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RESEARCH ARTICLE

Intestinal Lymphedema and Structural Changes in Crohn's Disease

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ABSTRACT

Background and Aims: In 1954 Warren and Sommers asserted that Crohn's disease was "essentially an elephantiasis of the intestinal wall". They described a late phase of Crohn's disease and did not try to reconstruct the various stages of the disease. The aim of this study is to provide a step-by-step reconstruction of the anatomic changes that lead to "terminal ileitis", i.e., to define the pathologic process.

Methods: A pathologic examination of over 100 surgical specimens of Crohn's disease was performed. Findings were compared with those of the published literature in order to derive sequential stages in disease evolution.

Results: Terminal ileitis occurs in four different steps. The initial event is thought to be the obstruction of the ileal branch of the superior mesenteric lymphatic collector. It leads to progressive lymphatic stasis and lymphangiectasias of all the layers of lymphatics of the intestinal wall, i.e. an intestinal lymphedema (Step 1). Progressive accumulations of protein-rich lymph occur in intestinal wall, that in the mucosa result in aphthous ulcers. The increase in pressure in the intestinal lymphatic network causes rupture of superficial lymphangiectasias (Step 2). These early parietal changes allow bacterial contamination of the lymphedema which, over time, leads to chronic superficial enteritis and granulomatous lymphangitis (Step 3). Finally, fibrosis is reached (Step 4).

Conclusions: Crohn's disease is a lymphedema of the intestinal wall contaminated by intestinal contents, ending in fibrosis. The structural changes defy therapeutic resolution.

Keywords: Crohn's disease, lymphedema, lymphatics

Introduction

Many studies have described the histologic features of Crohn's disease (CD). In 1939 Hadfield said there was "obstructive lymphedema of the submucosa".[1] And in 1948 Warren and Sommers spoke of occlusion of the lymphatics in three layers of affected bowel (mucosa, submucosa, muscularis).[2] In 1954 they asserted that CD was "essentially an elephantiasis of the intestinal wall".[3] Later, in 1995, Mooney et al. illustrated granulomatous obstruction of intestinal lymphatics.[4]

Recently, Sundqvist et al. drew attention to the intestinal microbiota.[5] It's known that when the fecal stream is diverted Crohn's colitis improves [6]; and when the fecal stream is reestablished disease is reactivated.[7] The question is how do bacteria, bacterial products, and dietary antigens penetrate the intestinal wall? Various explanations that have been proposed include NSAIDs use,[8] leaky gut syndrome,[9] M cells uptake,[10] reduced Paneth cell alpha-defensins,[11] and proinflammatory cytokines.[12] It is my belief that "stream substances" elicit disease when the intestinal wall is boggy with lymphedema.

Pedica et al. examined the distribution of lymphatic vessels in the diseased ileum and colon of patients with CD.[13] This distribution was compared to that of healthy controls and of other bowel diseases. They employed immunohistochemistry with monoclonal antibody D2-40, which labels lymphatic endothelium, and found that the number of lymphatics was significantly greater in both ileal and colon specimens of CD (versus controls). They described also diffuse intestinal lymphangiectasia. Both these conclusions suggest that lymphatic abnormalities are at the seat of CD (**Fig. 1**).

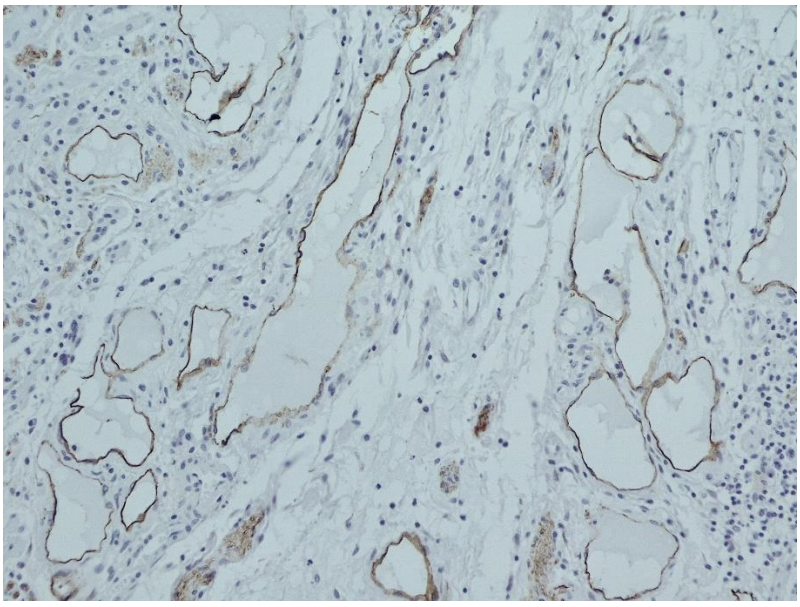

Figure 1. Ectatic lymphatics in submucosa in CD. IHC for D2-40, X400. (Courtesy of Dr. Herbert Van Kruiningen)

Since lymphangiectasias and fibrosis are typical microscopic features of CD, as well as of any lymphedema, like the lymphedema of the upper limb after breast surgery or congenital lymphedema of the lower limb,[14] CD can be seen a consequence of lymphedema of the intestinal wall. But if that's the case, what is the relationship between intestinal lymphedema and the other pathologic findings, characteristic of CD but not of lymphedema, such as aphthous ulcers, broad-based ulcers, transmural inflammation, fissures, fistulas, and strictures, that are responses unique to this tissue, this organ and its flora?

