Abstract  Interactions between metabolism and immunity play a pivotal role in the
development of obesity-associated chronic co-morbidities. Obesity involves
impairment of immune function affecting both the innate and adaptive immune
system. This leads to increased risk of infections as well as chronic low-grade
inflammation, which in turn causes metabolic dysfunction (e.g. insulin resistance)
and chronic disease (e.g. type-2 diabetes). Gut microbiota has emerged as one of the
key factors regulating early events triggering inflammation associated with obesity
and metabolic dysfunction. This effect seems to be related to diet- and obesity-associat
changes in gut microbiota composition and to increased translocation of
immunogenic bacterial products, which activate innate and adaptive immunity in
the gut and beyond, contributing to an increase in inflammatory tone. Innate
immune receptors, like Toll-like receptors (TLRs), are known to be up-regulated
in the tissue affected by most inflammatory disorders and activated by both specific
microbial components and dietary lipids. This triggers several signaling transduc-
tion pathways (e.g. JNK and IKKβ/NF-κB), leading to inflammatory cytokine and
chemokine (TNF-α, IL-1, MCP1) production and to inflammatory cell recruitment,
causing insulin resistance. T-cell differentiation into effector inflammatory or
regulatory T cells also depends on the type of TLR activated and on cytokine
production, which in turn depends upon gut microbiota-diet interactions. Here, we
update and discuss our current understanding of how gut microbiota could contrib-
ute to defining whole-body metabolism by influencing diverse components of the
innate and adaptive immune system, both locally and systemically.
Abbreviations

BMI  Body mass index
ER  Endoplasmic reticulum
ERK  Extracellular signal-regulated kinase
ERS  Endoplasmic reticulum stress
FetA  Fetuin-A
HFD  High-fat diet
IKK  Inhibitory κB kinase
IL  Interleukin
IL-1Ra  IL-1 receptor antagonist
iNOS  Inducible nitric oxide synthase
IR  Insulin receptor
IRF  Interferon regulatory transcription factor
IRS  Insulin receptor substrate
IRS-1  Insulin receptor substrate 1
LPS  Lipopolysaccharide
LTA  Lipoteichoic acids
M1  “Classically activated” macrophages
M2  “Alternative activated” macrophages
MAPKs  Mitogen-activated protein kinases
M-cells  Microfold cells
MCP  Monocyte chemotactic protein
MDP  Muramyl dipeptide
Meso-DAP  d-Glutamyl-meso-diaminopimelic acid
MHC  Major histocompatibility complex
NF-κB  Nuclear factor-κB
NKT  Natural killer T
NLRs  Nod-like receptor family
NOD  Nucleotide oligomerization domain
NOS2  Nitric-oxide synthase 2
PGN  Peptidoglycan
PI3K  Phosphatidylinositol 3-kinase
PI3-K  Phosphatidylinositol 3-kinase
RHM  Recruited hepatic macrophage
ROS  Reactive oxygen species
SAA3  Serum amyloid A3 protein
SFA  Saturated fatty acid
SOC  Suppressor of cytokine signaling
STAT3  Signal transducer and activator of transcription 3
TH1  T helper 1
TLRs  Toll-like receptor family
TNF  Tumor necrosis factor
Tregs  Regulatory T
ZO  Zonula occludens
Introduction

Obesity is associated with immune function impairment, affecting both the innate and the adaptive immune system. These alterations lead to an increased risk of infections and to a state of chronic low-grade inflammation, which is a major cause of metabolic dysfunction (e.g., insulin resistance, metabolic syndrome) and chronic disease (e.g., type 2 diabetes, fatty liver disease, cardiovascular disease, etc.).

Obesity is characterized by infiltration of macrophages and lymphocytes in the adipose tissue and other peripheral organs. This is accompanied by an imbalance in the cytokine network with increased production of pro-inflammatory cytokines, adipokines, acute-phase proteins and other immune mediators [1, 2]. These inflammatory mediators, as well as several transcription factors and kinases, are involved in inflammation-induced metabolic dysfunction such as insulin resistance [3].

Gut microbiota is likely to be one of the factors influencing our predisposition to develop obesity and associated comorbidities. Alterations in the gut microbiota structure have been related to obesity and metabolic dysfunction in murine models [e.g., 4–6] as well as in human observational studies [7, 8]. Differences in microbiota composition in obese animal models could be a consequence of diet and other environmental factors [6, 9–11] and of genotype (e.g., deletion of the leptin gene or its receptor [4, 5]). Notwithstanding, animals with the same genotype and under the same dietary influence (high-fat diet [HFD]) can also develop different metabolic phenotypes (either diabetic or non-diabetic) as a function of their specific gut microbiota profile. This finding suggests that gut microbiota per se determines the risk of developing metabolic dysfunction [12]. This relationship is also supported by studies showing that germ-free mice are protected against diet-induced obesity and by fecal transplantation experiments showing that when microbiota from twins discordant for obesity is transplanted in germ-free mice, these mice develop the corresponding phenotype whether they are fed a low-fat diet or high-saturated fat diet [11], although opposite results have also been published [10].

In humans, many studies associate alterations in gut microbiota structure and function with obesity and markers of metabolic risk, which may also be partly a consequence of diet [7, 13, 14], while effects of the genotype predisposing to obesity on the microbiota are largely unknown. Nonetheless, diet-induced gut microbiota alterations (e.g., an increase in Firmicutes and decrease in Bacteroidetes) seem to play a role in obesity by, for example, increasing energy harvest and lipid absorption [15, 16].

Gut microbiota is likely to be involved in body weight regulation by influencing the host’s metabolic and endocrine network, and the immune system [7]. Colonization of the newborn intestine has an enormous impact on the development of mucosal and systemic immunity, contributing to its ability to discriminate between harmful and innocuous antigens with important effects during early postnatal life through adulthood [17]. The innate immune system is one of the key regulators of the crosstalk between the host and its commensal and pathogenic intestinal bacteria.
Innate immune recognition of specific microbial components is mediated by families of pattern-recognition receptors (e.g. Toll-like receptor family [TLRs] and Nod-like receptor family [NLRs]) which, upon ligand binding, activate different signaling pathways. These can trigger inflammatory responses leading to pathogen clearance or attenuate intestinal inflammation, depending on the stimulus which may also vary depending on gut microbiota composition [19]. These receptors are also activated by dietary lipids and up-regulated in most tissues affected by inflammatory disorders (e.g. adipose tissue, liver, brain) contributing to the inflammatory process leading to insulin resistance [20, 21]. Lymphocyte differentiation into effector or regulatory T (Tregs) cells also depends on the type of TLR activation and cytokine production [19]. Therefore, obesity-associated alterations in lymphocyte distribution and their phenotype may also depend on gut microbiota-diet interactions [22]. Furthermore, recent animal studies report that the intestine, which is the tissue most exposed to “noxious” nutrients (saturated fatty acids) and to a high load of bacterial antigens, is where the inflammatory process associated with diet-induced obesity originates [23, 24].

