52 Inflammatory Causes of Obesity and Metabolic Diseases

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CONTENTS

52.1 INTRODUCTION

The dramatic rise in obesity is unavoidably linked to globally changing social trends toward increased energy intake and reduced energy expenditure. With the rise in obesity, a cluster of pathologies including diabetes, atherosclerosis, steatohepatitis, hypertension, dyslipidemia, and some cancers, collectively known as the metabolic syndrome, has been climbing to disturbing proportions. The obesity-related disorders have become the leading public health challenge for not only the developed countries but also in the developing ones, and without sparing the children. Hence, the research efforts to identify the causative molecular mechanisms and effective therapeutic targets to combat this epidemic have gained great momentum in the last decade. Genetic studies that investigated the individual differences in predisposition to obesity and related disorders could identify a few obesity susceptibility genes in humans that in isolation could explain the disease phenotype. These studies clearly demonstrated that the genetic makeup of the host is an important parameter in the complex interactions between energy intake, expenditure, and deposition in fat stores that underlie weight gain. However, the phenotypic outcomes related to genetic variation with smaller effect size are a function of dynamic interactions between complex dietary input and bewildering arrays of microbes, the natural and diverse occupants of the gut, determining the metabolic health or disease of the host and contributing to the pathogenesis of obesity, insulin resistance, diabetes, and steatohepatitis. In this chapter, we present an overview of the complex molecular interactions that take place between the environment, diet, genetic, and microbial participants at the metabolic and immune interface to incite metaflammation, the low-grade, chronic, and metabolically driven inflammatory changes underlying chronic metabolic disorders associated with obesity.

52.2 OBESITY AND METABOLIC DISEASE: INTEGRATION OF THE HOST, PATHOGENS, AND DIET

Genetic studies in humans and experimental findings from mice suggest that satiety, energy intake, expenditure, and deposition are regulated by the integration of central nervous system and peripheral organs, particularly the hypothalamus and through the hypothalamic actions of factors derived from metabolic organs such as the adipose tissue. The outcome of the studies on family-based linkage analyses of monogenic (Mendelian) disorders (with severe and early-onset obesity) and common forms of population-based obesity studies links excess body weight and adiposity to an extreme tilting of an "adipostatic set point" at which body fat stores are normally stabilized.[1] For example, mutations associated with extreme and early-onset obesity were discovered in leptin (*LEP*), leptin receptor (*LEPR*), and melanocortin 4 receptor (*MC4R*) genes, all of which target the hypothalamic regulatory circuits.[2–5] Genome-wide population genetic studies contributed to the identification of additional mutations in genes that also target the central nervous system or other endocrine pathways, although the effect size in general is rather small.[6–10] While the majority of genetic studies support a strong genetic component, the target pathways remain to be established in obesity in concert with environmental influences and interactions between peripheral endocrine signals. There is yet a lack of evidence of significant associations between obesity and genetic variations related to intrinsic factors such as the basal metabolic rate, energy expenditure, or the drive to exercise.[10,11] These are important areas of current research efforts in experimental systems as well as in humans, and they indicate the power of the homeostatic drive to establish equilibrium, which is resistant to most single assaults.

The dietary components that give rise to obesity and associated metabolic pathologies are discussed in detail elsewhere in this book and hence not covered here. However, the critical contribution, as well as the complexity, of the diet needs to be emphasized here. In addition to the dietary input and the genetic contribution of the host, which is unequivocal, trillions of accompanying gut microbiota (with overwhelmingly more cells, genes, and metabolites than the host) participate in metabolic homeostasis, through their direct products or by modulation of the dietary environment. The present chapter discusses this aspect in further detail below. Hence, the metabolic health and disease in free-living humans, as well as in experimental models, are functions of the integration of three major components: diet, genetic variation, and microbiome.

52.3 EVOLUTIONARY CONNECTIONS BETWEEN METABOLISM AND IMMUNITY

Overwhelming evidence supports a close functional and molecular integration between metabolic and immune systems that is crucial for systemic homeostasis and whose deregulation is causally linked to obesity and associated diseases such as insulin resistance, diabetes, fatty liver disease, and atherosclerosis.[12–15] Evolutionarily, survival in the face of insufficient or irregular food supply and abundant new pathogens may have been the catalysis for the coevolution of nutrient- and pathogen-sensing and response systems. Integration of these systems may once have ensured energy efficiency and storage in preparation for times of food deprivation or fighting off infections. Indeed, mounting a potent immune response is energetically costly; fever, expansion of immune cells, and their recruitment, phagocytosis, and humoral responses can all place a large bioenergetic demand on the organism.[16,17] For example, sepsis increases the basal metabolic rate by 40%. The increases of 1°C in body temperature during fever requires around 10% caloric expansion.[18] On the contrary, malnutrition and starvation as well as obesity all severely impair the integrity and proper regulation of the immune response. In rodents, significant reductions in total energy depots compromise the humoral immune response. The induction of an immune response in starving insects reduces their survival. Also, biological processes like reproduction, lactation, and thermoregulation have to compete with immune defense, particularly in conditions of energy limitations or deficit. Taken together, the immune system cannot operate properly when an organism is deprived of nutrients and energy or fails to deploy energy in proper temporal and spatial order (Figure 52.1).

Modern human reality, however, is not only one of nutrient deficiency but also pertains to energy and nutrient excess as evidenced by the obesity pandemic. Many components of the immune system including macrophages, mast cells, and T-cell-mediated immune responses, as well as neutrophil and natural killer cell activities, are altered in the obese state.[19,20] The energy surplus in obesity is associated with impaired immune responses and drives a chronic, sterile inflammatory state referred to as metaflammation (referring to metabolically orchestrated chronic inflammation).[21] Metabolic

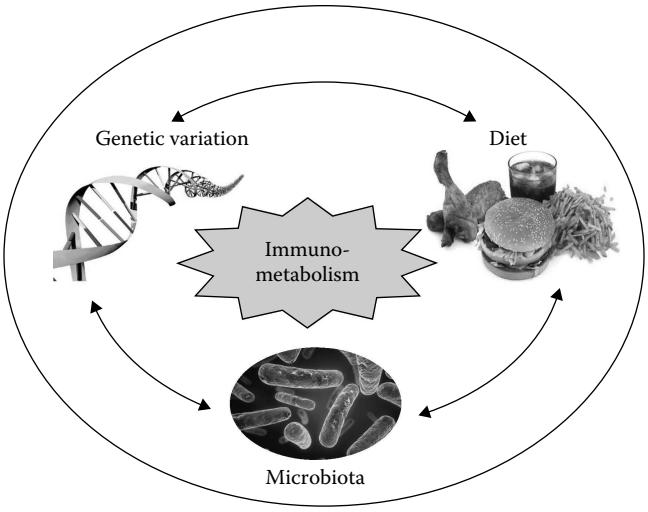

FIGURE 52.1 Immunometabolism—a network of complex interactions between metabolic and immune pathways in physiology and pathology. The complex molecular interactions that take place between genetic variation, diet and environment, and microbial occupants (of the gut) at the metabolic and immune interface incite metabolically driven, low-grade, chronic inflammatory changes named metaflammation underlying metabolic syndrome. This interface represents a rapidly growing field of research known as immunometabolism. The focus of immunometabolism research is on the close functional and molecular integration between metabolic and immune systems that is crucial for systemic homeostasis as well as these systems' deregulation that has been causally linked to obesity and associated diseases such as insulin resistance, diabetes, and fatty liver disease. The consideration of each component and their integrated output is necessary to link potential mechanisms with outcomes in a context-dependent manner.