Warren and Sommers did not explain this relationship and described only the late phase of CD without illustrating the earlier phases. Maybe their conclusions have been forgotten for these reasons. The aim of this study is to provide a step-by-step reconstruction of disease progression, in order to define the pathology that is represented by the term "Crohn's disease", expressing some personal perspectives, based on examination of over 100 surgical specimens.[15] To this end, I will refer both to terminal ileitis (the paradigmatic form of CD), and to untreated cases, because 6-mercaptopurine, azathioprine, and infliximab can profoundly influence the histologic lesions of CD.[16] What I have seen are four steps in the development of this pathologic process.

Step 1: Obstruction of the ileal branch of the superior mesenteric lymphatic collector. Within the anatomical arrangement of lymphatics, the lymphatic vessels of the small intestine include two independent networks. One is formed by the lacteals, which drain individual villi and connect with a network of lymphatics in the mucosal layer. The other by the lymphatics of the muscle layers. Both networks drain into contractile collecting lymphatics located at the mesenteric border of the intestine.[17]

Arrangement of the lymphatics of both small and large intestine parallels the arrangement of the respective blood vessels. The lymphatic vessels that drain the jejunum and the proximal ileum have an arrangement corresponding to the (arterial) vasa recta, that irrigate about one centimeter of the intestinal wall.[18] The lymphatics that drain the terminal ileum flow into the ileal lymphatic branch homologous with the juxta-ileal arterial arch that runs parallel to the terminal ileum. The juxta-ileal arterial arch is the right branch of the bifurcation of the superior mesenteric artery that originates 60-90 cm from the ileocecal angle (the left branch of the bifurcation anastomoses with the last arterial arch of the proximal ileum); it anastomoses with the colic branch of the ileocolic artery. On the right side the ileal lymphatic branch connects to the colic branch of the ileocolic lymphatic branch, while on the left side it connects to the origin of the superior mesenteric lymphatic collector (homologous with the superior mesenteric artery), which connects there to the lymphatic branches of the proximal ileum **(Fig. 2)**. The lymphatic vessels that drain the cecum and the proximal part of the ascending colon flow into the right colic lymphatic branch, then into the ileocolic

lymphatic collector, and finally into the superior mesenteric lymphatic collector (Fig. 2).[18,19]

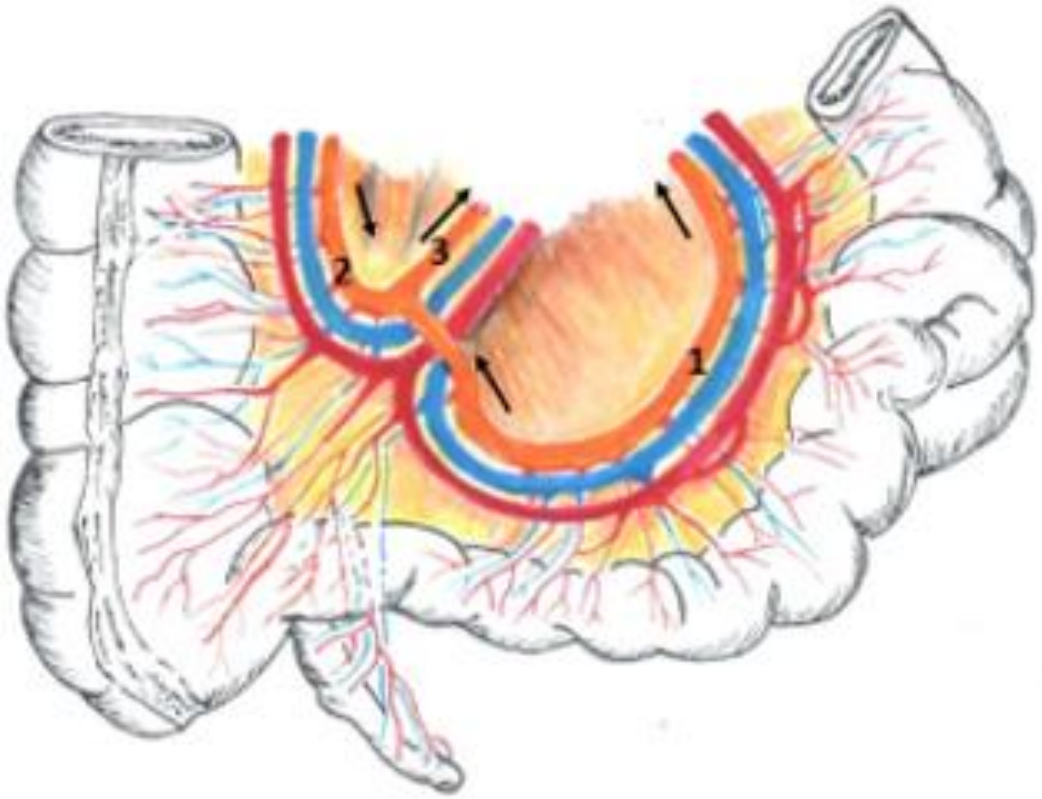

Figure 2. Normal anatomical arrangement of the lymphatic vessels of the terminal ileum, cecum, and ascending colon (based on anatomic illustrations of L. Testut and A. Latarjet[18]). 1. Ileal branch of the superior mesenteric lymphatic collector. 2. Colic branch of ileocolic lymphatic collector. 3. Ileocolic lymphatic collector. Black arrows show the direction of the normal lymph flow.

