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Review

The Intestinal Immune System in Obesity and Insulin Resistance

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Obesity and insulin resistance are associated with chronic inflammation in metabolic tissues such as adipose tissue and the liver. Recently, growing evidence has implicated the intestinal immune system as an important contributor to metabolic disease. Obesity predisposes to altered intestinal immunity and is associated with changes to the gut microbiota, intestinal barrier function, gut-residing innate and adaptive immune cells, and oral tolerance to luminal antigens. Accordingly, the gut immune system may represent a novel therapeutic target for systemic inflammation in insulin resistance. This review discusses the emerging field of intestinal immunity in obesity-related insulin resistance and how it affects metabolic disease.

Inflammation as an Underlying Cause of Obesity-Related Insulin Resistance

Obesity has reached epidemic proportions. The World Health Organization (WHO) estimates that over 1.9 billion people are overweight, of which 600 million are obese. Obesity is associated with numerous complications, including insulin resistance and type 2 diabetes, but also increased risks of cancer and cardiovascular and autoimmune diseases, among others.

Obesity is associated with low-grade chronic inflammation, which may be the precipitating factor for many of its associated complications. Increased circulating levels of tumor necrosis factor α (TNF-α), interleukin-1 (IL-1), and IL-6 in obese humans and in diet-induced obese (DIO) mice contribute to the development of insulin resistance and subsequent type 2 diabetes (Hotamisligil et al., 1993; Olefsky and Glass, 2010). This effect occurs as a result of increased serine phosphorylation of insulin receptor substrate 1 (IRS-1) and IRS-2 and activation of suppressor of cytokine signaling (SOCS), which reduces the insulin receptor's ability to transmit downstream signals in insulin-responsive tissues such as the liver, muscle, and adipose tissue (Biddinger and Kahn, 2006).

Inflammation in visceral adipose tissue (VAT) is a major driver of insulin resistance. VAT inflammation in obesity is a result of tissue accumulation of pro-inflammatory immune cells that include M1 macrophages (Lumeng et al., 2007; Weisberg et al., 2003; Xu et al., 2003), $CD8^+$ T cells (Nishimura et al., 2009), Th1 T cells (Winer et al., 2009), B cells (Winer et al., 2011), natural killer (NK) cells (Revelo et al., 2014; Wensveen et al., 2015), and neutrophils (Talukdar et al., 2012) and a reduction in the proportion of anti-inflammatory immune cells such as M2 macrophages (Lumeng et al., 2007), regulatory T cells (Tregs) (Feuerer et al., 2009; Winer et al., 2009), eosinophils (Wu et al., 2011a), and type 2 innate lymphoid cells (ILC2s) (Brestoff et al., 2015; Molofsky et al., 2013).

In addition to VAT, other organs display low-grade chronic inflammatory changes that may also contribute to insulin resistance. These organs include the liver, muscle, pancreas, brain, and small and large intestine. The gut contains an extensive immune system because it is exposed to microbial antigens and ingested antigens from the diet. However, only recently have inflammatory and immune cell changes in the bowel been investigated in depth as a link to obesity and insulin resistance (Garidou et al., 2015; Luck et al., 2015; Monteiro-Sepulveda et al., 2015).

This review describes evidence for and against immune cell-mediated, low-grade intestinal inflammation as an emerging feature and potential driving force behind the development of obesity-associated insulin resistance. It also briefly examines the mechanisms, including dysbiosis, changes in intestinal permeability, and alterations in oral tolerance, by which the intestinal immune system may affect systemic inflammation and insulin resistance. Moreover, clinical implications regarding the design of low-toxicity, gut-specific therapies targeting the gut immune system are also described.

Dysbiosis and Alterations in Intestinal Permeability in Obesity

The small and large intestines are home to over a trillion microorganisms consisting of hundreds of species. Increasing evidence links changes in intestinal bacteria to the development of obesity and glucose intolerance. Evidence for this notion stems from the initial observation that germ-free mice have reduced body fat and do not develop obesity or insulin resistance when placed on a high-fat diet (HFD) (Bäckhed et al., 2004, 2007). However, germ-free mice regain adiposity and develop insulin resistance and glucose intolerance 2 weeks after reconstitution with the gut microbiota of conventionally raised mice (Bäckhed et al., 2004). This effect occurred even with reduced food intake,

providing further evidence that gut bacteria are regulators of energy metabolism.

Obesity and metabolic syndrome are associated with an altered gut microbiota, known as dysbiosis (Turnbaugh et al., 2006). In mice and humans, metagenomic analysis showed that most bacteria in the distal gut and feces belong to two main bacterial phyla, Bacteroidetes and Firmicutes (Gill et al., 2006). Lean mice maintain a relative balance among these two bacterial phyla, but, in models of obese mice, an increased ratio of Firmicutes to Bacteroidetes is described most frequently (Ley et al., 2005; Turnbaugh et al., 2006). However, some studies show opposite results, suggesting that this issue is not fully resolved (Carvalho et al., 2012; Schwiertz et al., 2010). More recently, large-scale metagenomics studies in humans linked microbial gene signatures to features of metabolic syndrome (Karlsson et al., 2013; Le Chatelier et al., 2013; Qin et al., 2012). In one study, low bacterial richness, indicated by a low microbial gene count in fecal DNA, correlated with dyslipidemia, insulin resistance, and inflammation (Le Chatelier et al., 2013). Individuals with a high gene count had a higher prevalence of potentially anti-inflammatory species, such as *Faecalibacterium prausnitzii*, that are associated with increased production of short-chain fatty acids (SCFAs), including butyrate (Le Chatelier et al., 2013). In an accompanying study, restoring bacterial richness in individuals with a low gene count by diet-induced weight loss improved metabolic outcomes (Cotillard et al., 2013), suggesting that changes in the microbiota could be achieved through diet. Overall, decreased bacterial richness may be an indicator of inflammation and metabolic disease. Consistently, a small study in humans shows that intestinal transfer of fecal microbiotas from lean donors can improve insulin sensitivity and the abundance of butyrate-producing bacteria in recipients with metabolic syndrome (Vrieze et al., 2012). In mice, transfer of gut flora from obese to germ-free mice increased obesity more than gut flora from lean mice, demonstrating that obesity triggers the accumulation of "pathogenic" bacteria that promote its development (Turnbaugh et al., 2006). Consistently, antibiotic treatment of obese mice can reduce adiposity and adipose inflammation and improve glucose metabolism (Cani et al., 2008; Membrez et al., 2008). Alterations in microbial composition are also seen with weight loss, reduced adiposity, and improved metabolic parameters following gastric bypass surgery (Tremaroli et al., 2015; Zhang et al., 2009), further strengthening the link between obesity, dysbiosis, and metabolic disease.

Several mechanisms linking bacteria to the induction of obesity have been described. Gut bacteria suppress the lipoprotein lipase suppressor, also known as fasting-induced adipocyte factor (FIAF) or angiopoietin-like protein 4 (ANGPTL4), in intestinal cells, resulting in increased lipoprotein lipase activity and increased triglyceride storage in adipocytes and the liver (Bäckhed et al., 2004, 2007). The gut microbiota also controls the metabolism and regulation of bile acid profiles in the bowel that bind to farnesoid X receptor (FXR) and G protein-coupled bile acid receptor TGR5 (Swann et al., 2011; Thomas et al., 2009). Indeed, a gut-restricted FXR agonist reduces diet-induced weight gain, systemic inflammation, and hepatic glucose production (Fang et al., 2015). Finally, obesity-associated microbiotas may also be more efficient at harvesting energy from the diet by producing enzymes that degrade nutrients more efficiently (Turnbaugh et al., 2006).

