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Review Article

Intestinal Immune System in the Regulation of Obesity and Metabolic Syndrome-Therapeutic Implications-A Systematic Review

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Abstract

Obesity has reached epidemic proportions. WHO cites that > 1.9 billion people are overweight of which 600 million are obese. Obesity is associated with numerous complications like insulin resistance (IR), Type 2 Diabetes Mellitus (T2DM), along with increased numbers of cancers, cardiovascular and autoimmune diseases among others. Chronic low grade inflammation accompanies obesity. More and more data points to the involvement of intestinal immune system with inflammation of other metabolic tissues like liver and visceral Adipose tissue (VAT) also getting involved. Obesity leads to Altered intestinal immunity, which correlates with gut microbiota changes, changes in intestinal barrier function, innate and adaptive immunity residing in the gut along with oral tolerance to luminal antigens. Thus we conducted a systemic review of literature using Pubmed search engine using key MeSH terms like obesity; intestinal immunity; innate immune system, both innate and cellular; intestinal adaptive immune response and oral tolerance We found a total of 398 articles out of which we selected 120 articles for this review. No meta-analysis was done. Based on this research novel avenues get opened up for attempting to treat obesity and systemic disorders metabolic diseases related to obesity.

Keywords: *Obesity; Chronic Low Grade Inflammation; Intestinal Cellular and Adaptive Immunity; Intestinal Barrier Function; Changes in Intestinal Permeability; Lipopolysaccharides; Metabolic Endotoxaemia*

Introduction

Obesity is correlated with low grade chronic inflammation, that might be the precipitating factor for many of the associated sequelae. Increased circulating levels of tumor necrosis factor-α (TNF-α), Interleukin-1 (IL-1), and IL-6 both in obese humans and in diet induced obese (DIO), that add to the development of insulin resistance (IR) and ultimately type 2 diabetes Mellitus (T2DM) [1]. Increased serine phosphorylation of insulin receptor substrate (IRS1) and IRS-2 along with activated suppressor of cytokine signaling (SOCS), is behind the causation of these by decreasing the ability of insulin receptor for transmitting signals downstream in the insulin responsive tissue like liver, muscle and adipose tissue [2].

The inflammation that occurs in visceral adipose tissue (VAT) is a major force behind IR development. Tissue collection of proinflammatory immune cells which include M1 macrophages [3], CD8+T cells7 [4], Th1 T cells [5], B cells [6], natural killer (NK) cells and neutrophils and a decrease in the population of anti-inflammatory immune cells like M2 macrophages [3], regulatory T cells (Tregs) [5], eosinophils and type 2 innate lymphoid cells [ILC2s] [7].

Besides VAT, low grade chronic inflammation in other organs might add to the IR. The organs involved being liver, muscle, pancreas, brain, small and large intestine. Since the gastrointestinal (GIT) is exposed to microbial antigens and ingested antigens from diet, it contains massive amount of immune system. Despite that only recently the inflammatory along with immune cell changes in the gut have been researched in depth as a link to both obesity and IR [8-10].

The aim of this review is to give points for and against immune cell mediated, low grade chronic intestinal inflammation as a feature that can possibly obesity related IR development. Also, the mechanisms, besides that of dysbiosis, alteration in intestinal permeability, changes in oral tolerance, by which intestinal immune system might affect systemic inflammation and IR is touched.

Changes in intestinal permeability in obesity along with dysbiosis

It is well known that both small and large intestine house a trillion microorganisms that belong to over 100 species. Changes in intestinal bacteria has a role in development of obesity and glucose tolerance has been proven [11,12]. This belief started from Buckhead's early findings regarding germ free mice had decreased body fat and did not develop both obesity and IR when put on an HFD [13]. But following reconstitution with the gut microbiota of conventionally raised mice these very germ free mice gained the adiposity and developed IR and glucose intolerance within 2 weeks [13]. This occurred despite decreasing diet that added more data on role of GIT bacteria as being regulators of energy metabolism.

There is change in gut microbiota which has been associated with both obesity and metabolic syndrome (MetS) that is referred to as dysbiosis [14]. Metagenomic analysis showed that most bacteria in the distal gut and faeces are from 2 main bacterial phyla both in mice as well as humans, namely *Bacteroides* and *Firmicutes* [12]. A balance is maintained between these 2 phyla in lean mice, while in models of obese mice, a greater ratio of *Firmicutes: Bacteroides* has been observed commonly [14]. Yet some studies have reported opposite results [15] which points that this issue is still controversial. Large scale metagenomics studies in humans correlated microbial gene signatures with MetS [16]. Low bacterial prevalence, pointed to low microbial gene count in faecal DNA, that correlated with dyslipidemia, IR and inflammation as shown by Le-Chatellier in 2013 [16]. Those who had a high gene count had prevalence of potentially anti-inflammatory species like *Faecalibacterium prausnitzii*, which are associated with increased production of short chain fatty acids (SCFAs), which included butyrate [16]. Once bacterial numbers were increased in subjects with a low gene count by diet induced weight loss an improved metabolic outcome was seen in a study by Coutilajo., *et al*. in 2013 [17], that pointed that changes in the microbiota could be brought via diet. In toto bacterial richness might be an indicator of inflammation and metabolic diseases. In accordance with this, another small study in humans revealed that intestinal transfer of faecal microbiota from lean donors could improve insulin sensitivity along with richness of butyrate producing bacteria in recipients having MetS [18]. Transferring gut flora from obese to germ free mice increased obesity, then use of GIT flora from lean mice, showing that obesity signals the collection of pathogenic bacteria which promote its occurrence [14]. Hence, antibiotic therapy of obese mice can decrease adiposity and adipose inflammation and improve glucose metabolism [19]. Following weight loss and decreased adiposity and betterment of metabolic parameters by gastric bypass surgery, changes in microbial composition is also observed [20], that further adds weight to the interlinking of obesity, dysbiosis and metabolic diseases.

How changes in bacteria cause obesity, various mechanisms have been given; namely i) gut bacteria suppress the lipoprotein lipase suppressor also referred to as fasting induced adipocyte factor (FIAF) or angiopoietin like protein-4 (ANGPTL4), in intestinal cells, an increased lipoprotein lipase activity along with increased triglyceride storage in adipocytes and the liver [13]. ii) Further gut microbiota, control metabolism and regulation of bile acid profiles in the bowel which bind to the farsenoid X receptor (FXR) and G protein coupled bile acid receptors TGR5 [20]. Its been seen that, a gut restricted FXR agonist decreases diet induced weight gain, systemic inflammation, along with hepatic glucose production [21]. iii) Obesity associated microbiota might be more efficient in harvesting energy from the diet by producing enzymes which break down nutrients in a more efficient manner [14].