Therefore, a growing body of scientific evidence supports the notion that the crosstalk between the gut microbiota, diet and immune system activates mediators and signaling pathways, which influence whole body metabolism and disease. Here we update and discuss our current understanding of the specific role that the gut microbiota may play in obesity and metabolic dysfunction (insulin resistance) by influencing host innate and adaptive immunity.

Inflammation Associated with Obesity and Metabolic Dysfunction

Adipose Tissue Inflammation

Adipose tissue inflammation is likely the main contributor to inflammatory signals that lead to metabolic dysfunction (e.g. insulin resistance) in obesity. In fact, expression of pro-inflammatory cytokines in adipose tissue seems to be 100–1,000 times higher than in the liver of subjects with severe obesity and fatty liver disease [25]. Inflammation of this tissue is mainly attributed to macrophage infiltration, which may represent up to 40% of all cells. This is accompanied by inflammatory cytokine and adipokine production (e.g. tumor necrosis factor (TNF) α, interleukin [IL]-6 and IL-1β and leptin) [1, 2]. Macrophage migration is promoted by adipose tissue-produced chemokines, particularly monocyte chemo-tactic protein (MCP)-1. Adipose tissue inflammation is also characterized by an increased ratio of “classically activated” macrophages (M1) to “alternative activated” macrophages (M2). M1 are highly inflammatory macrophages via induction of pro-inflammatory cytokines and other factors (e.g. primarily TNF-α IL-1β, IL-6 and resistin [in humans] and inducible nitric oxide synthase [iNOS]). However, M2,
which are predominant macrophages in lean adipose tissue, exert anti-inflammatory effects via induction of IL-10 and IL-4 cytokine production [26, 27]. M2 also produce catecholamines to sustain adaptive thermogenesis, increasing thermogenic gene expression and contributing to fatty acid mobilization and energy expenditure in adipose tissue in a macrophage-dependent manner [28].

Figure 14.1 summarizes the mode of action of the main cytokines and adipokines and transcriptional factors produced by macrophages and adipose tissue in obesity-associated insulin resistance. TNF-α induces IKKβ/NF-κB and JNK activation promoting the phosphorylation of IRS-1 at serine sites that negatively regulate normal signaling through the insulin receptor/IRS-1 axis and suppress the transcription of adiponectin. IL-1β and IL18 induce insulin resistance by reducing IRS1 expression at a transcriptional level through an extracellular signal-regulated kinase (ERK) dependent mechanism and activating IKKβ/NF-κB. IL-6 may contribute to insulin resistance via induction of SOC proteins and inhibition of adiponectin transcription. Leptin contributes to inflammation by inducing activation of the MAPKs p38 and ERK and of STAT3, leading to pro-inflammatory cytokine production (TNF-α, IL-6 etc). Leptin also activates NOS2 production, leading to increased ROS. Adiponectin, improves insulin sensitivity by suppressing the NF-κB-dependent synthesis of TNF-α and IFNγ, and inducing the anti-inflammatory mediators IL-10 and IL-1RA, as well as by activating PPARγ, which inhibits the NF-κB pathway.
IRS-2 bind and activate phosphatidylinositol 3-kinase (PI3-K), which increases serine phosphorylation of Akt, leading to glucose transport in muscle and adipose tissue, glycogen synthesis in muscle and liver and lipogenesis in adipose tissue [29]. The proper signaling of this pathway may be disrupted by several mechanisms. These include serine phosphorylation of IRS proteins by protein kinases such as c-Jun N-terminal kinase (JNK) and inhibitory κB kinase (IKK)-β of the nuclear factor-κB (NF-κB) pathway, and decreased tyrosine phosphorylation of IRS-1 [29]. The JNK pathway can be activated by endoplasmic reticulum stress (ERS) activation, which occurs in both adipose tissue and liver [30]. Insulin signaling can also be impaired by increased secretion of pro-inflammatory cytokines of innate immunity, including TNF-α and IL-6, IL-1 and IL-18 as well as IL-17 and IFN-γ produced by T cells [30, 31]. Impaired production or action of adipokines such as leptin and adiponectin may also be involved [30].

TNF-α is overproduced exclusively by activated macrophages in the adipose tissue, and directly causes insulin resistance by acting locally on insulin target cells through paracrine mechanisms. Signaling via TNF-α activates intracellular kinases, such as cJNK and IKK, which inhibit insulin receptor signaling by serine phosphorylation of insulin receptor substrate 1 (IRS-1). Activation of transcription factors AP-1 and NF-κB also pro-inflammatory cytokine production by a feedback loop mechanism, whereby proinflammatory cytokine production is increased [31]. TNF-α also suppresses the transcription of adiponectin in adipocyte cell cultures, which explains the reduction in serum adiponectin levels in obese individuals. TNF-α production in adipose tissue can also contribute to increasing circulating levels, which can reach other peripheral tissues (e.g., muscle and liver) and contribute to systemic insulin resistance [31].

Several IL-1 family cytokine members, including pro-inflammatory (e.g., IL-1α, IL-1β, IL-18) and anti-inflammatory components (IL-1 receptor antagonist [IL-1Ra]), are produced by both immunocompetent cells and obese adipose tissue, and play an important role in metabolic inflammation [32]. Neutralization of IL-1 by IL-1Ra can improve hyperglycemia and glycemic control in humans, supporting a role for IL-1 in diabetes and related insulin resistance. Like other inflammatory cytokines, IL-1β and IL18 induce insulin resistance by reducing IRS1 expression at a transcriptional level through an extracellular signal-regulated kinase (ERK) dependent mechanism activating IKKβ/NF-κB [30]. IL-6 is a pro-inflammatory cytokine involved in regulating the acute phase response and in insulin resistance via induction of suppressor of cytokine signaling (SOC) proteins and inhibition of adiponectin transcription [30]. Although preclinical data are not fully conclusive as to whether IL-6 is beneficial or detrimental, in the context of hyperglycemia small clinical trials suggest a beneficial effect of anti-IL-6 therapy. The fact that anti-IL-1 therapies strongly decrease IL-6 levels, also suggests that neutralizing IL-6 is effective and plays a role in metabolic disease [32]. IL-6 also suppresses adiponectin transcription in adipocyte cell cultures such as TNF-α.