One major consequence of the altered microbial composition in obesity is increased intestinal permeability, which increases leakage of bacteria or bacterial products such as lipopolysaccharide (LPS) across the intestinal barrier (Amar et al., 2011a; Cani et al., 2007, 2008). Bacterial products trigger the innate immune system, resulting in chronic inflammation and metabolic disease. Four weeks of continuous LPS infusion recapitulates many metabolic abnormalities that occur during HFD consumption, such as increased fasting glucose and insulin, increased liver and adipose tissue and body weight, and adipose tissue inflammation (Cani et al., 2007). Bacterium-related leakage into blood and tissue, such as adipose tissue, can be detected as early as 1 week after starting a HFD and is dependent on the microbial pattern receptors NOD1 or CD14 (Amar et al., 2011a). LPS can also enter the systemic circulation and adipose tissue through uptake by chylomicrons (Ghoshal et al., 2009). High-energy intake, especially saturated fat, is correlated with endotoxemia in humans (Amar et al., 2008), and higher concentrations of bacterial 16S rRNA in the blood are correlated with increased abdominal adiposity and a higher risk for diabetes (Amar et al., 2011b).

The mechanisms by which diet-induced obesity is associated with increased intestinal permeability may involve reduced expression of epithelial tight junction proteins such as zonula occludens 1 (ZO-1) and occludin (Cani et al., 2008). Increased cytoplasmic sequestration of occludin has also been seen following HFD consumption in obesity-prone Sprague-Dawley rats (de La Serre et al., 2010), and abnormal distribution of occludin and ZO-1 is seen in *ob/ob* mice (Brun et al., 2007). Nonetheless, gut bacteria appear to be critical players in gut barrier dysfunction because antibiotic treatment prevented HFD-induced intestinal permeability (Cani et al., 2008). Interactions between diet and the microbiota also affect gut epithelial integrity and intestinal homeostasis. For example, non-digestible carbohydrates are fermented in the bowel to produce SCFAs, such as acetate, propionate, and butyrate, that bind to G protein-coupled receptors (GPRs) that suppress inflammation and improve barrier function and diabetes (Gao et al., 2009; Maslowski et al., 2009; Tremaroli and Bäckhed, 2012). Intestinal mucin produced by goblet cells also contributes to the maintenance of the gut barrier (Vaishnava et al., 2011).

Barrier function is also dependent on the intestinal immune system during HFD feeding and inflammatory disease. Interferon γ (IFNγ)-secreting immune cells are at least partially responsible for HFD-induced barrier permeability because HFD-fed IFNγ-deficient mice show reduced barrier permeability, and IFNγ directly reduced ZO-1 expression in intestinal epithelial cell lines (Luck et al., 2015). IL-1β can also increase intestinal epithelial tight junction permeability (Al-Sadi and Ma, 2007). Other immune cells may exert protective effects on intestinal barrier function, such as IL-22-producing innate lymphoid cells and Tregs, which dampen IFNγ-mediated immunity and promote mucin production and anti-microbial immunoglobulin A (IgA) (Cong et al., 2009; Wang et al., 2014). Eosinophils may exert a protective effect on the barrier by an unknown mechanism (Johnson et al., 2015). The role of the immune system in degrading or promoting intestinal barrier function has been studied extensively in a

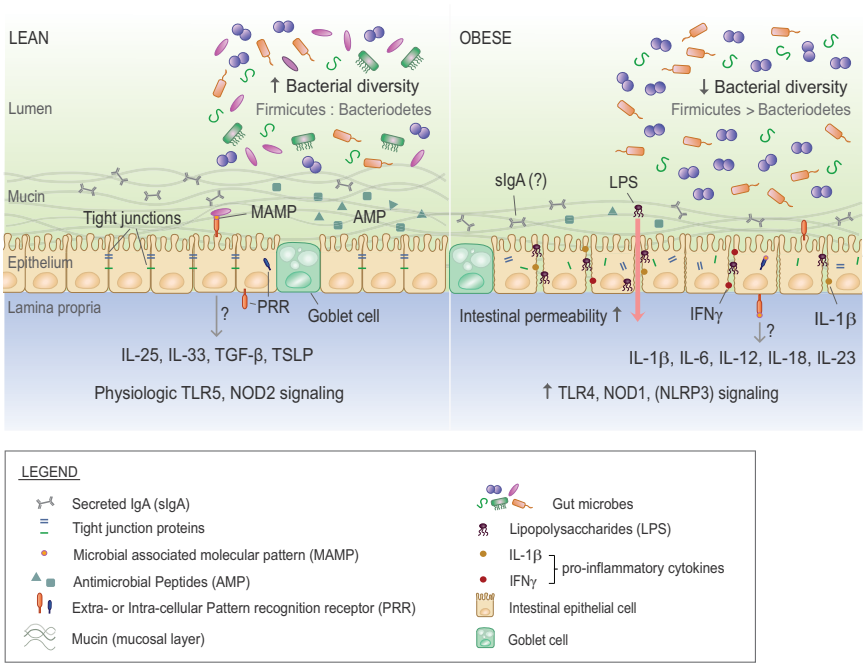

Figure 1. Changes to the Gut Microbiota and Intestinal Barrier Function during Obesity

Under normal physiological conditions, in the lean state, the gut microbiota is highly diversified. The intestinal barrier prevents pathogenic bacteria from penetrating the gut by the production of mucin by goblet cells, AMPs, and secreted IgA (sIgAs). Intestinal epithelial cells (IECs) produce tolerogenic responses to MAMPs of commensal bacteria bound to pattern recognition receptors (PRRs) by secreting anti-inflammatory mediators, including IL-25, IL-33, TGF-β, and TSLP. Some PRR signaling pathways important to eubiotic homeostasis include TLR5 and NOD2. During obesity, consumption of a HFD decreases the diversity of the gut flora and imbalances the ratio of bacterial species. HFD feeding also reduces the production of mucin and other anti-microbial factors, which allow for easier penetration of bacteria past the gut barrier. Invasive bacteria and bacterial products trigger innate NLR and TLR signaling, specifically TLR4, NOD1, and, possibly, NLRP3, to induce inflammatory responses. Binding of MAMPs of invasive bacteria to PRRs on the basal side of the IEC, in conjunction with inflammasome activation, promotes the release of pro-inflammatory cytokines, including IL-1β, IL-6, IL-12, and IL-18. In addition to IL-1β, another pro-inflammatory cytokine, IFNγ, produced by immune cells in response to the inflammatory milieu, weakens the epithelial barrier by decreasing expression or misplacement of tight junction proteins (ZO-1 and occludin). A weakened gut barrier allows leakage of bacterial products such as LPS across the barrier and into the systemic circulation. High levels of LPS and bacterial products cause endotoxemia and systemic inflammation that worsen metabolic disease.

variety of enteropathies that show dysbiosis and inflammation, including inflammatory bowel disease (IBD), common variable immune deficiency (CVID), or HIV, which are summarized elsewhere (Brown et al., 2013). There is a noticeable overlap in mechanisms behind HFD-induced versus inflammatory bowel disease-induced barrier dysfunction in which pro-inflammatory cytokines such as IFNγ and TNF-α play an important role. Figure 1 depicts microbial and barrier changes that occur in obesity.

General Inflammatory Changes in the Intestine with Obesity

The innate immune system of the bowel functions as the first line of defense against infection but allows for the maintenance of immune tolerance to normal bacterial flora. Components of the bowel innate immune system include luminal contents such as mucus, intestinal epithelial cells (IECs), including Paneth cells, which produce α-defensins, ILCs, and other rapidly responding immune cells, such as macrophages and neutrophils. Much of the innate immune response is governed by pattern recognition receptors such as transmembrane surface or endosome toll-like receptors (TLRs) and cytosolic nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) that bind to microbe-associated molecular patterns (MAMPs) expressed by normal gut flora or pathogens.