Changed microbial composition in obesity also has other big consequences in the form of greater intestinal permeability that causes leakage of bacteria, bacterial products like lipopolysaccharide (LPS) across intestinal barriers [22]. These, bacterial products stimulate the innate immune system chronic inflammation. Giving continuous LPS infusion for 4 weeks recalls most metabolic changes that occur post HFD consumption, like increased fasting glucose and insulin, raised liver, adipose tissue (AT) and body weight and AT inflammation [22]. This bacteria-related leaking into blood and tissues, like AT, can be found as fast as 1week following HFD initiation that is dependent on the microbial pattern receptors NOD1 or CD14 [12,23]. These LPS might enter the systemic circulation and AT via uptake by chylomicrons [24]. Increased energy intake, especially saturated fat is associated with endotoxemia in humans [25], along with concentration of bacterial 16S rRNA in the blood and associated with abdominal obesity along with risk of DM [26].

How diet induced obesity (DIO) is associated with intestinal permeability can involve decreased expression of epithelial tight junction proteins like zonula occludens 1 (ZO1) and occludin]. In obesity prone Sprague dawley (SD) rats sequestration of cytoplasmic occludin has also been demonstrated [26], along with abnormal distribution of occludin and ZO1 in db/db mice [27]. Anyway gut bacteria are key players in the gut barrier dysfunction since antibiotic treatment prevented HFD induced intestinal permeability [24]. Further diet and microbiota interactions affect epithelial integrity and intestinal homeostasis. E.g. nondigestible carbohydrates get fermented in the bowel to produce SCFA's like acetate, propionate and butyrate, that bind to G protein coupled receptors (GPCR), which suppress inflammation and improve barrier function and DM [28]. Mucin produced in intestinal goblet cells also helps in maintaining gut barrier.

Also, barrier function is dependent on the intestinal immune system at the time of HFD feeding and inflammatory disease. Interferon γ (IFNγ) secreting immune cells are to some extent cause the HFD induced barrier permeability, in view that HFD fed IFNγ deficient mice demonstrated decreased barrier permeability, and IFNγ directly decreased ZO1 expression in immune epithelial cells [11]. Also, IL-1β increased intestinal epithelial tight junction permeability [29]. Protective effects on intestinal barrier function might be executed by other immune cells, like IL-22 producing innate lymphoid cells and Tregs, that dampen IFNγ-mediated immunity and promote mucin formation along with antimicrobial immunoglobulin A (Ig A) [30]. By an unknown mechanism, eosinophils can be protective for the barrier [31]. Immune system's part in degrading or helping intestinal barrier function has been examined deeply in multiple enteropathies which express dysbiosis and inflammation, such as inflammatory bowel disease (IBD), Common variable immune deficiency (CVID) or HIV [32]. Marked overlap exists in mechanisms underlying HFD-induced vis a vis IBD-namely induced barrier dysfunction, where proinflammatory cytokines like IFNγ and TNF-α have an important role (Figure 1).

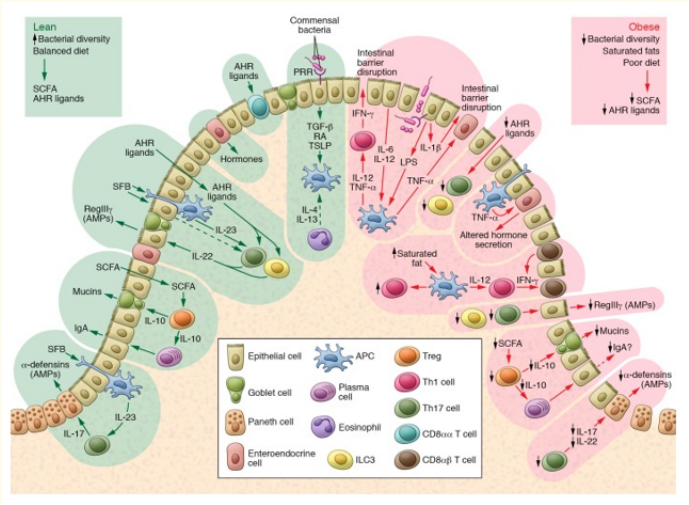

Figure 1

Obesity and general inflammatory changes observed in intestine

The innate immune system acts as the first line of defense against infection but simultaneously allows the maintenance of immune tolerance to normal bacterial flora. These bowel innate immune system components are luminal contents like mucus, intestinal epithelial cells (IECs), that include Paneth cells, that manufacture α-defensins, ILC's, along with other immune cells which respond rapidly like macrophages and neutrophils. Most of the innate immune system is regulated by pattern recognition receptors like transmembrane surface/endosome toll like receptors (TLR's) and cytosolic nucleotide oligomerization domain (NOD)-like receptors (NLR's) which bind to microbe associated molecular patterns (MAMP's) expressed by normal GIT flora or pathogens.

In normal homeostatic states, IEC's secrete mucin and antimicrobial peptides (AMP's) which control their actions with microbiota. The outer layer of mucus is colonized by the microbiota, while inner layer of mucus contains high levels of AMP's. Some of the microbiota are *Bifidobacteria* and *Bacteroides* spp. Which can metabolize glycans and produce SCFA's [33]. Normally during eubiotic state, MAMP's induce secretion of anti-inflammatory mediators like IL-25, IL-33. Transforming growth factor β (TGFβ), and thymal stromal lymphopoietin (TSLP), that promote tolerance and barrier function in the bowel. Since obesity has low levels of Intestinal barrier breach, changes in Intestinal cytokines associated with DIO and IR have been related to these Intestinal changes in research work done.