Adipocytokines also play different immune roles in the monocyte–macrophage components of the innate immune system, and in T cells of the adaptive immune system [3]. While adiponectin is generally considered anti-inflammatory, leptin and
resistin are considered pro-inflammatory adipocytokines. Through interaction with its receptor (AdipoR1/R2), adiponectin suppresses the NF-κB-dependent synthesis of TNF-α and interferon-γ (IFNγ), and induces IL-10 and IL-1RA production. Adiponectin also induces apoptosis of monocytes and inhibits phagocytosis by macrophages. Adiponectin also decreases T-cell proliferation, reducing the potential alloimmune T-cell response. Nevertheless, adiponectin also has a pro-inflammatory effect in specific circumstances that could be explained by different roles played by the different full-length and globular forms of adiponectin in inflammation and immunity, which are not fully understood yet. In monocytes/macrophages, the mitogen-activated protein kinases (MAPKs) p38 and extracellular-signal-regulated kinase (ERK), signal transducer and activator of transcription 3 (STAT3), are activated by leptin via its OBRb receptor. This activation leads to pro-inflammatory cytokine production, including TNF-α, IL-6 and IL-12. In addition, leptin activates nitric-oxide synthase 2 (NOS2) production, leading to increased reactive oxygen species (ROS), and enhances macrophage phagocytosis, and activation, proliferation and migration of monocytes. Leptin also influences the adaptive immune system, for example by increasing production of the Th helper 1 (Th1) cytokines IL-2 and IFNγ, and suppressing Th2 cytokine IL-4 production in T-cell proliferation assays with mouse cells; however, its role in humans is less clear. In monocytes/macrophages, resistin also activates p38, ERK and phosphatidylinositol 3-kinase (PI3K). This adipocytokine also increases the production of TNF, IL-1β, IL-6 and IL-12, contributing to inflammation.

PPARγ is an additional transcriptional factor and a genetic sensor of fatty acids, which is required for fat development and exerts insulin-sensitizing effects. In adipose tissue, PPARγ is also required for the maturation of M2 macrophages and induces adiponectin synthesis. PPARγ expressed by macrophages also inhibits TLR- and IFN-γ-mediated inflammatory responses and is essential for normal skeletal muscle and liver insulin sensitivity [30].

B cells and T lymphocytes also infiltrate the adipose tissue, which can contribute to inflammation and metabolic dysfunction. The sequence of recruitment of each cellular type into adipose tissue is unclear but their functional roles are known to some extent. B cells [33], CD8+ cytotoxic T cells [34], CD4+ Th1 cells [35] and CD4+ Th17 [36] may promote insulin resistance, whereas CD4+ regulatory T (Treg) cells reduce inflammation, likely contributing to improve insulin sensitivity [37, 38]. Treg cells are drastically reduced in obese adipose tissue paralleled to B cell increases [34, 38]. Tregs regulate the macrophage phenotype, inhibiting their polarization into M1-type and preventing macrophage recruitment into tissues [35, 38]. Depletion of Treg cells via administration of diphtheria toxin is also accompanied by substantial decreases in insulin-stimulated insulin-receptor (IR) tyrosine phosphorylation in epididymal fat and liver, supporting a role of this cellular population in glucose metabolism, at least in these tissues [38]. A recent study in mice on a HFD also suggests that CD19+ B lymphocytes are quickly recruited into adipose tissue and activate pro-inflammatory macrophages and T cells, thus adversely influencing glucose metabolism [33]. Adipose tissue-associated B cells can induce major histocompatibility complex (MHC)-dependent pro-inflammatory
cytokine release, including IFN-γ, from resident CD4+ and CD8+ T cells, which in turn modulate macrophage polarization. The role of B cells in obesity-associated metabolic dysfunction is also supported by the increased insulin sensitivity found in B-cell-deficient mice on a HFD compared with wild-type mouse controls [33]. CD4+ IFN-γ producing cells can also participate in adipose tissue inflammation and insulin resistance. IFN-γ promotes insulin resistance by (1) reducing insulin-stimulated uptake of glucose in adipocytes parallel to reducing serine/threonine-specific protein kinase Akt phosphorylation and down-regulating the insulin receptor, IRS-1 and the glucose transporter Glut4, which impair insulin signaling; (2) polarizing, activating and stimulating M1 macrophages in adipose tissue and up-regulating T-cell and monocyte chemoattractants (e.g. IP-10 and RANTES and MCP-1 and MCP-2); and (3) inducing STAT1 phosphorylation and SOCS1/3 expression in adipocytes [36]. Increased CD8+ T cell infiltration or increased CD8+/CD4+ ratio have also been described as another critical event driving adipose tissue inflammation since it can contribute to producing critical pro-inflammatory cytokines such as IFN-γ [34, 39]. The CD4+ Th17 cells, producing IL-17, are also detected in visceral adipose tissue, but at low frequencies. IL-17 produced by Th17 cells is a pathogenic mediator of inflammation in numerous autoimmune disorders, for example by triggering NF-κB activation and cytokine release. However, the role of Th17 cells in obesity-related insulin resistance is still unclear and requires further investigation [36].

Liver Inflammation

Two macrophage populations are identified in liver: a resident macrophage population (Kupffer cells) and a recruited hepatic macrophage (RHM) population, which migrated upon weight gain under the influence of the liver-derived MCP-1 and can represent 30–70 % of all hepatic macrophages in obesity [40, 41]. Both types of macrophage populations seem to contribute to chronic hepatic inflammation and insulin resistance. Natural killer T (NKT) cells, which can respond to lipid antigen, may be involved in obesity and glucose tolerance, but evidence from animal models is inconsistent [42, 43]. While mice fed a HFD showed increased expression of NKT cells (defined by CD3+NK1.1+) in adipose tissue, amounts decreased in the liver [44]. Hepatic insulin resistance is a driving force in the pathogenesis of type 2 diabetes Mellitus, coupled with excessive fat storage that ensures liver inflammation. Activation of transcription factor NF-κB and downstream inflammatory signaling pathways systemically and in the liver are considered key events in the etiology of hepatic insulin resistance and also in β-cell dysfunction, although the molecular mechanisms involved are only partly understood [45].
Central Nervous System Inflammation

The central control of energy balance and adjustment of food intake and expenditure mainly occurs in the hypothalamus, where there is a complex interplay between insulin, leptin and other neuro-regulators (e.g. serotonin) partly via IRS/PI3K signaling, which negatively regulates energy balance, reduces food intake and improves insulin signaling [46]. Obesity is associated with hypothalamic inflammation and production of pro-inflammatory cytokines that cause central leptin resistance, leading to reduced central regulation of food intake and energy expenditure. Central nervous system inflammation also contributes to systemic insulin resistance, particularly in the liver, via a brain-liver neuronal signal [47]. In animal models, inhibition of either TLR4 or TNFα reduces hypothalamic inflammation, which is accompanied by reduced hypothalamic resistance to leptin, and improved hepatic insulin signal transduction, reduced steatosis and reduced gluconeogenesis. All these effects are mediated by parasympathetic signals delivered by the vagus nerve. Circulating IL-6 is also known to activate the hypothalamic-pituitary-adrenal axis, which is associated with central obesity, hypertension, and insulin resistance [48].