It is my belief that ileitis begins with the obstruction of the ileal branch of the superior mesenteric lymphatic collector. This claim has not been verified in the literature, except for a case of surgically treated idiopathic hypoproteinemia illustrated by Waldmann et al.[20] This onset site, secluded and far removed from the intestine, may be the reason why the mystery of the etiology and histopathogenesis of CD have not yet been solved. The following findings confirm this statement.

First, CD occurs in segments that vary in length. The length is directly proportional to the obstructed lymphatic collector that drains that affected intestinal segment. Therefore, in CD jejunal and proximal ileal lesions are short in length because the lymphatic vasa recta drain only a short tract of the jejunal and

proximal ileal intestinal wall. In terminal ileitis lesions are long because the ileal lymphatic branch drains a long segment of the terminal ileum. In terminal ileitis with right colitis lesions are even longer because the ileocolic lymphatic collector drains terminal ileum and part of right colon.[15]

Second, the discrete demarcation between healthy and diseased intestine in terminal ileitis is like the one observed in ileal ischemia caused by embolism of the terminal branch of the superior mesenteric artery. Despite the obvious diversity of the macroscopic intestinal lesions between these pathologies (cobblestone appearance and thickened wall in CD, hemorrhagic suffusion and edematous wall in ileal ischemia), this demarcation suggests a vascular cause also for CD, that is, a lymphatic obstruction.

Third, Heatley et al. noted that "the site of obstruction appears by lymphangiography to be situated distally in the lymphatic pathway, away from the intestinal wall".[21] This finding explains the observations by Crohn et al. who, studying specimens obtained from patients who had been ill for at least a year, noted that the mesentery was greatly thickened and fibrotic.[22]

Fourth, the obstruction of the lymphatic collectors is the only anatomic explanation for the increase in number and dilatation of the lymphatics of the all layers of the intestinal wall.[13]

Fifth, "Crohn's rosary" and lymphangiectasias of the subserosa are evidence that the obstruction of the lymphatic network is upstream of the intestinal wall.

Sixth, animal models also argue for a role of mesenteric lymphatics rather intramural lymphatics. Reichert and Mathes produced experimental chronic intestinal lymphedema by injecting irritating and sclerosing materials into the mesenteric and subserosal lymphatics of the bowel, and used the adjacent bowel with undisturbed lymphatic drainage as a control. So, the pathologic changes of the intestinal wall were due entirely to the lymphatic obstruction. Comparing the microscopic findings of "regional enteritis" and experimental intestinal lymphedema, they stated that the greatly thickened muscular and submucosal layers, which were edematous, and the engorged and thrombosed lymphatics and lacteals of experimental lymphedema were identical to those of CD.[23] Kalima et al. injected formalin into the ileal lymph nodes of pigs. By destroying mesenteric lymphatic flow, they obtained an experimental model very similar to CD.[24]

Finally, the long latency between recognized abnormal gut permeability and overt disease[25] can be explained by the primary obstruction of the ileal lymphatic branch, which can compensate for the hindered lymphatic outflow of the terminal ileum for a long time through the formation of collateral circulation and neo-lymphangiogenesis.

All these findings prove that in terminal ileitis the lymphatic obstruction occurs in the ileal

branch of the superior mesenteric lymphatic collector, and then that the cause of CD lies outside and not inside the intestine.

Step 2: Rupture of superficial lymphangiectasias and development of apthous ulcers.

The increased pressure inside the obstructed intestinal lymphatic network yields progressive lymphangiectasia. Lymphangiectasia involves lymphatics of all the layers of intestinal wall, from lacteals to subserosa. Lymph stasis occurs within the lumen of lymphatics and in all the layers of the intestinal wall, most obvious in the submucosa. Progressive accumulations of protein-rich lymph appear in the mucosa and submucosa, first adjacent to the lymphatic capillaries, then diffused into the tunics, as illustrated in electron microscopic study of CD by Kovi et al. **(Fig. 3).**[26] Lymphatics cannot perform their physiologic function of lymph transport and neo-lymphangiogenesis occurs in an attempt to compensate. Lymphatic edema also provokes swelling of the adipose tissue that surrounds the surface of the intestine (fat-wrapping).

The progressive dilatation of superficial lymphangiectasias leads to flattening of the epithelial cell layer of the intestinal villi, that finally rupture. The rupture of superficial lymphangiectasias causes outflow of lymph into the intestinal lumen. Lymph leakage, which results in the reduction of endolymphatic pressure, and the ability of GI bacteria to move against the current support the seepage of intestinal fluids into the dilated and atonic intestinal lymphatic network **(Fig. 3 and 4).**

Figure 3. Illustration of the Steps 1 and 2. Superficial lymphangiectasia of a lacteal that finally breaks (red circles and arrows). Diffuse accumulation of protein-rich lymph in the mucosa that finally causes breaks at lymphoid follicle (blue circles and arrows). Submucosal and subserosal lymphangiectasias. Edematous thickening of the intestinal wall (mostly of the submucosa).

