Creeping fat in Crohn's disease: Innocuous or innocuum?

Creeping fat in Crohn's disease: Innocuous or *innocuum*?

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Summary:
In Crohn's disease, the characteristic expansion of mesenteric tissue on sites of intestinal inflammation is known as creeping fat. In a recent issue of *Cell*, Ha et al. report that translocation of gut bacteria to the mesenteries is associated with the formation of creeping fat.

Crohn's disease (CD) was first described in 1932 by Burrill Crohn and colleagues (Crohn et al., 1932). In this early report, the authors observed that patients with inflammation of the terminal ileum presented with a thickening of the mesenteries. This phenomenon, known as "creeping fat", is an intriguing and distinguishing feature of CD. Why the mesenteries "creep" through the abdomen and wrap around the intestines of many patients with CD has remained a medical mystery. Furthermore, whether creeping fat is pathogenic or protective is not clear. Ha et al. (Ha et al., 2020) unveil that CD is associated with increased translocation of mucosal-associated gut bacteria into the mesenteric adipose tissue, leading to expansion of this fat tissue, and suggest that this creeping fat may limit systemic bacterial dissemination.

Inflammation-induced barrier dysfunction is a prominent feature of CD lesions, driving multiple immunological and physiological changes associated with the pathologies found in CD (Chang et al., 2017; Pastorelli et al., 2013). Ha et al. begin their study by developing a robust and controlled pipeline to process and analyse the microbiota found in the intestinal mucosa and mesenteric tissues from patients with CD or ulcerative colitis, a gut inflammatory condition not associated with creeping mesenteries. In addition, healthy tissue from the ileal mucosal and mesenteric fat from non-inflammatory bowel disease patients undergoing colon surgery were also analysed. Using metagenomic sequencing, the authors revealed that bacterial translocation from the gut to neighbouring mesenteric adipose tissue frequently occurs, irrespective of disease status. However, bacterial translocation to the mesenteric fat was more pronounced in CD. Analysis of the composition of the microbial community did not reveal any specific clustering differences in the microbiota from mesenteric tissues and from mucosal tissues. Rather, mesenteric derived bacteria aligned phylogenetically to the microbial members of the adjoined mucosal tissue. Interestingly, the diversity of bacteria recovered from the creeping mesenteric tissue was reduced compared to control tissues, reflecting the decreased microbial diversity found in mucosal tissues affected by CD. Taken together, these findings indicate that the microbiota from the mesenteric adipose tissue arose from bacteria translocating from the neighbouring gut (**Figure 1**). Recent findings from two other groups investigating the presence of bacteria in adipose tissue of obese individuals further suggest that mesenteric adipose tissue is a primary site of bacterial translocation and that its composition is

reminiscent of the gut microbiome (Anhê et al., 2020; Massier et al., 2020). Nevertheless, these three studies have the caveat of analysing patients with pathologies with various levels of underlying inflammation. Therefore, further studies are needed to define the potential role of bacterial translocation to mesenteric fat tissue in healthy individuals.

Ha *et al.* were able to recover live bacterial isolates from most mesenteric adipose tissue samples, covering a total of 84 species. Five of these bacterial species, including *C. innocuum*, were exclusive to the creeping fat from CD patients. Relative abundance of these 5 bacterial species in the metagenomic dataset could discriminate between creeping mesenteric tissue from CD patients and healthy mesenteric. They next investigated what was driving the expansion of *C. innocuum* in CD patients. Sequencing of *C. innocuum* from creeping fat and neighbouring mucosal tissue showed genetic strain variation with acquisition of genes for evading killing by innate immune cells in creeping fat. Core genomic features of *C. innocuum* included multiple genes for lipid catabolism and a preference for lipid-derived metabolic substrates suggesting a preference for lipid-rich environments. Using gnotobiotic mice gavaged with human-derived *C. innocuum*, the authors provide striking preliminary evidence for *C. innocuum* translocation to the mesenteries, driving noticeable expansion of mesenteric adipose tissue and increased expression of genes involved in adipogenesis. This phenomenon was enhanced in mice that received dextran sulfate sodium in drinking water to induce intestinal injury. Due to COVID-19-associated restrictions, the dataset is limited to two mice per group and thus needs to be expanded, but provides proof-of-concept data that human-derived *C. innocuum* drives creeping fat formation in CD.

