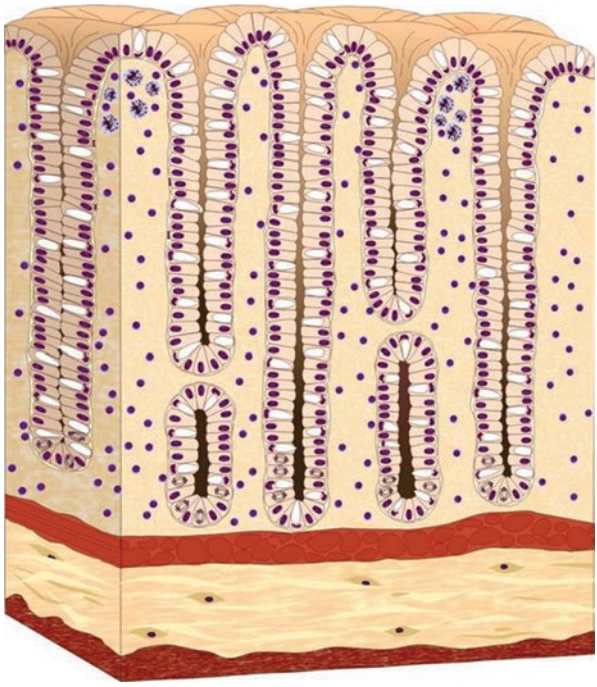


Fig. 2.9 Normal colon with superficial macrophages. Cells of the monocyte-macrophage lineage are normally present in the upper part of the lamina propria

Cells of the monocyte-macrophage lineage are usually not conspicuous in normal mucosal samples of the colon (Fig. 2.9). Histiocytes or macrophages are also seen in the lamina propria of the ileum but in fewer numbers than lymphocytes or plasma cells, and their characteristics are not entirely the same as in the colon. Most are located near the tips of the villi. As all other tissue macrophages, intestinal macrophages are derived from bone marrow stem cells through a very complex cascade of differentiation events that, among others, requires the presence of interleukin (IL)-1, IL-3, and IL-6. When present they are normally found in the upper part of the lamina propria, underneath the superficial small blood vessels just below the subepithelial collagen layer. This localization allows them to participate in the regulation of inflammatory responses to bacteria and antigens breaching the epithelium. In addition, they protect the mucosa against pathogens and scavenge dead cells and debris. The cytoplasm of the macrophages commonly contains dense inclusions of varying sizes and shape. It is weakly PAS (periodic acid-Schiff) positive [12]. In order to maintain mucosal homeostasis, resident intestinal macrophages are typically CD14-negative and thus regarded as anergic. They do not produce pro-inflammatory cytokines. However, in any case of intestinal infection or inflammation, blood CD14-positive monocytes are rapidly recruited, accumulate in the lamina propria, and actively fight against invading microorganisms by direct phagocytosis and degradation, as well as release of inflammatory mediators [13, 14]. Macrophages in ulcerative colitis mainly act within the intestinal mucosa. In Crohn's disease, macrophages can also be found in the muscularis and the mesenteric fat tissue compartment. Immune histochemical studies have shown that the macrophages or his-

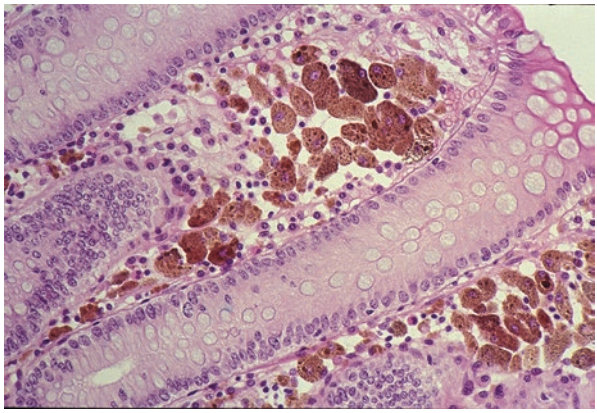


Fig. 2.10 Pigmented macrophages in the lamina propria are a hallmark of pseudomelanosis coli, usually due to laxative abuse (×20)

