

Diagnosing Lymphoproliferative Disorders of the Gastrointestinal Tract

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In this era of ever increasing endoscopic sampling of the gastrointestinal tract the pathologist is presented the opportunity to diagnose both inflammatory and neoplastic lymphoid proliferations arising within this organ system at an early stage, sometimes as unexpected lesions perceived only during the procedure. Correct diagnosis of these proliferations is often difficult for two reasons: the small size of the lesion (or the biopsy) and the capacity for benign proliferations of the immune apparatus of the gut to microscopically mimic lymphoma. In this presentation a systematic approach will be recommended by which confident distinction of these processes can usually (but not always!) be accomplished.

Applications of the WHO Classification to Lymphomas of the GI Tract

While all lymphomas encountered in the GI tract are to be found in the WHO classification of 2001 (1), only certain types occur there as *primary* tumors with any frequency: **diffuse large B-cell lymphoma**, **extranodal marginal zone ("MALT")**, **mantle cell**, **follicular**, and **Burkitt** types. As in other sites, T-cell lymphomas are relatively rare, but the one which is distinguished for its origin in the gut is **enteropathy type T-cell lymphoma**.

The Immune Apparatus of the Gastrointestinal Tract

To apply current concepts of lymphomas to the gut - as neoplasms corresponding to normal cell counterparts of the immune system - we must first identify the functional immune elements of this system. Although the upper aerodigestive tract has abundant mucosa-associated lymphoid tissues, the esophagus and stomach are "normally" almost devoid of such immune apparatus, presumably because of the rapid transit of food and the chemically hostile environment for microorganisms provided by salivary and gastric secretions. Only in pathologic conditions, such as viral or fungal esophageal infections, reflux esophagitis or Helicobacter gastritis, does one encounter *acquired mucosa-associated lymphoid tissue* in these sites. By contrast, the large and small bowel *normally* possess mucosa-associated lymphoid tissue (MALT), most visibly concentrated in the terminal ileum (Peyer's patches) and appendix. The cellular elements of this immune apparatus can be divided into **three functionally interactive compartments**.

***Mucosal Lymphoid Aggregates* -**

Follicular elements with specialized overlying mucosal epithelium are varyingly termed mucosal lymphoid aggregates, lymphoepithelial complexes or, in the ileum, Peyer patches. While the follicular architecture is largely homologous to that in lymph nodes, there are two exceptions. First, the follicular mantle zone is eccentrically concentrated along the mucosal aspect (the "dome cells"). Second, because there are no afferent lymphatics for delivery of antigen, a specialized, attenuated zone of mucosal epithelium overlies such aggregates. This features membranous epithelial cells or *M cells*, which are able to actively transport antigens from the luminal surface to the interstitial intramucosal fluid compartment where they can be opsonized by phagocytic and/or dendritic cells for antigen processing (2).

The lymphocyte response within mucosal lymphoid aggregates is similar to that in lymph nodes, entailing T-helper and receptor B-cell interaction, B-cell clonal expansions within follicle centers and the production of immunosecretory plasma cells, predominantly of IgA class with secretory component attached for transepithelial delivery to the gut lumen. There are elaborate and diverse systems of circulation of lymphoid cells within and from the MALT, including efferent lymphatic circulation of plasma cells and memory B-cells to mesenteric lymph nodes, and hematogenous circulation via high postcapillary venules of T-cells. Cellular adhesion molecules are probably an important mechanism in controlling such cellular trafficking (3).

Lamina Propria Lymphoid Cells -

The lamina propria compartment contains an extremely heterogeneous cell population. Small numbers of macrophages and dendritic cells represent the afferent (antigen processing) component, whereas most cells are effector types: plasma cells and T-cells, predominantly T-helper/inducer. Eosinophils and mast cells are normally present in small number, particularly in small intestine, but become numerous in many inflammatory conditions, such as parasitic infections, celiac disease, and primary inflammatory bowel disease.

Intraepithelial Lymphocytes -

Perceived only with careful scrutiny are the intraepithelial lymphocytes, universally present in small numbers but relatively more concentrated in small bowel. These are predominantly of cytotoxic T-cell type, probably mediating cell killing in response to viral and other infections. Conspicuous proliferation of this immune cell compartment is seen in celiac disease, lymphocytic ("microscopic") colitis and recently reported cases of "lymphocytic gastritis".