In the work done early to study low grade bowel inflammation at the time of HFD feeding and/or obesity measurement of total intestinal levels of proinflammatory cytokines like TNF-α, IL-1β and IL-12p40, which are manufacture in high amounts by innate immune cells. As per most publications HFD promotes inflammation in the distal small bowel. Ding., *et al*. demonstrated that ileal TNF-α, mRNA in conventional but not germ free mice increased at 6 and 16 weeks of HFD feeding. This rise was associated with gain in weight, adiposity and increased plasma insulin and glucose levels. No inflammatory changes observed in germ free mice demonstrates that it is the microbiota which induce intestinal inflammatory changes. Confirmation was done in nuclear factor kappa light enhancer of activated B cells (NF-kB-EGFP reporter mice since HFD induced NF-κB expression throughout the small intestine, as early as 2weeks of diet, and this effect remained maintained throughout HFD exposure [34]. This was seen in bowel immune cells, mainly in Peyers patches, lymphoid aggregates, and CD3+cells along with epithelial and endothelial cells and rare neuroendocrine cells, but were not observed in F4/80+intestinal macrophages.

Obese SD rats showed increased myeloperoxidase, in contrast to obesity resistant SD rats [26]. Further TLR4 activation was increased in intestinal epithelial cells in obesity prone rats. Correlation of the inflammatory changes was observed with dysbiosis, decreased intestinal alkaline phosphatase activity (that is a brush border enzyme which detoxifies LPS), abnormal tight junction expression along with increased intestinal permeability. Since all rats received an HFD, intestinal inflammation gets linked to obesity development instead of only HFD exposure as per this research work.

In proximal, mid and distal small intestine in HFD fed C57BL/6J mice, gene expression arrays were carried out at 2, 4 and 8 weeks demonstrated changed expression in some inflammatory genes, of which are time and location specific [35]. E.g. CCL-5, that is a chemokine which attracts proinflammatory immune cells, mainly Th1 cells, got upregulated only in distal small intestine at 4 and 8 weeks post HFD feeding [35]. Reversely macrophage migration inhibitory factor (MIF), that is a proinflammatory cytokine with chemokine like properties, got upregulated in the whole small intestine and at most time points during full HFD feeding [35]. MIF-deficient mice are protected from HFD induced IR [36]. Recent studies have demonstrated a decrease in ileal mRNA levels of IL-22, IL-17A, IL17F and IL-10, parallel to changes in immune make up of ileum during obesity, known to maintain epithelial barrier integrity, following 10 and 30 days of HFD feeding in mice and after 1 week in rat (IL-10) [10,37]. Why differential expression of inflammatory genes occur in relation to the gut at different times and location is not clear, but knowing that microbes demonstrate species specific changes along different areas of the intestine, it is feasible that these changes might impart selective pressures on immune cell function.

Analysis of human specimens from the jejunum of lean and obese subjects, RT-PCR demonstrated rise in pro inflammatory cytokines in the combined lamina propria (LP) and epithelial fraction, including IL-23, TNFα, TGF-β, CCL-5 and IFN-γ [12]. Other inflammatory mediators that include cyclo-oxygenase-2 (COX2) were also increased, mainly in the epithelial fraction. These results are in accord with another gene profile analysis study where IFNγ and IL-1β were increased in the duodeni of IR obese subjects [38].

Analysis of gene expression in total small bowels of mice after 7 days of HFD feeding did not observe increase in IL-1β, TNFα, or monocyte chemoattractant protein (MCP1) expression [31]. In previous studies most significant inflammatory changes were visualized after 2 - 4 weeks, it might be that 1 week is very early for getting significant inflammatory changes in the intestine, in view of gut microbial factors. After exposure to an HFD, microbial changes can be seen within 24h, both in mice as well as humans, but even 10 days of HFD feeding couldn't change the microbial enterotype [12]. Further since this study used whole bowel analysis-early gene expression changes in different parts of small intestine, e.g ileum might not have been found [31].

From colon, less consistent data was seen. TNFα levels did not vary following 1 week of HFD feeding [31]. In NF-kB-EGFP reporter mice, a HFD induced NF-kB expression throughout colon by 2 weeks of HFD feeding that was maintained throughout the entire exposure of HFD exposure (> 16 weeks) despite TNF-α mRNA levels were not noticeably increased at any time point [34]. 14 weeks following HFD feeding, the proximal colon showed increases in some inflammatory cytokines, like IL-1β, and IL-12 p40, but not IL-6 or TNFα [12]. Conversely another study demonstrated 6.6 fold increase in TNFα but not IL-6, in the proximal colon between 8 - 12 weeks of HFD feeding. Gene arrays and RTPCR following 17wks of HFD feeding showed a 72% increase in TNFα [12]. Same study also demonstrated a 41% increase in IL-18 in the colon following long term HFD feeding.

In toto these data show that DIO changes general cytokine expression throughout the small intestine and possibly in the colon starting around 2 weeks post-HFD consumption and becomes of significance with prolonged HFD exposure or obesity of both mice and humans. Thus, low grade bowel inflammatory changes are an early manifestation of early HFD feeding which precedes detectable systemic metabolic disease [34].

Right now the strongest inflammatory link appears to be in the jejunum and ileum of the small intestine and proximal colon, all of which have high levels of commensals. Since different bacterial species are rich in different parts of bowel, it stands to be seen which species are drivers of inflammation that is associated with HFD and obesity. Besides those done by Monteiro-Sepulveda in 2015 in jejunum, more studies are needed for validating low grade bowel inflammatory changes in obese humans especially in the distal intestine [10]. In a study by Pendyala 2011, diet induced weight loss in obese adults decreased colon mucosal cytokines, immune cells and proinflammatory networks while improving plasma glucose and lipid levels [39]. Conversely 2 human studies that utilized fecal calprotectin as a general marker of intestinal inflammation found no differences between lean and obese adults [12]. But it is important to understand that calprotectin is mainly released by neutrophils and is increased in diseases of a more florid type of active colitis, like IBD and infectious colitis [40].

Intestinal innate immune system in obesity

Role of intestinal TLR s and NLRs in diet induced metabolic disease

In view of HFD induced inflammatory changes in the bowel being dependent on the microbiota, upstream interactions between MAMP and damage-associated molecular pattern (DAMP) sensors, namely TLRs and NLRS, on intestinal epithelial cells and resident immune cells start this process. E.g. TLR 4 is expressed by intestinal epithelial cells, that binds LPS to induce NF-kB-expression in a time and dose dependent manner [12]. NOD2 and NLRP2 are pattern recognition receptors (PRR) of the NLR family which gets expressed by intestinal epithelial cells [41]. Ceramide or saturated fatty acids, but not unsaturated fatty acids, activate the NLRP3 or its inhibition by omega 3 fatty acids protect against HFD-induced metabolic changes [44]. Further composition of gut microbiota also gets influenced by dietary lipids

which then modulate adipose inflammation via TLR interactions. Mice fed a lard based diet presented a heightened inflammatory phenotype, while mice fed fish oil were protected from AT inflammation [45]. Here the action was partly dependent on GIT microbial products production of CCL2 by adipocytes through TLR4, MyD88, and TIR domain containing adapter inducing interferonβ (TRIF signaling) [45].