inflammation is now recognized as a major underlying factor for reduced insulin sensitivity, abnormal glucose and lipid metabolism, and the development of type 2 diabetes and steatohepatitis, particularly, but not exclusively, in the context of obesity.[21,22] Historically, the earliest indication of the association between inflammation and insulin resistance goes back to the observations made in the late 1950s when an elusive "insulin antagonist" activity was described in the serum of a patient suffering from foot gangrene and infection; when this patient's serum was transferred to mice, it could antagonize the hypoglycemic effects of insulin.[23–25] In the 1980s, Feingold and Grunfeld demonstrated that infection or administration of tumor necrosis factor α (*TNF*α), a pro-inflammatory cytokine, caused dyslipidemia and metabolic abnormalities.[26,27] Bagby and Lang also demonstrated the impact of *TNF*α administration in inducing insulin resistance.[28,29]

The first evidence regarding the presence of inflammation in obesity and its contribution to the pathogenesis of insulin resistance and type 2 diabetes came in the early 1990s by the discovery of adipose tissue inflammatory changes associated with obesity in experimental models[30] and subsequently in humans.[31,32] Other studies showed that exposure to inflammatory cytokines in adipocytes or liver cells caused insulin resistance by blocking postinsulin receptor signaling.[33–35] Importantly, a large number of independent studies

demonstrated that insulin sensitivity could be improved in obese mice or rats by neutralization of *TNF*α using molecular or genetic approaches to block the function of this pathway.[30,36–43] Many more studies since have provided support that chronic inflammation in metabolic tissues plays a central role in the pathogenesis of insulin resistance, diabetes, steatohepatitis, and cardiovascular disease.[13–15,44,45] Important advances also came from understanding the immune components that contribute to metabolic inflammation (discussed later in Section 52.8 in further detail) and discovery of key signaling pathways that produce metaflammation.[13,15,46–49] Today, the studies starting with the obesity-induced adipose tissue inflammation have greatly expanded, leading to the establishment of the field of immunometabolism.

52.4 FEATURES AND MECHANISMS OF METABOLIC INFLAMMATION

It is critical to note that obesity leads to an aberrant form of immunity or metaflammation in a specific and unique energetic context; the inflammation in obesity occurs in the presence of excess nutrients and energy in metabolic tissues, predominantly the adipose tissue but also in many other critical organs such as liver, pancreas, and the central nervous system.[21,50] However, this inflammation does not feature an increase in energy expenditure or basal metabolic rate and raises the possibility that this may in fact have originated from an adaptive mechanism, perhaps under intermittent exposure to food, to prevent sustained insulin sensitivity, hence promotion of adipose expansion and obesity.[34] Many cytokines, including *TNF*α, interleukin-6 (IL-6), IL-1β, and monocyte chemoattractant protein-1 (MCP-1), are increasingly expressed and secreted from the adipose tissue of obese subjects as well as from other metabolic target cells and tissues.[51,52] Their local concentrations can be high with strong autocrine/paracrine influence (such as inhibiting insulin receptor signaling and recruitment and activation of immune cells), but their levels in systemic circulation remain low in comparison to classic inflammatory situations such as sepsis, infection, or trauma.[51] Furthermore, signaling pathways that may be responsible for the production of these cytokines, such as inhibitor of kappa B kinase (IKK), c-jun terminal kinase (JNK), and protein kinase RNA-activated (PKR) pathways, are also upregulated in the metabolic tissues in obesity, and their ablation through genetic or chemical approaches proved to be beneficial for insulin sensitivity or metabolic homeostasis in obese mice.[15,53–56] Unlike classic inflammation, obesity-induced metaflammation is uniquely characterized by energy conservation. Of note, blocking JNK, IKKε, and PKR derepresses energy expenditure while preventing the inflammatory responses, thereby generating the most consistent and substantial impact on systemic metabolic improvements.[53–55]

The intricate links between nutrient-sensing and pathogen-sensing systems have been weaved into the architecture of metabolic organs. For example, the fruit fly's fat body is a single organ that coordinates both the immune and metabolic responses.[57] Similar functional, temporal, and physical contiguity of energy and nutrient stores for normal function of immune cells can be found in higher organisms too. Metabolically active hepatocytes are found side by side with macrophage-like Kupffer cells in the liver.[21] The mesenteric adipose tissue is embedded with lymph nodes and harbors various immune effector cells. The activation of local immune response triggers selective lipolysis of the perinodal fat tissues, suggesting the purposeful colocalization of immune cells with energy stores in this example may be to meet the excessive energy demands of mounting an immune response.[58] While the interaction between resident immune cells and the stromal components is essential for tissue homeostasis in general, this kind of proximity to rich local energy sources can become particularly important during infection, coupled to suppression of appetite, weight loss, and the consequent systemic deficit in deliverable fuels.[20] Additionally, the perinodal adipose tissue has a high content of polyunsaturated fatty acids and could supply them to neighboring immune cells for the generation of lipid-based immune mediators such as prostaglandins and leukotrienes.[59] The intimate and dynamic relationship between immune cells and local energy depots could thus influence both the immune response and metabolism as is abundantly evident in chronic metabolic diseases such as obesity and diabetes. These intricate links also appear to be emphasized in some chronic inflammatory diseases, which are accompanied by adipose tissue remodeling such as the panniculitis associated with inflammatory bowel disease or the HIV-associated adipose tissue redistribution.[60,61] It is thus not surprising that metabolic abnormalities are also common in many chronic inflammatory diseases. For example, patients with psoriasis, a systemic autoimmune disease influencing the skin, or rheumatoid arthritis, an autoimmune disease mainly affecting the joints, carry markedly elevated risk of developing insulin resistance, diabetes, and atherosclerosis. There is also compelling emerging data that blocking inflammatory pathways, most efficiently by *TNF*α neutralization, can result in a marked reduction in the incidence of diabetes among subjects with psoriasis and rheumatoid arthritis.[62]

In addition to colocalizing anatomically, the genetic similarities and functional overlap that can be seen in metabolic and immune cells are striking and further accentuated with obesity. Despite originating from distinct lineages, macrophages and adipocytes resemble each other in relation to specific functions and genomic expression profiles. For example, preadipocytes, similar to macrophages, express nicotinamide adenine dinucleotide phosphate oxidase and can support phagocytosis.[63] Adipocytes express the pattern recognition receptors, toll-like receptor (TLR), PKR, inflammasome components, and T-cell receptors that can be activated by nutrients, pathogens, and lipopolysaccharide (LPS); activate inflammatory signaling cascades in a cell autonomous manner; and secrete various cytokines and chemokines when metabolically stressed and regulate the responses of immune cells.[64] The transdifferentiation of preadipocytes into macrophage-like cells has also been observed. Consistently, transcriptional profiling reveals a striking resemblance between preadipoctyes, adipocytes, and macrophages. The list of shared genes is further

increased when macrophages are transformed to lipid-laden, pro-atherogenic foam cells.[12,63,65] Similar to adipocytes, intestinal epithelium was also found to reactivate an ancient capacity to respond to pathogens. In a recent study, it was shown that intestinal epithelium upregulates its interferon-inducible immune response pathways at the expense of its metabolic functions when faced with microbiota under conditions when the adaptive immune system is impaired.[66]

