REVIEW

Immune cells and metabolic dysfunction

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Abstract Throughout evolution, effective nutrient sensing and control of systemic energy homeostasis have relied on a close physical and functional interaction between immune and metabolically active cells. However, in today's obesogenic environment, this fine-tuned immunometabolic interface is perturbed. As a consequence, chronic inflammatory conditions and aberrant activation of immune cells have emerged as key features of obesity-related metabolic disorders, including insulin resistance, cardiovascular complications, and type 2 diabetes, whereas a major research focus has been placed on the adipocyte–macrophage interaction in the context of metabolic dysfunction; recent studies have not only expanded the scope of relevant immune cells in this setting but also highlight the impact of distinct metabolic organs, including the liver, on immunometabolic control, metabolic disease development, and potential anti-inflammatory therapeutic options in obesity-driven pathologies. This review will thus summarize recent progress in this emerging area of metabolic research.

Keywords Adipose tissue · Liver · Inflammation · Immune cells

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Obesity, inflammation, and insulin resistance

Epidemiological studies estimate that by the year 2030, 2.2 billion people worldwide will be overweight and 1.1 billion will be obese [1]. Obesity as such represents the core component of the so-called metabolic syndrome which is a cluster of metabolic disorders, including insulin resistance, obesity, fatty liver disease, hyperglycemia, dyslipidemia, and hypertension, mainly caused by caloric excess, sedentary lifestyle, and a genetic predisposition. Progression of the aforementioned disorders can precipitate into end-stage diseases such as type 2 diabetes, atherosclerosis, and eventually even cancer. Consequently, components of the metabolic syndrome are tightly associated with increased mortality as observed in these patients [2].

Inflammation is now recognized as a key feature of metabolic dysfunction. Dating back to the 1990s, a critical role for inflammatory mediators, i.e., tumor necrosis factor alpha (TNFα), has been described in the manifestation of obesity and associated complications most notably insulin resistance. While adipose tissue of obese humans and mice is characterized by elevated numbers of inflammatory macrophages and the induction of circulating TNFα levels [3], early studies already have shown that antibody-mediated TNFα neutralization or genetic loss-of-function mouse models for TNFα improved insulin sensitivity during diet-induced obesity [4]. Also, critical molecular effectors of inflammatory signaling such as the transcription factor nuclear factor kappa B (NFκB), its upstream kinase IKK, or the jun-N-terminal kinase (JNK) were subsequently found to be aberrantly activated under obese conditions, and prevention of NFκB/JNK signaling ameliorated substantial parts of metabolic dysfunction as associated with insulin-resistant obesity [5, 6]. Consistent with these findings, salicylate treatment indeed improved insulin sensitivity and glucose homeostasis in both humans and mice [7], again underlining the close interaction between inflammatory and metabolic cues in the control of energy homeostasis. Indeed,

metabolic and inflammatory cells have acted in close physical proximity throughout evolution starting from the *Drosophila* fat body which combines major mammalian metabolic cell functions, including equivalents to adipocytes, hepatocytes, and various hematopoietic as well as immune cells. Over 600 million years of evolution, the intra-organ communication in flies has diverged into distinct organ compartments (i.e., adipose tissue and liver), however, still reflected by the close proximity between adipocytes, hepatocytes and dedicated tissue macrophages, and other immune cells. In fact, this tightly coupled interaction between the metabolic and immune compartments within adipose and liver tissue has been hypothesized to represent a critical interface for nutrient sensing, inter-organ communication, and metabolic control [8].

Once considered an inert energy storing depot, adipose tissue is now considered a potent endocrine organ with critical importance for overall energy homeostasis. Indeed, adipocytes secrete proteins involved in inflammation, appetite regulation, blood pressure control, and energy balance [9]. Under normal physiological conditions, adipose tissue function is tightly coupled to the systemic adaptation to varying conditions of food availability; upon fasting, stored adipose tissue lipids can be rapidly released and used for energy by peripheral organs, particularly including skeletal and cardiac muscle and liver; however, excessive adipose tissue is associated with increased risk of insulin resistance, cardiovascular disease, and cancer [10].

Given its central location within the systemic circulation, the liver serves as one of the body's critical organ for maintenance of systemic energy homeostasis [11]. Indeed, the liver represents the critical control relay in the reception of small molecules arising from food digestion or degradation of endogenous sources, their metabolic conversion or storage, and the final (re-)distribution to the periphery. Consequently, as the predominant interconversion point for energy substrates in mammals, the liver plays an essential role in the adaptive metabolic response during daily/periodic fasting–feeding cycles [11–13]. In this regard, defects in hepatic insulin signaling have been demonstrated to importantly contribute to the development of systemic peripheral insulin resistance [14]. Mice bearing a targeted disruption of the insulin receptor gene in liver display hyperglycemia, hyperinsulinemia, and impaired glucose tolerance [15]. Also, inhibition of the PI3K/Akt-dependent insulin signaling pathway in liver by the Akt-inhibitor tribbles homolog 3 leads to hyperglycemia and glucose intolerance [16].