Decreased Immunological Surveillance Associated with Obesity

Obesity is also associated with alterations in the immune defense mechanisms, thus leading to increased risk of infection and decreased response to vaccination. This constitutes an important cause of morbidity and mortality in obese subjects. Epidemiological human studies demonstrate that obese subjects are at a greater risk of nosocomial infections after surgery [49]. Obesity is also an independent risk factor for increased morbidity and mortality related to infection by influenza A (H1N1) virus [49]. Obesity also seems to compromise the efficacy of vaccination against viral infections, as demonstrated in murine models of obesity [50].

The mechanisms underlying obesity-associated risk of infection have been studied in murine models of genetically or diet-induced obesity. In leptin-deficient murine models of obesity (ob/ob or db/db) both innate and adaptive immune systems are adversely affected. Leptin activates monocytes/macrophage chemotaxis, phagocytic activity and cytokine production and, consequently, these functions are impaired in leptin-deficient mice [51]. In fact, ob/ob mice showed impaired immunological protection against different bacterial pathogens due to defective phagocytic activity [52]. Leptin deficiency in mice also leads to an impairment of DC function, characterized by increased production of immunosuppressive cytokines and decreased stimulation of allogenic T cells [53, 54]. T cell reactivity is also impaired in HFD-fed mice that are transgenic for a TRC recognizing a peptide from ovalbumin, indicating that similar defects in immunity occur
in diet-induced obesity. T cells from HFD-fed naïve transgenic mice exhibit an inflammatory response against in vitro antigen/mitogen stimulation, which could contribute to chronic obesity-associated low-grade inflammation [55]. In contrast, antigen-experienced T cells from ovalbumin immunized HFD-fed mice produce a Th2 cytokine profile and have reduced proliferation capacity. DCs from HFD-obese mice are also less able to present antigens to T cells, which may influence T cell polarization [55]. All these findings explain this increased susceptibility to infections and hypo-responsiveness to vaccination in obese subjects.

Influence of Gut Microbiota on Inflammation Associated with Obesity and Metabolic Dysfunction via Regulation of Innate Immunity

Gut microbiota is considered one of the factors contributing to chronic-low grade inflammation associated with obesity and metabolic dysfunction (e.g. insulin resistance). The mechanisms by which gut microbiota influences this process are not well understood, but could be related to alterations in gut microbiota composition. Such changes could increase bacterial components that might activate innate immunity locally in the gut and systemically, and increase translocation of immunogenic bacterial products via different routes, thus contributing to inflammation.

The innate immune system is one of the key regulators of the crosstalk between the host and the microbiota (commensal and pathogenic microbes). Innate immune recognition of specific microbial components (e.g. LPS, DNA, etc.) is mediated by families of pattern-recognition receptors, like the TLR family and NOD-like receptor family, which are expressed in epithelial cells and antigen presenting cells (DCs and macrophages). Upon ligand binding, different signaling pathways (e.g. NF-κB, MAPKs/JNK and the interferon regulatory transcription factor [IRF]) are activated, leading to the expression of inflammatory genes encoding cytokines, cytokine receptors, immuno-regulatory proteins, adhesion molecules and stress-associated proteins [18]. These molecules also induce the recruitment of other immune cells (T cells, basophils, neutrophils, DCs and NK cells) that trigger inflammatory responses and can lead to pathogen clearance [18]. These signaling pathways are also responsible for maintaining tolerance to commensal bacteria which, unlike pathogens, attenuate intestinal inflammation via different mechanisms (e.g. inhibiting NF-κB, inducing regulatory T cells, etc.; [19]). TLRs are up-regulated in most tissues affected by inflammatory disorders and activated by dietary lipids, and thus mediate the crosstalk between the gut microbiota, the host innate immune system and whole body metabolism [56]. A schematic representation of the mechanisms by which gut microbiota and dietary lipids could contribute to “metabolic” inflammation by activating innate immunity in the gut and systemically is shown in Fig. 14.2.
**LPS and TLR4 Signaling**

Lipopolysaccharide (LPS) from the cell wall of Gram-negative bacteria is currently thought to play a role in both immunity and metabolism through the TLR4/MyD88/NF-κB signaling pathway. Increased LPS plasma levels are associated with an elevated body mass index (BMI) and high-fat feeding, postprandial inflammation.
and risk of type-2 diabetes and atherosclerosis in humans [57, 58]. In animal models, increased LPS in plasma (termed “metabolic endotoxemia”) has been causally linked to adiposity and obesity-related insulin resistance and inflammatory liver diseases, such as non-alcoholic fatty liver disease and non-alcoholic steatohepatitis [59, 60]. Mice fed a HFD exhibit a significant increase in plasma LPS, associated with changes in the gut microbiota, obesity, inflammation and glucose metabolic dysfunction. The role of circulating LPS per se in metabolic dysfunction is demonstrated by LPS infusion. On reaching the same plasma LPS levels as those measured in HFD-fed mice they reproduce the same phenotype of HFD-fed mice. The role of LPS and TLR4 is proven in mice deficient in CD14, a key molecule in TLR4 signaling activated by LPS, showing that these mice are resistant to inflammation in adipose deposits, liver and muscles, induced by both HFD and chronic LPS administration [60]. The fact the gut microbiota is involved in LPS-induced metabolic dysfunction has been shown by administering antibiotics (norfloxacin and ampicillin) to two different mouse models of insulin resistance (genetically and diet induced), which led to gut microbiota depletion parallel to reduced serum LPS levels, low grade inflammation, obesity and type-2 diabetes [61]. Comparisons between germ-free and conventional mice have also demonstrated the direct role of the gut microbiota in triggering colonic serum amyloid A3 protein (SAA3) expression, which could contribute to inflammation via LPS signaling [62]. This effect is demonstrated to be partially mediated via TLR/MyD88/NF-κB signaling, by comparing wild-type with Myd88 deficient mice, with the latter gaining less weight on a Western HFD and lower epididymal fat pad and liver masses [62]. A recent study highlights the importance of diet-gut microbiota interactions in this process, reporting that a HFD causes deregulation of the gut microbiota composition (increasing the Firmicutes to Bacteroidetes ratio) leading to increased fecal endotoxin and colonic inflammation, parallel to increased plasma LPS and systemic inflammation. This intestinal inflammation was characterized by increased expression of proinflammatory cytokines, TLR4, iNOS and COX-2, activation of NF-κB and reduced the expression of tight junction-associated proteins claudin-1 and occludin. This study also demonstrates that TLR4 mediates inflammation associated with adiposity and obesity induced by a HFD comparing wild-type with TLR4-deficient mice [24]. Comparing germ-free and conventionally colonized mice has also demonstrated that gut microbiota colonization leads to impaired glucose metabolism and increased macrophage accumulation, and polarization towards a pro-inflammatory M1 phenotype in white adipose tissue in mice fed a standard diet, without HFD feeding [63]. In the same study mice were colonized with an Escherichia coli strain, producing immunogenic LPS or not, demonstrating that macrophage recruitment requires LPS, whereas impairment of systemic glucose metabolism is not exclusively LPS-dependent and may involve an additional mechanism [63]. The fact that protection of TLR4-deficient mice from obesity-induced insulin resistance does not require germ-free conditions, also suggests the microbiota is not the only factor activating this signaling pathway triggering metabolic disease [64, 65]. Altogether, these findings demonstrate that LPS-induced TLR4 signaling constitutes one of the links between the gut
microbiota and inflammation that leads to metabolic dysfunction, which is also influenced by diet.