Typically, under homeostatic conditions, IECs secrete mucins and anti-microbial peptides (AMPs) that control their interactions with the microbiota. The inner layer of mucus is enriched in AMPs, whereas the outer layer is highly colonized by the microbiota, some of which (including *Bifidobacterium* and *Bacteroides* spp.) can metabolize mucin glycans to produce SCFAs (Fukuda et al., 2011). Under eubiotic homeostasis, MAMPs typically induce secretion of anti-inflammatory mediators, including IL-25, IL-33, transforming growth factor β (TGF-β), and thymic stromal lymphopoietin (TSLP), which promote tolerance and barrier function in the bowel (Maynard et al., 2012). In contrast, upon pathogen invasion or barrier breach, MAMPs stimulate IECs, macrophages, and dendritic cells to produce pro-inflammatory cytokines, including IL-1, IL-6, IL-12, IL-18, and/or IL-23 (Maynard et al., 2012). Because obesity is characterized by low levels of intestinal barrier breach, studies have correlated changes in intestinal cytokine profiles with diet-induced obesity and insulin resistance.

Early studies assessing low-grade bowel inflammation during HFD feeding and/or obesity began by measuring the total intestinal levels of pro-inflammatory cytokines, such as TNFα, IL-1β, and IL-12p40, that are produced heavily by innate immune cells. Most studies report that a HFD promotes inflammation in the distal small bowel. The results from Ding et al. (2010) show that ileal TNF-α mRNA in conventional, but not germ-free, mice increased at 6 and 16 weeks of HFD feeding, and this increase correlated with weight gain, adiposity, and plasma insulin and glucose levels. The absence of inflammatory changes in germ-free mice illustrates that the microbiota is a driving force behind intestinal inflammatory changes. These results were confirmed in nuclear factor κ light chain enhancer of activated B cells (NF-κB)-EGFP reporter mice because a HFD induced NF-κB expression throughout the small bowel as early as 2 weeks of diet, and the effect was maintained throughout HFD exposure (Ding et al., 2010). Expression was detected in bowel immune cells, especially in Peyer's patches, lymphoid aggregates, and $CD3^+$ cells, along with epithelial and

endothelial cells and rare neuroendocrine cells, but not in F4/80$^+$ intestinal macrophages.

In an outbred rat study, obesity-prone Sprague-Dawley rats exhibited an increase in ileal myeloperoxidase compared with obesity-resistant Sprague-Dawley rats (de La Serre et al., 2010). TLR4 activation was also increased in intestinal epithelial cells in obesity-prone rats. These inflammatory changes correlated with dysbiosis, reduced intestinal alkaline phosphatase activity (a brush border enzyme that detoxifies LPS), abnormal tight junction expression, and increased intestinal permeability. Because all rats were given a HFD, this study links intestinal inflammation to the development of obesity rather than to just HFD exposure.

Gene expression arrays on proximal, mid-, and distal small bowel in HFD-fed C57BL/6J mice at 2, 4, and 8 weeks showed altered expression in some inflammatory genes, of which some are time- and location-specific (de Wit et al., 2008). For example, CCL5, a chemokine that attracts pro-inflammatory immune cells, especially Th1 T cells, was upregulated only in the distal small bowel at 4 and 8 weeks post-HFD feeding (de Wit et al., 2008). In contrast, macrophage migration inhibitory factor (MIF), a pro-inflammatory cytokine with chemokine-like properties, was upregulated in the entire small bowel and at most time points during HFD feeding (de Wit et al., 2008). Interestingly, MIF-deficient mice are protected from HFD-induced insulin resistance (Finucane et al., 2014). Consistent with changes in the immune makeup of the ileum during obesity, recent studies have shown reductions in ileal mRNA levels of IL-22, IL-17A, IL-17F, and IL-10, known to maintain epithelial barrier integrity, after 10 and 30 days of HFD feeding in mice and after 1 week (IL-10) in rats (Garidou et al., 2015; Hamilton et al., 2015). The significance of differential expression of inflammatory genes in terms of location in the gut and timing is unclear, but, given that microbes show species-specific changes along different regions of the intestine, it is plausible that these changes could impart selective pressures on immune cell function.

Recently, in a comprehensive analysis of human specimens from the jejuna of lean and obese subjects, RT-PCR data showed increases in various pro-inflammatory cytokines in the combined lamina propria (LP) and epithelial fraction, including IL-23, TNF-α, TGF-β, CCL5, and IFNγ (Monteiro-Sepulveda et al., 2015). Other inflammatory mediators, including cyclooxygenase 2 (COX2), were also increased, mainly in the epithelial fraction. These results complement another gene profile analysis study in which IFNγ and IL-1β were increased in the duodeni of insulin-resistant obese subjects (Veilleux et al., 2015).

One study that analyzed gene expression in total small bowels of mice after 7 days of HFD feeding did not find increases in IL-1β, TNF-α, or monocyte chemoattractant protein 1 (MCP-1) expression (Johnson et al., 2015). In the context of previous works where most significant inflammatory changes were seen after 2-4 weeks, it may be that 1 week is too early to elicit significant inflammatory changes in the bowel, possibly because of gut microbial factors. Following exposure to a HFD, microbial changes can be seen within 24 hours in both mice and humans, but even 10 days of HFD feeding was unable to alter the microbial enterotype (Turnbaugh et al., 2009; Wu et al., 2011b). Moreover, because this study used whole-bowel analysis, early gene expression changes in different portions of the small bowel, for example the ileum, may not have been detected (Johnson et al., 2015).

Data from the colon are less consistent. TNF-α levels in the colon did not change after 1 week of HFD feeding (Johnson et al., 2015). In NF-κB-EGFP reporter mice, a HFD induced NFκB expression throughout the colon by 2 weeks of HFD feeding, and this was maintained during the entire course of HFD exposure (more than 16 weeks) even though TNF-α mRNA levels were not increased notably at any time point (Ding et al., 2010). After 14 weeks of HFD feeding, the proximal colon exhibited increases in some inflammatory cytokines, such as IL-1β and IL-12p40, but not IL-6 or TNF-α (Li et al., 2008). In contrast, another study showed a 6.6-fold increase in TNF-α, but not IL-6, in the proximal colon between 8–12 weeks of HFD feeding (Lam et al., 2012). Gene arrays and RT-PCR after 17 weeks of HFD feeding showed a 72% increase in TNF-α (Liu et al., 2012). The same study also showed a 41% increase in IL-18 in the colon following long-term HFD feeding.

Overall, these data indicate that diet-induced obesity alters general cytokine expression throughout the small bowel and, probably, in the colon starting around 2 weeks post-HFD consumption and becomes more significant with prolonged HFD exposure or obesity in both mice and humans (Table 1). Therefore, low-grade bowel inflammatory changes are an early manifestation of HFD feeding that precedes detectable systemic metabolic disease (Ding et al., 2010).

Currently, the strongest inflammatory link appears to be in the jejunum and ileum of the small bowel and the proximal colon, all of which are associated with high levels of commensal organisms. Because different bacterial species are enriched in different parts of the bowel, it will be interesting to determine which species drive bowel inflammation associated with a HFD and obesity. In addition to the recent studies of the human jejunum (Monteiro-Sepulveda et al., 2015), further studies are required to validate low-grade inflammatory changes in the intestines of obese humans, especially in the distal bowel. In one study, diet-induced weight loss in obese adults reduced colon mucosal cytokines, immune cells, and pro-inflammatory networks while improving plasma glucose and lipid levels (Pendyala et al., 2011). On the contrary, two human studies using fecal calprotectin as a general marker of intestinal inflammation found no differences between lean and obese adults (Brignardello et al., 2010; Tiihonen et al., 2010). However, of note, calprotectin is largely released by neutrophils and is typically increased in diseases of a more florid type of active colitis, such as inflammatory bowel disease and infectious colitis (Chen et al., 2012).