On the contrary, immune cells also express metabolic programs and are equipped to actively monitor nutrients and energy sources to dictate the inflammatory capacity of the effector cells. For example, macrophages express the receptor for advanced glycation end products that recognize lipids and nucleic acids produced as a result of oxidative stress and hyperglycemia; activate lipid synthesis and storage programs through the activity of transcription factors like peroxisome proliferator-activated receptor (PPAR), liver X receptor (LXR), and sterol regulatory element-binding protein (SREBP); and generate lipid droplets as they transform to foam cells.[12,67] Furthermore, PPAR and LXR not only activate transcription of genes involved in lipogenesis, but they also limit inflammatory output.[68,69] PPARγ regulates the phenotypic switch between M1 and M2 in macrophages recruited to the adipose tissue in obesity. These studies also showed that PPARγ-induced M2 polarization is protective against insulin resistance in diet-induced obesity; mice with macrophage-specific deletion of PPARγ exhibit increased insulin resistance and obesity.[70] A recent study also demonstrated an immunoregulatory role for SREBP-1a through upregulating components of the inflammasome complex, further supporting the link between inflammation and lipid metabolism.[71] These observations underscore the coevolution of transcriptional networks to respond to a wide range of challenges from nutrient status to pathogens. Whether functional or genetic similarities exist between other metabolic cells and other immune effectors is not as well characterized. However, all immune cells rely on active metabolism in preparation for and during immune response. Glucose and lipids are important for fueling the proliferation of immune cells like lymphocytes.[72] In addition, glucose and lipids energize an immune attack by macrophages and neutrophils, particularly phagocytic activity, which consumes copious amounts of energy and requires a high rate of lipid turnover.[73] Lipids can also be important in the generation of lipid-based immune mediators secreted from these cells, and by altering membrane organization and domains, they can impair immune function.[74,75] Finally, lipids and other nutrients can serve as signals or even ligands to engage specific immune pathways.[12,54,76,77] In summary, the immune and the metabolic systems intercept at many levels, from molecules to cells to organs, and this can serve for the benefit or the detriment of the organism, depending on the context within which they interact.

52.5 ORIGINS OF INFLAMMATION IN OBESITY

The traditional initiators of inflammatory response are pathogens (such as microbes, viruses, and parasites) or tissue damage. These components engage inflammatory signaling pathways and induce a response that could contain the hazard and establish tissue homeostasis. The proximal mechanisms responsible for obesity- or high-fat diet-induced metaflammation leading to metabolic pathologies are not fully understood and remain an important area of research. Nutrients may initiate inflammation from within the metabolic cells (such as adipocytes, hepatocytes, myocytes, and pancreatic cells) or in immune cells (such as macrophages, mast cells, and lymphocytes) recruited to metabolic organs or both. One potential mechanism involves nutrients directly engaging inflammatory responses on the cell membrane through immune receptors. For instance, high concentrations of saturated fatty acids stimulate signaling through the TLR, a pattern recognition receptor pathway in adipocytes and macrophages. However, whether this is based on a direct interaction with the TLR receptor or indirectly through fatty acid metabolites like ceramide has not been determined.[78] Recently, a role for fetuin in lipid–ligand interaction with the TLR has been described, lending support to direct engagement of innate immune pathways by nutrients or some specialization for nutrients, however, distinguishing it from other signals.[76] Obesity, with high levels of saturated fatty acids, is associated with heightened TLR signaling, and the genetic ablation of TLR4 in mice was found protective against weight gain on chow diet.[79] Bone marrow transplantation chimeras for the *TLR4*$^{-/-}$ and the *Myd88*$^{-/-}$ genotype in mice display protection against insulin resistance on a high-fat diet.[80] Later studies presented a mixed picture, however, where either *TLR4*$^{-/-}$ on a different background or mice lacking the primary mediator of TLR and IL-1R, the myeloid differentiation primary response protein 88 (Myd88), were not protected against insulin resistance when compared to wild-type, control mice on a high-fat diet.[81–84] In light of recent findings, these conflicting results are likely because of the colonization of the gut microbiota that differs among facilities, handling, and specific diets, as directly demonstrated in a recent report.[85] In fact, divergent metabolic outcomes in animal models with single-gene or pathway modifications are not uncommon and most likely reflect the contribution of diet and microbiota to the impact of the underlying genetic manipulation. Further studies are needed to determine the contribution of specific pathways downstream of TLRs and other sensing and signaling molecules that are triggered by nutrients, how and which combination of these factors determines the outcomes.

In addition to membrane-based activation of inflammation, nutrients could engage inflammatory signal transduction pathways in metabolic and immune cells. For example, obesity leads to increased ceramide synthesis and production of reactive oxygen species (ROS) from the endoplasmic reticulum (ER) and the mitochondria. Both ceramide and ROS may activate the cytosolic inflammasome, a multiprotein complex that consists of nucleotide-binding domain (NOD)–, leucine-rich repeats–, and pyrin domain–containing family member protein, the adaptor molecule apoptosis-associated speck-like protein containing a caspase recruitment domain, and pro-caspase-1. This complex is

normally activated by pathogen-derived molecular patterns and cleaves pro-caspase 1, thereby activating it. The active caspase-1 then cleaves pro-IL-18 and pro-IL-1β.[86] Indeed, the activation of the inflammasome and elevated IL-1β levels are well documented in obesity in human and experimental models. Furthermore, genetic deficiency for caspase-1 and NLRP3 (and other inflammasomes including NOD1 and NOD2) improves systemic glucose homeostasis and insulin sensitivity.[87] Recently, a novel and critical role for the double-stranded RNA-dependent protein kinase (PKR) was defined in inflammasome activation.[88] Importantly, PKR deficiency in mice also leads to major metabolic phenotypes including protection against high-fat-diet-induced weight gain, inflammation, and insulin resistance and preservation of metabolic health in obesity.[54] The discovery of PKR's integral role in the inflammasome may provide an important advance in the understanding of coupling innate immune responses to metabolic stimulus and may lead to further understanding of the signals that initiate immune responses in a metabolic context and integrate insulin signaling, translational control, and immune response.

The integration between nutrients and inflammation may also potentially occur in the nucleus. Obesity is associated with an accelerated aging phenotype of the adipose tissue that impacts inflammation and insulin action. It was recently shown that cellular tumor antigen p53 is not only activated in response to shortening telomeres that happens during aging but also by excessive caloric intake and high-fat diet characterized by oxidative stress, senescence-like changes, and elevated p53 levels in the adipose tissue. Adipose tissue–specific p53 deficiency (*TRP53*$^{-/-}$) in mice protected against insulin resistance and adipose tissue inflammation induced by genetic or diet-induced obesity.[89] Overexpression of p53 in the adipose tissue resulted in inflammation and insulin resistance. Bone marrow transplantation from wild-type to adipose tissue–specific *TRP53*$^{-/-}$ mice partially improved insulin sensitivity, demonstrating the important contribution of macrophage p53 to systemic glucose homeostasis. The authors of this particular study showed that telomere shortening (that occurs in aging) is linked to metabolic deterioration; using telomerase-deficient mice, the authors demonstrated that increased DNA damage and senescence markers in the adipose tissue correlate with insulin resistance and inflammation.[89] These observations suggest that obesity-induced, aging-like changes in the adipose tissue are linked to inflammation, insulin resistance, and metabolic dysfunction through an important nuclear regulator of senescence, p53, and thus offer an additional mechanism by which metabolic derangements can trigger immune response, such as is the case in obesity.

52.6 IMPACT OF MICROBIOME ON SYSTEMIC METABOLISM

In recent years, a large body of evidence emerged demonstrating the critical importance of microbial communities in the regulation of systemic metabolism. The gut microbiota that play a critical role at the juncture of the environment and the host belong to four major bacterial phyla: the gram-negative Bacteroidetes and Proteobacteria and the gram-positive Actinobacteria and Firmicutes.[90] Early studies showed genetic obesity (in leptin-deficient mice) correlates with a reduced ratio of Bacteroidetes to Firmicutes.[91] Diet (high-fat and high-polysaccharide) generates a similar change that can be reversed with antibiotics or weight loss.[92] In most but not all human studies, a reduced Bacteroidetes/Firmicutes ratio in obesity has also been observed.[93–95] Some of the discrepancies may have been due to confounding factors such as varying diets, antibiotics usage, housing conditions, and environmental factors between the breeding facilities that could impact the initial colonization of the experimental groups as well as phenotypic outcomes.[85,96] Future studies based on the analysis of metagenomic-derived functional biomarkers rather than phylogenetic ones could help refine the data and define stronger associations with specific populations. What is consistent among the studies is that both diet, the primary nutritional source for the intestinal bacteria, and the genetic makeup of the host directly influence the microbial populations and their metabolic consequences, adding yet another level of complexity. In other words, in addition to the direct effects of diet and microbiota on their own, the energy extraction from the diet, as well as the byproducts of metabolism, could be influenced by the interactions between them. Remarkably, the metabolic phenotype associated with the microbiota could be transferred to germ-free mice in both genetic and diet-induced obesity.[92,97] Furthermore, a dietary shift from low to high fat swiftly altered the gut microbiota in humanized mice (colonized with human intestinal microbiota).[98] In the future, it may be possible to directly test the metabolic consequences of the disappearing human gut microbial complexity in mice by utilizing these humanized models. More studies in humans are also needed to determine the relevance to human disease of these conclusions derived from mouse studies.