tiocytes in the lamina propria of the human intestine are a heterogeneous population. They express usually CD68/PG-M1. They can show a positive staining with antibodies directed against the S100 protein, and they are frequently HLA-DR (HLA = human leukocyte antigen) positive. Some of the cells have a strong membrane adenosine triphosphatase activity but weak acid phosphatase, while others, especially in the colon, have a strong acid phosphatase activity [15]. In healthy mucosal conditions, the resident macrophages of the gut will continuously be replenished through the recruitment of new circulating monocytes [16]. They are quite easy to identify when exo- or endogenous material accumulates or when they become very numerous. Most lesions result from a proliferation of histiocytes with either engulfed infectious agents or cellular or extracellular debris. Their cytoplasm frequently shows dense inclusions of different size and shape. Based on these inclusions, intestinal macrophages can be categorized into two main groups, i.e., pigmented and nonpigmented macrophages. Pigmented lesions include melanosis or pseudomelanosis coli, atmospheric dust, barium deposits, and hemosiderosis (Fig. 2.10). Accumulation of nonpigmented (foamy) macrophages presents a differential diagnostic issue of muciphages, lysosomal storage diseases, and infections including Whipple's disease (extremely rare in the colon but observed in the small intestine) and *Mycobacterium avium* complex infection (Fig. 2.11). Muciphages are mucin-rich phagocytes resulting from mucosal damage, mainly seen in the terminal phase of repair after previous injury and/or in situations of low-grade injury [17]. They may also be observed in the normal mucosa, particularly in the rectum (40–68% of all rectal biopsies). The most common localization of these muciphages is the superficial lamina propria or the basal mucosa (Fig. 2.12). Positive histochemical staining for PAS combined with diastase digestion is strongly suggestive, like expression of CD68 and lysozyme. The main type of mucinic acid is sialomucin. The nature of the cytoplasmic inclusions in foamy macrophages is highly variable. The deposits may be microorganisms, normal mucins or lipids, or abnormal glycoproteins or glycolipids. For this reason different staining procedures (PAS, Oil Red O) are required, eventually associated with electron microscopy study or polymerase chain reaction (PCR) [16].

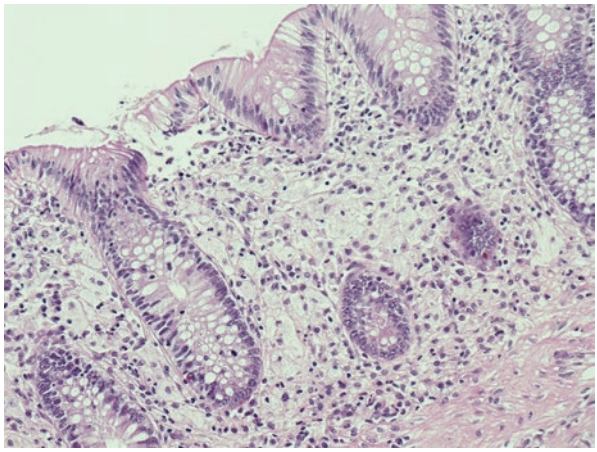


Fig. 2.11 Microphotograph showing the presence of numerous foamy macrophages, suggestive of lysosomal storage disorder. Oil Red O stain on frozen sections confirmed the presence of lipid material (×10)