Hyperplastic and Neoplastic Immune Proliferations of the Gastrointestinal Tract

Lymphoid proliferations, both hyperplastic and neoplastic, will be considered in relation to the normal counterpart cell types.

Lymphoid Aggregates

Localized (Focal) Hyperplasia:

Benign hyperplastic enlargement of the gut lymphoid complexes can be localized ("focal") or diffuse. Not surprisingly, the commonest site of the localized form is in the small bowel, particularly the terminal ileum, where Peyer's patches are normally present. Presumably such focal massive enlargement is due to an inapparent antigen challenge, e.g. microbial. The criteria for the distinction from malignant lymphoma essentially come down to the recognition of functional immune compartments (germinal centers, mantle layers, plasmacytosis, etc.). Enlargement of mucosal lymphoid aggregates also occurs in the colon and rectum, where it is commonly mistaken for adenomas by the endoscopist (4).

Diffuse Hyperplasia:

In the absence of any demonstrable underlying immunodeficiency, diffuse lymphoid hyperplasia is a relatively common and innocuous disorder, often being detected incidentally. Presumably this represents widespread response of mucosal lymphoid aggregates throughout small and large bowel to some unknown stimulus. By contrast, diffuse nodular lymphoid hyperplasia *with* immunodeficiency is a more persistent clinically symptomatic disorder, with small but significant risk of transformation to malignant lymphoma.

Follicular Lymphomas:

Predictably, with the conspicuous role played by follicle centers within the mucosal lymphoid aggregates, lymphoid neoplasms corresponding to this compartment constitute a significant portion of primary gut lymphomas. Because follicular lymphomas are in general advanced stage at presentation, proof of their actual origin in the gut depends upon relative bulk of distribution of disease. Radiologically and grossly the process can be misinterpreted as mantle-cell lymphoma due to its capacity to mimic lymphomatous polyposis (but usually in a more localized region of bowel or stomach) (5).

Microscopically, the distinction between B-marginal zone (MALT) lymphoma and follicular lymphoma may be difficult because of the phenomenon of "follicular colonization" (see below). The distinction from benign hyperplasia (focal or diffuse) is greatly facilitated by immunostaining for bcl-2 protein.

Mantle-Cell Lymphomas:

Over the past decade mantle-cell lymphoma has attained clear definition, with both phenotypic and genetic criteria. Originally recognized by Lennert and his co-workers in Kiel as "centrocytic" lymphoma, and more vaguely in America as "intermediately differentiated lymphocytic lymphoma", this neoplasm is now characterized as a spectrum of low-intermediate to high-intermediate grade B-cell tumors occurring predominantly in older males (6). There is a striking tendency for involvement of G-I tract, often as the spectacular phenomenon of lymphomatous polyposis. This lymphoma grouping manifests CD5 coexpression and nuclear immunohistochemical staining for cyclin-D1.

Comparison of Immunophenotype of Low-Grade Lymphomas

Tumor	CD20	CD5	CD3	CD23	bcl-1	bcl-2	bcl-6
Small lymphocytic	+/-	+	-	+	-	+	-
Mantle-cell	+	+	-	-	+	+	-
Follicular	+	-	-	+	-	+	+
MALT type	+	-	-	-	-	+/-	-
T-cell (enteropathy)	-	+	+	-	-	-	-

Large B-Cell Lymphomas:

The commonest type of primary lymphoma of the gut is diffuse large B-cell lymphoma (7). As is the case with primary extranodal presentations in other sites, the single most important prognostic factor is *stage*, in particular stage IE vs stage IIE or higher (8). Therefore, the sampling of regional lymph nodes is a prognostically useful component of any surgical procedure. Some diffuse large B-cell lymphomas of gut appear to represent high-grade transformations of preexisting lower grade lymphomas, such as B-marginal zone (MALT) type or mantle cell type (see below) (9). However, in most cases there is no evidence of such transformation, and it is assumed that the same sort of pathogenetic events which give rise to primary nodal large B-cell lymphoma underlie such tumors in the gut.