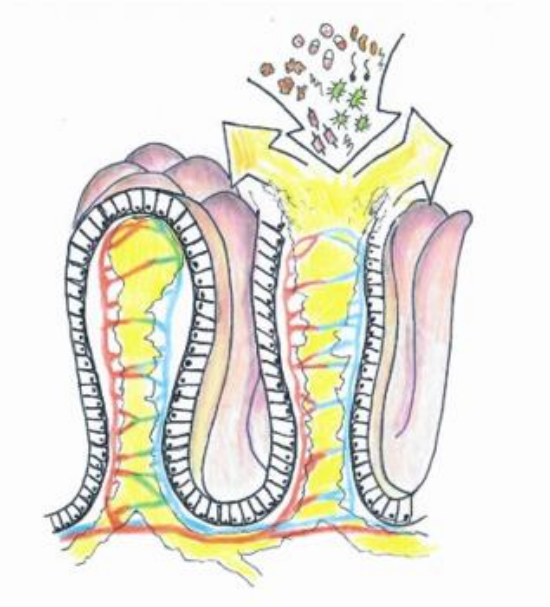

Figure 4. On the left, superficial lymphangiectasia of a lacteal. On the right, a broken superficial lymphangiectasia with the outflow of the stagnant lymph into the intestinal lumen (yellow arrows) and seepage of intestinal fluids into the atonic lymphatic network of the intestinal wall (white arrow).

At the same time, in the mucosa, the accumulations of protein-rich lymph between the epithelium and the lamina propria are infected by the intestinal contents through the M cells or by cell to cell junction penetration. It is noteworthy that M cells provide a route of entry into the mucosa for various pathogens.[27,28] Nevertheless, M cells alone are not enough to cause CD. If CD was caused only by the transport of pathogens into the mucosa through M cells, it would be a much more widespread disease. Something more is needed to cause CD, and it could be the accumulations of protein-rich lymph between the epithelium and the lamina propria. They can be easily infected by pathogens transported through M cells. Moreover, bacteria and their products could progress between epithelial cells whose tight junctions have been stretched by edema.

Once infected, these formations break into the intestinal lumen, giving rise to aphthous ulcers that may undergo healing as well as evolve into larger broad-based ulcers over time. Even if they resolve, accumulations of protein-rich lymph are destined to reoccur as long as lymphatic obstruction persists.

Step 3: Contamination of the intestinal lymphedema.

The early structural changes of the intestinal wall allow intestinal contents to get in contact with the intestinal mucosa in two areas: aphthous ulcers, and the broken superficial lymphangiectasias of the lacteals. Contamination develops progressively.

Aphthous ulcers first**,** and broad-based ulcers later, are the areas of mucosal surface where the intestinal mucosal barrier is destroyed. Here, contact occurs between the intestinal contents and the lamina propria, thus triggering an inflammatory and immunological reaction. This reaction gives rise to a superficial enteritis similar to the spontaneously remitting enteritis observed in any infectious intestinal process. It is characterized by lymphocytic and plasma cell aggregates. Because of the presence of some insoluble and more resistant antigens, epithelioid cells and multinucleated giant cells appear. In some areas affected by enteritis, necrosis may occur and cause infiltration by granulocytes. Chronic enteritis causes a polypoid surface. Functionally, this superficial enteritis protects the intestinal wall from the many pathogens of the intestinal contents, by acting as a cellular and molecular neo-barrier (as demonstrated by studies showing the presence of high cytokine levels in CD intestinal wall[29]). The neo-barrier replaces the epithelial layer that deteriorated. However, unlike spontaneously remitting enteritis in which the mucosa returns to normal after its initial cause is removed, in CD enteritis becomes chronic because lymphatic obstruction and then chronic stasis of the lymph persist **(Fig. 5).**