The Role of Intestinal Innate Immunity in Obesity

Intestinal TLRs and NLRs Control Diet-Induced Metabolic Disease

Because HFD-induced inflammatory changes in the bowel are dependent on the microbiota, the upstream interactions of MAMP and damage-associated molecular pattern (DAMP) sensors, namely TLRs and NLRs, on intestinal epithelial cells and resident immune cells initiate this process. For example, intestinal epithelial cells express TLR4, which binds LPS to induce NF-κB expression in a time- and dose-dependent manner (Cario et al., 2000). NOD2 and NLRP3 are well studied pattern recognition receptors of the NLR family that are expressed by intestinal

Table 1. General Cytokine Changes in the Small and Large Intestine during Diet-Induced Obesity

	Small Intestine			Large Intestine		
Cytokine Expression	Duodenum	Jejunum	Ileum	Proximal	Distal	Citations
Increased						
NF-κB	●	●	●	●	●	Ding et al., 2010
MIF	●	●	●			de Wit et al., 2008
IL-23		●				Monteiro-Sepulveda et al., 2015
TNF-α		●	●	○	○	Ding et al., 2010; Monteiro-Sepulveda et al., 2015; Lam et al., 2012; Liu et al., 2012
CCL5		●	●			de Wit et al., 2008; Monteiro-Sepulveda et al., 2015
IFNγ	●	●		●		Monteiro-Sepulveda et al., 2015; Veilleux et al., 2015; Garidou et al., 2015
MPO			●			de La Serre et al., 2010
IL-12p40				●		Li et al., 2008
IL-1β	●		●	●		Li et al., 2008; Veilleux et al., 2015; Hamilton et al., 2015
IL-18				●	●	Liu et al., 2012
Decreased						
IL-10			●	●		Garidou et al., 2015; Hamilton et al., 2015
IL-17A			●			Garidou et al., 2015; Moya-Pérez et al., 2015
IL-17F			●			Garidou et al., 2015
IL-22			●	●		Garidou et al., 2015

High-fat diet consumption or obesity alters the production of chemokines and cytokines by innate and adaptive immune cells and intestinal epithelial cells throughout the small bowel (duodenum, jejunum, and ileum) and colon (distal and proximal). For the colon, the study measuring IL-18 did not specify proximal vs. distal. ●, expression in this location of the intestine; ○, discrepancies in expression between studies; empty column, the parameter was not assessed or not changed; MPO, myeloperoxidase; CCL5, chemokine (C-C motif) ligand 5.

epithelial cells (Hisamatsu et al., 2003; Kummer et al., 2007). Saturated fatty acids or ceramide, but not unsaturated fatty acids, can activate the NLRP3-ASC inflammasome, and total body deficiency of NLRP3 or its inhibition by omega-3 fatty acids protect against HFD-induced metabolic disease (Vandanmagsar et al., 2011; Wen et al., 2011; Yan et al., 2013). Dietary lipids can also influence gut microbiota composition, which then modulate adipose inflammation through TLR interactions. A recent study has shown that mice fed a lard-based diet presented a heightened inflammatory phenotype, whereas mice fed fish oil were protected from adipose tissue inflammation (Caesar et al., 2015). The mechanism in this case was partially dependent on gut microbial products leading to the production of CCL2 by adipocytes via TLR4, MyD88, and TIR domain-containing adapter-inducing interferon β (TRIF) signaling (Caesar et al., 2015).

Abnormalities in some TLRs and NLRs result in dysbiosis and predispose to colitis, nonalcoholic steatohepatitis (NASH), and metabolic disease. When fed a methionine-choline-deficient diet, mice deficient in NLRP3 or the inflammasome adaptor protein ASC, show increased hepatic steatosis and NASH, which was dependent on dysbiosis, TLR4, and TLR9 (Henao-Mejia et al., 2012). In another example, mice deficient in TLR5, which recognizes bacterial flagellin, develop obesity and features of metabolic syndrome (Vijay-Kumar et al., 2010). This phenotype was linked mechanistically to alterations in the gut flora because disease could be transferred to wild-type, germ-free mice. Finally, HFD-fed mice deficient in NOD2, which recognizes bacterial cell wall peptidoglycan and regulates microbial homeostasis, show dysbiosis, increased bacterial translocation, and insulin resistance (Denou et al., 2015). In contrast, HFD-fed, NOD1-deficient mice are protected from insulin resistance (Schertzer et al., 2011).

A specific pathologic role for intestinal epithelial TLR signaling has been shown recently by Everard et al. (2014). Here, intestinal epithelial-specific deletion of the TLR adaptor MyD88 partially protects against diet-induced obesity, metabolic dysfunction, and inflammation. This protection is dependent on the gut microbiota, which could transfer protection to germ-free recipients and is linked mechanistically to increases in anti-inflammatory endocannabinoids, microbial peptides, and intestine-resident Tregs. TLRs and NLRs are therefore critical in maintaining intestinal and microbial homeostasis and contribute to HFD-induced bowel inflammation and subsequent metabolic abnormalities.

Cellular Changes in the Innate Immune Compartment Accompany Obesity

Because innate immune cells express molecular pattern receptors, it would not be surprising to see alterations in some of these cells or their cytokines with exposure to a HFD. ILCs are rapid responders activated by cytokines and molecular patterns and do not express variable antigen-specific receptors. They are currently categorized into three broad groups, ILC1, ILC2, and ILC3, based on transcription factors and the cytokines they express. ILC3s are abundant in the bowel and are a source of IL-22 (Spits et al., 2013).

IL-22 is a member of the IL-10 family expressed by ILCs (mainly group 3) and Th17 and Th22 cells and is critically involved

in host defense, tissue regeneration/repair, maintenance of intestinal epithelial integrity, and homeostasis of commensal organisms (Sonnenberg et al., 2012; Zheng et al., 2008). IL-22 may be involved in maintaining mucosal immunity during obesity and in regulating weight gain and glucose homeostasis (Wang et al., 2014). Specifically, obesity and a HFD reduced ILC production of IL-22 following antigen challenge or infection. Although no difference in metabolic parameters was seen in IL-22 knockout (KO) mice after 1 month of HFD feeding, IL-22 receptor (IL-22R1) KO mice exhibited increased weight gain and insulin resistance. Furthermore, injection of recombinant IL-22 (fused to the Fc portion of mouse IgG2a, IL-22-Fc) improved both body weight and metabolic parameters. The beneficial effects of IL-22 included changes in bowel permeability, reduced serum LPS, and improved metabolism in liver and adipose tissue, suggesting that IL-22 may be important in regulating systemic metabolic disease.

Consistently, the percentages of IL-22-producing NCR^+CD4^- (also specified as $NKp46^+CD4^-$) ILC3s are reduced in the LP of HFD-fed mice compared with lean mice, which correlated with reduced epithelial barrier integrity, increased serum LPS, and anti-LPS IgG in HFD-fed mice (Luck et al., 2015). The cause for this reduction of IL-22-producing NCR^+CD4^- ILC3 cells is not known. However, IL-23 can activate ILC3s to produce IL-22 and reduced expression of IL-23 in obese mice after *Citrobacter rodentium* infection suggests that there may be a defective IL-23-to-IL-22 axis in obesity (Wang et al., 2014; Zheng et al., 2008). Nevertheless, the reduction in the percentage of NCR^+CD4^- ILC3s may at least partially contribute to increases in intestinal permeability associated with a HFD.

Along with ILCs, the proportions of γδ T cells in the bowel change with HFD feeding in one study (Luck et al., 2015). γδ T cells are enriched in the intraepithelial lymphocyte fraction and respond mostly in a major histocompatibility complex (MHC)-independent fashion. After 3 weeks of HFD feeding, IL-17-producing γδ T cells increased in the colon but not small bowel. However, by 12 weeks of HFD feeding, both the colon and small bowel showed increases in IL-17-producing γδ T cells. Another study found no changes in the total amount of γδ T cells in the jejunal mucosa of obese human subjects (Monteiro-Sepulveda et al., 2015).