Burkitt and "Burkitt-Like" Lymphomas:

Among the most aggressive, most proliferative of all human neoplasms are those collectively designated as "small noncleaved cell type" in the Working Formulation, and as Burkitt or "Burkitt-like" in the WHO classification (1). The classic presentation is as an obstructing tumor of the terminal ileum, presumably arising within Peyer's patches, but primary G-I tract sites elsewhere in small or even large intestine occur. Air-dried imprint preparations are of great value in assuring cellular detail satisfactory for accurate classification. In classic Burkitt lymphoma nuclei are *relatively* uniform, medium (not small) sized with thick chromatin rims and prominent nucleoli (2 to 4). There is deeply basophilic cytoplasm with clear vacuoles representing lipid droplets. Although the "Burkitt-like" variant was proposed as a "provisional" type in the REAL classification, the trend (e.g., in the new WHO system) is to establish this, despite poor reproducibility. This entity consists of

cases with greater pleomorphism than Burkitt type proper but with all other features such as proliferative rate (at least 90% nuclear staining for Ki-67) (10).

These neoplasms are endemic in settings with neonatal EBV exposure and malaria, and in HIV-positive individuals, but also occur sporadically without EBV association in high socioeconomic settings without immunodeficiency. There is usually a c-myc translocation to one of the immunoglobulin encoding regions (8;14 or 2;8 or 8;22).

Postransplantation Lymphoproliferative Disorders:

Among the most spectacular and challenging cases for clinician and pathologist alike are those explosive proliferations arising in the setting of immunosuppressed transplant recipients. Such processes often arise in extranodal sites, in particular within the organ transplants themselves, or in the G-I tract. The majority have a highly proliferative polymorphous appearance which suggests true neoplasia, but which often responds to diminished immunosuppression. Recognition of this syndrome, as distinct from conventional high-grade neoplasms, depends upon the clinical history and microscopic identification of telltale clues, including geographic necrosis, polymorphism, abundant dispersed apoptotic cells, variable plasmacytic maturation, and the presence of occasional Reed-Sternberg-like multilobated immunoblasts reminiscent of acute infectious mononucleosis. Almost all such proliferations are EBV-positive, as is demonstrable with immunostains for latent membrane protein or with in situ hybridization for EBER. Clonality studies are not predictively useful, since monoclonal proliferations, defined either by molecular or Ig light chain phenotyping, are not necessarily malignant, i.e. irreversible (1). Recent molecular studies suggest that oncogene translocation is the determinant factor for malignancy, but the required molecular methods require too much time to be of current practical value. Usually an empiric trial of reduced immunosuppression will inform the managing physician as to the underlying nature of the process.

Acquired Immune Cellularity in the G-I Tract

Originally, Isaacson and colleagues likened the peculiar, complex, heterogeneous microscopic appearances of long-localized, highly favorable low-grade gastric lymphomas to normal MALT, particularly the follicular and mantle ("dome-cell") elements and coined the term MALT-type lymphomas. Over time, MALT-type lymphomas in other extranodal sites were identified and it became apparent that sites of predilection were normally devoid of lymphoid tissue. Simultaneously, Isaacson and others became aware of the association of *Helicobacter* gastritis with gastric MALT-type lymphoma (11). Then a new complication in our concept of "malignancy" was born: the London group demonstrated clinical remission of early MALT type lymphomas after antimicrobial therapy for *Helicobacter* (11).

Follicular Gastritis and Helicobacter Pylori:

Initially, withdrawal of the instigating foreign antigen results in interruption of the B-cell induction and clinical remission. However, eventually clonal B-cell expansions achieve independence from T-cell helper/inducer effect and become *locally* irreversible. If the B-cell clones achieve proliferative pathways no longer dependent upon autoantigen stimulation they can disseminate, becoming conventional systemic lymphomas. Thus, the concept of *acquired MALT* has become essential in the histogenesis of these tumors.

Low-Grade B-Cell Lymphoma of MALT-Type:

Thought originally to be "pseudolymphomas" because of the follicle center prominence, these tumors were initially considered follicle center neoplasms by Isaacson. Subsequently their derivation from the B-marginal zone was identified. This compartment is variable and elusive, being composed of a mixture of small lymphocytes, "centrocyte-like" small cleaved cells often with clear monocytoid cytoplasm, plasmacytic and plasmacytoid cells and occasional large lymphoid cells resembling those in follicle centers. The ratio of these different cell types varies within a single case and from case to case, but in low-grade tumors the overall mitotic rate is low. Follicle centers are invariably present, and are often atypical in appearance, resembling neoplastic follicles on the basis of large size, monotony of cell type, or atypia of large basophilic cellularity. This is the phenomenon of "follicular colonization", probably reflecting the functioning of antigen response inherent in low-grade MALT-type lymphomas. Lymphoepithelial lesions represent the neoplastic B-cells homing into gut epithelium, often with

eosinophilic epithelial metaplasia. Recent molecular genetics studies suggest two types of MALT gastric lymphoma: one with abnormalities of chromosome 3 and another with t(11;18). Lymphomas with chromosome 3 abnormality tend to respond to Helicobacter eradication, but, if allowed to persist, have a tendency to progress to higher grade lymphoma. Those with t(11;18) do not respond to antimicrobial therapy, but do not progress to higher grade.

