HHS Public Access

Author manuscript

Gastroenterology. Author manuscript; available in PMC 2023 February 13.

Published in final edited form as:

Gastroenterology. 2017 March ; 152(4): 908–910. doi:10.1053/j.gastro.2017.01.022.

Defective Lymphatics in Crohn's Disease: Tertiary Lymphoid Follicles Plug the Gap

EÓIN N. MCNAMEE,

Mucosal Inflammation Program, School of Medicine, University of Colorado, Anschutz Medical Campus, Aurora, Colorado

JESUS RIVERA-NIEVES

Inflammatory Bowel Disease Center, Division of Gastroenterology, University of California San Diego *and* San Diego VAMC, La Jolla, California

Randolph GJ Bala S, Rahier JF, et al. Lymphoid aggregates remodel lymphatic collecting vessels that serve mesenteric lymph nodes in Crohn disease. Am J Pathol 2016;186:3066-3077.

The gut-associated lymphoid tissues are the guardians of the enteric immune system and integrate dietary and microbial stimuli to maintain immunologic tolerance. However, during periods of persistent chronic inflammation, such as in Crohn's disease (CD), a dysfunctional lymphatic system is a pathologic hallmark. Indeed, the predominant histopathologic features of CD are lymphocytic lymphangitis, occluded lymphatic vessels, and inflammatory granulomas. These trademark features of chronic disease are found in or around ectopic tertiary lymphoid tissues (TLT) in the inflamed lamina propria (Gut 2008;57:1-4). In addition, the presence of TLT at the base of aphthous ulcers is the earliest endoscopically evident lesion in CD and their appearance heralds recurrent disease within the neoterminal ileum after ilectomy (Gut 1984;25:665-672). To date, the function of intestinal TLT and their impact on the etiology and pathogenesis of CD remains enigmatic, with limited experimental evidence as to their role. How TLT integrate into the pathophysiology of CD remains a critical question, yet clinical investigation into their development and function remains limited.

Randolph et al aimed to assess the integrity of mesenteric lymphatic vasculature in CD. First, they confirmed the previous findings generated from immunopathologic studies of intestinal mucosa, indicating defective lymphatic function during active disease. To achieve this, patent blue dye was injected into the serosa of ileal loops before surgical resection, highlighting afferent lymphatic vessels draining the lamina propria. This technique nicely demonstrated a deviation of normal lymphatic vessels in active CD, compared with non-CD counterparts and indicated a remodeling of the vasculature in the mesentery. Next, they used a 3-dimensional system to image whole mount sections from a patient cohort undergoing ilectomy for stricturing CD (to obtain cm^3 tissue coverage). Surprisingly, this work revealed advanced remodeling of the lymphatic vascular tracks, with the presence of ectopic lymphoid follicles within the collecting vessel line. A border of adipocytes and no evident capsule, suggesting that these organized structures were not sentinel lymph nodes but TLT instead. The work by Randolph et al highlights that, although most studies have

reported on lymphatic capillaries in the intestinal wall, where collecting vessels remove solutes and immune cells, the primary functional site of lymphatic dysfunction may be the larger vessels in the mesentery, which interface with sentinel lymph nodes.

Comment.

Our current understanding of the pathophysiology of CD points toward a complex and heterogeneous disease with an array of genetic and immunologic defects. Yet, a consistent and primary pathologic hallmark is the development of ectopic TLT and defective lymphatic vasculature. However, our understanding of the potential clinical significance of targeting lymphatics as a therapeutic strategy in CD is limited, given the paucity of preclinical and clinical studies investigating the biology of lymphatics in the setting of inflammatory bowel disease.

A pressing need raised by Randolph et al is to generate imaging techniques to investigate lymphatic function and identify the presence of TLT within mesenteric lymphatics of patients with CD, aiding in the assessment of clinical course and treatment response. Although the appearance of mesenteric TLT is a pathologic hallmark of treatment-refractory fibrostenotic disease at ilectomy, it is not known how and when they appear during the earlier stages of CD, or whether they are responsive to therapeutic intervention. Equally important is to achieve an understanding of the regional differences within the intestine (ie, ileal versus colonic CD). Surprisingly, an exhaustive pathologic characterization of TLT within the mucosa and mesentery of patients with CD is still lacking, hampering our ability to assess if the colon or ileum are more permissive for lymphatic vessel dysfunction or TLT development. The heterogeneous disease phenotypes and clinical courses of inflammatory bowel disease will surely have varying effects on regional lymphatic vessels and on sentinel lymph node function. In preclinical mouse models, isolated lymphoid follicles and TLTs within the mucosa develop in response to inflammation and microbial stimuli, dissipating with antiinflammatory interventions (Gut 2013;62:53-62; J Immunol 2007;178:5659-5667). Whether the mesenteric TLTs identified by Randolph et al are regulated by the same immune cues or if they can be modulated by therapeutics (eg, vedolizumab) is not known.

The anatomic location of mesenteric TLT may give clues as to their function. It is debatable whether mucosal TLT are generated de novo within the chronically inflamed intestine or whether they represent hyperplasia and maturation of preexisting isolated lymphoid follicles. However, in the setting of the observations made by Randolph et al, ectopic TLT within afferent mesenteric lymphatics may either highlight an attempt to restore lymph node function (by generating new follicles and germinal centers) or alternatively indicate sites of vessel damage where TLTs form locally to contain disseminating microbes.