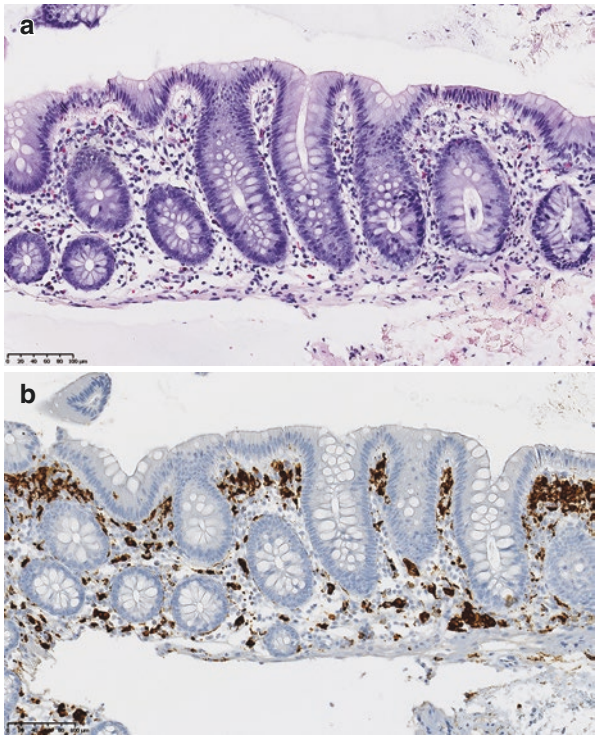


Fig. 2.12 Microphotograph showing macrophages mostly in the upper part of the mucosa forming a more or less continuous layer. Their cytoplasm shows cellular debris (**a**) HE (×10), (**b**) CD68 (×10)

Eosinophils are normally present in the lamina propria of the colon as well as of the ileum (Fig. 2.13). They differentiate from hematopoietic progenitor cells into mature eosinophils in the bone marrow. Eosinophil migration from the bone marrow into the peripheral circulation is primarily regulated by IL-5. Circulating eosinophils interact with the endothelium in the gastrointestinal tract by a regu-

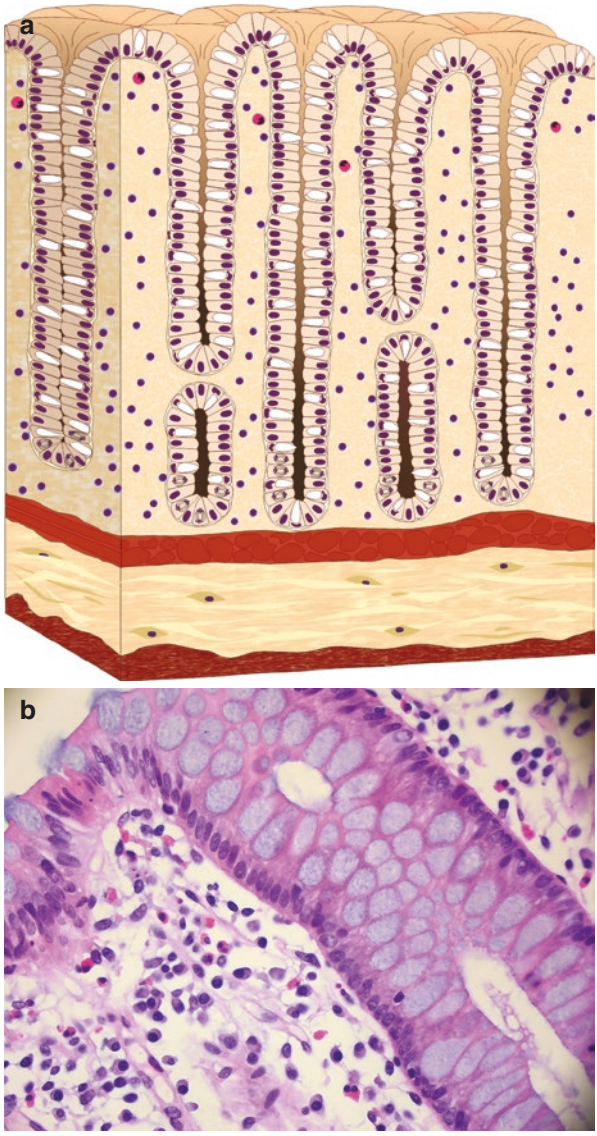


Fig. 2.13 Eosinophils are normally present in the mucosa of the colon in variable numbers as shown in the schematic presentation (**a**) and in the microphotograph (×40) (**b**)

lated process involving the coordinated interaction between adhesion molecules, chemokines, and their receptors. Eosinophils initially home to the gut in the prenatal period, independently of the bacterial flora. The mature eosinophils are terminally differentiated cells with limited survival in the tissue, in the absence of survival-promoting cytokines such as IL-5. In the gastrointestinal tract, they reside for at least 1 week. The number of eosinophils is usually low. For the colon, most authors propose a diagnostic threshold of 20 eosinophils per high-power field present as focal aggregates or more diffuse in the lamina propria and muscularis