It is tempting to speculate that inflammation and insulin resistance have developed as adaptive traits which might provide an evolutionary advantage for organisms under specific conditions. Proper inflammatory response is extremely important for fighting invading pathogens and recognizing transformed tumor cells. It is also an energetically costly process, and therefore, insulin resistance develops in the site of inflammation to fuel immune cells. Nowadays, in obesogenic environments, an originally beneficial response has transformed to one of the key contributors to metabolic dysfunction [17]. Given the critical importance of both adipose tissue and liver for overall nutrient handling and metabolic control, intra-adipose/hepatic communication pathways between metabolic and inflammatory cells are crucial determinants of energy homeostasis and/or metabolic dysfunction, especially in the context of obesity-related insulin resistance and type 2 diabetes. Given excellent recent reviews on components of the adaptive immune system and metabolic control [18, 19], a particular focus will be placed on the innate immune system and its impact on metabolic dysfunction.

Innate immune system cells and adipose tissue (dys)function

Innate or nonspecific immune response represents first line of defense against invading pathogens, and virtually, all types of innate immune cells have been identified in adipose tissue and been implicated in maintaining of adipose homeostasis or in inflammation-induced metabolic disease (Fig. 1). Most work on obesity low-grade inflammation has been focused on macrophage infiltration, and function in adipose tissue and several excellent reviews have been recently published elsewhere [19–21]. However, a role of other immune cells has recently emerged as an important topic. In addition to macrophages, cells from innate and adaptive branches of immune system seem to play a prominent role in maintaining adipose tissue homeostasis and are involved in promoting or suppressing inflammation upon obese conditions.

Neutrophils

In classical immune response, neutrophils are the first immune cells present at the site of inflammation and further help to recruit macrophages [22]. Similarly in obesity, they infiltrate adipose tissue as soon as 3 days after initiation of high-fat feeding and precede infiltration of macrophages [23]. Neutrophils secrete several types of proteases that are known to be involved in inflammation [24, 25]. Expression of elastase, a well-characterized peptidase, was increased in adipose of high-fat fed mice compared to chow-fed animals, and it participated on inflammation-induced insulin resistance via degradation of insulin receptor substrate 1 [24]. Upon pharmacologic or genetic loss of function of neutrophil elastase, mice showed reduced inflammation of adipose tissue with lower macrophages infiltration and improved insulin sensitivity and glucose tolerance [24]. Moreover, increased levels of neutrophils were detected in circulation of obese adults [26, 27] and children [28], which further emphasizes their function in metabolic homeostasis.
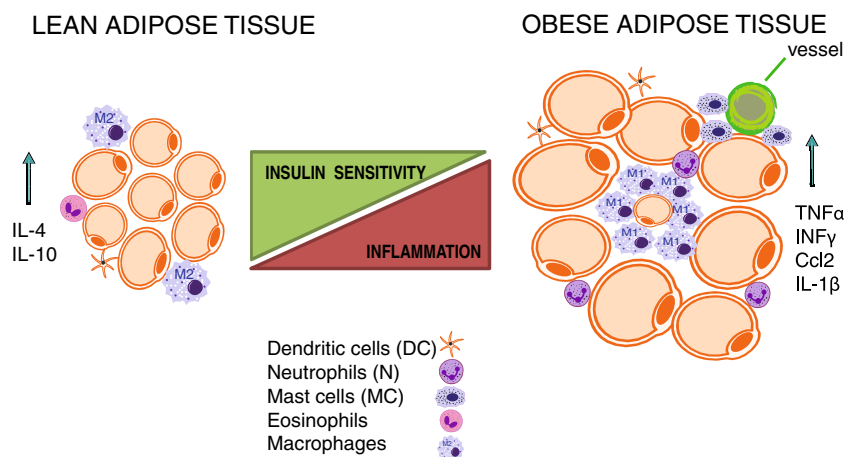
LEAN ADIPOSE TISSUE

OBESE ADIPOSE TISSUE

Fig. 1 A schematic representation of changes in adipose tissue innate immune cells populations in obesity. Lean adipose tissue is characterized with low occurrence of innate immune cells and predominantly anti-inflammatory cytokines are secreted. With obesity, insulin sensitivity decreases proportionally to increased inflammation of the adipose tissue. Obese adipose tissue is associated with increased infiltration of immune cells and inflammatory cytokines production. *IL-4* interleukin 4, *IL-10* interleukin 10, *TNFα* tumor necrosis factor α, *IFNγ* interferon γ, *CCL2* chemokine (C-C motif) ligand 2, *IL-1β* interleukin 1β

Basophils

Basophils are the rarest granulocytes in human blood and are involved in regulation and modulation of allergic inflammation. The role of basophils in obesity remains poorly understood, and their association with detrimental obesity outcomes is not clear [29–31]. To date, any reports examining basophil infiltration into adipose tissue have not been published.