Dysbiosis results as a result of any abnormalities in TLR s and NLRs, that predisposes to colitis, nonalcoholic steatohepatitis (NASH), and metabolic disease. On feeding a methionine-choline deficient, mice deficient in NLRP3, or the inflammasome adaptor protein ASC demonstrate hepatic steatohepatitis and NASH, that are dependent on dysbiosis, TLR4 and TLR9. Mice deficient in TLR5, that recognizes bacterial flagellin, develop obesity along with features of MetS [12]. Mechanically this phenotype was related to the changes in gut flora since disease could be transferred to wild type, germ free mice. Moreover, mice deficient in NOD2, on feeding HFD diet, recognizes bacterial cell wall peptide and recognizes bacterial cell wall peptidoglycan and regulates microbial homeostasis, display Dysbiosis, increased bacterial translocation, and IR. Conversely HFD fed, NOD1- deficient get protected from IR [12].

Everard., *et al*. demonstrated a specific pathological role for intestinal epithelial TLR signaling [11]. Intestinal epithelial specific deletion of the TLR adaptor MyD88 protected partially against DIO, metabolic dysfunction and inflammation. The protection here is reliant on the gut microbiota, that could transfer protection to germ free recipients and is linked mechanistically to increases in anti-inflammatory endocannabinoids, microbial peptides and intestinal Tregs. Hence TLR s and NLRs play a key role in maintaining intestinal and microbial homeostasis and contribute to HFD-induced bowel inflammation and ultimate metabolic abnormalities [11].

In obesity cellular changes in innate immune system

As Innate Immune cells express molecular PR, it won't be surprising to observe changes in some of these cells/their cytokines and molecular patterns and don't express variable antigen specific receptors. They are classified into 3 broad groups, ILC1, ILC2 and ILC3, on the basis of transcription factors along with cytokines they express. Abundant ILC3s are present in bowel, which produce IL-22 [7].

IL-22 belongs to the IL-10 family expressed by ILC'Ss (mainly group 3) and Th17 and Th22 cells and is crucially involved in host defense, tissue regeneration/repair, maintenance of intestinal epithelial integrity, and homeostasis or commensal organisms [6]. Mucosal immunity during obesity might be maintained by IL-22 and in regulating weight gain along with glucose homeostasis [6]. Both obesity and HFD decrease ILC production of IL-22 specifically, following antigen challenge or infection. Though no difference in metabolic parameters was observed in IL-22 knockout (KO) mice 1 month following HFD feeding. IL-22 Receptor (IL-22R1) KO mice displayed greater weight gain and IR. Moreover, injecting recombinant IL-22 (fused to the Fc portion of mouse IgG2a, IL-22-Fc) caused improvement in both body weight along with metabolic parameters. IL-22 effected changes in intestinal permeability, decreased serum LPS along with improving metabolism in liver and AT, that pointed that IL-22 might be an important cytokine in controlling systemic metabolic diseases.

Percentages of IL22 producing NCR+CD4- (also called NKp46+CD4-) ILC3s are decreased in the LP of HFD -fed mice compared with lean mice, that is correlated with decreased epithelial barrier integrity, increased serum LPS and anti-LPS-IgG in HFD fed mice [9]. Cause of this decrease in IL22 producing NCR+CD4-ILC3 cells is unknown. But IL-23 can activate ILC3's for producing IL22 and decreased expression of IL23 in obese mice following *Citrobacter rodentium* infection IL22 suggests that there might be a defective IL23 to IL22 axis in obesity [30]. Still the decrease in NCR+CD4- ILC3s might at least partly increased intestinal permeability that is associated with HFD.

Besides ILC's the proportion of γδ T-cells in the intestine change along with HFD feeding [18]. Increased γδ T-cells are present in the intraepithelial lymphocyte fraction and respond mostly in a major histocompatibility complex (MHC) independent manner. 3 weeks following HFD feeding, IL-17 producing γδ T-cells increased in the colon but not small intestine. But by 12 weeks of HFD feeding, both colon and small intestine displayed increases in IL-17 producing γδ T-cells. However, Monteiro-Sepulveda 2015 did not find any changes in the total amount in obese human subjects [10].

In the mucosal surfaces mucosa associated invariant T (MAIT) cells ate innate like T-cells which are abundant, that includes GIT and regulate inflammatory responses by rapid production of cytokines. These cells are restricted by the nonpolymorphic, MHC class 1 related protein MR1, and are activated by bacteria via the detection of riboflavin metabolites bound to MR1. Obese and/r T2DM patients have reduced circulating MAIT cells, with an associated rise in Th1 and Th17cytokine-producing profiles in inflamed tissue like VAT. Though changes in such cells have not been studied in detail in the GIT it can be proposed that these cells could also be responsible for intestinal inflammatory changes during obesity.

Following 1 week of HFD feeding a decrease in number along with proportion of eosinophils was seen [31]. The decrease was attributed to the fat content in the HFD since ob/ob mice that were given a normal control diet didn't demonstrate a reduction in eosinophils. Anyhow decreased eosinophils correlated with increased paracellular permeability across the intestinal epithelium mainly in the ileum of HFD fed mice. Importance of HFD feeding-associated decrease of eosinophils along with if they are maintained for 1 week of HFD needs more study.