LPS can impair metabolic functions when reaching tissues involved in glucose and lipid metabolism, such as the liver and the adipose tissue, by stimulating TLRs expressed in infiltrated immune cells (e.g., macrophages and dendritic cells) and in obese adipose tissue. In adipose tissue, LPS-stimulated TLR4 activates p65/p50 and p68/p52 NF-κB signal transduction pathway, inducing the expression of inflammatory mediators such as IL-6, TNF-α, and SAA3 protein, possibly impairing insulin sensitivity as explained in a previous section (Fig. 14.1). This signaling pathway can also induce endoplasmic reticulum (ER) stress and JNK activation accompanied by increased IRS-1 serine 307 phosphorylation in the liver, muscles, and adipose tissue, leading to a reduction in insulin sensitivity and signaling [29]. TLR4 signaling also increases expression of iNOS, which reacts with cysteine residues to form S-nitrosothiol adducts, inducing S-nitrosation/S-nitrosylation of the insulin signaling pathway, leading to insulin resistance in the liver, muscles, and adipose tissue. Circulating LPS can also activate monocyte chemo-attractant protein MCP-1, mediating migration of monocytes to peripheral tissues and contributing to the inflammatory process [66].

High intake of saturated fat, which results in increased levels of circulating free-fatty acids and/or lipid accumulation in muscles and liver, is also known to be directly involved in the inflammatory process leading to insulin resistance [21]. Saturated fatty acids (SFAs) trigger both the expression of TLRs and their activation, which may contribute together with the microbiota-derived products to the increased induction of inflammatory cytokines in different tissues, such as adipose tissue and liver [67]. SFAs activate innate immunity components, such as TLR4 and 2 and the inflammasome, thereby triggering kinase activation (JNK and IKK) and inflammatory cytokine production, inhibiting insulin signaling and action [68, 69]. A protein called fetuin-A (FetA), which is a major carrier of free-fatty acids in serum, acts directly as an endogenous ligand of TLR4, thus activating its signaling pathway, promoting insulin resistance in peripheral tissues [70]. In contrast, polyunsaturated free-fatty acids (e.g. Ω-3 fatty acids) can inhibit TLR4 signaling. LPS stimulation also increases cytokine-mediated plasma lipid levels by increasing VLDL lipoprotein synthesis in the liver and inhibiting lipoprotein lipase. In fact, mobilization of lipid stores is considered a mechanism to fuel the host’s response against infections; moreover, lipoproteins also seem to help fight against infection by binding and neutralizing LPS [71].

It is still unclear which gut microbiota components constitute a source of LPS in animal models of obesity and in observational human studies. LPS originating from E. coli is reportedly sufficient to promote glucose and insulin intolerance and macrophage accumulation in white adipose tissue when mono-colonizing the gut of germ-free mice [63]. In our own studies, compared with standard-diet-fed mice, HFD-fed mice showed increased numbers of Enterobacteriaceae, which were reduced by B. pseudocatenulatum CECT 7765 administration, parallel to amelioration of metabolic dysfunction; however, LPS translocation was not measured [6]. By contrast, in HFD-fed mice increases in Proteobacteria (which include
enterobacteria) and reductions in Firmicutes and Bacteroidetes by vancomycin administration have been related to reduced body weight gain, TNF-α production and metabolic dysfunction [72]. Other animal studies demonstrated that gut microbiota alterations associated with genetically or HFD-induced obesity do not involved Gram-negative bacteria, which could contribute to LPS increases [24, 73], but reductions in Gram-positive bacteria [74]. A couple of recent human studies support the idea that increased proportions of Proteobacteria are associated with inflammatory and metabolic disease risk markers [8, 13] while other studies do not support such an association [7]. This controversy could partly be due to the influence of confounding factors and differences in methodologies used for microbiota analyses. Furthermore, gut barrier dysfunction associated with diet-induced obesity can lead per se to increased LPS translocation without significant alterations in gut microbial ecology.

Bacterial products may be translocated via different mechanisms, including transcellular and paracellular pathways. LPS could translocate via a transcellular epithelial pathway together with chylomicrons formed to incorporate dietary long-chain fatty acids in the form of triglycerides, which are finally released into the mesenteric lymph. This LPS translocation mechanism also requires TLR-4 expression by epithelial intestinal cells [75]. In blood, LPS-enriched chylomicrons exchange LPS with other lipoproteins, a process that requires the LPS-binding protein (LBP) and involves the soluble CD14 receptor, facilitating LPS transport to different tissues and blood vessels. LPS can also translocate by a transcellular pathway through intestinal-epithelial microfold cells (M-cells), which are more permeable and responsible for uptake of bacteria and bacterial antigens by the underlying lymphoid tissue, with a preference for Gram-negative bacteria [76]. Murine models of HFD-induced obesity have also demonstrated that live Gram-negative commensal intestinal bacteria (E. coli) can translocate to the blood and adipose tissue [77]. This translocation is dependent of innate immunity pattern-recognition receptors (TLR4 and Nod1) demonstrated by the fact it is blocked in mice lacking CD14 or Nod1 but increased in Myd88 knockout and ob/ob mice. This ‘metabolic bacteremia’ is thought to be mediated by DCs and reversed by administration of Bifidobacterium animalis subsp. lactis 420, which also improves the animals’ overall inflammatory and metabolic status. This study also suggests that leptin plays a role in intestinal bacterial adherence and translocation in the intestine since leptin treatment reduces translocation in ob/ob mice [77].