Eosinophils

Eosinophils are commonly associated with allergic reactivity and parasitic infection. Despite being elevated in serum of obese humans and animals [32], their numbers in adipose tissue decline with obesity [33]. Eosinophils are a major source of interleukin (IL)-4 in adipose tissue, thereby sustaining adipose macrophages under alternatively activated M2 phenotype and protecting tissue from inflammation [33]. Eosinophil-deficient mice on high-fat diet showed augmented adiposity, impaired glucose tolerance, and increased proportion of classically activated macrophages [33], while the total number was not changed. This suggests eosinophils function in regulation of macrophages polarization and thereby influence insulin sensitivity.

Mast cells

Mast cells play a prominent role as effectors in allergic reactions and have been implicated in wound healing and tissue remodeling. Mast cells preferentially localize to mucosal and connective tissues, acting as first responders to viral and bacterial pathogens. They originate in bone marrow, but unlike the other innate immune cells, do not mature until they reach the target tissue [34]. In addition, a population of

mast cells' progenitors have been previously detected in adipose tissue [35], suggesting that in addition to bone marrow, adipose tissue might be another source of mast cells. Counts of mast cells in lean adipose tissue are relatively low, but increase with fat accumulation in humans as well as in animal models [36, 37], which suggest a contribution to diet-induced obesity and insulin resistance. Indeed, mast cell-deficient mice fed a Western diet gained less body weight, showed decreased adiposity, improved glucose tolerance, and attenuated adipose tissue inflammation compared to control counterparts [36]. Administration of mast cell stabilizers, Cromolyn (DSCG) and Zaditor (ketotifen), which are used in the clinical treatment of allergies, led to analogous results [36]. Lower body weight in mast cell-depleted animals might be attributed, at least in part, to increased thermogenesis, as they showed higher resting metabolic rate and increased expression of key thermogenic protein UCP1 in brown fat [36].

The exact mechanism by which mast cells contribute to pathophysiology of obesity is not yet fully understood, but secretion of pro-inflammatory cytokines IL-6 and interferon γ (IFNγ) are likely to contribute. Furthermore, mast cells promote obesity via stimulation of adipogenesis [38]. Mast cells produce a vast spectrum of bioactive molecules including prostaglandin D2 [39], which is metabolized to 15-deoxy-δ-PGJ2, the major endogenous ligand of peroxisome proliferator-activated receptor γ (PPARγ) [40, 41], a well-known inducer of adipocyte differentiation [40, 42]. Therefore, mast cells might be able to induce adipogenesis by prostanoids production. It has been proposed that mast cells also promote angiogenesis in adipose tissue by production of certain proteases [36, 43]. This notion would be further supported by mast cell localization next to the microvasculature and positive correlation of mast cells and microvessels number during obesity development [36].

Natural killer cells

Natural killer (NK) cells are large granular lymphocytes with potent ability to activate antigen-independent cytotoxic response upon viral infection and participate in antitumor response. Their function in adipose tissue has not been clearly defined. NK cells are abundant in adipose tissue forming about 30 % of the cells in stromal vascular fraction [44]; however, the results concerning quantity of NK cells in adipose tissue and its changes upon obesity are inconsistent showing decrease [44–46] or no change [47] in animal and human studies or even increase in human visceral adipose tissue from obese subjects [48]. Their counts seem to be regulated by other leukocytes, as B and T cells deficiency resulted in a pronounced increase in NK cell number in adipose tissue [47]. Furthermore, an important regulator of NK cells function, IL-15, was suggested to influence NK cells number in adipose tissue. Transgenic mice overexpressing IL-15 have more NK cells in adipose tissue and showed lean phenotype, while IL-15 deficiency was associated with increased body weight and less NK cells in adipose tissue [45, 49].

Leptin, a hormone secreted specifically by adipocytes, is implicated in body weight and appetite regulation. Its levels are increased in obesity accompanied by leptin resistance [50]. Moreover, leptin plays a prominent role in immunity [51] as it enhances inflammatory response during infection, promotes production of many cytokines, e.g., IL-1β, IFNγ, IL-6, TNFα [52–54], activation of macrophages [55] and proliferative and anti-apoptotic effects [56], and thus provides plausible link between obesity and immune dysfunction.

Indeed, obesity leads to decreased cytotoxicity of NK cells [57] decreasing their ability to fight against infected and transformed cells. Leptin might be likely candidate involved in the process as long leptin receptor was found to be expressed on subpopulation of human circulating NK cells. Proportion and also total number of NK cells in leptin receptor deficient db/db mice were declined as well as their cytotoxic activity [58], suggesting leptin signaling to be involved in NK cells development and activation.

It has been shown that short-term leptin stimulation of primary human NK cells increased a secretion of IFNγ and cell-dependent cytotoxic lysis of tumor cells [59]. However, the opposite is true for a long-term leptin stimulation, which better mimics obese conditions, as it led to dampened secretion of inflammatory and cytotoxic agents by NK cells [59]. Therefore, leptin resistance might impair central immune function and possibly mediates cancer susceptibility of obese people.