An intriguing question remains as to the function of these ectopic follicles and whether they are contributing to either a protective or pathologic immune environment. Are TLT generating secretory immunoglobulin (Ig)A to “man the border” following epithelial barrier defects and infiltration of pathobiont bacteria (similar to isolated lymphoid follicles)? Because IgA is not protective against invasive bacteria, they may be generating antibacterial IgG in an attempt to neutralize inflammation driven by invasive species. This process has

been previously observed in mouse models with mesenteric fat-associated lymphoid clusters (Nat Immunol 2015;16:819-828) and in the setting of experimental colitis on a background of failed antimicrobial immunity ($ROR\gamma t^{-/-}$ mice; J Exp Med 2011;208:125-134). Although extensive immunophenotyping of TLT was limited in this report, dense clusters of B cells with defined germinal centers were not observed by Randolph et al and CD20 immunopositive B cells within TLT presented with a diffuse pattern. However, TLT follicles were evident with segregated T-cell and B-cell compartments and mature high endothelial venules (as evidenced by $PNAd^{+}$ vessels), indicating immune maturity.

This latest report by Randolph et al recognizes the limitations of current mouse models, further supporting the need for experimental models that recapitulate the histopathologic features of CD. However, it is worth noting that the authors may not be aware that the $TNF^{\Delta ARE}$ model has transmural pathology restricted to the ileum, florid TLT in mucosa, and marked induction of mesenteric fat-associated lymphoid clusters (Gut 2013;62:53-62: Nat Immunol 2015;16:819-828). Whether TLT are present within lymphatic vessels of the mesentery and contribute to vessel occlusion and remodeling in this model is yet to be identified. Furthermore, the *SAMP1YitFc* and *SAMP1* mouse strains further develop ileal restricted TLT and the appearance of “creeping fat” around the mesenteric vessels (Inflamm Bowel Dis 2010;16:743-752). A global caveat to using mouse models is that the lymphatic pump does not function in a similar manner to higher vertebrates and cannulating mouse mesenteric lymphatic vessels might not be yet feasible technically (Ann N Y Acad Sci 2010;1207 Suppl 1:E69-74). Thus, functionally assessing lymph flow in relation to ileal inflammation and ectopic TLT may require development of new murine (eg, rat) or primate models of CD.

Is there a therapeutic window to repair damaged afferent lymphatics and restore mucosal integrity during CD? From a therapeutics perspective, recent elegant work has demonstrated the efficacy of de novo lymphangiogenesis as a viable intervention to ameliorate colonic inflammation (J Clin Invest 2014;124:3863-3878). Danese et al used an adenoviral system to overexpress vascular endothelial growth factor-C, driving functional lymphatic vessel generation in the intestine that attenuated pathology in both dextran sodium sulfate-induced and $IL\text{-}10^{-/-}$ colitis in mice. However, in light of the observations made by Randolph et al in fibrostenotic stricturing CD, it remains to be seen whether de novo generated lymphatic vessels can really integrate with functional sentinel lymph nodes in the mesentery to restore immune tolerance. Recent work assessing the impact of infection with *Yersinia pseudotuberculosis* demonstrated that infection induced damage to afferent lymphatic vessels and resulted in mesenteric bacterial leak, which persisted long after the infection was cleared, in addition to developing fibrotic mesenteric lymph nodes and compromised regional immunity (Cell 2015;163:354-366). Thus, permeable lymphatic vessels, fibrotic lymph nodes, and chylomicron leak may represent a point of no return for tissue integrity and function in CD.

A concern for the therapeutic dissociation of mesenteric TLT with the intent of reversing the vascular remodeling process is a potentially detrimental effect on regional antimicrobial immunity. For example, lymphotoxin-β receptor signaling is a critical cytokine cue for the development and organization of secondary lymphoid tissues and a multitude of studies have

used lymphotoxin-β receptor blockade to disaggregate mature TLTs in various preclinical models of infection and autoimmunity. However, limited studies have reported on the subsequent impact on posttreatment pathology. Although lymphotoxin-β receptor inhibition will dissociate TLT in virtually all preclinical models reported, it has dramatic effects on lymph node structure and function including disruption of germinal centers, high endothelial venule repression (Immunity 2005;23:539-550) and altered dendritic cell ratios (J Immunol 2008;180:238-248). Although these experiments serve as proof-of-principle studies, the site-directed delivery of therapeutics that target TLT structure and function in CD, with limited effects on antimicrobial immunity may prove challenging. The report by Randolph et al emphasizes the need to investigate in detail the molecular signatures and regional differences of TLT from patients with inflammatory bowel disease, in an effort to identify novel therapeutic targets. Building on this work and understanding how mesenteric TLT integrate into enteric immunity (by controlling lymphocyte, dendritic cell, and antigen trafficking patterns) is sure to advance our understanding of TLT function and their role in CD pathophysiology. Given the marked lymphatic dysfunction in CD, further efforts in this area will certainly expand our understanding of the pathogenesis of the disease.