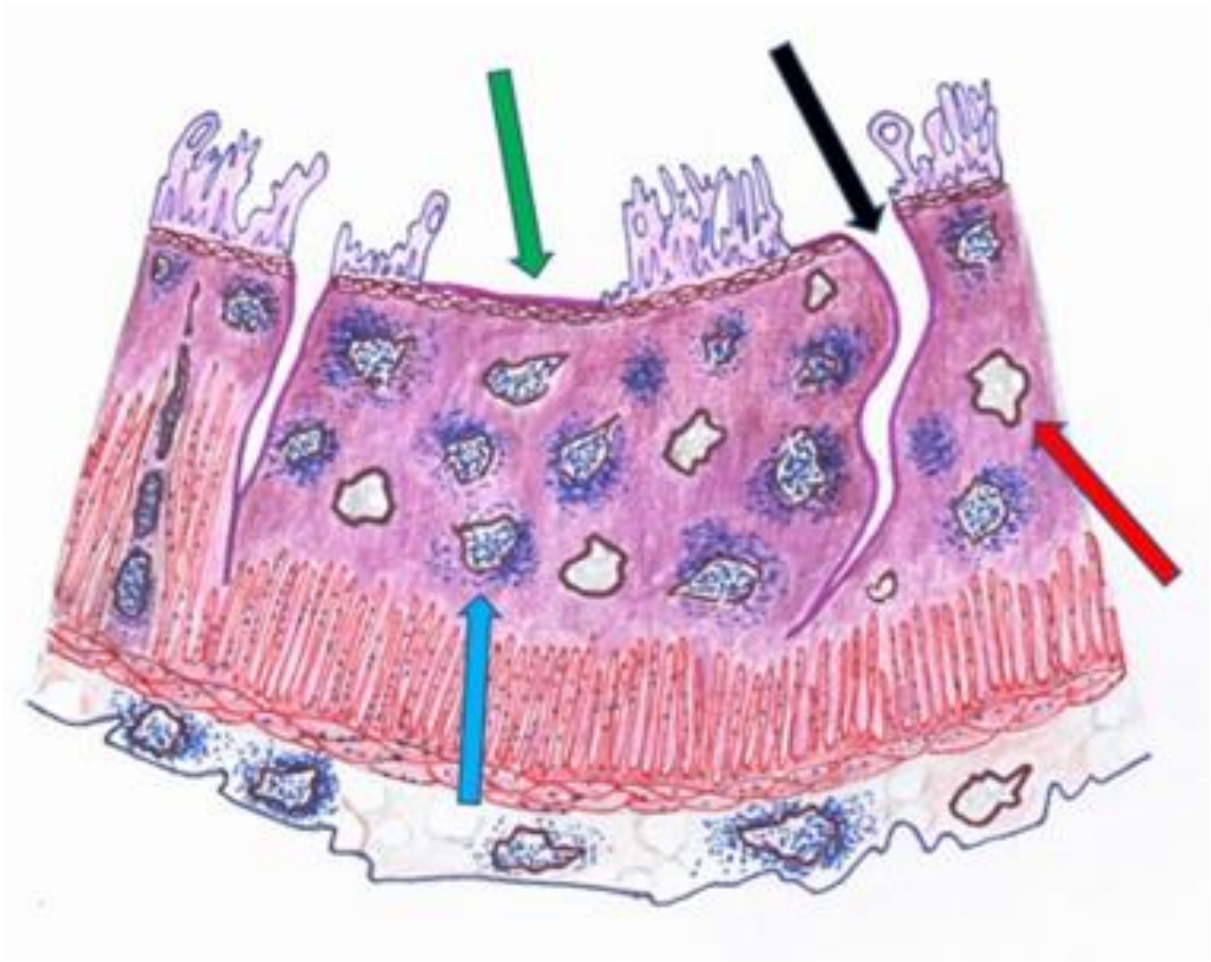

Figure 5. Illustration of the Steps 3 and 4. Superficial enteritis at the stage of broad-based ulcers (green arrow). Broken superficial lymphangiectasias result in fissures (black arrow). One of the many nodules that form transmural inflammation (blue arrow). Lymphangiectasia of the submucosa (red arrow). Crohn's rosary of the subserosa.

The seepage of intestinal fluids into the dilated (and atonic) lymphatic network of intestinal wall through the broken superficial lymphangiectasias **(Fig. 4)** also triggers an inflammatory and immunological reaction, which crams lymphangiectasias with an endolymphatic lymphocytic aggregation, similar to lymphocytic "thrombi" **(Fig. 6)**. These lymphocytic aggregations partly obscure intestinal lymphangiectasias and make all the layers of the diseased intestinal wall seem occupied by randomly scattered nodular formations. This feature constitutes transmural inflammation **(Fig. 5)**. When endolymphatic pressure increases and dilated vessels break, lymphocytic aggregates surround damaged lymphatics.