Natural killer T cells

Family of natural killer T (NKT) cells represents a bridge between innate and adaptive immunity and comprises several types of NKT cells. Best characterized are type I NKT cells, so-called invariant NKT cells, which are also most abundant in adipose tissue. NKT cells share characteristics of NK cells and simultaneously express invariant T cells receptor and CD3—T lymphocyte markers. They are able to skew immune response to inflammatory or anti-inflammatory direction, depending on stimuli provided under specific conditions either nutritional or immunological. NKT cells recognize and are activated by lipid antigens presented by MHC class I-like molecule CD1d on antigen-presenting cells [60]. Thus, not surprisingly, they have been found enriched in adipose tissue [44, 61]; however, function of NKT and their influence of inflammatory process in adipose have not been well characterized yet. Model of diet-induced obesity showed inconsistent results. While significant enrichment of adipose tissue with NKT cells upon high-fat feeding was detected [62, 63]; the others showed completely opposite results demonstrating negative correlation of NKT cells amount with adiposity [61, 64], BMI, and insulin resistance [65] and percentage of NKT cells was reduced also in ob/ob mice, a well-established genetic obesity model [61, 66]. Additionally, in some studies, number of NKT cells remains comparable between high-fat and low-fat feeding [67], but cells were shown to be more active with high-fat feeding and produce more pro-inflammatory cytokines [67]. Contrast to mice, NKT cells are scarce in human adipose tissue [68, 69] and are found even in lower frequencies with obesity [68]. Using mouse models lacking NKT cells have not shed more light on the problematic. β2 microglobulin knockout mice lacking NKT cells fed high-fat diet demonstrated ameliorated glucose tolerance and polarization of macrophages towards M2 phenotype when compared to high-fat fed controls [62]. In contrast, opposite results have been achieved with another mouse strain with depleted NKT cells, namely the CD1d knockout mouse [61, 66], where positive correlation between insulin sensitivity and NKT cell abundance was demonstrated [65]. Furthermore, in this study, a link between NKT cells and improved glucose homeostasis has been further supported with experiments encompassing NKT cells activation with α-galactosylceramide (αGalCer, a potent glycolipid agonist derived from marine sponge not found in mammals). After αGalCer injection and NKT cells activation, obese mice fed high-fat diet showed improved glucose tolerance and macrophages polarization shifted to M2 phenotype [65]. Interestingly, αGalCer injection did not have any effect in lean mice [65], which contrasted results gained by Wu et al. showing expansion of NKT cells in adipose after αGalCer treatment and raised pro-inflammatory cytokines secretion [67].

The question remains why some studies demonstrated NKT cells to increase production of pro-inflammatory cytokines upon lipid activation accompanied with worsen obesity-associated pathogenesis while the others showed

improved obesity-associated conditions. One of the reasons might be the usage of different mouse strains, different lipid content, and composition of diets or feeding period. Future investigations will be necessary to clarify NKT cells role in adipose tissue function.

Dendritic cells

Dendritic cells (DCs) are a heterogeneous antigen-presenting cell population containing conventional or myeloid, plasmacytoid, and newly characterized inflammatory DCs. They contribute to pathogen defense and are involved in recruitment of macrophages into the site of inflammation and priming of naïve CD4+ T cells [70–72]. However, there are not many data available about DCs and their function in obesity-mediated low-grade adipose tissue inflammation and associated insulin resistance. DCs were detected in adipose tissue of mice and humans [73–75], and their number was increased with obesity [74, 75]. Further, DCs are implicated in recruitment of macrophages into adipose during overnutrition [75], and mice lacking DCs showed to be resistant to high-fat induced obesity [75]. DCs are capable of presenting antigens to CD4+ T cells, which then become effector cells showing Th1 immune response in lean animals. In contrast, adipose tissue of obese or type 2 diabetic mice display a switch from Th1 to Th17 response [74]. Th17 immune response has been recently shown to deteriorate autoimmunity [76] and might play an important role in metabolic process; however, this notion would need further examination.

The liver: an immune organ

A coordinated network of cells, tissues, and organs comprise the innate immune system, which remains the first line of defense against pathogenic and or damaged "self" signals. Anatomical location and dual blood supply of the human liver (20 % oxygenated blood from the hepatic artery and 80 % nutrient rich blood from the portal vein) ensures constant exposure to various antigens, pathogenic stimuli, and toxins; underscoring the organ's relevance in immunological response [77]. Innate immune cells of the liver include both phagocytic and lymphocytic subsets. Kupffer cells (resident hepatic macrophages) in concerted effort with lymphocytic natural killer cells, dendritic cells, and natural killer T cells modulate liver immune status. Pathogen-associated molecular patterns or damage-associated molecular patterns are received by pattern recognition receptors (PRRs) [e.g., Toll-like receptors (TLRs)] expressed by cells of the innate immune system to compliment nonspecific killing mechanisms and other innate immune barriers [77, 78]. Experimental animal models accompanied by human clinical studies demonstrate involvement of the aforementioned immune cells (amongst

others) during basal and injurious conditions within the hepatic microenvironment. Specifically, cytokine balance/imbalance controlled by cells of the innate hepatic immune system critically regulates disease pathogenesis, including those evolving from metabolic syndrome [79].