To gain immunological and cellular insights into how bacterial translocation might promote creeping fat formation, the authors performed single-cell RNA sequencing (scRNA-seq) on paired samples of creeping fat and mesenteric adipose tissue associated with healthy mucosa. Creeping fat showed a signature of immune cell activation, fibrosis and adipogenesis as well as an expansion of the B and T cell compartment. Previous work by Gwen Randolph and colleagues elegantly documented the presence of multiple B and T cell immune structures forming in the creeping fat of CD patients (Randolph et al., 2016). The expansion in B and T cell compartments reported by Ha et al. may be linked to the formation of these lymphoid structures.

The macrophage compartment was the most expanded in creeping fat, with macrophages showing expression of genes involved in tissue repair and bacterial killing. Contrary to what is observed upon addition of lipopolycsaccharide (LPS), *C. innocuum* did not induce inflammatory macrophage differentiation when added to *in vitro* culture of bone marrow derived macrophages. Rather it favoured mannose receptor (CD206) expression and the release of factors inducing the expression of collagen I and VI by fibroblasts (Figure 1). As it stands, these observations do not allow for any conclusions to be drawn on the precise identity and functional phenotype of the macrophages activated by *C. innocuum* in creeping fat. These will have to be investigated in more detail to elucidate the mechanisms involved in the expansion and fibrosis of mesenteric adipose tissue in response to translocation by *C. innocuum*.

Increased gut permeability is a common trait of inflammatory bowel diseases. Ha et al. observed similar loss of expression of genes important in the maintenance of the gut barrier function in patients with CD or ulcerative colitis. However, in patients with CD, loss of gut barrier function was not associated with increased systemic translocation of bacteria, as assessed by levels of circulating LPS-binding protein and soluble CD14, which suggested that creeping fat protected against bacterial translocation. The creeping of mesenteric fat on the small intestine of CD patients is not unlike the faculty of the omental adipose tissue to wrap around diseased organ in the peritoneal cavity. The omentum is a large visceral adipose tissue, shaped like an apron covering most of the viscera. In the same way surgeons use the creeping fat to localise the inflamed intestine in CD, surgeons follow the omentum to find the inflamed appendix in patients with appendicitis. In a murine model of peritonitis, neutrophils form very large aggregates on omental FALCs, allowing the capture of peritoneal contaminants and limiting peritoneal contamination and systemic spread. In patients with appendicitis, bacterial contamination is found in the omentum, but not in the fluid of the peritoneal cavity, suggesting that the omentum successfully contained bacterial translocation (Jackson-Jones et al., 2020). Whether the formation of neutrophil aggregates on creeping fat also contributes to contain bacterial translocation in CD patients cannot be deducted from transcriptional analysis and will require further study. The mesenteries and omentum harbour small immune structures called fat-associated lymphoid clusters (FALCs) enriched in innate-B cells secreting natural antibodies (Bénézech et al., 2015). These natural antibodies are mostly germline encoded, polyreactive and play a key role in the early control of infection as well as the colonisation of the gut by the microbiota. Whether, the lymphoid structures observed in CD are FALCs or tertiary lymphoid structures is unknown. Both may support adaptive immune responses, and/or natural antibody secretion to impact the establishment of bacteria in creeping fat and thus may constitute therapeutic targets in the treatment of CD.

In summary, the study by Ha et al. provides evidence that creeping fat is a protective response of the mesenteries to limit systemic dissemination of gut bacteria. This response is induced by the specific translocation of a subset of intestinal bacteria including *C. innocuum*. However, there is a price to this protective response. As the mesenteric adipose tissue creeps on the intestine, both become severely fibrotic, facilitating the formation of gut strictures. Preventing *C. innocuum* translocation to the mesenteries may provide a way to treat adverse fibrosis in CD patients. Overall, visceral adipose tissue is emerging as a key target of gut bacteria translocation, which prompts further examination of the interplay between translocating bacteria and immune cells in adipose tissues as this may underly a number of disease process including inflammatory bowel disease, peritonitis and obesity.