How do the changes in the gut microbiota contribute to obesity and metabolic deterioration? Again, this paradigm is a prime example of the interactions between the genetic makeup of the host, diet, and the identity, composition, function, and output of the pathogen populations. For example, it is possible that certain microbial species are more efficient than others in extracting energy and contributing to weight gain.[97] The gut microbiota can convert nondigestible carbohydrates (fibers) into short-chain fatty acids (SCFA). These diet-derived SCFA products can be oxidized to provide energy for the host and delivered directly to the liver through the portal vein.[99] The increased flux of fatty acids could lead to steatohepatitis, followed by insulin resistance in the liver and gradual impairment of systemic glucose metabolism.[100] Whether these products are directly transported or need to interact with trafficking proteins is not known, and their exploration presents formidable experimental challenges.

In an alternative mechanism, high-fat diet and certain pathogens instigate inflammatory changes and epithelial dysfunction and thus elevate the overall permeability in

the gut. The resulting leakiness of the gut could lead to an increased delivery of gut microbiota or their metabolites to the mesenteric fat bordering on the gut.[101–103] What these microbial-derived factors are and which host metabolic targets they modify remain unanswered questions of profound interest. Recent studies elaborated angiopoietin-like protein 4 (Angptl4) as one host target, intestinal expression of which can be inhibited by the gut microbiota.[104] Angptl4 can increase plasma triglyceride levels by inhibiting lipoprotein lipase-mediated lipolysis of lipoproteins and consequent fatty acid mobilization toward adipose tissue.[105,106] Furthermore, hypothalamic actions of Anglpltl4 also suggests a potential role in central regulation of energy balance.[107] Another host target could be the endocannabinoid (EC) system engaged through LPS released by the gut microbiota.[103] A greater EC tone affected by the gut microbiota may negatively influence appetite, satiety, and adiposity.

Exposure to microbial products such as lipopolysaccharide, flagellin, or others can also engage innate immune response through the TLR or other sensing molecules. Because of inflammation and increased gut permeability, the bacterial flagellin can engage its specific TLR5 receptor on the basolateral surface of the gut epithelia to initiate an immune response against the pathogen, which is necessary for containment. Indeed, the TLR5-deficient (*TLR5*$^{-/-}$) mice displayed increased chronic inflammation, adiposity, insulin resistance, and hyperlipidemia, whereas antibiotic treatment could reduce the bacterial load and reverse the metabolic parameters to healthy levels. In this particular study, even the lean mice exhibited insulin resistance due to excess inflammatory responses in metabolic tissues.[108] Furthermore, both the gut microbiota and the associated metabolic dysfunction could be transferred from the *TLR5*$^{-/-}$ mice to germ-free, *TLR5*$^{+/+}$ mice.[108] The gut microbiota from the *TLR5*$^{-/-}$ mice differed in composition but not in the relative proportions between the major phyla.[108] However, these observations were recently challenged by another study, which did not observe inflammation of the gut or systemic metabolic dysfunction in two separate colonies of *TLR5*$^{-/-}$ mice. This latter study did confirm improper immune response to flagellated bacterial species.[109] These disparate results may have been because of the colonization of the gut microbiota that differs among facilities, handling, and specific diets and may illustrate the complexities of these experimental systems and their sensitivity to each component in place that can modify the phenotype. For example, earlier studies in germ-free mice showed that TRL2 deficiency (*TLR2*$^{-/-}$) is protective against insulin resistance and obesity. However, when grown in conventional facilities, these mice developed insulin resistance, metabolic dysfunction, and obesity. These changes in the metabolic phenotype of the *TLR2*$^{-/-}$ mice in conventional facilities accompanied changes in the gut microbial populations that resembled, at least in part, those found in obese mice and humans. In addition, the metabolic phenotype could be reversed by antibiotic treatment and transferred to wild-type mice with fecal transplant of the *TLR2*$^{-/-}$ mice's gut microbiota.[85] Collectively, these results underscore the metabolic impact of the gut microbiota that can override or modify the genetic preconditioning of an organism, at least under certain conditions, drawing attention to these interactions between dietary input and metabolic and immune responses that possibly underlie many conflicting phenotypic outcomes of similar genetic models in different studies.

Finally, the mesenteric fat, embedded with lymph nodes and in close contact with the resident immune cells and factors, is essentially an innate immune barrier and is capable of producing an inflammatory response to the incoming microbial challenge from the gut.[110] The proinflammatory cytokines, adipokines, and fatty acids released from the mesenteric fat could enter the liver through the portal vein and in turn destabilize hepatic metabolism. Consequently, steatosis and insulin resistance develop in the liver. Interestingly, the liver phenotype driven by dysbiosis, a microbial imbalance within the digestive tract, and inflammation appears to be heavily, if not completely, driven by *TNF*α action and could be rescued by blocking this pathway.[111] The development of extrahepatic insulin resistance and the growing metabolic pressure resulting from insulin resistance on the pancreas can bring about full-blown diabetes. What these microbial metabolites or factors are and their molecular targets responsible for this kind of a profound metabolic shift will become an important area for future research.

In conclusion, the three central factors contributing to the nutrient-driven metabolic and immune responses and the phenotypic outcomes include the genetic heritage of the host and genetic variation between subjects, host–pathogen interactions, and dietary exposure and diet composition (Figure 52.2).

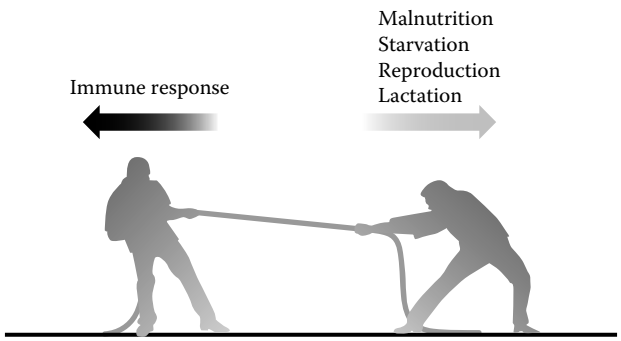

FIGURE 52.2 The competing functions of the organism at times of energy deficit and surplus. The mounting of a potent immune response is an energetically costly endeavor. Conditions such as malnutrition, reduction in energy depots, and starvation severely impair the immune system. Conditions of energy deficit lead to significant competition between immune defense and biological processes of central importance for the organism like reproduction, lactation, and thermoregulation. On the other side of the coin, the immune system is also not equipped to adapt to chronic energy surplus and associated molecular and cellular alterations, which cause it to exhibit malfunction. The best example for the latter is illustrated in obesity and associated metabolic inflammation setting the grounds for a cluster of metabolic diseases.