Regarding effect of HFD feeding on intestinal macrophages and dendritic cells (DC) subsets not much is known. Besides the classical CD11c- macrophages, there are a number of CD11c+ macrophages or DC cell subsets that exist in the gut. CD11c+CX3CR1hiF 480+ monocyte derived macrophages are generally sessile cells and anti-inflammatory in nature [7]. CD11c+CX3CR1intDCs are more inflammatory and can produce IL-12, inducible nitric oxide synthase (iNOS), and TNF-α, which have the capacity to migrate to local lymph nodes and activate Th1 responses [7]. CD11c+CD11b+CD103+DCs are present in the LP and are important in oral tolerance and Treg responses [7]. No changes were found in the total grouped small intestine macrophages and DC's by 1 week of HFD feeding in Johnsons study. But the relative proportion of these cells is partially increased in LP in view of decrease in eosinophils [31]. In HFD -fed, NF-kB-EGFP reporter mice, co-immunofluorescence with F4/80 did not co-localize HFD - induced EGFP expression to macrophages, that pointed that intestinal macrophages might not get activated [34]. Another study that utilized broad MHCII+CD19-gating antigen presenting cells (APC's), APC's from 10 - 30 day HFD-fed mice displayed decreased levels of activation markers like CD86 and a decreased action of inducing Th 17 T-cell differentiation *in vitro* but also demonstrated upregulation of some genes that were supposed to be involved in pathways of inflammation induction like Narp3 [8]. But what are the changes in the proportion and functions of distinct subsets of macrophages and DC's present in GIT have not been checked. It might be early to say these cells have a role in promoting or inhibiting low grade inflammation of the intestine following HFD.

A study by Monteiro-Sepulveda., *et al*. in 2015 on lean and obese humans which divided obese patients into 3 groups (obese diabetics [ObD], Obese nondiabetics having a co-morbidity [Ob] and Metabolically healthy obese [MHO] studied the changes of innate immune cells in jejunum [9]. Unlike animal studies, all obese patients demonstrated an increase in total macrophage density [as checked by CD68 staining] as well as increased mature DC's and NK cell numbers in the Ob and ObD group but not in MHO and lean groups.

Desai., *et al*. recently tried to examine the metabolic role of JAK2, that is a crucial downstream mediator of different cytokines and growth factors in the pathogenesis of obesity-associated inflammation and IR. During HFD-feeding macrophages specific JAK2knockout (M JAK2-/-) mice gained less body weight compared to wild type littermates control (M JAK2+/+) mice and were protected from HFD induced systemic IR. Histologic analysis showed smaller adipocytes and qPCR analysis revealed upregulated expression of some adipogenic markers in VAT of HFD-fed M JAK2-/-) mice. Decreased crown like structures in VAT along with decreased mRNA expression of some macrophage markers and chemokines in liver and VAT - of HFD - fed M JAK2-/-) mice. Peritoneal macrophages from M JAK2-/-) mice and Jak2 knockdown in macrophage cell line RAW 264.7 cells expressed lower levels of chemokines expression and decreased phosphorylated STAT3. But leptin -dependent effects on augmenting chemokines expression in RAW 264.7 cells did not need JAK2. Concluding that their findings demonstrated that macrophages JAK deficiency improved systemic insulin sensitivity and decreased inflammation in VAT and liver in response to metabolic stress [7].

Role of neutrophils is also not clear. Examining HFD fed mouse or corresponding human specimens did not find any basis for active ileitis or colitis (histological activity defined by number of infiltrating neutrophils [9]. Apparently, neutrophils have limited role in HFD induced inflammation of the intestine although more data is required for meaningful conclusions.

Intestinal adaptive immune response in obese

Besides the innate immune system, HFD changes the composition of adaptative immune cells in the LP of the (small ileum and distal jejunum) and large bowel (colon). By 3 weeks of the HFD feeding in mice the % age of Tregs in the colon, but not in the small intestine was decreased [9]. Though changes in proinflammatory CD4+or CD8+Tcells were not significant after 3 weeks by 12 weeks post HFD feeding. Not like IL-17 producing γδ T-cells, the portion of IL-17 producing γδ T-cells was not affected by HFD -feeding in this study [9].

The effect of shorter duration of HFD feeding in mice for 30 days and found decreased frequency and numbers of Th 17 producing cells in ileum [17]. This decrease was associated with HFD induced reductions in specific commensals bacteria like segmented filamentous bacteria (SFB) and *Porphyromonas gingivalis*, that is known to stimulate IL-17 production. Increased percentages of Th1 cells while HFD feeding course might also add to increased permeability by changing the production of AMP's, mainly Reg3β and Reg3γ that are critical for mucosal barrier defense [8]. This effect possibly acts along with raising IFNγ responses to worsen barrier function and initiate metabolic endotoxaemia and systemic low grade inflammation. Differences in the Th 17 cell numbers between different studies are possibly because of differences in the length of HFD exposure or how abundant certain intestinal microbiota, like Th 17 producing SFB, that vary in amount in different facilities and vendors of mice [7]. Analysis of Th 17 cells is further affected by the lineage plasticity of Treg cells [7].

Resection specimens from a small number of human specimens of obese subjects raised T-bet (Th1, ILC1) and CD8+Tcells and deceased Foxp3 (Treg) cells in both small intestine and colon in contrast to lean humans [9]. In a larger cohort of jejunum specimens, total mucosal CD3+Tcells were raised in the intraepithelial fraction as shown in increased LP and intraepithelial CD8+Tcells mainly, CD8αβTcells, in obesity [10]. Increase in intraepithelial CD8+Tcells is of interest as it places cytokines from these cells in direct proximity to change the barrier function of epithelia cells. Also, in this study an increase inTh17/Th22 and IFNγ producing CD8+Tcells in the jejunum LP with obesity. The increase in Th17/Th22 cells in obesity might be secondary to a presence of compensatory response to barrier breach for maintaining integrity. Many cytokines that are derived from activated intestinal T cells, that include IL17A, IL-22, IFN γ and TNF-α, have the potential to modulate enterocyte insulin sensitivity *ex vivo*, and it has to be observed if such changes do affect enterocyte function [10]. Repeating these analysis in larger cohorts in humans from other segments of intestine is important. Further changes in LP B cell population following HFD are also to be done in future tests, but in the present jejunum study LPCD20+cells did not vary between lean and obese patients [10].

Also, in humans and mice the histological low grade inflammation in the intestine appears to be more subtle as compared with inflammation in metabolic tissues like VAT and liver. E.g. macrophage infiltrates in VAT during HFD feeding are dramatic and easily seen in the form of crown like structures, while no changes are seen in the bowel on histology [10]. Hence inflammation in the gut manifests possibly by many ways, that include changes in immune cell numbers and their localization along with changes in functional states of immunity. There is a homeostatic shift towards inflammatory cell polarity in the GIT during obesity without the large acute and chronic inflammatory infiltrates typically visualized in active infection or IBD.