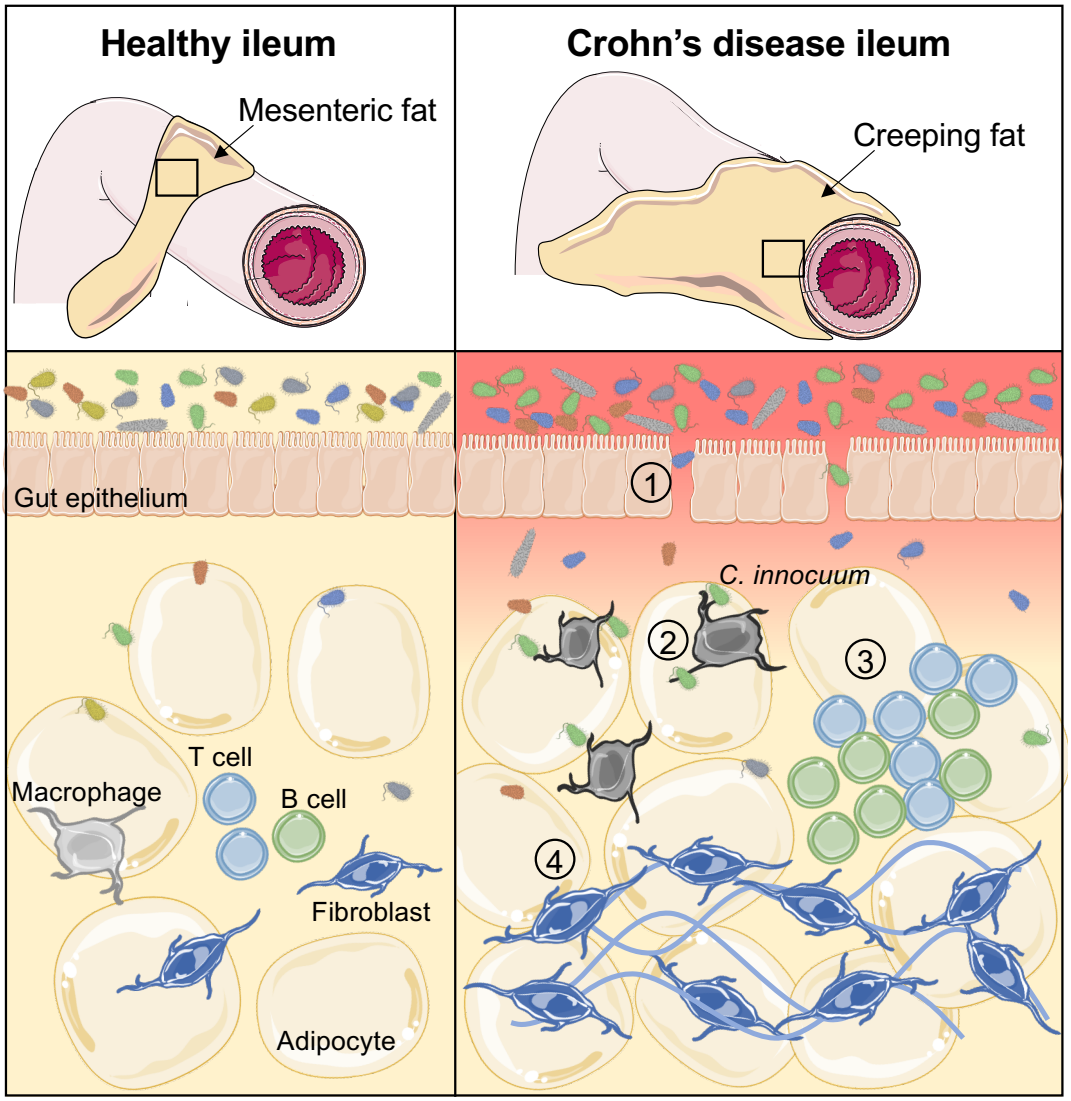

Figure 1: *C. innocuum* translocation to the mesenteric adipose tissue drives creeping fat formation

Translocation of gut bacteria naturally occurs in the mesenteries. In Crohn's disease, translocation of bacteria from inflamed ileum is increased with over-representation of the bacteria *C. innocuum* (1). This is associated with immune activation of the mesenteric adipose tissue and increased recruitment of macrophages (2) and B and T cells (3). *C. innocuum* favours the differentiation of macrophages driving fibrosis (4), adipogenesis and ultimately the formation of creeping fat.

Acknowledgements: PS is supported by a British Heart Foundation-University of Edinburgh PhD studentship (RE/18/5/34216).

The authors declare no competing interests.

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