52.7 ORGANELLE FUNCTION IN INFLAMMATORY SIGNALING AND METABOLIC DETERIORATION

An important primer for metaflammation in obesity is the chronic metabolic overloading of anabolic and catabolic organelles leading to impairment of their function. One such organelle, the ER, serves as a critical intracellular metabolic hub for protein, lipid, and calcium metabolism and lipid droplet formation.[112] The vital functions of ER are maintained by a conserved, adaptive stress response that emanates from its membranes and is known as the unfolded protein response (UPR). Several diverse stimuli including accumulation of unfolded proteins, hypoxia, and toxins can induce the UPR.[113] UPR can also be triggered by acute or chronic excess of nutrients (including fatty acids and free cholesterol) or their deficiency (such as glucose). ER communicates its distress by engaging three signaling branches initiated by the pancreatic ER kinase (PERK), inositol-requiring transmembrane kinase/endonuclease 1 (IRE1), and activating transcription factor 6 (ATF6).[21,114,115] IRE1 possesses dual activity consisting of a kinase, which autophosphorylates itself, and an endoribonuclease, which leads to mRNA processing. The major target of the IRE1 endoribonuclease activity is an important transcriptional regulator of the UPR, the X-box-binding protein 1 (XBP1).[116–118] In synergy with IRE1, the ATF6 branch leads to transcriptional upregulation of XBP1 expression.[119] Together, XBP1 and ATF6 maintain a complex transcriptional program vital for the execution of UPR that involves upregulation of ER-resident chaperones to promote folding and components of the protein degradation apparatus.[113,115] If ER stress cannot be resolved, the UPR can engage apoptotic pathways and lead to cell death[48,120] (Figure 52.3).

The ER and the UPR play a significant role in both the physiological and pathological responses of immune cells. For example, some aspects of the UPR are important for the maturation of immune cells such as dendritic cells, lymphocytes, and plasma cells, XBP1 being a central regulator for the latter.[115] ER stress can impair adaptive immune responses

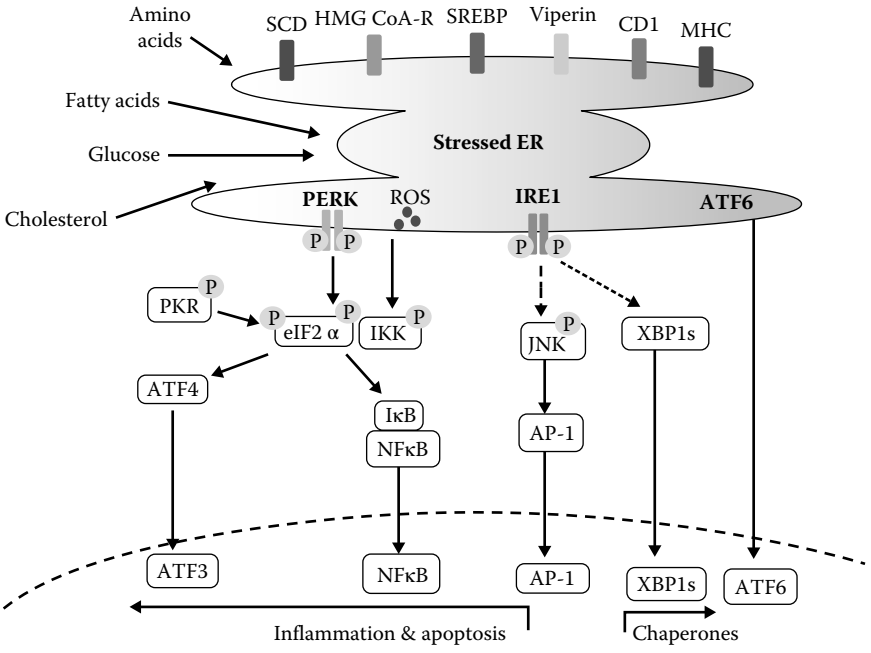

FIGURE 52.3 (See color insert.) The endoplasmic reticulum as a potential hub for metabolism, energy, immune, and stress responses. In addition to its well-known roles in protein quality control, folding, secretion, and calcium homeostasis, the endoplasmic reticulum (ER) also plays a critical role in lipid metabolism and lipid droplet formation through harboring central players such as the cholesterol-sensitive transcription factor sterol regulatory element-binding protein-1 (SREBP-1); the key regulating enzyme in cholesterol synthesis 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoAR); and in fatty acid desaturation through stearoyl CoA reductase (SCD) on its membranes. Studies have now shown that lipid droplets are released from specialized regions of the ER and regulated by proteins like viperin, also associated with ER membranes. Many links also exist between ER responses and glucose metabolism (not depicted here). These myriads of functions are maintained by an adaptive stress response system that emanates from ER membranes, known as the unfolded protein response (UPR). Various stressful situations including accumulation of unfolded proteins, hypoxia, toxins, and acute or chronic excess of nutrients (including fatty acids and free cholesterol) or their deficiency (such as glucose) can activate the UPR. Upon induction, the UPR engages three signaling branches initiated by the pancreatic ER kinase (PERK), inositol-requiring transmembrane kinase/endonuclease 1 (IRE1), and activating transcription factor 6 (ATF6). The major target of the IRE1 endoribonuclease activity is the X-box binding protein 1 (XBP1), which together with the ATF6 branch mounts a complex transcriptional program vital for the execution of UPR. The UPR can also engage inflammatory cascades such as JNK and the IKK–NF-κB pathway, leading to the production of cyclooxygeneases and other pro-inflammatory mediators. Induction of the double-stranded RNA-dependent protein kinase (PKR) can activate the inflammasome, leading to pro-inflammatory responses, and interfere with insulin action.

by reducing the processing and presentation of peptides by major histocompatibility complex (MHC)–associated antigen presentation.[121] Chronic ER stress, induced by overnutrition in obesity, for example, can also potentially serve as a primer for inflammatory and stress responses in both immune cells (such as macrophages) and metabolic cells (such as adipocytes or beta cells). During the course of obesity, unresolved stress in the ER can engage inflammatory response systems through several mechanisms. To begin with, the stressed ER and the mitochondria can produce significant amounts of ROS that can lead to oxidative damage.[122] ROS, whether originating from the mitochondria or the ER, has been shown to activate several stress and inflammatory pathways and contribute to the development of metabolic deterioration in obesity.[123,124] Regardless of the origin, in a chronic disease process, the stress can spread from one organelle to another through inter-organelle contact sites (such as the ER mitochondria encounter sites [or ERMES] found between the ER and the mitochondria), which can transmit vital information in the form of proteins or lipids or through the endomembrane system.[21,125] Thus, the ER and the mitochondria can have a strong impact on each other's function; however, it remains to be seen whether these interactions have any role in the functional deficiencies of these organelles seen in obesity and the metabolic pathologies.

Alternatively, the UPR can directly engage inflammatory cascades and stimulate cytokine production. For example, the IRE branch can stimulate JNK kinase activity, through its association with the adaptor protein *TNF* receptor–associated factor 2 (TRAF2), leading to production of pro-inflammatory gene expression by the transcription factor activator protein-1.[118] Active JNK1 can also phosphorylate the serine residues on IRS proteins and inhibit postinsulin receptor signaling. Consistent with these findings, JNK1-deficient mice exhibit reduced inflammatory cytokine levels and were protected from developing insulin resistance on a high-fat diet.[53] Inhibiting JNK by chemical or peptide inhibitors also improved insulin sensitivity in cultured cells such as macrophages, adipocytes, and whole animals.[126–130]

