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SHORT COMMUNICATION

Lymphoid Aggregates Remodel Lymphatic Collecting Vessels that Serve Mesenteric Lymph Nodes in Crohn Disease

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Early pathological descriptions of Crohn disease (CD) argued for a potential defect in lymph transport; however, this concept has not been thoroughly investigated. In mice, poor healing in response to infection-induced tissue damage can cause hyperpermeable lymphatic collecting vessels in mesenteric adipose tissue that impair antigen and immune cell access to mesenteric lymph nodes (LNs), which normally sustain appropriate immunity. To investigate whether analogous changes might occur in human intestinal disease, we established a three-dimensional imaging approach to characterize the lymphatic vasculature in mesenteric tissue from controls or patients with CD. In CD specimens, B-cell–rich aggregates resembling tertiary lymphoid organs (TLOs) impinged on lymphatic collecting vessels that enter and exit LNs. In areas of creeping fat, which characterizes inflammation-affected areas of the bowel in CD, we observed B cells and apparent innate lymphoid cells that had invaded the lymphatic vessel wall, suggesting these cells may be mediators of lymphatic remodeling. Although TLOs have been described in many chronic inflammatory states, their anatomical relationship to preestablished LNs has never been revealed. Our data indicate that, at least in the CD-affected mesentery, TLOs are positioned along collecting lymphatic vessels in a manner expected to affect delivery of lymph to LNs. *(Am J Pathol 2016, 186: 3066–3073; http://dx.doi.org/10.1016/j.ajpath.2016.07.026)*

In the bowel, the regulation of inflammatory and immune responses must be finely balanced to cope with the large microbial load present in the gut lumen. It is thought that the etiology and pathophysiology of inflammatory bowel disease relate to inflammatory and immune alterations that, in turn, connect with changes in the microbiome or interactions with microbial products.[1] A recent study that followed mice for weeks after *Yersinia pseudotuberculosis* infection revealed that long after the infection was completely cleared, immune dysregulation persisted.[2] This persistence was because of tissue insult that lingered and continuously deviated the immune response, normally programmed in draining lymph nodes (LNs), in part by inhibiting dendritic cell trafficking to the LNs. Inhibition of dendritic cell trafficking to LNs has also been found to underlie ileitis in the SAMP1/YitFc mouse model.[3] In the case of infection-induced impairment of dendritic cell migration, the inhibition was apparently caused by compromised lymphatic integrity that damaged lymphatic collecting vessels in the mesentery and left them highly hyperpermeable, such that

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the contents of lymph spilled out into the mesentery, including migratory dendritic cells, rather than progressing to downstream mesenteric LNs.[2]

An interesting consideration that results from this work is whether related phenomena might contribute to inflammatory bowel disease in humans. Indeed, lymphatic dysfunction has been long discussed, although often overlooked, in Crohn disease (CD).[4,5] In particular, the sites where lymphatic dysfunction may be most relevant are scarcely studies in inflammatory bowel disease (IBD) models. That is, most analysis is performed in the intestinal wall, where lymphatic capillaries take up immune cells and solutes from the interstitium. The study in mice following *Yersinia* infection implicated the larger lymphatic vessels in the mesentery that interface with LNs. These vessels, called collecting vessels, are not known to take up cells or solutes but instead they function to actively pump lymph, via the action of specialized muscle cells and valves that promote unidirectional flow, along afferent collecting vessels that drain into LNs and then through efferent collecting vessels that emerge from LNs.[6] The permeability of the collecting vessels has recently been shown to be regulated by a subset of dendritic cells.[7] Furthermore, classic studies in dogs by Adair et al[8] revealed another means by which collecting lymphatic vessel hyperpermeability develops. They observed that afferent lymph, with its typical low protein content, is filtered in the LN so that water is absorbed into the venous vasculature and efferent lymph emerges nearly as concentrated as plasma.[8] However, increased efferent collecting vessel pressure changed the filtration properties of the LN and ultimately led to markedly leaky afferent lymphatics.[9]

Lymphatic collecting vessels are surrounded by fat throughout the body. In the mesentery, they run through the copious mesenteric adipose tissue. In CD, this fat expands beyond its usual anatomical restriction to the mesentery, such that during its expansion, it creeps up on to the intestinal wall, giving it the name creeping fat. Indeed, creeping fat is a hallmark of the inflamed CD-affected tissue, but its etiology is unexplained.[10] One possibility connected to the discussion of lymphatic vessels is that creeping fat is driven by the spillage into the mesentery of the fatty chylomicrons carried in lymph[11] by highly permeable or leaky lymphatic vessels.

Lymphatic vessels in the human mesentery have scarcely been studied, in either normal or diseased states, generating an obstacle to assessing the potential relevance of these studies in mice and dogs to human disease states. Herein, we developed an approach to characterize the human mesenteric lymphatic vasculature in normal and CD-affected mesentery. We identify the existence of tertiary lymphoid organs (TLOs), known to be present in many chronic inflammatory diseases, although not previously described in CD mesentery, as structures that remodel the collecting lymphatic vessel path to and from LNs, supporting the possibility that flow of lymph through the mesentery is altered in human CD.

Materials and Methods

Patient and Specimen Information

We studied tissue specimens from 17 operated on CD patients (11 women and 6 men; mean age = 45 ± 15 years) bearing disease with a diagnosis during adulthood involving stricturing in ileum-involved disease and 6 control tissues. Control tissue was derived from intestinal tissue removed for non-IBD indications, including distal from cancerous lesions or tissue from cadaveric donors. Specimens were provided as paraffin-embedded blocks of the mesentery, or whole fixed tissue was obtained after cases were signed out by pathologists at Washington University School of Medicine (St. Louis, MO). Patient identification was deidentified, and each tissue was assigned a random number by an honest broker (administered through Washington University's Digestive Diseases Research Core Center) who also recorded relevant patient information without identifiers. Use of these tissues and approval for the study was given by the Institutional Review Board of Washington University School of Medicine.

Patent Blue Tracing

Patent blue dye tracing of lymphatics during surgery was performed with patient and institutional consent from Clinique Universitaire Saint-Luc (Brussels, Belgium) (EudraCT N 2008-001746-12). Following the method as described,[12] the surgeon injected a solution of patent blue dye, a dye used for lymphangiography, in the serosa on the anti-mesenteric border of the ileum just before resection.

Whole Mount Imaging

In the course of routine handling by pathologists, tissue derived from surgical resections of CD-affected ileum was fixed in formalin. On transfer to our laboratory, specimens were examined grossly and slices of the mesenteric adipose tissue and intestinal wall were made with the aid of manual tissue holders containing 2-mm cutting grids. Except where noted, slices were made tangential to the serosal surface of mesentery (Figure 1, slices labeled "a" were those containing serosal surface epithelium), and records were kept with respect to location of each slice relative to the terminal ileum and to the serosal surface. Because we observed the highest density of lymphatic capillaries and collecting vessels nearer to the serosal surface, slices along the serosal surface were typically analyzed first, especially those nearer the intestinal border, with some of the border left attached to retain orientation. A typical slice was approximately 2 cm in the x and y directions, respectively, with a z-axis thickness of approximately 2 mm. Up to 60 slices were often made