Systemic metabolic disturbances and innate hepatic immune activation

Indices of metabolic syndrome are known causative factors for development of liver steatosis or nonalcoholic fatty liver disease (NAFLD). Excessive fat accumulation and increased hepatic triglycerides can arise from delivery of free fatty acids from lipolysis of visceral fat, from de novo lipogenesis, or from dietary consumption of high-fat high-sugar foods/beverages [80]. Portal vein delivery of fatty acids impairs hepatic extraction of insulin and stimulates gluconeogenesis/triglyceride synthesis. Metabolic syndrome/obesity has become a global pandemic as such occurrence rates of NAFLD are alarmingly high in the USA and abroad, where NAFLD currently ranks as the most common liver disease in the Western world [81]. Classic chronic liver disease pathogenesis includes progression from NAFLD to nonalcoholic steatohepatitis (NASH), a more severe state of injury characterized by steatosis, increased inflammation, and elevated parenchymal cell damage [82]. Increases in lipid peroxidation and reactive oxygen species continually evoke cytokine overproduction, further propelling cellular dysfunction in fatty liver disease. As such, approximately 20 % of NASH patients will progress to end-stage liver disease (cirrhosis), highlighting the importance of hepatic manifestations of metabolic syndrome [81]. As basic science research in the field has expanded, the immunological component has received much attention resulting in numerous studies that clearly define contributions of the innate immune response in NAFLD and NASH [83] (Fig. 2).

Kupffer cells

Nonspecific phagocytosis in the liver is predominantly mediated by Kupffer cells (KCs), which account for over 80 % of fixed-tissue macrophages within the human body and approximately 20 % of nonparenchymal hepatic cells [77]. Zonal distribution analyses indicate KCs possessing increased lysosomal activity and greater phagocytic capacity are sequestered to the periportal tract, where blood flow from the portal vein delivers bacterial products transported from the gut [84]. KCs are directly linked to the liver's response to infection, toxins as well as other stressors and are known to display characteristic macrophage polarization. As active phagocytes, KCs secrete various inflammatory cytokines in response to intravascular debris and serve to eliminate bacterial cells and other particulates via cell surface receptor complexes, notably