In another mechanism, IRE1 and PERK arms activate the IKK–nuclear factor-κB (NF-κB) pathway, leading to the production of cyclooxygenases and other pro-inflammatory mediators.[131,132] PERK activation has been associated with the degradation of IκB and nuclear transport of NF-κB. On the other hand, IRE1 activates IκB-kinaseβ [IκKβ] indirectly, through association with TRAF2. Signals through TLR2 and TLR4, but not intracellular TLRs, were recently shown to activate IRE1, through TRAF6, to induce XBP1 processing and the secretion of pro-inflammatory cytokines such as IL-6, *TNF*α, and interferon-β.[133] This study and an earlier one also showed that spliced XBP1 could bind the promoter of *TNF*α and inducible nitric oxide synthase *(iNOS)* genes, implicating XBP1 in the regulation of these pro-inflammatory cytokines.[133,134] However, clear mechanistic links between inflammatory pathways and UPR remain to be established in metabolic diseases. Recently, obesity was found to be associated with the activation of an important pathogen sensor, the double-stranded RNA-dependent protein kinase (PKR), in concert with the induction of UPR in metabolic tissues. PKR can be responsive to ER stress, but the mechanisms of its activation remain unclear. PKR deficiency in mice that fed a high-fat diet is protective against weight gain, metabolic deterioration, and insulin resistance. Lipid-induced insulin resistance also requires PKR, both in vitro and in vivo, and PKR interacts with and leads to IRS1 phosphorylation.[54] PKR can interact with and modulate the inflammasome activation and potentially contribute to inflammasome-mediated metabolic deterioration. Moreover, recent studies point out regulation of the inflammasome by a proximal ER stress sensor, IRE1 itself.[135] Collectively, these studies emphasize the integral role played by the ER in connecting the pathogen-sensing, inflammatory, and metabolic systems.[54]

The UPR is highly receptive of the nutrient status of cells. Historically, the deletion of yeast IRE1 is associated with auxotrophy for inositol.[113] Another set of UPR proteins, namely, the glucose-regulated proteins, were discovered by their induction in glucose-deprived conditions, a first implication of the nutrient-sensing function of the ER.[136] Either lowering or increasing glucose levels can activate the UPR. The PERK arm plays an important role in systemic glucose homeostasis, particularly through its actions in the liver and pancreas.[137,138] Glucose acutely regulates IRE1 activity, and when this becomes chronic, IRE1 can impinge on inflammatory cascades mediated by JNK or IKK.[113] UPR-induced transcriptional programs are also directly linked to glucose synthesis and breakdown.[139] Moreover, the ER assumes a central role in the synthesis of phospholipids and cholesterol. The UPR contributes to the lipogenic program, for example, through the activities of ATF6 and XBP1, although a great deal of uncertainty exists among reported phenotypes of the genetic mouse models with respect to lipid metabolism.[140,141] An essential sensor for intracellular cholesterol levels, the SREBP, is situated on the ER membrane.[113] Cholesterol deprivation activates this transcription factor, which upregulates key enzymes in the cholesterol synthetic pathway. Furthermore, elevated free cholesterol or free fatty acids induce ER stress and initiate all three arms of the UPR, but the molecular mechanisms for how the ER senses these changes remain unclear.[142,143]

In obesity or dyslipidemic conditions, exposure to high levels of nutrients such as saturated fatty acids and free cholesterol leads to ER stress and activation of the UPR in several metabolically active sites including the liver, hypothalamus, atherosclerotic plaque, and adipose tissue.[144] Recent studies have elaborated the causal role of ER stress in the liver and adipose tissues to the development of systemic insulin resistance and type 2 diabetes. A genetic haploinsufficiency model for the *Xbp1* gene in mice leads to elevated ER stress levels and promotes glucose intolerance, insulin resistance, and obesity. In contrast, treating obese mice with chemical chaperones has been shown to release ER stress and improve all of these metabolic parameters.[145,146] Moreover, prolonged ER stress can spur a homeostatic mechanism called autophagy to recycle damaged cellular components and organelles. In recent studies, the failure of autophagy by

genetically altering master regulators of this mechanism in obese or atherosclerotic mouse models was associated with increased inflammation, insulin resistance, and lesion progress, respectively.[147–149] Recently, autophagy was also linked to the metabolic benefits of exercise, particularly on insulin action, providing an additional layer of integration between this response and metabolic homeostasis.[150]

How do nutrients stress the ER in the first place? Recently, the metabolically stressed ERs from obese mice were carefully examined to understand the mechanisms of nutrient-induced ER stress. These studies showed that in the stressed ER from obese mice, when compared to control nonobese groups, protein synthesis is suppressed and chaperone content is not significantly changed. However, the metabolically stressed organelle exhibits prolific lipid synthetic capacity, and the specific alterations in ER lipid composition are associated with the inhibition of sarco/ER ATPase (SERCA) activity. Furthermore, reversing the lipid compositional changes or hepatic overexpression of SERCA resolved ER stress in vivo and improved glucose homeostasis and insulin sensitivity in mice.[151,152] In a recent study coupling polysome profiling to microarray analyses, the mammalian translatome was recaptured in vivo. This study showed the steady-state liver translational profile from an obese animal represented a fasting profile of liver from lean animals, suggesting the liver may become insensitive to the excessive flux of nutrients during obesity. The liver, in particular, also displays aberrations in alternative pathways of bile acid metabolism. Both the liver and the muscle profiles also emphasize mitochondrial defects. These findings suggest that translational dysfunction is another important aspect of obesity that may contribute to the associated metabolic dysregulation.[153]

Several studies elaborated on the mechanisms of ER stress-induced insulin resistance and weight gain. For example, the IRE1 arm of the UPR can inhibit insulin receptor signaling through activating JNK1, which then phosphorylates serine residues on IRS1.[145] JNK can also be activated through PKR and calcium/calmodulin-dependent kinase signaling, each of which has been shown to disrupt metabolism and insulin signaling.[154] Another mechanism has been proposed where XBP1 interacts with forkhead box protein O1, leading to its proteosomal targeting and degradation.[155] ER stress can also lead to leptin resistance in the hypothalamus that is associated with increased weight gain in mice on a high-fat diet, which can be reversed by the administration of chemical chaperones.[156] These findings highlight ER's important position as an interface between metabolism and immunity whose dysfunction plays a causal role in the development of obesity and chronic metabolic disease.[145,157–159] In summary, organelle stress and inflammation both contribute to the development of obesity-associated insulin resistance and atherosclerosis. However, it remains to be determined which is the preceding factor and how the metabolic consequences are determined through each of these potential mechanisms. Furthermore, a critical remaining question is whether inflammation itself can be proximal to disruption of ER function or its adaptive capacity and if so, in what capacity? Future studies chemically or genetically targeting intermediate genes in both UPR and inflammatory pathways should be instrumental in delineating the order of events and their isolated or combined effects. Regardless, therapeutic targeting of organelle dysfunction offers potential new opportunities for the management of chronic metabolic diseases and rare genetic diseases that display defective ER function and diabetes, such as the Wolfram syndrome (also called DIDMOAD; Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, and Deafness) and the Wolcott–Rallison syndrome (WRS).[160,161] The Wolfram syndrome occurs because of a rare mutation leading to defective wolframin gene, ER function, and insulin secretion. The WRS is a rare autosomal recessive disease characterized by neonatal/early-onset, nonautoimmune, insulin-requiring diabetes associated with skeletal dysplasia and growth retardation, and it is caused by mutations in the *PERK gene*. Two chemical chaperones that are known for their ability to improve insulin sensitivity and systemic glucose homeostasis in obese mice, tauroursodeoxycholic acid (TUDCA) and phenylbutyric acid (PBA), were recently evaluated in humans. One study with TUDCA demonstrated it could increase insulin sensitivity in the liver and the muscle in obese humans.[162] PBA treatment in humans also prevented lipid-induced insulin resistance and pancreatic beta-cell dysfunction.[163] These promising outcomes warrant studies of therapeutic agents with capacity to improve ER function and folding capacity in wider clinical trials and other settings.[164]