mucosae. However, normal values for tissue eosinophils in the colon vary widely. Location of the biopsy is an important variable. In humans, the appendix, cecum, and ascending colon contain the highest numbers. Lamina propria eosinophils are, on average, three times more numerous in the ascending compared with the descending colon. The normal value may range from <10 per high-power field in the rectum to >30 in the cecum. Geographical differences have also been observed with a 35-fold increase in samples from asymptomatic patients in New Orleans compared to Boston. Furthermore, mucosal eosinophils are slightly more numerous and may be in an intraepithelial position in samples obtained in April and May, corresponding to periods of high pollen counts. The distribution of the eosinophils is usually patchy. They are only rarely seen in an intraepithelial position, usually in crypts in the ascending colon. The function of eosinophils is not entirely known. They may play a role in organogenesis and tissue repair as well as a protective role, but through the release of substances such as major basic proteins (MBP-1 and MBP-2), eosinophilic cationic protein (ECP), and eosinophil peroxidase (EPO), they can have a toxic effect and a pro-inflammatory influence [18–20].

Mast cells develop from CD34-positive or c-kit-positive progenitor cells of the bone marrow. They contain abundant specific basophilic metachromatic granules in their cytoplasm. They constitute 2–5% of mononuclear cells in the gastrointestinal lamina propria, with an average level of 13 cells per high-power microscopic field in the colon (Fig. 2.14) [21]. They are less numerous than eosinophils. They can be visualized with special stains such as toluidine blue or with immune histochemistry

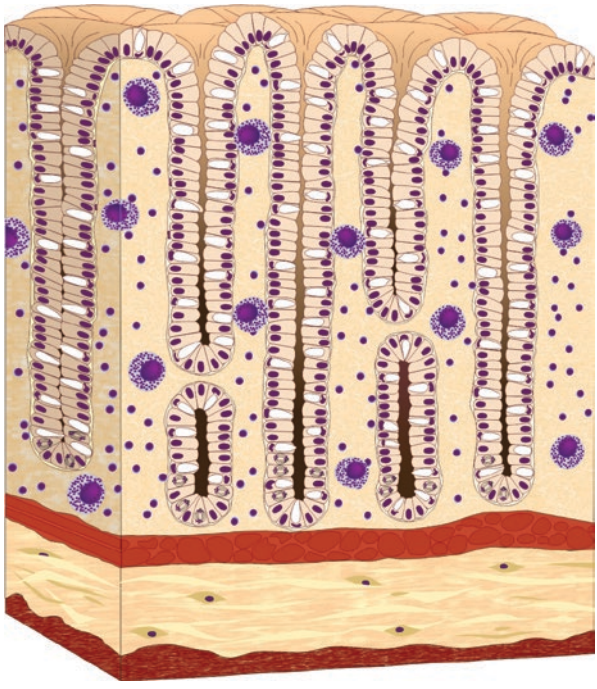


Fig. 2.14 Mast cells are normal components of the lamina propria cellular infiltrate in a perpendicular section

using antibodies directed against mast cell tryptase or c-kit (CD117). Increased normal or decreased numbers of mast cells have been noted in patients with inflammatory bowel disease, collagenous colitis, and gluten-sensitive enteropathy. Increases have also been linked to the irritable bowel syndrome and other forms of chronic diarrhea.

Neutrophils or neutrophilic granulocytes are normally not present outside the lumen of capillaries. More than three neutrophils in the lamina propria outside capillaries would be abnormal [22]. Neutrophils are also formed from stem cells in the bone marrow and normally reside in the blood stream. They are one of the first cells to be recruited to a site of injury and are the hallmark of inflammation. They migrate through the endothelial lining and interstitial tissue following chemical signals such as interleukin-8 in a process called chemotaxis. Outside the blood vessels, they are short-lived (1–2 days) although survival can be promoted environmental factors such as growth factors and cytokines released in the inflammatory process. Neutrophils express and release cytokines which can amplify the inflammatory reaction. They are capable of ingesting microorganisms or particles. Neutrophils are also capable of releasing various proteins (contained in three types of granules). These include a variety of enzymes such as metalloproteinases (gelatinase or MMP-9), myeloperoxidase, collagenase, and various others. By the release of these substances, neutrophils can induce breakdown of the fibrovascular stroma and induce damage. The presence of neutrophils in the mucosa of the colon is therefore considered as a sign of "disease activity."