How does one distinguish benign Helicobacter-associated gastritis from low-grade MALT-type lymphoma? In small endoscopic biopsies with great care! A grading system is a useful approach, ranging from negative (no gastritis) to gastritis without evidence of lymphoma, to atypical suspicious for lymphoma, to consistent with lymphoma. Criteria for deciding the level of suspicion for lymphoma center upon the degree of displacing atypical lymphoid infiltrate expanding the lamina propria.

Immune Cellularity of the Lamina Propria

Hyperplasia of this component occurs *universally* with *any* immune stimulus, since this compartment represents a functional interchange zone between luminal epithelium and the lymphoid aggregates.

Microscopic (Lymphocytic) Colitis:

This entity, or at least grouping, is of unknown pathogenesis but may be autoimmune in some instances. It involves both intraepithelial *and* lamina propria elements, predominantly T-cell, and seems to carry with it little risk of neoplastic transformation.

Immunoproliferative Small Intestinal Disease:

While earlier a well known entity in the Mediterranean and other Mideast locations, this disease spectrum is almost unseen in North America. Microscopically, there is massive expansion of lamina propria by plasmacytosis with eosinophilia. In concept this process may be analogous to MALT-type lymphomas of the acquired mucosal lymphoid aggregate compartment, with chronic foreign antigen stimulation engendering eventual B-cell clonal selection and autonomy.

Plasmacytoma (Extramedullary):

Plasma cells are normally a prominent component of the lamina propria in both small and large bowel, and therefore the occasional development of primary gastrointestinal plasmacytomas is predictable. The commonest site of extramedullary plasmacytoma is in the *upper* aerodigestive tract. Low-grade plasmacytomas are difficult to distinguish in small endoscopic biopsies from plasmacytic hyperplasia. Fortunately, the abundant cytoplasmic immunoglobulin is conveniently studied by paraffin immunostaining as to whether there is light chain restriction. The distinction between well differentiated plasmacytoma and MALT-type lymphoma with predominant plasmacytic differentiation is sometimes arbitrary. Lymphoepithelial lesions weight the process towards MALT-type lymphoma.

Peripheral T-Cell Lymphomas of No Special Type:

The vast majority of primary gut lymphomas are B-cell phenotype, however, rarely a neoplasm corresponding to the T-helper cells of lamina propria arises in stomach or bowel. Such tumors are unassociated with celiac disease or other enteropathy, are protean and polymorphous in cellular composition, and are usually disseminated and clinically highly unfavorable.

Intraepithelial Lymphocytes of Intestinal Mucosa

Nontropical Sprue (Gluten Enteropathy):

Increased intraepithelial lymphocytes accompany villous blunting in celiac disease. These are predominantly cytotoxic T-cells, no doubt playing a critical role in the pathogenesis of this disease process.

Ulcerative Jejunitis:

This process is a manifestation of advanced celiac disease and now is thought to represent early lymphomatous progression, with clonal T-cell expansions, in this disease setting (12). Refractory sprue is a term implying early, autonomous biologic behavior, and some authors consider these lesions “cryptic” enteropathy-type lymphomas.

Enteropathy-type T-Cell Lymphoma:

Lymphomas of complex and widely varying cellular composition may arise in the background setting of celiac disease, although the majority of such tumors, while associated with microscopically demonstrable villous atrophy do not have a clinical history of malabsorption symptoms. These neoplasms are often obscured by accompanying ulcerative jejunitis, and many feature abundant eosinophils and plasma cells, as well as histiocytes (a feature which initially resulted in misinterpretation of the process as "malignant histiocytosis of the small intestine". These tumors show an immunophenotype comparable to normal intraepithelial lymphocytes: CD3+, CD7+, CD8+/-, CD103+ and expressing cytotoxic granule markers TIA-1 and perforin (13). These lymphomas are early to disseminate and carry a highly unfavorable prognosis.

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