Thus, studies which examine both adaptive and innate immune responses in the gut on HFD -feeding consistently report changes in inflammatory parameters (Figure 2). Not all studies agree on the exact timing and sequence of inflammatory events that occur. Reason being variations in environmental factors (like diet, animal housing conditions, animal vendor, microbiota) and gets further complicated by changes in the readout of inflammation used (like changes at genetic or molecular visa vis cellular level). A study by Ussar., *et al*. in 2015 showed that metabolic phenotypes might be strongly affected by the host genetic background and environment when comparing results and discrepancies between studies [44].

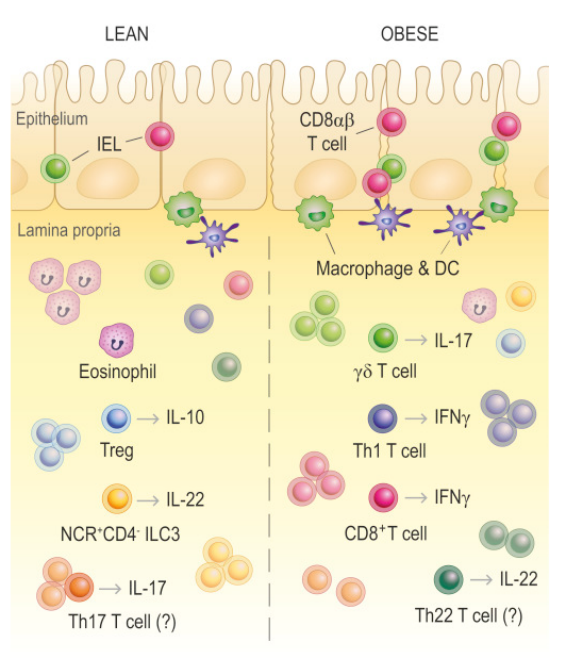

Figure 2

Obesity related IR-role of oral tolerance

Oral fed antigens can inhibit immune responses both in gut and systemically which is called ''oral tolerance''. Oral Tolerance to food antigens probably occurs by loading of antigen on CD 103+DC s in the intestinal LP, while tolerant responses to commensal bacteria occur mainly in the gut associated lymphoid tissue (GALT) [45]. Oral antigen loading onto CD103+DCs might involve transfer of the sampled luminal antigen by CX3CR1+macrophages. Antigen loaded CD103+DCs migrate to mesenteric lymph nodes where they include Foxp3+Tregin a retinoic acid and TGFβ dependent manner. The committed Tregs then home back to the bowel LP and promote local tolerance and reduction of inflammation [7]. How these local responses decrease systemic immune suppression is not clear, although some Tregs might leave the intestine via lymphatics and get seeded into distant nodes [45]. Further abundant tolerance inducing APC's in the liver might also add to the systemic oral tolerance [7].

Defective oral tolerance may be linked to DIO. Feeding ovalbumin (OVA) antigen in obese mice induces preferentially proinflammatory Th1 -skewed anti-OVA IgG2a/c antibodies upon systemic challenge, while feeding of same antigen in lean mice induces a tolerogenic response with preferential production of IgG1 [7]. Thus the belief that a HFD and obesity promote inflammation, that can potentially affect oral tolerance is corroborated by above. This inflammatory environment possibly occurs in major tissues that are related to oral tolerance, which include bowel, mesenteric lymph nodes, and liver.

The immune response to soluble oral antigen might be important in controlling inflammation in obesity in both systemic and local metabolic tissues which directly affect systemic IR. These oral antigens that include, OVA, can be found in the blood of humans and mice following eating and can induce the expression of T-cell activation markers in mesenteric and peripheral lymph nodes [7]. Under poor immunological tolerance conditions if mice are fed dietary antigens it causes local alterations in CD4+T cell numbers in VAT along with

glucose intolerance [7]. Thus, it is feasible that the breakdown of oral tolerance response observed during obesity, in addition to abnormal gut immunity might low grade systemic and metabolic tissue inflammation and metabolic dysfunction.

Targeting intestinal inflammation for metabolic disease management

On exposure to a chronic HFD, both innate and adaptive intestinal immune systems get changed that is accompanied by changes in inflammatory gene and cytokine changes. But total contribution of these changes to metabolic diseases had been only recently investigated. Overall role of the gut immune system was checked in mice having β-7 integrin deficiency [18]. β-7 integrin associated with α-4 integrin to form LPAM-1 that is essential in tracking immune cells into the LP of the small bowel and colon. Hypoplasia of GIT lymphoid tissue associated with considerable decreased number of leucocytes is observed in β-7 integrin-deficient mice [7]. These β-7 integrin deficient mice on HFD feeding display normal HFD weight gain but get protected from metabolically diseases associated with simultaneous decrease in VAT inflammation along with hepatic steatosis. This protective effect of β-7 integrin deficiency in obesity might be secondary to decreased intestinal permeability, probably related to a decrease in IFNγ producing immune cells although not totally clear [9]. Further mice deficient in intestinal epithelial TLR signaling displayed decreased intestinal inflammation along with raised Tregs and get protected from metabolic diseases [12]. Low amounts of 2 commonly used emulsifiers, carboxy methylcellulose and possibly polysorbate-80 changed the gut microbiota composition along with its encroachment to bowel epithelium [7]. Further low grade inflammation that is associated with obesity got decreased with these food additives. Gut microbiota composition changes were crucial since metabolically disease phenotype could get shifted to germ free mice with use of fecal transplantation. In this human jejunum study, accumulation of T-cells correlated with systemic inflammation, obesity, dyslipidemia and a number of liver parameters [10].

With this finding that low grade intestinal inflammation accounts for metabolic abnormality development, gives the chance of using gut targeted anti-inflammatory therapies that have minimal side effects for IR. Mesalamine is one therapy, that has been utilized to treat IBD as the first line treatment of IBD for > 30 yrs. Mesalamine is a salicylic acid derivative having PPARγ agonist along with anti-inflammatory properties [46]. Mesalamine therapy in C57BL/6 HFD mouse model, abrogated low grade intestinal inflammation by decreasing the numbers of IFN γ secreting Th1 and CD8+Tcells and IL-17 producing γδ T cells in the small intestine and colon, while raising Treg cells. Further Mesalamine abrogated HFD -induced metabolic disease and increased insulin sensitivity in VAT, liver, and muscle without having any effect on weight gain [9]. Further Mesalamine decreased VAT inflammation and increased VAT Tregs almost 3 times. Moreover, oral Mesalamine also affected oral tolerance, decreased systemic endotoxaemia, along with promoting microbial diversity [9]. Mesalamine might further prevent TNFα induced decrease in GLP-1 secretion by human intestinal cells.