Fibroblasts are distributed randomly throughout the lamina propria or in a sheath surrounding the crypts and tightly apposed to the subepithelial basement membrane. Fibroblasts are responsible for the synthesis of collagen, a major protein of the extracellular matrix. The normal intestinal wall contains type I (68%), type III (20%), type V (12%), type IV, and type VII collagens. Type I, III, IV, and V collagens are present in the lamina propria. Type V collagen is also present in the submucosa. Type VII collagen is confined to the basement membranes of intercryptal surface epithelium in a punctate manner [23].

Arteriolar branches from the submucosal plexus penetrate the muscularis mucosae and then break up in a leash of capillaries in the mucosa. The capillaries ascend along the glands and reach the surface of the mucosa where they form a honeycomb pattern around the openings of the glands, just beneath the surface epithelium. This pattern can be visualized with CLE imaging showing the hexagonal appearance with a regular-ordered network of capillaries demarcating the luminal crypt orifice [24]. With CLE fluorescein sodium 10% imaging of the normal colon, the surface crypt architecture is classically represented by ordered and regular crypt orifices covered by a homogeneous epithelial layer with visible "black-hole" goblet cells within the subcellular matrix.

In the colon, a lymphatic plexus is normally present immediately superficial to, within, and below the muscularis mucosae. Lymphatics may extend upward for a short distance, but they are not seen above the level of the lowest one-sixth of the crypts [25]. In samples from patients with IBD, lymphatics can occur in the more superficial third of the lamina propria, probably as a result of lymphangiogenesis [26].

In the small intestine, each villus contains a centrally located lymphatic channel and an arteriovenous capillary network.

The muscularis mucosae, the layer which separates the mucosa from the submucosa, is fully developed between 17 and 20 weeks of gestation. This layer is composed of smooth muscle cells organized in a very thin layer of outer longitudinal and inner circular smooth muscle cells. These are plump, bipolar, spindle-shaped cells with a central nucleus. In the muscle coat, they are densely packed together, running roughly parallel to each other and separated from each other by spaces measuring only a few tens of nanometers and mainly occupied by collagen fibrils.

Perikarya of nerve cells, so-called ganglion cells, can occasionally be found in the normal colonic mucosa as well as in the normal ileal mucosa. They can appear as single cells or in clusters and were found in up to 20% of mucosal biopsies [27].

The Submucosa

In endoscopic biopsies usually, only a small part of the submucosa is present. It is a layer of dense or loosely arranged connective tissue that supports the mucosa and connects it with the underlying muscularis propria. Blood vessels and lymphatics are running through the submucosa. In its upper part, underneath the muscularis mucosae, the submucosal (Meissner) nerve plexus, which develops around 8–12 weeks, can be observed. The plexus is composed of small ganglia and nerve fibers. Lymphoid aggregates can occasionally be seen at the junction with the mucosa.

Regional Variability in Histologic Pattern

Although the aspect of both the mucosa and the submucosa remains the same all along the colic cadre, the right and left colon and the rectum display subtle morphologic differences in the ratio of types of epithelial cells (colonocytes versus goblet cells), thickness of basal layer, presence of Paneth cells, crypt length, number of intraepithelial lymphocytic cells, etc. The cecal mucosa has more absorptive cells and fewer goblet cells as compared to the left colon and the rectum. This difference reflects the variability of a dominant function in each colonic area: absorption in the right colon and formation of mucin necessary for transit in the left colon. The difference between the right and left colon may also be important for the history of colonic cancer. The normal distal rectum shows variations in crypt architecture with mild crypt distortion and shortened crypts. This feature should not be misdiagnosed as quiescent colitis. The rectal basement membrane is thicker (median 10 μ) than the colonic basal membrane (median 5 μ) [28]. If the pathologist does not know this, he may misdiagnose a microscopic colitis. A progressive decrease in the cellular components of the lamina propria is normal from the right to the left colon. Usually,