Alcohol ingestion and HFD, common in obese subjects, can also lead to increased intestinal permeability, which is reflected in alterations of tight-junction integrity and related proteins. This might facilitate the translocation of LPS and other bacterial components by a paracellular pathway [74]. Alterations in gut microbiota composition could also contribute to increasing paracellular permeability via alterations in tight-junctions, which could be secondary to excessive activation of inflammatory cytokine production (e.g. TNF-α; [78]). Thus, HFD-fed diabetic mice show alterations in gut bacteria, associated with increased intestinal permeability, characterized by reduced expression of genes coding for two tight
junction proteins ZO-1 and occludin, while antibiotic-treated mice recover normal intestinal epithelial integrity. This reveals the specific role of the microbiota, which seems to be greater than the role of diet in gut permeability [79]. A selective increase in *Bifidobacterium* spp. by feeding *ob/ob* mice with a prebiotic (oligofructose) also reduces the impact of the HFD-induced metabolic endotoxaemia, inflammatory tone and metabolic dysfunction and improves intestinal permeability, demonstrating that these effects are partly mediated by gut microbiota-induced changes [59]. The protective effects of the prebiotic on gut barrier function could also be explained by the reduction in plasma cytokines, known to promote tight-junction disruption, including TNFα, IL1β, IL1α, IL6 and INFγ [59]. These effects could also be attributed to the trophic effect of bacterial fermentation products (short-chain fatty acids [SCFAs] including butyrate) on the gut, leading to increased villus height and crypt depth and thickened mucosal layer [59, 80]. Researchers have also reported that prebiotic-microbiota-induced changes are associated with increased endogenous production of the glucagon-like peptide-2 (GLP-2), whose production may improve mucosal barrier function by increasing the rate of crypt cell proliferation and villus elongation, and reduce apoptosis [59]. In a more recent study, administration of the mucin-degrading bacterium *Akkermansia muciniphila* has also been shown to reverse metabolic endotoxemia and high-fat diet-induced metabolic disorders in mice obesity models, via restoration of gut barrier function and inflammation by increasing the intestinal levels of endocannabinoids (e.g. 2-arachidonoylglycerol and 2-oleoylglycerol) and mucus thickness [81].

**TLR-2, Lipoteichoic Acids and LPS**

TLR2 recognizes lipoteichoic acids (LTA) from Gram-positive bacteria and also LPS from Gram-negative bacteria, acting synergically with TLR4. Although TLR4-LPS activation is necessary to trigger an innate immune response, TLR2 participates in the up-regulation of genes encoding TNF-α and in the connection between innate and adaptive immunity [82].

In addition, TLR2 can be activated by saturated fatty acids [20]. Thus, TLR2 in conjunction with TLR4 can synergically contribute to insulin resistance in different tissues and constitute one of the links between gut microbiota components and metabolic dysfunction.

The role of TLR2 in metabolic dysfunction was directly evidenced by comparing effects of HFD on *Thr2(−/−)* mice and *Thr2(+/+)* mouse controls, showing that knock-outs were protected from the adverse metabolic effects of diet [83]. Glucose tolerance, insulin sensitivity, and insulin secretion were markedly improved, particularly in female *Thr2(−/−)* mice. This was paralleled by increased fat-burning rates in *Thr2(−/−)* mice as well as reduced tissue inflammation [83]. The specific role of gut microbiota was shown in studies demonstrating that TLR2-deficient mice, under germ-free conditions, were protected from HFD-induced insulin
resistance, whereas they were not under conventional conditions. TLR2-deficient mice conventionally colonized developed metabolic syndrome parallel to a threefold increase in Firmicutes and a slight increase in Bacteroidetes, accompanied by decreased Proteobacteria compared to wild-type controls [84]. This phenotype was reproduced when microbiota from conventionally reared TLR2-deficient mice was transplanted to *Bacillus*-monoassociated wild-type lean mice, and was subsequently reversed by antibiotic treatment. These findings prove that gut microbiota can define a specific phenotype regardless of the predisposing genotype for a specific condition. Increased LPS plasma levels may induce insulin resistance by interfering with insulin signaling, as in other models, but insulin resistance in TLR2-deficient mice has particular characteristics. There was activation of TLR4 in liver, muscles, and adipose tissue, associated with endoplasmic reticulum (ER) stress and JNK activation, but no activation of the IKKβ-IκB/NFκB pathway, probably due to lack of TLR4-TLR2 interactions in the knock-outs. While chronic activation of TLR4 by low doses of LPS is sufficient to increase JNK activation, the activation of the IKK/IκB/NF-κB pathway may also depend on the interplay of TLR2 and TLR4 [84].

TLR2 is also involved in regulating intestinal barrier function via modulation of tight-junctions. TLR2 deficiency leads to barrier dysfunction, reflected in decreased expression of the tight-junction protein zonula occludens (ZO)-1 in the ileum, which leads to increased gut permeability and increased LPS translocation and inflammation even in mice fed standard rodent chow. These effects are paralleled to *Bifidobacterium* spp. decreases while their increase leads to reduced gut permeability. Gut microbiota transplantation from TLR2-deficient mice to *Bacillus*-monoassociated wild-type mice also reduces ZO-1 expression in the ileum, proving the role of gut microbe-TLR2 interactions in this phenomenon [84].

**TLR5 and Flagellin**

TLR5 is expressed in the intestinal mucosa, recognizes flagellin and, upon ligand binding, induces an inflammatory response with TNFα production, contributing to defenses against infection. However, TLR5 may protect against metabolic syndrome as genetically deficient TLR5 mice exhibit hyperphagia and develop the main features of metabolic syndrome, including hyperlipidemia, hypertension, insulin resistance, and increased adiposity [85]. These metabolic dysfunctions correlated with changes in gut microbiota composition (diversity and phylotypes related to murine bacteria). Also gut microbiota transferred from TLR5-deficient mice to wild-type germ-free mice conferred many features of metabolic syndrome to recipients, demonstrating the role of the microbiota in this particular metabolic phenotype via interaction with the innate immune system. Metabolic syndrome in TLR5-deficient mice was exacerbated by a HFD. Food restriction prevented obesity, but not insulin resistance in the TLR5-deficient mice, suggesting that the latter effect is primarily dependent on TLR5-gut microbiota interactions [85].
**TLR9 and DNA**

TLR9 recognizes special DNA sequences ( unmethylated CpG motifs) and activates innate immunity. Translocation of bacterial DNA to the blood stream has been identified in animal models of metabolic dysfunction and also associated with the onset of diabetes and cardiovascular disease risk in humans [86]. Therefore, TLR9 activation constitutes another possible route by which bacterial components may contribute to metabolic diseases.