Fig. 2 Immune cell-mediated NAFLD pathogenesis. The pathogenesis of NAFLD is often described by the "two-hit" hypothesis. Excessive consumption of dietary fats along with potential underlying metabolic disruption, genetic or otherwise, stimulates an increase in fatty acid synthesis with concurrent increases in triglycerides, β-oxidation, and disruption of glucose homeostasis. Simple steatosis coupled with additional injury or a "second-hit" may progress the state of injury to NASH marked by tissue inflammation. Immune cell activation observed in NAFLD, and elevated inflammatory cell infiltrate observed in NASH, marres the hepatic microenvironment with inflammatory cytokines, with subsequent increases in lipoapotosis, oxidative stress, and insulin resistance. Innate immune cells are critical mediators of this process; those highlighted in this review are diagrammed above with notable secretory factors and receptors. *DC* dendritic cell, *KC* Kupffer cell, *NK* natural killer cell, *NKT* natural killer T cell
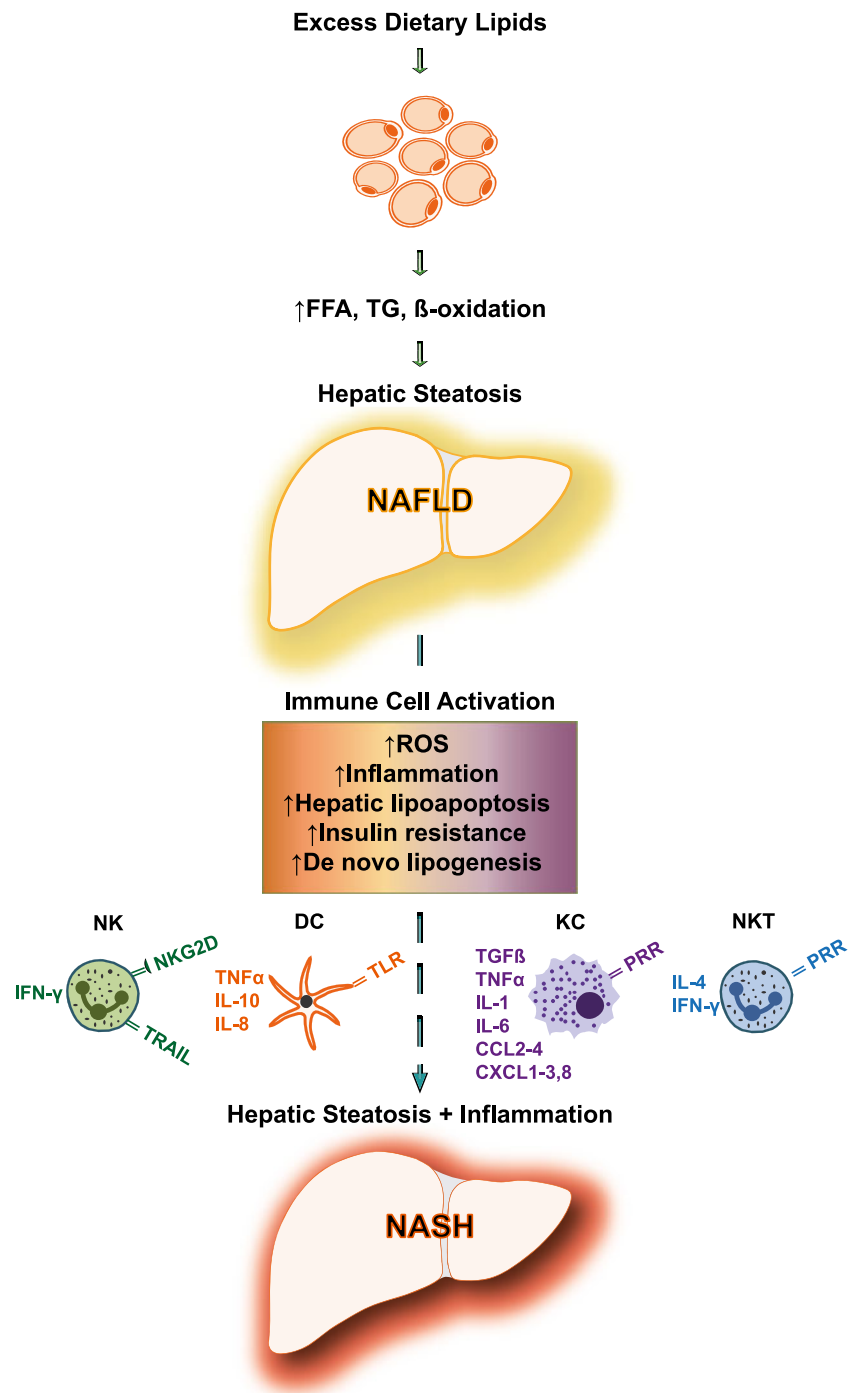
complement receptors (ICAM-1 binding adhesion receptors and TLRs facilitating recognition of polysaccharides) [77, 78, 85]. Overproduction of these inflammatory mediators by KCs, including TNFα, IL-1 and 6, MIP1α, TGFβ, and RANTES can lead to parenchymal injury/necrosis and subsequently activate inflammatory cascades in neighboring cells, including upregulation of pro-inflammatory/pro-fibrotic signaling with the hepatic stellate cell population. Concrete links have previously been established between KCs activation and the

pathogenesis of NAFLD and NASH, indicating reduction of this cell population attenuates histological signs of steatosis, inflammation, and necrosis [86–89]. More recently, elegant studies by Tosello-Trampont et al. demonstrate depletion of KCs (either pre- or post-methionine-choline deficient [MCD] diet) triggers a significant hepatic influx of CD11b^{int}Ly6C^{hi} pro-inflammatory blood monocytes during development and progression of NASH [90]. Interestingly, in this rodent model of NASH development, KCs are polarized to the M1

macrophage phenotype and, along with the blood-derived monocytes, account for the main cellular source of TNFα. Elevation in hepatic TNFα promotes recruitment of Th1 inflammatory cells through subsequent elevation in MCP-1 levels [90]. Additionally, TNFα is known to regulate intrahepatic lipid metabolism through various mechanisms (e.g., insulin resistance), further amplifying NASH pathology [81]. Visceral adiposity observed in clinical NAFLD and NASH is often accompanied by increased gut permeability [91]. Thus, elevated gut-derived endotoxin levels trigger KC activation (via TLR4), which may also contribute to the classical M1 phenotype often observed [91]. While KC numbers have been shown to increase as a result of liver injury, it is clear from the recent work of Leroux and colleagues that differential phenotype is also observed in the setting of increased lipogenesis [92]. KCs isolated from high-fat diet fed mice were characterized by increased cell size and lipid droplet retention. Specifically, lipid droplets enriched with ceramides and diacylglycerols were reported, the former already known to stimulate macrophage activation and apoptosis. In this same study, increased inflammatory signaling was accompanied by an induction of lipogenic gene expression in KCs from fatty livers, which was attenuated by inhibiting the first step in lipogenesis (via suppression of acetyl-CoA carboxylase) [92]. In contrast to the deleterious M1 KC phenotype, previous studies have shown alternatively polarized M2 KCs to ameliorate insulin resistance in diet-induced obesity [93, 94]. Additionally, while the mechanism of KC activation/ polarization remains unclear, recent studies have shown that neurotransmitter serotonin and cognate receptors may skew macrophage polarization. Specifically, KCs preferentially express serotonin receptor 5HT2B, and upon agonist activation, KCs display the characteristic M2 phenotype (anti-inflammatory, pro-cell growth, and tissue repair) [95]. Serotonin levels are decreased in patients with metabolic syndrome [96]; thus, it would reason to speculate that the pronounced M1 pro-inflammatory phenotype of KCs observed in NAFLD/NASH may be in some way linked to serotonin levels in the peripheral blood supply. Additionally, oxidative stress due to elevated ROS has been implicated as the secondary insult in the two-hit hypothesis of NAFLD [79]. KCs are a significant source of hepatic redox disruption via both direct and indirect ROS generation, and these effects on disease pathology and activation of adaptive immunity have been reviewed previously [79].