Oral anti-CD3 plus gluco-sylceramide (an NKT cell target antigen) induced T cell production of IL-10 and TGFβ in the mesenteric lymph nodes and bowel was related to decreased fasting glucose, VAT inflammation, liver enzymes along with improved hepatic steato hepatitis in ob/ob mice. An oral anti-CD3 monoclonal antibody has shown good results in early clinical studies in patients with NASH along with impaired fasting glucose.

One can also target intestinal immunity by manipulating the intestinal microbiota. Different commensal stains have beneficial anti-inflammatory effects within the gut during metabolic disease, many of them increase intestinal permeability. E.g. *Bifidobacterium pseudocatenulatum* CECT 7765 administration in HFD -fed mice for 14 weeks reduced the expression of TLR4 and the intestinal inflammatory cytokines TNFα, MCP-1, IL-6, and IL-8 along with improving gut-barrier function [7]. A mucin utilizing bacterium, *Akkermansia muciniphila*, decreases blood glucose by changing the level of endocannabinoids, that in turn directly regulates intestinal barrier function, inflammation and the release of gut hormones [11,12]. Besides *A. muciniphila, F. prausnitzii*, that is another commensal linked to improving glucose regulation, produces SCFA, that includes butyrate, and can directly increase the differentiation of Tregs in the colon by transcriptional regulation. A microbial anti-inflammatory molecule (MAM) which suppresses intestinal inflammation, is also produced by. *F. prausnitzii*, giving alternate ways by which GIT microbiota manipulate intestinal immune system. Different other ways by which local effects on intes-

tinal bacteria with overall anti-inflammatory effects on intestinal immunity is by using polyphenols, omega-3 fatty acids and probiotics, which include oligofructose and inulin. Together these findings suggest HFD-bowel inflammation, changes in gut immune system and microbe-immune interactions as promoters of metabolic diseases which can get potentially targeted by therapy.

Further Revilo., *et al*. [47] emphasized on how starving intestinal inflammation using the amino acid sensor general controlled non repressed kinase (GCN2) on the basis of work done by Ravindran., *et al*. [48] controlled gut inflammation had opened new doors on obesity research (Figure 3). During low amino acid levels the integrated stress response (ISR) gets initiated by GCN2. GCN2 phosphorylates the translational inhibitor eukaryotic initiation factor 2 α (eIF-2α), that translational arrest along with restoration of amino acid homeostasis. Ravindran., *et al*. showed that in mice GCN2 controls intestinal inflammation by suppressing inflassome activation. Increased activation of ISR was seen in intestinal APC's and epithelial cells during amino acid starvation or intestinal inflammation. Genetic deletion of Gcn2 (also called eIF-2α4) in CD11c+APC'S or IEC's caused an increased intestinal inflammation and Th17 responses, because of increased inflammasome activation and IL-1β production. This was caused by reduced autophagy in Gcn2 (-/-) intestinal APC's and IEC's increased Reactive oxygen species (ROS), a potent inhibitor of inflammasomes. Hence conditional ablation of Atg5 or Atg7 in intestinal APC's caused enhanced ROS and Th 17 responses. Moreover, *in vivo* blockade of ROS and IL-1β caused inhibition of Th 17 responses and decreased inflammation in Gcn2 (-/-) mice. Acute amino acid starvation suppressed intestinal inflammation via a mechanism which couples amino acid sensing with control of intestinal inflammation via GCN2 [48].

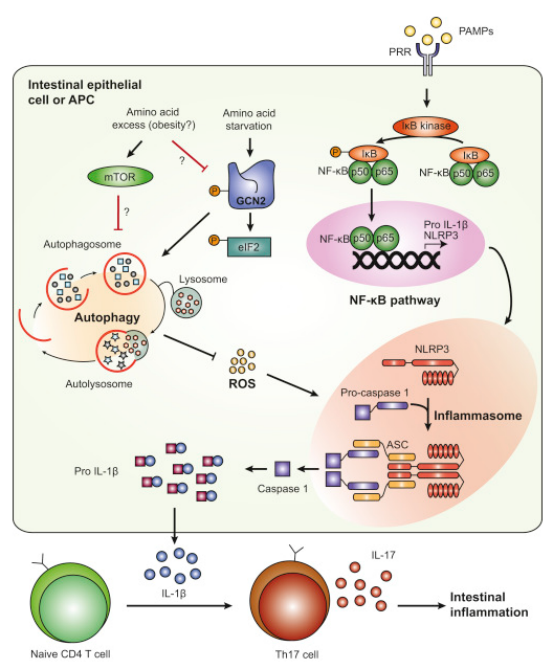

Figure 3

Further Lin., *et al*. screened 20 plant based extracts to assess for preferential production of IL-10 compared to TNF-α, specifically targeting metabolic tissues, including the VAT. They assessed the therapeutic potential of the strongest anti-inflammatory compound, indigo, in the C57BL/6J DIO mouse model with supplementation for upto 16 weeks by measuring changes in body weight, glucose and insulin tolerance and gut barrier function. They also utilized flow cytometry, quantitative PCR, ELISA, histology to measure changes to immune

cell populations and cytokine profiles in the VAT and liver 16Sr RNA sequencing to examine gut microbial differences induced by indigo supplementation. They found, an aryl hydrocarbon receptor (AHR) ligand agonist, as a potent reducing agent of IL-10 and 1L-22, that protects against HFD-induced IR and fatty liver disease in the DIO. Therapeutic actions were mechanistically linked to reduced inflammatory immune cell tone in the intestine, VAT and liver. Specifically, indigo increased lactobacillus and elicited IL-22 production in the gut, that improved intestinal barrier permeability and decreased endotoxaemia. These changes were associated with increased IL-10 production by immune cells which are present in VAT and liver. Thus indigo is a naturally occurring AHR ligand having anti-inflammatory properties which effectively protect against HFD-induced glucose dysregulation. Compounds derived from indigo or those with similar properties could represent novel therapies for diseases associated with obesity related metabolic tissue inflammation.