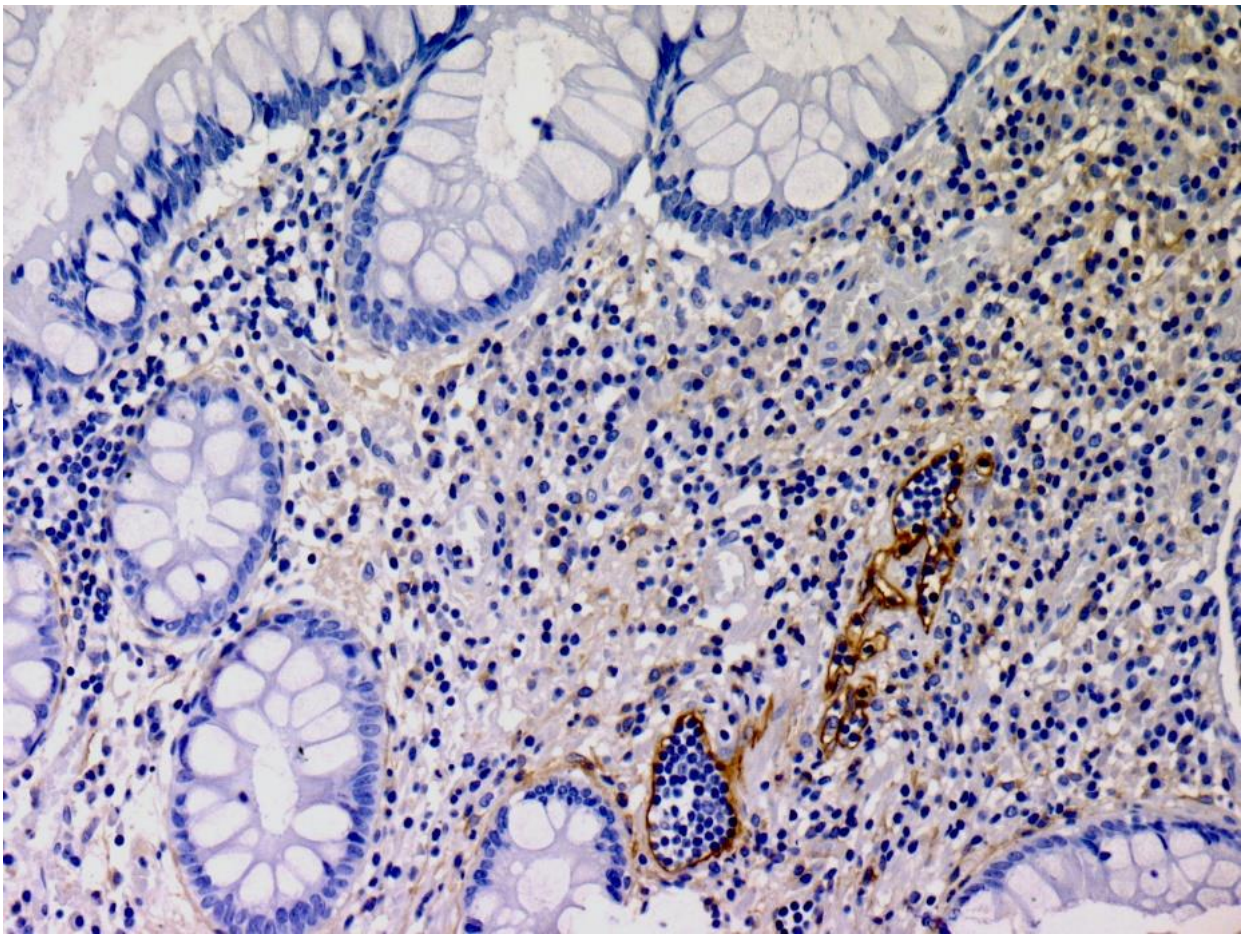

Figure 6. Ectatic lymphatics with endolymphatic lymphocytic aggregation in CD. ICH for D2-40 x 400 (Courtesy of Dr. Federica Pedica)

As occurs in the ulcerated mucosa, epithelioid cells and multinucleated appear because of some and more resistant antigens that penetrated in the dilated lympathic intestinal network, giving rise to "granulomatous lymphangitis"[3] that obscures lymphatic vessels **(Fig. 7)**. Granulomas contribute to the obstruction of intestinal lymphatics.[30]