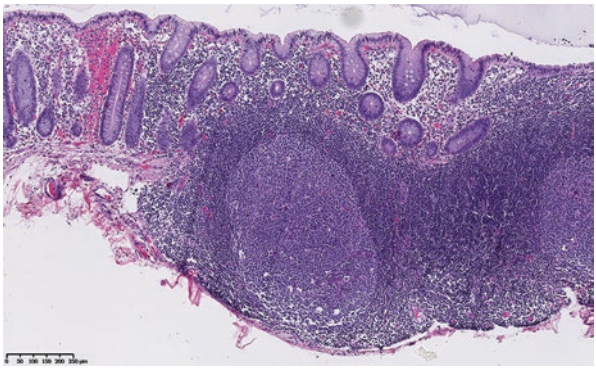


Fig. 2.15 Crypts appear in a diagonal or near-horizontal axis when they are adjacent to lymphoid aggregates (×10)

the cecum is more cellular than the other segments of the colon [29]. Intraepithelial lymphocytes are more frequent in the right colon than in the left, as well as in areas of mucosa overlying lymphoid follicles. A contrario, macrophages (muciphages) are more frequent in the distal colon. Paneth cells are normally present within the right colon but are indicative of chronic mucosal injury for the left colon [30, 31]. Variation in the usual pattern of the mucosa is observed at innominate grooves or adjacent to lymphoid aggregates. In this condition, crypts appear in a diagonal or near-horizontal axis (Fig. 2.15).

These significant differences have to be known by the pathologists in order not to overdiagnose colitis. It is also a reason to encourage endoscopists to send biopsies separately with the full identification of biopsy origin. The knowledge of the anatomic location of the colonic specimens is key in the interpretation.

Variability of Microscopic Features

Histopathology represents a snapshot in time of a complex and dynamic biologic process that shows the normal variations and variations induced by the duration and activity of disease processes. The mucosa of the alimentary tract is indeed a microworld composed of various cell types forming an organized and dynamic community which is well equipped for a variety of functions but rapidly changing. Organized community means that the cell types can be described structurally in different compartments including the epithelial cells lining the surface and the crypts and the lamina propria cells present in a stroma together with connective tissue cells, vascular structures, and smooth muscle cells in the muscularis mucosae. Dynamic community means that there is continuous cell renewal for most cell types including epithelial cells, lymphocytes, and monocytes. In the colon, epithelial cell turnover ranges between 2 and 8 days. Cell renewal is influenced by exogenous and endogenous factors. An adequate immune response implies migration of immune cells, cell recognition, and interaction of cells requiring adhesion and de-adhesion of cells. Proteins incorporated in the cell wall are important for these cellular

interactions. These proteins have been called adhesion molecules. They can be classified into families including the "immunoglobulin superfamily" with members such as the major histocompatibility (MHC) class II antigens, CD4, CD8, ICAM (intercellular adhesion molecule), and VCAM (vascular cell adhesion molecule); the "integrins" such as LFA (lymphocyte function-associated antigen), involved in the interaction between lymphocytes and other cells; and the "selectins" such as ELAM (endothelial-leukocyte adhesion molecule) involved in neutrophil-endothelial adhesion [32, 33].

Because of the multitude of exogenous influences, the mucosa is well equipped for defense, and many cells present in the intestinal mucosa play a role in this defense system and will adapt to a changing environment [6]. These changes can be observed during inflammation. The intensity and pattern of the changes depend however upon host and environment. The cellular inflammatory reaction, for instance, can show differences when samples from immune-competent and immune-depressed patients are concerned. The reaction will be different in transplant patients compared with immune-competent patients. Neutropenia, for instance, can occur during aplastic anemia or chemotherapy for malignant disease or in hematologic diseases. One complication of neutropenia that may occur is an acute necrotizing inflammation in the cecum and terminal ileum. This condition is known as "neutropenic colitis" (synonyms: necrotizing enteropathy, typhlitis, and the ileocecal syndrome).Several reports have identified various bacteriological agents as causative factor. Microscopic examination shows extensive mucosal and variable submucosal necrosis and paucity of a neutrophilic response because of the underlying disease [34].

In a similar way, a variety of anti-inflammatory drugs can influence the features of inflammation [35]. This is why it is important for the pathologist to have clinical information concerning treatment, immune status of the patient, and duration of the disease.

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