Conclusion

Multiple organ interconnected network influences the development of obesity and related metabolic disorders. HFD-induced changes in the intestine have emerged as critical factors in the development of obesity and IR, that appears logical since intestines are the 1st organ that comes in contact with dietary compounds. A model which integrates the bowel and metabolic diseases, including VAT, as drivers of metabolic disease (Figure 4).

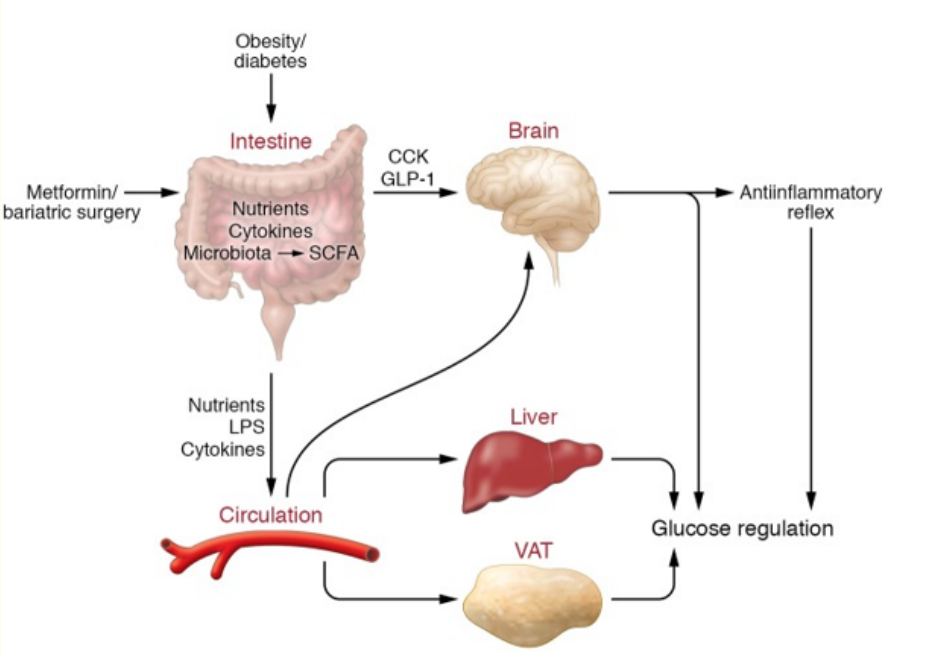

Figure 4

Saturated fatty acids and cholesterol from HFD or western diets pass through the GIT, these components affect the gut microbiota, thus directly stimulate intestinal TLR's and NLR's. Chemokines, cytokines like IL-1β get produced by activated IEC'S that can increase intestinal epithelial permeability. Hence bacteria and bacterial products, that include LPS, leaks across the barrier into the bowel LP, and into the systemic circulation and VAT. Genetic and/or environmental factors like decreased NLRP6 and NLRP3 function or compounds like dietary emulsifiers further promote bacterial and bacterial product leakage [46]. Further dietary emulsifiers decreased intestinal mucus produc-

tion, increase bacterial adherence, and a more pro-inflammatory microbiota. Genetic factors include polymorphisms in immunologic loci, like TLR4 or IL-10, which may impinge on mucosal barrier function. Endotoxins, the leaked components along with alterations in bacterial metabolites, like SCFA in the bowel induce low grade chronic inflammatory changes characterized by increased numbers of IFNγ secreting Th1 and CD8+T cells and decreased proportions of Tregs, -IL-22 producing NCR+D4ILC3 cells and eosinophils. The proinflammatory environment created by these cells changes further perturbs intestinal permeability like IFNγ and ultimately create a positive feedback loop that finally worsens metabolic endotoxaemia, dysbiosis, and IR. Though many studies support the concept of an inflammatory gut during DIO, lack of important inflammatory cytokines that are known to maintain gut barrier integrity that include IL-17, might also manifest with systemic endotoxaemia and IR [17]. Thus, this concept of cytokine presence in the gut and how that impinges on gut barrier function and endotoxaemia might be the ultimate factors that dictate the role of intestinal immunity in IR.

On obesity development, the hypoxic and stressed VAT adipocyte death along with shedding of cellular debris that promotes a VAT immune response. Additionally, leaked bacterial proinflammatory components and soluble antigens migrate from gut into AT to further to further promote proinflammatory changes in VAT, some in a TLR2 and CCL2 dependent manner [45]. Though the antigenic targets in AT which drive this inflammation is yet to be confirmed [8]. Possibility exists that links gut immune system to distal tissues and lymphoid organs like VAT. Bowel immune antigens participate in this process are possible because T cell responses to intestinal OVA may be seen in AT following oral ingestion, and HFD fed mice exhibit impaired oral tolerance to luminal antigens [9]. Conversely immune trafficking network might exist which links immune system to distal tissues and lymphoid organs like VAT. Bowel immune cell, that include CD4+T cells and Tregs, have been demonstrated to possess broad trafficking abilities to lymphoid organs at distant sites, like inguinal lymph nodes and spleen [49]. Further Morton., *et al*. showed that bowel derived Th-17 cells can emigrate to spleen and autoinflammation arthritis [49]. Hence it is feasible that gut immune cells could potentially migrate to other tissues which include VAT.

Also one needs to study how HFD-induced gut inflammatory changes influence the neuroendocrine systemin the bowel. E.g. chronic exposure to TNF-α can decrease the secretion of the intestinal incretin hormone GLP-1 by both murine and human ileal L cells. Hyperglycemia is decreased by anti-TNFα therapy in HFD-fed mice and prevents the decrease of GLPI secretion in primary intestinal cultures. Hence alterations in incretins might be another mechanism by which intestinal inflammation manipulates systemic glucose intolerance. Further investigating how intestinal immunity impinges on non-incretin hormones, including secretin. E.g. obesity is associated with increased peripheral hormone serotonin, of which neuroendocrine cells in the ileum are a large source. Mice having tryptophan hydroxylase deficiency 1, that produces serotonin, are protected from obesity and metabolic abnormality.

Hence growing literature suggests an increasing findings of HFD to low grade intestinal inflammation, inflammatory changes involve changes in intestinal epithelial barrier, the microbiota, and population of gut residing innate and adaptive innate immune cells. These inflammatory changes in the gut are of importance since they represent potential therapeutic targets for metabolic diseases. Therapies which are locally active in the gut, having limited side effects need to be designed and hence give a novel and safe means to treat metabolic disease [50].

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