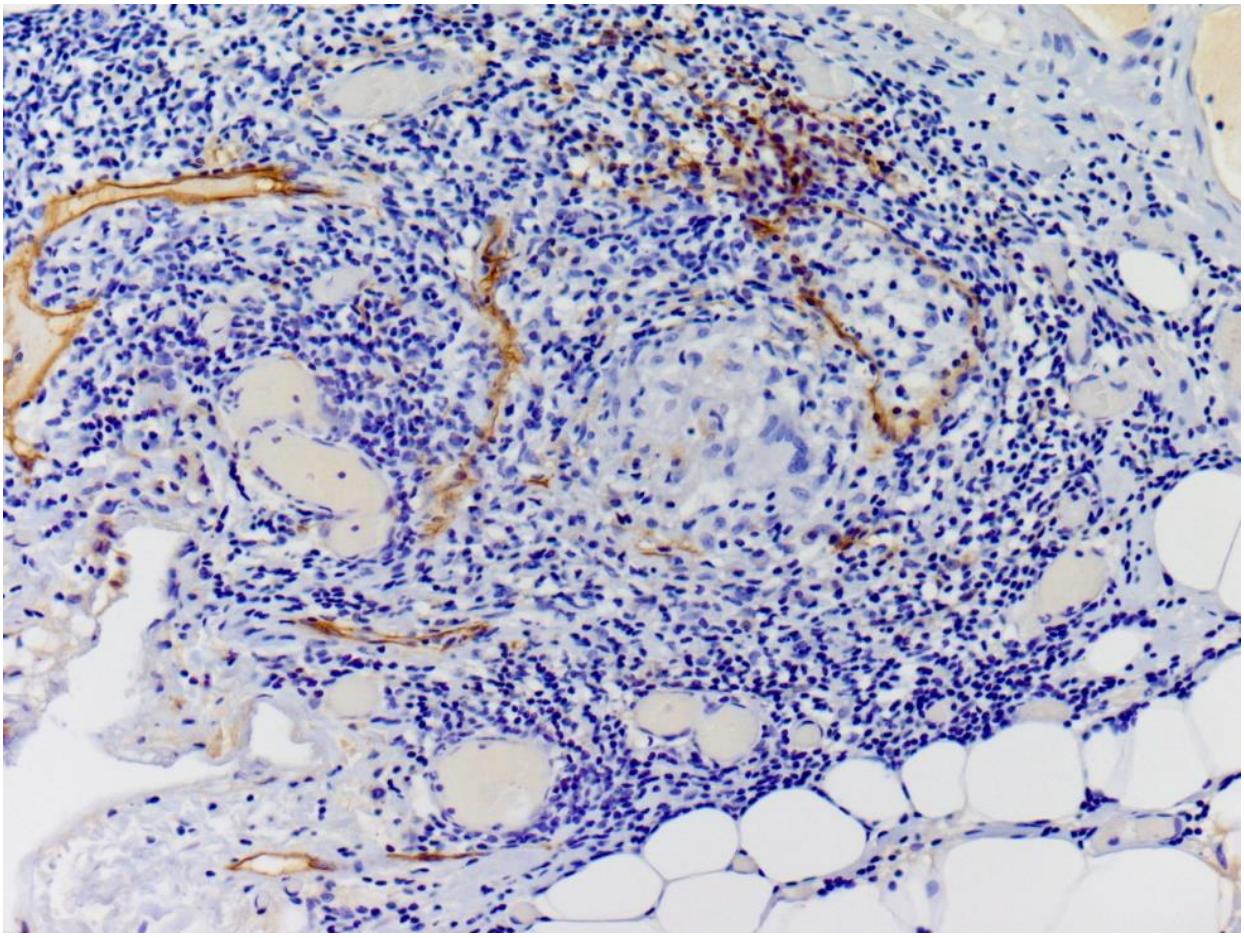

Figure 7. Ectatic lymphatics associated with a granuloma in CD. ICH for D2-40 x 400 (Courtesy of Dr. Federica Pedica)

Transmural inflammation appears as randomly scattered nodules because the microtome that cuts the surgical specimen of diseased intestinal wall meets the dilated vessels with peri- and endo-lymphatic lymphocytic thrombi and granulomas at several points, creating the appearance of nodules scattered in the intestinal wall. Transmural inflammation is transparietal because the anatomic course of lymphatics through the various layers of the intestinal wall is transparietal.

Lymphoid aggregations and granulomas oriented in a line located at the junction of the muscularis propria and subserosa, form "Crohn's rosary". They obstruct the lymphatics that emerge from the intestinal wall and which in turn form the roots of the ileal lymphatic branch.

Van Kruiningen's photographs demonstrate that lymphocytic thrombi occur upstream of granulomatous obstructions in lymphatics.[30,31] The blood vascular changes observed first by Knutson et al,[32] and then by Wakefield et al.[33] represent secondary vasculitis in an inflamed intestinal segment.

In this complex pathologic process (which develops over a long time), it is also necessary

to consider the lymph that is constantly produced by the diseased intestine. What happens to the lymph? Some lymph can flow slowly towards the superior mesenteric lymphatic collector as shown by the in vivo surgical experience.[34] But if the diseased intestine cannot discharge all the lymph it produces, the intestinal wall would thicken causing a severe reduction of the intestinal lumen. This usually does not happen because part of the dilated lymphatic network discharges stagnant lymph into the intestinal lumen through the ruptured lymphangiectasias of the lacteals. The lymphatic vessels that open into the intestine are "flushed" by an incessant flow of lymph fluid towards the intestinal lumen, becoming fissures.

Step 4: Fibrosis of the intestinal wall.

Fibrosis is the final phase of CD. It is due to the gradual transformation of stagnant lymph into dense connective tissue, via an increase in collagen production.[35] Bowel wall edema may also lead to mucosal hypoxia, increasing inflammatory infiltrate and fibrosis.[36] Over time, fibrosis causes progressive thickening of the intestinal wall and the progressive reduction of the intestinal lumen.

Hypertrophy and hyperplasia of nerve fibers and alterations of neuronal cell bodies occur in the parasympathetic ganglia of the submucosa and plexus myentericus. Nerve fiber hypertrophy is associated with one or more inflammatory cell types and granulomas in both the submucosal and myenteric plexus.[37] It remains unknown whether these nerve changes precede or follow the inflammation.[38] Immunosuppressive therapies appear ineffective on inflammation of the enteric nervous system.[39] It's known that CD transforms the terminal ileum into a thickened tube, stiffened by fibrosis and with a small lumen. Encased by fibrosis, the muscularis propria has reduced ability to contract and to cause effective peristalsis. Severi et al. described alterations in smooth muscle cell morphology and contractile activity.[40] Nervous impairment may be the result of excessive stimulation of smooth muscle cells in an attempt to restore peristalsis.

Summarizing, my findings and those of others quoted support the hypothesis that CD is a lymphedema of the intestinal wall contaminated by the intestinal contents. It is my belief that it is a consequence of the obstruction of mesenteric lymphatic collectors. This pathologic process develops over a long time.[16] Findings of the lymphedema (lymphangiectasias, accumulations of protein-rich lymph, edema of the intestinal wall) overlap with consequences of lymphedema (broken superficial lymphangiectasias, aphthous ulcers, broad-based ulcers), and are obscured by the simultaneous presence of chronic inflammation (superficial enteritis, transparietal inflammation), healing phenomena (healing of aphthous ulcers, regeneration of the epithelium), attempts to compensate for lymphatic stasis (lymphatic neo-angiogenesis, discharge of stagnant lymph through fissures), and defense processes (lymphocytic aggregation with activation of the immune

system and its mediators). All these findings can occur at the same time or at different times, in different evolutionary phases, in one or more parts of the intestinal segments where lymphatics are obstructed, until finally fibrosis occurs in the whole challenged segment. **(Fig. 8)**.