TLR9 is involved in non-alcoholic fatty liver disease, steatohepatitis and fibrosis, as shown by comparing wild-type and TLR9-deficient mice. In a nonalcoholic steatohepatitis murine model, induced by a choline-deficient amino acid-defined diet, TLR9 signaling induced IL-1β production by Kupffer cells, leading to steatosis, inflammation, and fibrosis [87]. Steatohepatitis and fibrosis were also reduced in mice deficient in MyD88, an adaptor molecule for TLR9 and IL-1R signaling [87]. However, the aforementioned studies did not specifically evaluate relationships with the gut microbiota.

**NOD1/2 and Peptidoglycan**

Nucleotide oligomerization domain (NOD) proteins NOD1 and NOD2 are members of the NOD-like receptor (NLR) family in mammals. These are cytosolic pattern recognition receptors, expressed not only in immune but also in metabolic tissues, which play a role in detecting intracellular microorganisms. These receptors propagate inflammatory signals in response to bacterial peptidoglycan (PGN). NOD1 detects d-glutamyl-meso-diaminopimelic acid (meso-DAP)-containing PGN found principally in Gram-negative bacteria, whereas NOD2 detects muramyl dipeptide (MDP) present in all bacteria, though more abundant in Gram-positive bacteria [88]. NOD1 is expressed in all cell types and required for NF-κB activation by Gram-negative bacterial infection, once the bacteria have bypassed TLR activation [89]. NOD2 is expressed in monocytes/macrophages and DCs and induced in intestinal epithelial cells by TNF-α. NOD2 mutations have also been associated with defective IL-10 production, and Crohn’s disease in humans [90].

PGN levels are lower in the serum of germ-free and antibiotic-treated mice [91]. Germ-free mice are protected from HFD-induced insulin resistance and antibiotic treatment in conventionally colonized mice attenuates the HFD-induced metabolic dysfunctions. Altogether this suggests that PGN is a potential factor linking innate immunity and metabolic dysfunction [91]. The fact mice deficient in NOD1 and NOD2 peptidoglycan receptors are protected from HFD-induced inflammation and insulin intolerance is evidence of causality. Activation of NOD1 causes acute systemic insulin resistance, as demonstrated in mice injected with mimetics of meso-diaminopimelic acid-containing PGN or the minimal bioactive PGN motif, which activate NOD1 and NOD2, respectively. Ex vivo, NOD1 ligand
can cause pro-inflammatory cytokine secretion and impaired insulin-stimulated glucose uptake in adipocytes and also cause inflammation and insulin resistance in primary hepatocytes from wild type, but not NOD1(−/−), mice [92]. PGN motifs acting on NOD2, but not those acting on NOD1, induce muscle cell-autonomous insulin resistance [91]. NOD1 mediates insulin resistance by acting on adipocytes/hepatocytes, and NOD2 by acting on myocytes, through mechanisms activating common pathways such as the MAPKs (p38, JNK, ERK1/2) pathway, expression and production of proinflammatory cytokines/chemokines, and impairment of insulin signaling at the level of IRS-1. However, we do not know why these metabolic tissues utilize divergent intracellular innate immune sensors [88]. Overall, it can be concluded that NOD1-activating PGN causes peripheral insulin resistance, involving the complex crosstalk between hepatic and adipose tissues, which is indirectly manifested in skeletal muscles. In contrast, NOD2-activating bacterial PGN motifs cause a milder insulin resistance that affects skeletal muscle.

NLRP6 and NLRP3 Inflammasomes

Inflammasomes are signaling platforms that sense diverse microbial products as well as stress and damage-associated endogenous signals. Inflammasome complexes can be formed by members of the NOD-like receptor family or the PYHIN family AIM2. Upon formation, inflammasomes trigger proteolysis of caspase-1, which cleaves the cytokine precursors of IL-1β and IL-18 to initiate a pro-inflammatory and antimicrobial response. Research has linked inflammasome activation to metabolic disorders, including atherosclerosis, type 2 diabetes, liver disease and obesity [69].

Inflammasome-deficiency is associated with changes in gut microbiota composition, parallel to exacerbated hepatic steatosis and inflammation through influx of TLR4 and TLR9 agonists in the portal circulation, leading to enhanced hepatic TNF-α expression, which drives disease progression [93]. Co-housing of inflammasome-deficient mice with wild-type mice, implying microbiota exchanges by coprophagy, results in exacerbation of hepatic steatosis and obesity in wild-type mice. These findings demonstrate that defective NLRP3 and NLRP6 inflammasome sensing alters interactions between the gut microbiota and the host innate immune system, possibly contributing to metabolic complications [93].

Influence of Gut Microbiota in Macrophage Infiltration in Peripheral Tissues

Different studies show that gut microbiota, and its modulation by dietary intervention, could influence migration and infiltration of macrophages into peripheral tissues, this being a major feature of obesity-induced metabolic dysfunction. For
example, in ob/ob mice fed a normal diet, prebiotic administration and the consequent increase in intestinal bifidobacterial numbers, reduced several serum inflammatory and anti-inflammatory cytokines (IL-1β, TNF-α, IL-18, and IL-15) as well as the main chemokine (MCP-1) involved in monocyte/macrophage migration and infiltration in the adipose tissue [60].

When mice with HFD-induced obesity and gut microbiota imbalances were administered *B. pseudocatenulatum* CECT 7765, there was a reduction in serum inflammatory cytokines and chemokines of the innate immune system (IL-6 and MCP-1) and a decline in macrophage infiltration in adipose tissue, presumably due to lowered MCP-1 production [6]. These changes in inflammatory markers were accompanied by improvements in glucose tolerance and insulin sensitivity in HFD-fed mice administered *B. pseudocatenulatum* CECT 7765, as well as partial restoration of HFD-induced gut microbiota imbalances [94]. These finding reveal that gut microbiota modulation might help to ameliorate metabolic dysfunction via regulation of macrophage chemoattractants.

Gut microbiota alterations induced by chronic treatment with olanzapine are also suspect to be involved in infiltration of macrophages in adipose tissue and metabolic dysfunction associated with the consumption of this antipsychotic. This hypothesis has been proven by showing that gut microbiota alterations induced by antibiotic administration (neomycin, metronidazole and polymyxin B) to chronically olanzapine treated female rats reduces metabolic alteration caused by olanzapine alone, including body weight gain, uterine fat deposition and plasma free fatty acid levels and macrophage infiltration of adipose tissue [95].

**Influence of Gut Microbiota on Adaptive Immunity Alterations Associated with Obesity and Metabolic Dysfunction**

Fewer studies report the possible influence of the gut microbiota on the adaptive immune system and its role in the chronic low-grade inflammation associated with metabolic disorders. However, proof of concept of the role played by gut microbiota can be found in studies demonstrating the beneficial effects of intervention with specific bacterial strains on adaptive immune function in animal models of obesity. These beneficial effects seem to be mediated mainly by inducing Tregs, which express the transcription factor Foxp3 and act by limiting proliferation of effector CD4+ T cells, which are often critical in regulating intestinal inflammation [96].