Mucosa-associated invariant T (MAIT) cells are innate-like T cells enriched in mucosal surfaces, including the gut, and regulate inflammatory responses by rapid production of cytokines. These cells are restricted by the non-polymorphic, MHC class I-related protein 1, MR1, and are activated by bacteria through the detection of riboflavin metabolites bound to MR1 (Kjer-Nielsen et al., 2012). In patients with severe obesity and/or type 2 diabetes, circulating MAIT cells are decreased, with an associated increase in Th1 and Th17 cytokine-producing profiles in inflamed tissues such as VAT (Magalhaes et al., 2015). Although changes in such cells have not been investigated in detail in the gut, it is interesting to speculate that these cells could also contribute locally to intestinal inflammatory changes during obesity.

Eosinophils were reduced in both number and proportion after 1 week of HFD feeding (Johnson et al., 2015). This reduction was linked to the fat content in the HFD because obese *ob/ob* mice given a normal control diet did not show a decrease in eosinophils. Nonetheless, reduced eosinophils were correlated with increased paracellular permeability across the intestinal epithelium, predominantly in the ileum of HFD mice. The significance of HFD-associated reduction of eosinophils and whether these changes are maintained longer than 1 week of HFD requires further study.

Little is known about the influence of a HFD on intestinal macrophage and dendritic cell (DC) subsets. Aside from classical $CD11c^-$ macrophages, there are a number of $CD11c^+$ macrophage or DC cell subsets present in the gut. $CD11c^+CX3CR1^{hi}F4/80^+$ monocyte-derived macrophages are generally sessile cells and anti-inflammatory in nature (Schulz et al., 2009). $CD11c^+CX3CR1^{int}$ DCs are more inflammatory and can produce IL-12, inducible nitric oxide synthase (iNOS), and TNF-α, with the capacity to migrate to local lymph nodes and activate Th1 responses (Rivollier et al., 2012). Finally, $CD11c^+CD11b^+CD103^+$ DCs are present in the LP and are important in oral tolerance and Treg responses (Sun et al., 2007). One study did not find any changes in the total number of grouped small bowel macrophages and DCs by 1 week of HFD feeding. However, their relative proportion in the LP is increased, partially because of a reduction in eosinophils (Johnson et al., 2015). In HFD-fed, NF-κB-EGFP mice, co-immunofluorescence with F4/80 did not co-localize HFD-induced EGFP expression to macrophages, suggesting that bowel macrophages may not be activated (Ding et al., 2010). In another study utilizing broad $MHCII^+CD19^-$-gating antigen-presenting cells (APCs), APCs from 10- or 30-day HFD-fed mice had reduced levels of activation markers such as CD86 and a reduced ability to induce Th17 T cell differentiation in vitro but also showed upregulation of certain genes implicated in inflammatory pathways, including Nlrp3 (Garidou et al., 2015). However, changes in the proportions and functions of distinct subsets of macrophages and DCs present in the gut have not been assessed. Therefore, it may be too early to exclude a role for these cells in promoting or inhibiting low-grade bowel inflammation following HFD feeding.

A recent study of lean and obese humans that stratified obese patients into three groups (obese diabetics [ObD], obese non-diabetics with a co-morbidity [Ob], and metabolically healthy obese [MHO]) assessed innate immune cell changes in the jejunum (Monteiro-Sepulveda et al., 2015). In contrast to animal studies, all obese patient subsets exhibited an increase in total macrophage density (measured by CD68 staining) as well as increased mature DCs and NK cell numbers in the Ob and ObD groups but not the MHO and lean groups.

The role of neutrophils is also unclear. Analysis of HFD-fed mouse or corresponding human specimens did not detect any evidence for active ileitis or colitis (where histological activity is defined by having any number of infiltrating neutrophils) (Luck et al., 2015). On face value, these findings suggest that neutrophils have a limited role in HFD-induced bowel inflammation, although more data are needed to draw meaningful conclusions.

The Intestinal Adaptive Immune Response in Obesity

In addition to the innate immune system, HFD alters the composition of adaptive immune cells in the LP of the small (ileum and distal jejunum) and large bowel (entire colon). By 3 weeks of HFD feeding in mice, the percentage of Tregs in the colon, but not in

the small bowel, was reduced (Luck et al., 2015). Although changes in the proportions of pro-inflammatory $CD4^+$ or $CD8^+$ T cells were not yet significant after 3 weeks, by 12 weeks post-HFD feeding, the proportion of IFNγ-producing Th1 cells and $CD8^+$ T cells increased in both the small bowel and colon. A corresponding decrease in Tregs in the small bowel and colon was also present after 12 weeks of HFD feeding. Unlike IL-17-producing γδ T cells (mentioned above), the proportion of IL-17-producing Th17 cells was not altered by HFD feeding in this study (Luck et al., 2015).

Another study investigated the effects of a shorter duration of HFD feeding in mice for 30 days and identified reduced frequency and numbers of Th17 cells in the ileum (Garidou et al., 2015). This reduction was related to HFD-induced decreases in specific commensal bacteria such as segmented filamentous bacteria (SFB) and *Porphyromonas gingivalis*, known to induce IL-17 production. The same study also identified an increase in the percentages of Th1 cells, which reinforces the notion of an IFNγ response in DIO mice. Early reductions in intestinal Th17 cells during the course of HFD feeding may also contribute to increased intestinal permeability by altering the production of AMPs, specifically Reg3β and Reg3γ, which are crucial for mucosal barrier defense (Garidou et al., 2015). This effect likely acts in conjunction with the rising IFNγ response to worsen barrier function and trigger metabolic endotoxemia and systemic low-grade inflammation. Variations in the change in Th17 cell numbers between studies are likely due to differences in the length of HFD exposure or abundances of certain intestinal microbiota, such as Th17-inducing SFB, which vary in quantity in different facilities and vendors of mice (Ivanov et al., 2009). Analysis of Th17 cells is further confounded by the lineage plasticity of Treg cells (Gagliani et al., 2015).

In another study, HFD mice treated with early penicillin led to reduced levels of ileal Th17 cells as well as reduced AMPs, Reg3γ, and β-defensin 1. These changes were associated with reductions in intestinal SFB, altered intestinal barrier function, and increased adiposity, highlighting how changes in the gut microbiota can impinge on intestinal adaptive immunity and downstream development of obesity and metabolic disease (Cox et al., 2014).

In a small cohort of resection specimens from humans, obese individuals showed increased T-bet (Th1, ILC1) and $CD8^+$ T cells and reduced Foxp3 (Treg) cells in both the small bowel and colon compared with lean humans (Luck et al., 2015). In a larger cohort of jejunum specimens, total mucosal $CD3^+$ T cells were increased, with a greater increase in the intra-epithelial fraction, as reflected in increased LP and intra-epithelial $CD8^+$ T cells, especially CD8αβ T cells, in obesity (Monteiro-Sepulveda et al., 2015). The increase in intra-epithelial $CD8^+$ T cells is especially interesting because it positions cytokines from these cells in direct proximity to alter the barrier function of epithelial cells. This study also showed an increase in Th17/Th22 and IFNγ-producing $CD8^+$ T cells in the jejunum LP with obesity. The increases in Th17/Th22 cells seen in obesity may occur possibly as a compensatory response to barrier breach to maintain integrity. Interestingly, many cytokines derived from activated intestinal T cells, including IL-17A, IL-22, IFNγ, and TNF-α, also have the potential to modulate enterocyte insulin sensitivity ex vivo, and it remains to be seen how such changes influence enterocyte function (Monteiro-Sepulveda et al., 2015). It will be important to repeat such analyses in larger human cohorts from other segments of the bowel. Changes to LP B cell populations following HFD are also awaiting future investigation, but, in the aforementioned human jejunum study, LP $CD20^+$ cells did not differ between lean and obese patients (Monteiro-Sepulveda et al., 2015).