Natural killer cells

Originally termed Pit cells, NK cells reside within the hepatic sinusoid functioning as hepatic lymphocytes with qualities distinct from that of peripheral NK cells, T and B cells, due to antigen receptor expression deficiency [97]. Re-population rates remain high under normal physiological conditions, with cellular turnover every ~1–2 weeks. Extrahepatic replenishment of NK cells is controversial, but likely attributable to bone marrow-derived stem cell, with tissue-enrichment of NK cells attributed to sinusoidal endothelial cell–NK cell adhesion. Hepatic NK cells have the ability to directly and/or indirectly kill pathogens, stressed parenchymal and nonparenchymal cells, and tumor cells within the hepatic microenvironment [78]. Regulatory functions of NK cells have been reported with regard to Kupffer cells, T and B cells, as well as dendritic cells via production of various chemokines, cytokines, and growth factors. NK–target cell interactions are dictated by the presence of NK cell receptors, notably NKG2D, and target cell ligand expression [98, 99]. In addition to cell-activating ligands, activation of KCs is induced by various cytokines and chemokines, predominantly IFNα/β and CCL2 [100]. Several studies have reported key regulatory functions of NK cells in various liver pathologies, including HBV, HCV, and alcoholic liver disease (recently reviewed here [97, 101]); however, far less has been reported on the involvement of this cell type in NAFLD and NASH. Significant increases in NK cell presence has been reported in diabetes and obesity with corresponding increases in NK cell ligand expression. MIC A/B, ligands for NK cell receptor NKG2D, are increased in response to liver injury and have recently been reported to be upregulated along with NKG2D and other NK cell-associated mediators in livers of obese patients [102]. Expression of NK cell death receptors (TRAIL, CD95/FASL) was also significantly increased in livers of NASH patients compared to NAFLD and healthy controls, indicating an associating between activated hepatic NK cells and NASH pathogenesis [102]. Conflictingly, O'Shea and colleagues reported obese patients to have significantly fewer circulating NK cells compared to healthy controls [103]; however, peripheral blood NK cell counts may not accurately reflect the hepatic compartment. While the specific role of NK cells in NAFLD/NASH remains unclear, inferences may be drawn from studies examining NK contributions in liver fibrosis and HCV, both of which commonly present with underlying steatosis [97]. Production of cytotoxic mediators by NK cells can induce hepatic injury, but can also inhibit fibrogenesis through direct killing of the collagen-secreting hepatic stellate cell and production of IFNγ. These findings were recapitulated in the setting of HCV, wherein NK cells isolated from HCV-infected patients were able to induce apoptosis in activated HSCs, pointing to a beneficial role of this cells subset in progressive disease [104]. Paradoxically, a recent study from Gomez-Santos et al. indicates that NK cells actively promote a classical Th1 (pro-inflammatory) response in early stages of NAFLD as evidenced by increased TRAIL expression and cytotoxic activity [105]. Overall, these reports indicate divergent roles of hepatic NK in early vs. late stage disease, which should be a primary consideration when developing therapeutic strategies.

Messengers across the divide: dendritic cells and NKT cells

Dendritic cells

In concert with Kupffer cells, DCs are the first to detect invading pathogens and are classified as professional antigen-presenting cells regulating immunity and tolerance. DCs also provide an important link between the innate and adaptive immune systems through priming of T cell responses. DCs are subdivided into two major populations: plasmacytoid (IFN producing CD123^{+}) and myeloid (CD11c^{+}), both of which are present in human liver [78, 106]. Interestingly, properties and functions of dendritic cells vary among tissue types and to a greater degree among species (e.g., human DCs vs. rat), making it difficult to identify DC populations by cell surface markers. DCs initiate innate immune responses to eliminate foreign microbes, similarly to KCs, dendritic cells express several PRRs, including TLRs. Due to constant exposure to bacterial LPS, downregulation of TLR4 is observed; thus, somewhat limited direct responsiveness to danger signals and increased tolerogenic properties through expression of IL-10 is observed [107]. Upon detection and uptake of invading microbes, activated DCs have the capability to migrate to draining lymph nodes to promote NK cell activation in addition to their ability to modulate T cell response and activate neighboring macrophages [106]. In experimental models of cholestatic disease, accumulation of DCs has been reported; however, less is known concerning the function of DC in hepatic metabolic syndrome and progression to NASH. High-fat diet alone has been reported to increase hepatic DCs [108], and in a recent study, DC-depleted mice present with decreased liver macrophages as well as resistance of weight gain and metabolic disturbances from high-fat diet [75]. Henning et al. has recently reported NASH-associated dendritic cells present a more mature phenotype characterized by increased expression of co-stimulatory molecules and cytokines [109]. Depletion of CD11c^{+} DCs in a murine model of NASH markedly increased intrahepatic inflammation, with expansion of activated Kupffer and monocyte populations observed. Additionally, DCs blunted expansion of CD8^{+}T cells and inflammatory monocytes. These elegant studies by Henning and colleagues indicate a positive role for DCs in NASH potentially via clearance of necrotic and apoptotic cell debris [109]. These data support previous studies examining a role for DCs in a fibrotic milieu [107], which suggests this cell type may be important in the progression of liver disease.