In mice with Western diet-induced obesity (characterized by a CD4(+) Th17-biased immune profile and changes in microbial communities) the administration of *L. reuteri* ATCC 6475 shifted this pro-inflammatory immune cell profile and prevented abdominal fat pathology and age-associated weight gain [22]. The bacterial effects were mediated by induction of Foxp3(+) Tregs and IL-10 in colonic
mesenteric lymph nodes, without significantly influencing gut microbiota composition. Furthermore, these microbe-related beneficial effects were transferable into naïve recipients by adoptive transfer of purified *L. reuteri*-induced CD4(+) Foxp3+ T cells [22].

In mice with HFD-induced obesity, *B. pseudocatenulatum* CECT 7765 supplementation increased cytokine production of the adaptive immune system, including the anti-inflammatory cytokine IL-4 [6]. In the context of obesity, this cytokine together with IL-13 contribute to macrophage differentiation into M2 macrophages, which secrete the anti-inflammatory cytokine IL-10, thus helping to control inflammation and promote normal insulin sensitivity [97]. IL-4 also mediates Th2 lymphocyte differentiation and inhibits production of inflammatory cytokines such as IL-1β, TNF-α and IL-6.

*A. muciniphila*, a newly discovered mucus-degrading bacterium of the human gut, improves glucose tolerance in HFD-fed mice by inducing Foxp3 Tregs in the white adipose tissue. This effect has been related to reduced gene expression of pro-inflammatory cytokines (IL-1β and IL-6) but not to changes in M1/M2 or CD4/CD8 T cell ratios, altered by HFD [39].

A recent study investigating the possible protective effect of *H. pylori* in diet-related disorders reported that it favorably modulates glucose metabolism and suppressed weight gain in db/db mice (lacking the long isoform of the leptin receptor) and mice with diet-induced obesity, particularly when animals were colonized by a non-pathogenic strain negative for *cag PAI* (cytotoxin-associated gene pathogenicity island) [98]. The effects were mediated by up-regulation of gastric PPAR γ-responsive genes (i.e., *CD36* and *FABP4*) parallel to decreased white adipose tissue macrophages and increased adipose tissue Tregs, since the effects were impaired in mice deficient in PPAR γ in immune and epithelial cells [98].

Although the precise mechanisms by which microbiota exerts these Treg inductive effects are unknown, short-chain fatty acids (SCFAs) derived from gut microbiota fermentative activity are one of the possible actors [96]. SCFAs contribute to regulating the size and function of the colonic Treg pool, specifically inducing Foxp3+IL-10-producing Tregs but not colonic Foxp3+TGFβ+cTregs, colonic Th17 and Th1 or MLN cell and splenic Tregs [96].

TLR2-deficient mice also have lower Tregs in visceral adipose tissue, suggesting this pattern-recognition receptor may also contribute to regulate insulin resistance, an effect that in turn can be influenced by gut microbiota molecules recognized by this receptor [84].

**Influence of Gut Microbiota on Decreased Immunological Surveillance Associated with Obesity and Metabolic Dysfunction**

There is scarce research into the potential role of gut microbiota in immunological dysfunction, leading to weakened host responses against infections and vaccination. Recent studies have demonstrated that when mice with HFD-induced obesity are
fed *B. pseudocatenulatum* CECT 7765 or *Bacteroides uniformis* CECT 7771, the oxidative burst of macrophages, which reflects their role in phagocytosis, is increased parallel to restoration of HFD-induced microbiota imbalances [6, 94]. Administration of *B. pseudocatenulatum* CECT 7765 or *B. uniformis* CECT 7771 to HFD-fed mice also improved the ability of DCs to activate T-lymphocyte proliferation, a function also adversely affected by HFD-induced obesity in mice [6, 94]. These findings indicate that modifying the gut microbiota may contribute to restoring host defense mechanisms impaired by diet-induced obesity in mice.

Studies of rodents with genetic deficiency in leptin or leptin receptors, reveal obesity-related deficits in macrophage phagocytosis via alterations in phospholipase activation and reduced pro-inflammatory cytokine secretion (e.g. TNF-α and IL-6) in vivo and in vitro. These effects may be due to leptin deficiency as exogenous leptin up-regulated both phagocytosis and proinflammatory cytokine production by macrophages [51]. In leptin-deficient models of obesity (*ob/ob* mice), DCs and their role in T cell priming is also adversely affected. DCs from *ob/ob* mice are less able to activate allogenic T cells in vitro. The impaired functionality of DCs may be related to increased secretion of the immunosuppressive cytokine TGF-β, rather than to changes in expression of activation markers, which could be due to the absence of leptin in *ob/ob* mice [53]. Leptin can improve DCs functions and survival, driving naïve T cell polarization toward a Th1 type phenotype by activating NF-κB and exerting an antiapoptotic effect via up-regulation of gene expression (bcl-2 and bcl-xL). These results demonstrate the ability of leptin to improve DCs and T cells functions and to promote DC survival [54], which could be important in defense against pathogens and in response to vaccination. Decreased leptin plasma concentration in food-deprived animals or malnourished humans impairs immune functions similarly to those detected in leptin-deficient mice.

Similar immune defense mechanism dysfunctions have been demonstrated in a murine model of HFD-induced obesity, the most common form of obesity characterized by hyperleptinemia, presumably due to leptin resistance and, therefore, lack of adequate leptin functionality [6, 94]. Alterations in macrophage, DCs and T-cell function, identified in both models of obesity (genetically or diet-induced), may also be related to obesity-associated alterations in glucose uptake and metabolism, possibly affecting immune cells which are sensitive to insulin because glucose is their major energy source [20].

**Conclusions and Future Perspectives**

Scientific evidence supports a role of gut microbiota in immunological dysfunctions associated with obesity and metabolic disease, including intestinal and systemic chronic low-grade inflammation, and diminished responses against infections and vaccination. The interdependency of diet and gut microbiota is evident in that diet constitutes a major factor influencing gut microbiota structure and function.
Moreover, both dietary lipids and gut microbes can exacerbate inflammation by activating similar pattern-recognition receptors and signaling pathways of the innate immune system. Furthermore, it has been evidenced that intestinal inflammation is an early event preceding obesity and metabolic disease and the fact that this can be altered by dietary-modulation of the gut microbiota paves the way for novel preventive dietary intervention strategies, designed to combat these disorders. In this context, it is essential to identify the exact immunological processes that are sensitive to gut microbiota interactions within a specific dietary context and to gain a better understanding of the role gut microbiota plays in early responses particularly of the adaptive immune system to high calorie diets.

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References


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