It should also be noted that, in humans and mice, the histological low-grade inflammation in the intestines appears to be more subtle compared with inflammation in metabolic tissues, including VAT and liver. For instance, macrophage infiltrates in VAT during HFD feeding are dramatic and easily visible as crown-like structures, whereas no such change is seen in the bowel on histology (Luck et al., 2015). This suggests that inflammation in the gut likely manifests in multiple ways, including changes to immune cell numbers and their localization as well as altered functional states of immunity. Homeostatic shifts toward inflammatory cell polarity in the gut during obesity occur without the large acute and chronic inflammatory infiltrates typically seen in active infection or IBD.

Collectively, studies that look at both the adaptive and innate immune responses in the gut upon HFD feeding consistently report changes in inflammatory parameters, as summarized in Figure 2. Of note, not all studies agree on the exact timing and sequence of inflammatory events that take place. This discrepancy is perhaps due to differences in environmental factors (e.g., diet, animal housing conditions, animal vendor, microbiota) and is complicated by the different readouts of inflammation used (e.g., changes at the genetic or molecular versus cellular level). In fact, a recent study by Ussar et al. (2015) has demonstrated that metabolic phenotypes may be strongly influenced by the host genetic background in some animal strains (i.e., C57BL/6 or 129SvEv/ImJ mice), whereas others are more vulnerable to environmental reshaping of the gut microbiota (i.e., 129SvEv/Tac mice) (Ussar et al., 2015). Therefore, it is important to consider factors such as genetic background and environment when comparing results and discrepancies between studies.

Oral Tolerance as a Potential Contributing Factor to Obesity-Related Insulin Resistance

The ability of orally fed antigens to inhibit immune responses, both in the gut and systemically, is referred to as ''oral tolerance.'' Oral tolerance to food antigens is thought to occur primarily by loading of antigen on $CD103^+$ DCs in the bowel LP, whereas tolerant responses to commensal bacteria occur prominently in the gut-associated lymphoid tissue (GALT) (Pabst and Mowat, 2012). Loading of oral antigen onto $CD103^+$ DCs may involve transfer of the sampled luminal antigen by $CX3CR1^+$ macrophages (Mazzini et al., 2014). Antigen-loaded $CD103^+$ DCs migrate to mesenteric lymph nodes, where they induce $Foxp3^+$ Tregs in a retinoic acid- and TGF-β-dependent manner (Sun et al., 2007; Worthington et al., 2011). These committed Tregs then home back to the intestine LP and promote local tolerance and reduction of inflammation (Hadis et al., 2011). It is unclear how these local responses induce systemic immune suppression, but some Tregs may leave the bowel via lymphatics and seed to distant nodes (Pabst and

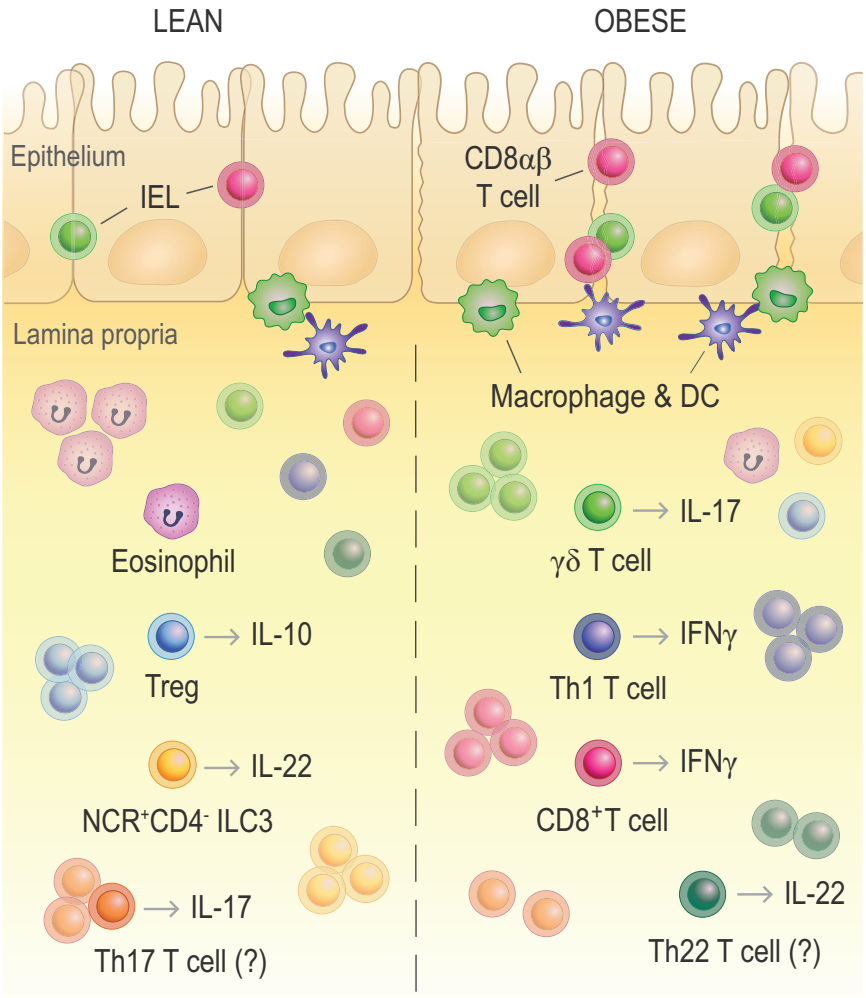

Figure 2. Alterations in Innate and Adaptive Immune Cell Populations Contribute to Low-Grade Chronic Inflammation during High-Fat Diet Feeding

In addition to the intestinal epithelial cells and intraepithelial lymphocytes (IELs), innate and adaptive immune cells in the gut LP regulate intestinal inflammatory responses during obesity. In a lean gut milieu, anti-inflammatory innate immune cell subtypes, including tolerogenic macrophages and DCs, eosinophils, and IL-22-producing NCR^+CD4^- ILC3s maintain homeostasis and generate commensal-tolerating responses. Tregs regulate gut homeostasis by producing the anti-inflammatory cytokine IL-10. In the obese state, immune cell populations in the LP shift toward a pro-inflammatory phenotype by increasing γδ T cells, Th1 cells, and $CD8^+$ T cells that secrete pro-inflammatory cytokines, including IL-17 and IFNγ, respectively. Th17 cells are reduced during the early stages of high-fat diet feeding in the ileum and are closely linked to the host microbiota. In contrast, Th17 and Th22 cells may also be increased, at least in the jejunum at a later stage of obesity. Intraepithelial αβ $CD8^+$ T cells largely accumulate in the obese state. It is unknown whether distinct macrophage and DC subsets are altered during the course of obesity, although increases in total macrophages and mature dendritic cell density have been described in the obese human jejunum. Overall, these inflammatory changes contribute to altered intestinal permeability and worsened downstream obesity-related insulin resistance.

Mowat, 2012). This process may also be related to the abundant tolerance-inducing antigen-presenting cells in the liver, which is another site of oral tolerance (Thomson and Knolle, 2010).

Diet-induced obesity in mice has been linked to defective oral tolerance. Oral feeding of ovalbumin (OVA) antigen in obese mice induces preferential pro-inflammatory Th1-skewed anti-OVA IgG2a/c antibodies upon systemic challenge, whereas feeding the same antigen in lean mice induces a typical tolerogenic response with preferential production of IgG1 (Mito et al., 2006). These results are consistent with the notion that a HFD and obesity promote inflammation, which can potentially affect oral tolerance. This inflammatory environment likely occurs in major tissues linked to oral tolerance, including the intestines, mesenteric lymph nodes, and liver.