In my earlier pathologic studies, I could not assess the mesenteric lymphatic collectors because the surgical specimens consisted of the intestinal wall and only a small portion of the mesentery. Examination of the mesentery of the terminal ileum would be useful to identify the intestinal lymphatic collectors and to study them microscopically.

This histopathogenesis should induce physicians to perform lymphoscintigraphy or contrast lymphography to study the lymphatic system of CD patients.

It is possible that initial obstruction of lymphatics could be caused by an infectious agent able to trigger obliterative endolymphangitis as suggested by Van Kruiningen,[41] by a low-grade infection as suggested by Heatley et al,[22] by a primary disease of the mesenteric lymph nodes,[42] or even by congenital or genetic diseases as is the case for lymphedema of the lower limbs.[43] It will be important to seek agents that might cause this process. Certainly, only by understanding the CD pathological process we will be able to cure CD.

In conclusion, this work confirms that in the pathogenesis of "terminal ileitis" a central role is played by damaged lymphatic vessels. The study explains the various histologic findings characteristic of this disease, how they occur and how they are related to one another.

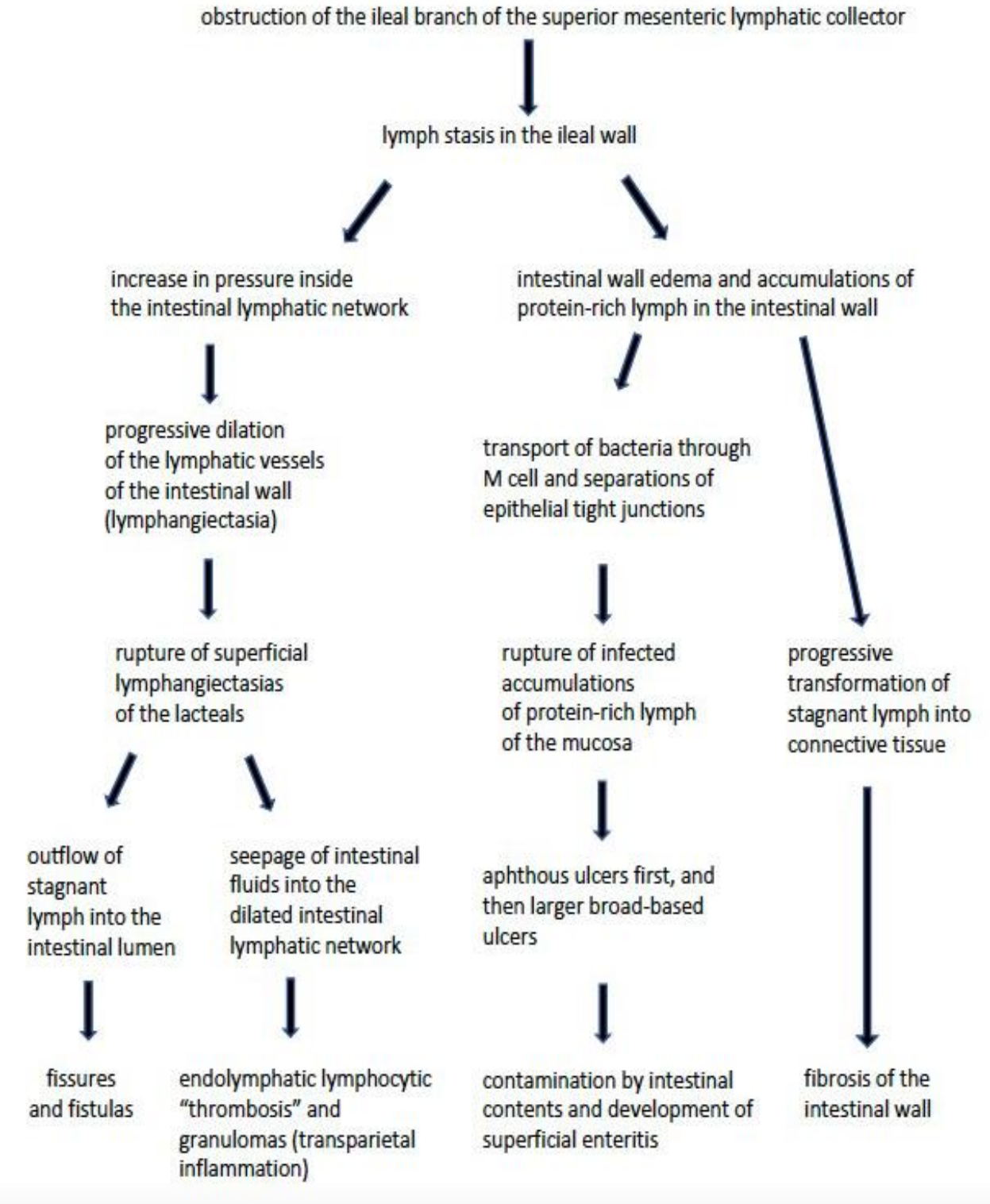

Figure 8. Pathway of the pathologic process of terminal ileitis.

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