Natural killer T cells

Similar to hepatic DCs, NKT cells provide an important line of communication between the innate and adaptive arms of the immune response [97, 110]. NKT cells, which express NK cell

characteristics, express a variable pattern of T cell receptor and NK cell markers and recognize lipid antigen CD1d [97]. Intrahepatic NKT cells (reported to develop in the thymus) are positive for both CD3 and CD56 and are classically divided as types I, II, or III (CD1-independent), with all subtypes providing critical intravascular immune surveillance. Hepatic antigen-presenting cells will present microbial glycolipid antigens to NKT cells, stimulating secretion of Th1 or Th2 cytokines, which subsequently activates neighboring innate immune cells and adaptive T cells [97, 101]. A distant role for NKT cells in NAFLD progression is beginning to take shape, with NKT cell depletion reported in fatty liver, but high accumulation in NASH-related fibrosis [111]. This reduction in hepatic NKT cell content is reportedly mirrored in the periphery in patients with NAFLD [112]. High-fat diet and obesity are known to trigger apoptosis of NKT cells through induction IL-12 and increases in immature myeloid cells, which alone can direct NKT cell death. Invariant NKT cells (iNKT, type I cells) have been widely studied in adipose tissue derived from lean and obese mice [113]. Lynch et al. have shown high enrichment of iNKT cells in human and murine adipose and liver tissue. In an obese model, depletion of iNKT cells was observed, which correlated with the presence of macrophage infiltration [61]. Mice deficient in iNKT cells presented with enhanced weight loss, fatty liver, and insulin resistance. Hepatic NKT cells also have the ability to secrete osteopontin (OPN) and sonic hedgehog (Hh), both of which are known to promote NAFLD/NASH and fibrosis progression. Elegant studies from Syn et al. reported attenuated Hh and OPN expression and dramatically blunted liver damage in mice depleted of NKT and fed the MCD diet, indicating that hepatic NKT cells drive the progression of NASH via production of OPN and Hh ligands [114]. These studies support previous evidence that type II NKT cells initiate hepatic inflammation and exacerbate obesity leading to insulin resistance [110]. In addition to being influenced by Hh signals, NFκB signaling may also be critical to NKT regulation. NFκB1-deficient mice, which develop enhanced NASH, displayed increased hepatic NKT recruitment compared to wild-type mice [115]. NKT cell recruitment was associated with increased IL-15 expression, a cytokine that influences NKT maturation and survival. Interestingly, while NFκB loss increased NKT cell recruitment and subsequently enhanced IFNγ production, liver fibrosis (which should be attenuated by IFNγ) was still observed indicating the appreciable complexity of innate NKT-mediated responses [115].

Outlook

In light of the vast complexity of cell phenotypes/functionality, which is highly dependent on etiology and disease state, it is

evident that future work is needed to validate whether (innate) immune cell subsets can be exploited for therapeutic intervention in metabolic disorders. Classical anti-inflammatory strategies to treat insulin resistance and other obesity-mediated disorders include high-dose salicylate administration [7], TNFα neutralization [116], and inhibition of interleukin-1 signaling [117, 118], however, associated with varying effectiveness and degree of clinical improvements. In this respect, also the main class of anti-diabetic drugs, PPARγ agonists of the TZD family, has been found to exert anti-inflammatory effects, but was also associated with severe (cardiovascular) side effects [119]. Novel approaches have begun to characterize the inflammatory infiltrate in NAFLD and NASH patients to uncover markers of inflammatory cells that correlate to disease severity. Unsurprisingly, recent assessment of immune cell profiles in pediatric NAFLD indicates increased CD136+ Kupffer cell number correlates to severity of disease, while the opposite effect is observed in CD3+ cells [120]. Additionally, in adult patients with NASH, distribution of naive, memory, LT helper, and cytotoxic subpopulations in the periphery are also skewed revealing a distinct profile [121]. While these studies are small in number, expansion is expected also into other relevant organ entities, including adipose tissue and skeletal muscle, which may provide more substantial data from larger patient cohorts with appropriate controls. It is clear that each immune subpopulation is unique in function within the corresponding microenvironment, and that depletion and/or expansion of certain cell types can alter disease progression. Thus, future research in immunometabolism can be expected to further define avenues to anti-obesity and anti-diabetic therapies.

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