The immune response to soluble oral antigen may be important in regulating inflammation in obesity in both systemic and local metabolic tissues that directly affect systemic insulin resistance. Oral antigens, including OVA, can be detected in the blood of humans and mice after eating and can induce expression of T cell activation markers in mesenteric and peripheral lymph nodes (Zinselmeyer et al., 2005). Feeding of dietary antigen to mice under conditions of poor immunological tolerance leads to local changes in $CD4^+$ T cell numbers in VAT and glucose intolerance (Wang et al., 2010). Therefore, it is plausible that the breakdown of the oral tolerance response observed during obesity, fueled by aberrant gut immunity, may be a contributor to low-grade systemic and metabolic tissue inflammation and metabolic dysfunction.

Intestinal Inflammation as a Promoter and Therapeutic Target of Metabolic Disease

Both the innate and adaptive intestinal immune systems are altered with detectable inflammatory gene and cytokine changes when exposed to a chronic HFD. However, the overall contribution of these changes to metabolic disease has only been investigated recently. An overall role for the gut immune system was assessed in mice with β7 integrin deficiency (Luck et al., 2015). β7 Integrin pairs with α4 integrin to form LPAM-1, which is essential in trafficking immune cells into the LP of the small bowel and colon. β7 Integrin-deficient mice show hypoplasia of gut lymphoid tissue and markedly reduced numbers of leukocytes (Wagner et al., 1996). HFD-fed β7 integrin-deficient mice show normal HFD weight gain but are protected from metabolic disease with corresponding reductions in VAT inflammation and hepatic steatosis. Although the mechanisms are unclear, the protective effects of β7 integrin deficiency in obesity may lie in reduced intestinal permeability, possibly because of a reduction in IFNγ-producing immune cells (Luck et al., 2015).

Mice deficient in intestinal epithelial TLR signaling show reduced intestinal inflammation with increased Tregs and are protected from metabolic disease (Everard et al., 2014). In another recent study, low concentrations of two commonly used emulsifiers, carboxymethylcellulose and polysorbate-80, altered the composition of the gut microbiota and increased its encroachment to the bowel epithelium (Chassaing et al., 2015). These food additives also induced low-grade bowel inflammation associated with the development of obesity and metabolic syndrome. Gut microbial factors were critical because the metabolic disease phenotype could be transferred to germ-free mice with fecal transplants. In the aforementioned human jejunum study, mucosal accumulation of T cells correlated with systemic inflammation, obesity, dyslipidemia, and a number of liver parameters (Monteiro-Sepulveda et al., 2015).

The recent finding that low-grade bowel inflammation participates in the development of metabolic abnormalities raises the possibility of using gut-targeted anti-inflammatory therapies with minimal side effects for insulin resistance. One such therapy is mesalamine, which has been the first-line maintenance therapy for IBD for over 30 years. Mesalamine is a salicylic acid derivative with PPARγ agonist and anti-inflammatory properties (Rousseaux et al., 2005). In the C57BL/6 HFD mouse model, mesalamine therapy reversed low-grade inflammation in the bowel by reducing the frequencies of IFNγ-secreting Th1 and $CD8^+$ T cells and IL-17-producing γδ T cells in the small bowel and colon while increasing Tregs. Moreover, mesalamine ameliorated HFD-induced metabolic disease and increased insulin sensitivity in VAT, liver, and muscle but had no effect on weight gain (Luck et al., 2015). In addition, mesalamine reduced VAT inflammation and increased VAT Tregs almost 3-fold. Furthermore, the drug also affected oral tolerance, reduced systemic endotoxemia, and promoted microbial diversity (Luck et al., 2015). Mesalamine may also prevent TNF-α-induced reduction of GLP-1 secretion by human intestinal L cells (Gagnon et al., 2015).

In another bowel-targeted therapy, oral anti-CD3 plus glucosylceramide (an NKT cell target antigen) induced T cell production of IL-10 and TGF-β in the mesenteric lymph nodes and bowel and was associated with reduced fasting glucose, VAT inflammation, liver enzymes, and improved hepatic steatosis in *ob/ob* mice (Ilan et al., 2010). An oral anti-CD3 monoclonal antibody has shown promise in early clinical studies in patients with NASH and impaired fasting glucose (Lalazar et al., 2015).

Intestinal immunity could also be targeted by modulating the intestinal microbiota. Several commensal strains exert beneficial anti-inflammatory effects within the gut during metabolic disease, many of which improve intestinal permeability. For example, administration of *Bifidobacterium pseudocatenulatum* CECT 7765 in HFD-fed mice for 14 weeks decreased the expression of TLR4 and the intestinal inflammatory cytokines TNF-α, MCP-1, IL-6, and IL-18 and improved gut barrier function (Moya-Pérez et al., 2015). *Akkermansia muciniphila,* a mucin-utilizing bacterium, lowers plasma glucose by altering the levels of intestinal endocannabinoids, which, in turn, directly regulate intestinal barrier function, inflammation, and the release of gut hormones (Everard et al., 2013). In addition to *A. muciniphila*, *F. prausnitzii*, another commensal linked to improvements in glucose regulation, is a producer of SCFA, including butyrate, which can directly increase the differentiation of Tregs in the colon by transcriptional regulation (Furusawa et al., 2013; Lukovac et al., 2014). *F. prausnitzii* may also produce a microbial anti-inflammatory molecule (MAM) that suppresses intestinal inflammation, providing alternative mechanisms by which gut microbiota modulate the intestinal immune system (Quévrain et al., 2015). Other strategies that have local effects on intestinal bacteria with overall anti-inflammatory effects on intestinal immunity involve the use of polyphenols, omega-3 fatty acids, and prebiotics, including oligofructose and inulin (Anhê et al., 2015; Dehghan et al., 2014; Lam et al., 2015). Taken together, these findings point to HFD-induced bowel inflammation, alterations in the gut immune system, and microbe-immune interactions as promoters of metabolic disease that can be potentially targeted by therapy.

Current Model and Conclusion

An interconnected network of multiple organ systems influences the development of obesity and related metabolic disorders. HFD-induced changes in the bowel are emerging as crucial initiators in the development of obesity and insulin resistance, which is logical because the bowel is the first organ to come in contact with dietary components. We currently favor a model that integrates the bowel and metabolic tissues, including VAT, as drivers of metabolic disease (Figure 3).

As saturated fatty acids and cholesterol from high-fat or Western diets transit through the bowel, these components alter the gut microbiota and directly stimulate intestinal epithelial cells via TLRs and NLRs. Activated IECs produce chemokines, alarmins, and pro-inflammatory cytokines, such as IL-1β, that can directly increase intestinal epithelial permeability (Al-Sadi and Ma, 2007). This allows bacteria and bacterial products, including LPS, to leak across the barrier into the bowel LP and into the systemic circulation and VAT. Genetic and/or environmental factors such as reduced NLRP6 and NLRP3 function or dietary emulsifiers further promote bacterial and bacterial product leakage (Chassaing et al., 2015; Wlodarska et al., 2014). Dietary emulsifiers result in reduced intestinal mucus production, increased bacterial adherence, and a more pro-inflammatory microbiota (Chassaing et al., 2015). Genetic factors include polymorphisms in immunological loci, such as in *TLR4* or *IL10*, that may impinge on mucosal barrier function (Belforte et al., 2013; Hua et al., 2013; Saxena et al., 2013). Leaked bacterial components, including endotoxins, and changes in bacterial metabolites, such as SCFA, in the intestine induce low-grade chronic inflammatory changes characterized by increased proportions of IFNγ-secreting Th1 and $CD8^+$ T cells and reduced proportions of Tregs, IL-22-producing NCR^+CD4^- ILC3 cells, and eosinophils. The pro-inflammatory environment created by these cell changes further perturbs intestinal permeability through cytokines such as IFNγ and subsequently creates a positive feedback loop that ultimately worsens metabolic endotoxemia, dysbiosis, and insulin resistance. Of note, although many studies support the notion of an inflammatory gut during diet-induced obesity, the lack of important inflammatory cytokines known to maintain gut barrier integrity, including IL-17, may also manifest with systemic endotoxemia and insulin resistance (Garidou et al., 2015). Therefore, the