52.8 METAFLAMMATION: OBESITY-INDUCED INFLAMMATION

The involvement of the immune system in obesity lacks resemblance to acute inflammation that is characterized by five classic signs including pain, heat, redness, swelling, and loss of function. The acute inflammatory reaction of the vascular tissues is a robust response of the immune cells to confine the injury or infection at its origin and resolves rapidly when the trigger is removed. However, none of these attributes of classic inflammation prevail in obesity-induced metabolic inflammation. Obesity leads to an atypical immune activation, also referred to as metabolic inflammation or "metaflammation," for it is triggered by metabolic cues and involves both immune (macrophages, mast cells, T cells, and eosinophils) and metabolic cells (such as adipocytes, beta cells, and hepatocytes).[21,22] This immune activation is almost undetectable at a systemic level, and the obesity-induced inflammation appears to be limited to metabolic tissues and remains unresolved over time. In particular, the obese adipose tissue shows signs of elevated inflammation, which includes infiltration of various immune effector cells and production of a variety of inflammatory mediators. Studies have shown that the stressed adipose tissue can alter the metabolic functions of the liver and muscle by releasing fatty acids, adipokines, and inflammatory cytokines. For example, the genetic ablation of JNK in the adipose tissue enhanced systemic insulin sensitivity and glucose metabolism, whereas

overexpression of MCP-1 in the adipose tissue resulted in systemic metabolic deterioration.[165–167] These findings thus argue that the adipose tissue is important for systemic metabolic deterioration as a primary site and through sustaining obesity-induced inflammation. It is, however, important to note that inflammatory alterations in obesity are prominent in many other critical metabolic sites, such as hypothalamus, pancreatic islets, and liver, and impact a wide variety of metabolic parameters such as insulin action and secretion, glucagon production, hepatic glucose production, and leptin and adiponectin sensitivity (Figure 52.4).[48] Finally, disruption of inflammatory or metabolic pathways in immune cells can also block inflammation and improve metabolism, demonstrating the critical role of immune response for metabolic control and illustrating the importance of the interactions between immune and metabolic cells.[168] It is, however, essential to note that inflammatory response is an integral component of adaptation, defense, repair, and homeostasis. Hence, it may be overly simplistic to label it "friend or foe" in an absolute manner, but rather consider this metabolic–immune interface an equilibrium where the outcome is highly dependent on where the organism resides in this continuum.

Obesity leads to enlargement of adipocytes, which becomes an abundant source of inflammatory cytokines including *TNF*α, IL-6, IL-1β, and MCP-1 as well as many lipid products.[169] Immune cells are recruited into the adipose tissue (as well as other metabolic organs).[43,170] Several immune signaling cascades are activated including TLR, IKK, JNK, and PKR and may be responsible for the cytokine production from the adipocytes and/or immune cells infiltrating adipose tissue.[48] The adipocytes are overburdened in their effort to metabolize the excess nutrients, and the resident immune cells are devoid of any significant metabolic capacity to deal with this level of nutrient assault and may die. When these stressed cells die, they invite further involvement of the immune system and thus sustain a chronic inflammatory reaction without chance for resolution.[34] The inability to resolve metaflammation in obesity is not mechanistically understood. The presence of macrophages in the obese adipose tissue was first recognized in 2003 and found in crown-like structures that form around the necrotic adipocytes.[46,47,171] Time course analysis of mice on a high-fat diet showed that macrophages arrive before the first signs of adipocyte necrosis. The adipocyte apoptosis in an inducible mouse model of lipoatrophy leads to macrophage recruitment into the adipose tissue. However, blocking adipocyte necrosis through the deletion of cyclophilin D did not stop macrophage infiltration or inflammation in the adipose tissue.[172,173] Furthermore, it was also found that human obesity did not increase adipocyte death.[174] Hence, these findings suggest that complex interactions beyond adipocyte necrosis may be required, such as genotoxic events, activation of other stress signaling cascades, or production of immunomodulators and layers of immune activation and cellular complexity for immune cell infiltration and other inflammatory responses of the adipose tissue during obesity, and these mechanisms are not yet fully understood.

Up to 60% of the adipose tissue in obesity can be populated with cells that stain positive for the macrophage marker F4/80 compared to about 10% in adipose tissue from lean

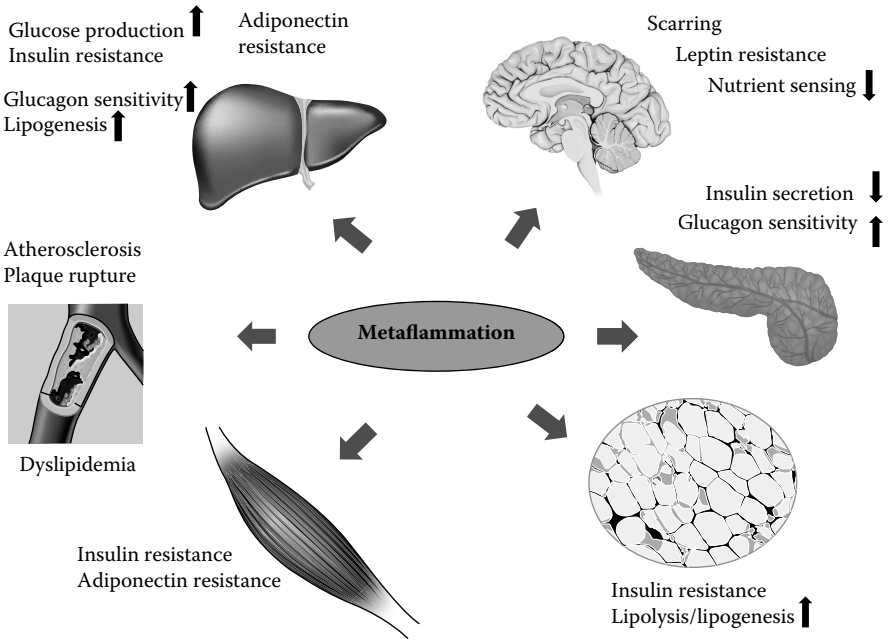

FIGURE 52.4 The impact of metaflammation on multiple organs leading to metabolic syndrome. The adipose tissue is important for systemic metabolic deterioration as a primary site of sustained obesity-induced inflammation. However, inflammatory alterations are prominent in many other critical metabolic sites including the hypothalamus, pancreas, liver, and skeletal muscle in obesity, impacting a wide variety of metabolic parameters such as insulin action and secretion, glucagon production and sensitivity, hepatic glucose production, and leptin and adiponectin sensitivity in the respective targets.

animals.[47] Interestingly, the macrophages in adipose tissue in obesity display characteristics of classical M1 phenotype (proinflammatory; nitric oxide synthase 2 and *TNF*α expression), while macrophages from adipose tissue of lean animals appear to belong to the alternatively activated M2 phenotype (anti-inflammatory; arginase 1 [ARG1]+, c-type lectin domain family member receptor [CD] 206+, CD301+, and IL-10 secreting).[175,176] The M1 type macrophages align around necrotic adipocytes, presumably to scavenge the lipids and necrotic material, and form crown-like structures.[171] The pro-inflammatory M1 macrophages and the immune mediators they secrete are thought to contribute to the pathogenesis of insulin resistance and systemic metabolic deterioration. Consistent with these observations, depletion of M1 macrophages in CD11c-DTR mice or preventing their chemotaxis to tissues in chemokine (C-C motif) receptor 2–deficient mice reduced adipose tissue inflammation and improved insulin sensitivity.[177,178] Furthermore, myeloid deficiency of IKKβ or JNK1 prevented myeloid cell–derived inflammation in adipose tissue and improved insulin action or glucose metabolism, although there are differences in the extent of contribution in different studies.[165,179–181] Adipose tissue–driven improvement in inflammatory and metabolic status is best explained in blocking multiple isoforms of JNK in the stroma rather than in the immune cells.[165,166,181] This is also evident in IKKε-deficient as well as in PKR-deficient models.[54,55] Taken together, these studies demonstrate how the recruitment and activation of macrophages to the adipose tissue contribute to systemic glucose homeostasis, but the interactions with the metabolic and stromal cellular components are also critical.