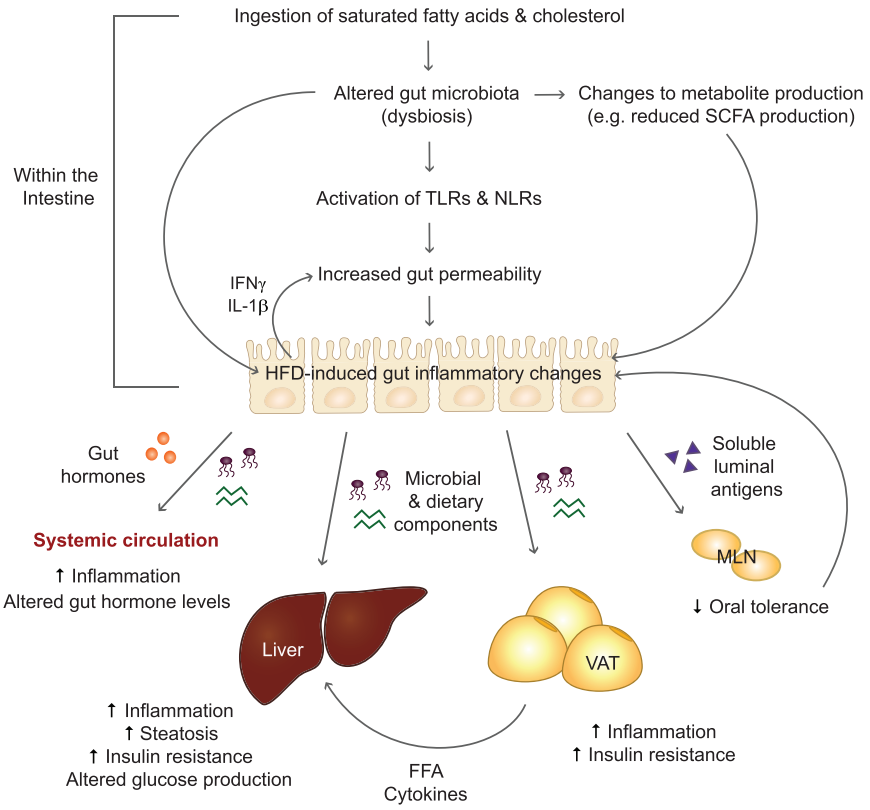

Figure 3. Diet-Induced Microbiota and Gut Inflammatory Changes as the Initiators of Metabolic Disease in a Multi-organ System

The ingestion of saturated fatty acids and cholesterol from high-fat or Western diets alters the composition of the gut microbiota, leading to dysbiosis. Dysbiosis favors the reduction of bacterial species that produce metabolites with anti-inflammatory properties, such as SCFAs, which, in turn, promotes inflammatory immune changes within the intestine. In addition, these microbial changes can trigger the innate immune system and activate TLRs and NLRs on IECs or innate immune cells to promote inflammation within the gut. The secreted pro-inflammatory mediators weaken the intestinal barrier, increasing intestinal permeability to luminal microbial or dietary components. Leakage of these components promotes inflammatory responses governed by innate and adaptive immune cell populations within the gut and leads to a state of low-grade chronic gut inflammation. Pro-inflammatory cytokines, including IFNγ and IL-1β, produced by immune cells or IECs feedback positively to increase gut permeability. Inflammatory cytokines may also alter the levels of gut hormones produced by intestinal cells, which affect the lowering of systemic blood glucose. Furthermore, leaked luminal microbial and dietary components enter the circulation to induce systemic and metabolic tissue inflammation. These components can transit to the liver to alter glucose production and increase inflammation, steatosis, and insulin resistance. Microbial and dietary components from the gut also leak into the VAT to worsen tissue inflammation and insulin sensitivity, which can, in turn, increase free fatty acid (FFA) and pro-inflammatory cytokine production by VAT. FFA may traffic to the liver and promote further inflammation. Leakage of or intolerance to soluble luminal antigens within the gut decreases oral tolerogenic responses in the mesenteric lymph nodes (MLNs) and, in turn, promotes inflammatory responses in the gut. Together, inflammatory changes in the gut induced by a HFD interact with multiple organs to ultimately worsen downstream metabolic disease.

context of the cytokine milieu in the gut and how it impinges on gut barrier function and endotoxemia may be the ultimate factors dictating the role of intestinal immunity in insulin resistance.

As obesity develops, hypoxic and stressed VAT leads to adipocyte death and shedding of cellular debris that promotes a VAT immune response. In addition, leaked bacterial components and soluble antigens migrate from the gut into adipose tissue to further promote pro-inflammatory changes in VAT, some in a TLR- and CCL2-dependent manner (Caesar et al., 2015). Although the antigenic targets in adipose tissue that drive this inflammation have still to be verified (Winer et al., 2011), the possibility that intestinal luminal antigens participate in this process are likely because T cell responses to intestinal OVA can be seen in adipose tissue following oral ingestion, and HFD-fed mice exhibit impaired oral tolerance to luminal antigens (Luck et al., 2015; Wang et al., 2010). On the other hand, an immune trafficking network may exist that links the gut immune system to distal tissues and lymphoid organs such as VAT. Bowel immune cells, including $CD4^+$ T cells and Tregs, have been shown to possess broad trafficking abilities to lymphoid organs at distant sites, such as the inguinal lymph nodes and spleen (Morton et al., 2014). The same study demonstrated that bowel-derived Th17 cells can emigrate to the spleen and contribute to autoinflammatory arthritis (Morton et al., 2014). Therefore, it is plausible that gut immune cells could potentially migrate to other tissues, including VAT.

It would also be intriguing to assess how HFD-induced gut inflammatory changes influence the neuroendocrine systems in the bowel. For instance, chronic exposure to TNF-α can reduce the secretion of the intestinal incretin hormone GLP1 by both murine and human ileal L cells (Gagnon et al., 2015). Anti-TNF-α therapy reduces hyperglycemia in HFD-fed mice and prevents the reduction of GLP-1 secretion in primary intestinal cultures. Therefore, alterations to incretins may be another mechanism by which intestinal inflammation modulates systemic glucose intolerance. It will also be interesting to investigate how intestinal immunity impinges on non-incretin hormones, including serotonin. For example, obesity is associated with increased peripheral serotonin, of which neuroendocrine cells in the ileum are a large source (Bertrand et al., 2011). Mice deficient in tryptophan hydroxylase 1, which produces serotonin, are protected from obesity and metabolic abnormality (Crane et al., 2015).

In conclusion, there is growing evidence linking HFD to low-grade intestinal inflammation. Inflammatory changes currently involve alterations in the intestinal epithelial barrier, the microbiota, and populations of gut-residing innate and adaptive immune cells. These gut inflammatory changes in obesity are of importance because they represent potential therapeutic targets for metabolic disease. The design and use of therapies that are locally active in the gut with limited systemic side effects could, therefore, provide a novel and safe means to treat metabolic disease.

AUTHOR CONTRIBUTIONS

D.W., H.L., S.T., and S.W. contributed to the design and writing of the manuscript and the generation of the figures and table.

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