Recent studies show infiltration of the adipose tissue in obesity by several other types of immune cells, which also have systemic effects on metabolism. During obesity, the number of T helper-1 lymphocytes (T_H1; secrete pro-inflammatory cytokines) and CD8+ T cells (cytotoxic T cells; secrete pro-inflammatory cytokines) are increased in the adipose tissue, while the T helper-2 (T_H2; secrete anti-inflammatory cytokines) and regulatory T helper-2 cells (Treg; secrete anti-inflammatory cytokines) are decreased.[182,183] However, some recent studies report contradictory findings regarding the type of T cells that are increased or reduced in obesity. What is the role of T lymphocytes in obesity-induced inflammation and metabolic deterioration? One study in mice that lacked T lymphocytes (mice deficient in recombination-activating genes-1 [RAG-1]) showed they developed worse inflammation and insulin resistance when compared to control mice on a high-fat diet, arguing that certain populations of lymphocytes may play a protective role against metabolic inflammation and disease.[182] In fact, adoptive transfer experiments in RAG-1 mice showed Treg and T_H2 lymphocytes but not T_H1, and CD8+ cytotoxic T cells suppressed metaflammation and restored glucose metabolism.[182] Furthermore, targeted induction of Treg cells (by IL-2/anti-IL-2 complex) reduces adipose tissue inflammation and enhances systemic insulin sensitivity in obese mice.[183] Consistently, depleting Treg cells (in the Foxp3-DTR mice) worsened inflammation and insulin resistance. It is generally accepted that Treg and T_H2 cells improve insulin sensitivity, while T_H1 and CD8+ cytotoxic T cells promote insulin resistance. In addition to the changes in T cells, the adipose tissue B lymphocytes are found to be elevated, as is serum immunoglobulin levels, over the course of weight gain.[184] B cells function in antigen presentation to T cells and are important for humoral immunity. Mice deficient in B cells or depleted from B cells by CD20-specific immunotherapy exhibit enhanced insulin sensitivity compared to wild-type mice on a high-fat diet.[184] This positive response may be due to reduction in the number of pro-inflammatory T cells and macrophages as seen in the adipose tissue or other sites in these mice. Since adipocytes express unique T-cell receptors and MHC, it is possible that these aspects of metaflammation are also orchestrated by metabolic cells and signals. Either B-cell transfer or serum IgG from obese mice, but not from the lean control mice, to the B-cell-deficient mice induced insulin resistance.[184] In summary, obesity leads to activation of both the innate immune system and the adaptive immune response during the progression of metabolic disease. An important question remains unanswered: What are these antigens recognized by the T cells and from where do they originate?

Mast cells and eosinophils are two other types of immune cells that have attracted research interest because they are found in higher numbers in adipose tissue in obesity. Recent studies showed that either genetic depletion or pharmacological stabilization of the mast cells reduces adipose tissue inflammation and improves systemic insulin sensitivity in obese mice.[185] Furthermore, the IL-4- and IL-12-producing, anti-inflammatory M2 macrophage-promoting eosinophils are reduced in the adipose tissue during obesity. The eosinophil-deficient mice exhibit enhanced inflammation and altered insulin sensitivity, suggesting that these cells play a protective role against chronic inflammation and metabolic disease.[186]

Another important feature of metaflammation is that it is a low-grade immune response. Several immune organs, including primarily the adipose tissue, as well as liver, pancreas, muscle, and brain, display increased inflammation and local cytokine concentrations. The reflection of these inflammatory changes to circulating cytokines levels is small, if any, in obese mice and humans.[51,187] Furthermore, metaflammation does not increase energy expenditure or basal metabolic rate. This is a central issue in the exploring the role of individual immune pathways on systemic metabolism and why these systems were so closely positioned in the first place. These features of metaflammation are in contrast to the classic systemic inflammatory reaction to pathogens or trauma, which uniformly increases metabolic rate and energy expenditure, suggesting that obesity-induced inflammation remains a local but effective response that alters systemic metabolic homeostasis but preserves weight. This cycle can also be disrupted, at least in genetic models such as in PKR- and *IKK*ε-deficient mice, which exhibit reduced metabolic inflammation and increased energy expenditure, hence resulting in systemic metabolic improvement as well as better weight control in experimental models.[54,55]

Finally, metaflammation is chronic and unresolved. Inflammatory cell infiltration escalates over the time course of weight gain, cycles in intensity, but never resolves in obesity. In mice, inflammatory alterations can be detected within hours or days, and cells begin to infiltrate the adipose tissue and stabilize as early as in weeks after high-fat diet, and they reach a climax around 26 weeks on such a diet.[46,47] These studies suggested inflammation increased in parallel to the enlargement of the fat pads and potentially coincided with adipocyte cell death.[171] In this context, one can even imagine some utility for inflammation. As discussed earlier in this section, the inflammation remains local and modest. Perhaps, this kind of a low-grade inflammatory response does not trigger the mechanisms to resolve it, or alternatively, metabolic signals that initiate inflammation are not able to also engage resolution pathways or even prevent their proper responses. On the contrary, the adipose tissue turnover (adipocyte necrosis, proliferation, and differentiation occur simultaneously in the obese fat pads) appears to be an attempt at maintaining tissue homeostasis in this particular target of metaflammation. Over time, at least some aspects of inflammation will result in fibrosis and scarring and may become irreversible. Such changes have been reported in the hypothalamus and adipose tissue and may impact the outcomes of genetic or chemical interventions, again depending on time and context.[188]

52.9 CONCLUSIONS

The contribution of inflammation to metabolic deterioration is unequivocal and much better understood at the mechanistic level because of the significant amount of research reported in the past two decades. Beginning with some early observations in septic shock and severe infection, continuing with the discovery of metaflammation and arriving at the knowledge about the gut microbiota's contribution to insulin sensitivity and other metabolic phenotypes, the two seemingly afar scientific spheres of immunology and metabolism are beginning to merge into the field of immunometabolism. In this chapter, we presented overwhelming evidence for the causal role of inflammation in metabolic dysfunction associated with obesity as well as some of the unsettled issues, disparities, and potential reasons. An important future direction involves effective translation of this knowledge to shape future therapeutic approaches to metabolic syndrome. To date, several approaches have been taken to target inflammation from antibody-based neutralization of inflammatory cytokines to nutritional modification of inflammation in humans. Early studies with *TNF*α-blocking antibodies reduced inflammation without significant enhancement in insulin sensitivity in a small group of obese and diabetic patients. More recently, metabolic improvement and glucose-lowering effects have been reported following *TNF*α neutralization in obese humans.[189] Insulin resistance seen in rheumatoid arthritis patients benefited from *TNF*α antibody treatments, although some studies have reported negative results as well.[190] Blocking IL-1 receptor was also reported to improve glucose metabolism, although there have been some negative findings.[15] More substantial effects have been seen upon treatment of obese subjects with salicylates. Based on several independent studies, salicylates had beneficial impact on metabolism and improved insulin sensitivity while reducing inflammation and blood lipid levels. However, the molecular target of salicylates remains to be determined for the therapeutic window reported in these studies.[191,192] Taken together, an effective anti-inflammatory strategy has not yet been applied and vigorously tested in humans. Ongoing and future studies should probably target several cytokines or combinations (e.g., *TNF*α and IL-1) or upstream regulators of metaflammation (such as JNK, IKKε, or PKR) that would impact multiple mediators, overcome potential redundancies, and regulate energetics for effective and long-lasting improvements in glucose metabolism and insulin action. Potential upstream targets could be the molecules that constitute the metaflammasome (metabolically induced inflammasome assembly), pathogen-sensing and/or stress kinases, or organelle function, as discussed in this chapter, which can produce favorable changes in systemic energy homeostasis while preventing the engagement of inflammatory responses. It is likely that such interventions should be coordinated with dietary exposures and modulation of gut microbiome for optimal and sustainable results.

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