The role of innate immune cells in obese adipose tissue inflammation and development of insulin resistance

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Summary

Obesity is characterised by a chronic state of low-grade inflammation in different tissues including the vasculature. There is a causal link between adipose tissue (AT) inflammation and obesity-related metabolic complications, such as the development of insulin resistance and subsequently of type 2 diabetes. Intense efforts in the recent years have aimed at dissecting the pathophysiology of AT inflammation. The role of both innate and adaptive immune cells, such as macrophages or cytotoxic T cells in AT inflammation has been demonstrated. Besides these cells, more leukocyte subpopulations have been recently implicated in obesity, including neutrophils and eosinophils, mast cells, natural killer cells or dendritic cells. The involvement of multiple leukocyte subpopulations underlines the complexity of obesity-associated AT inflammation. In this review, we discuss the role of innate immune cells in AT inflammation, obesity and related metabolic disorders.

Keywords

Obesity, adipose tissue, inflammation, review, leukocytes

Introduction:
The development and time course of obesity-driven adipose tissue inflammation

Metabolic disorders related to obesity, such as insulin resistance, the metabolic syndrome and type 2 diabetes are continuously increasing worldwide. Obesity-associated metabolic disorders contribute to atherosclerosis and cardiovascular diseases (1-3). An intriguing bidirectional link between obesity and inflammation has been uncovered in the last decade (4, 5); on the one hand, low-grade inflammation in multiple tissues may mediate the detrimental effects of obesity, whereas inflammation in the adipose tissue (AT) itself significantly contributes to the development of insulin resistance (6).

The first organ influenced by nutritional excess is the white AT, displaying fat deposition and growth of adipocytes (1, 7). The hypoxia associated with white AT expansion, as well as the activation of the endoplasmic reticulum stress and oxidative stress response pathways (8) orchestrate the production of proinflammatory cytokines like interleukin (IL)-6 or tumour necrosis factor (TNF), which can directly inhibit insulin action and promote insulin resistance (9-11). The proinflammatory environment of the obese white AT activates both resident leukocytes as well as triggers further infiltration of inflammatory cells (macrophages, mast cells, neutrophils, dendritic cells, lymphocytes), resulting in qualitative and quantitative alterations of the stromal vascular fraction of the white AT (12-16). Interestingly, increased caloric intake in a meal (either as glucose or fat challenge) can rapidly activate neutrophils and monocytes in the blood already 1 hour postprandially (17, 18), resulting in an increase of blood neutrophils and elevated expression of activation markers.

Intensive research in the recent years pertinent to obesity-related AT inflammation has resulted in a scenario, in which a prominent role in promoting AT inflammation is ascribed to AT macrophages, and particularly the pro-inflammatory M1 polarised macrophages (19). In addition, lymphocytes, such as CD4+ Th1 lymphocytes, CD8+ cytotoxic lymphocytes or B cells have been implicated in AT inflammation (12, 20-22). The contribution of other immune cells, especially of cells of innate immunity remains less well understood. The purpose of this review is to summarise and discuss the involvement of innate immune cells in obesity-related at inflammation. We will focus on the mechanisms governing the accumulation and function of innate immune cells in the course of obese white AT inflammation and insulin resistance development.
Macrophages

Macrophages differentiate in a tissue from recruited monocytes and show a high degree of heterogeneity (23). Macrophages, defined as F4/80+CD11b+ cells by flow cytometry, are resident in the lean white AT, representing 5% of the stromal vascular fraction (22). They are the dominant immune cell population accumulating in the obese white AT, being the major component of the chronic low grade inflammation in the obese white AT (16, 24, 25). In the course of diet-induced obesity the percentage of macrophages in the stromal vascular fraction gradually increases up to 20-30% (22). A major role of macrophages as players in AT inflammation and in the development of insulin resistance has been established. AT macrophages-derived proinflammatory mediators, such as IL-6 or TNF directly interfere with insulin signalling (9, 10, 16, 24).

AT macrophages in lean and obese animals have different functions and inflammatory potential along with distinct surface markers and cytokine signatures. In lean AT, macrophages are rather alternatively activated M2-polarised macrophages, characterised by the presence of CD206 (mannose receptor) and macrophage galactose-type C-type lectin 1 (MGL1), which are scavenger receptors mediating phagocytosis. M2 macrophages in the lean AT express anti-inflammatory genes, such as IL-10. Functionally, M2-like cells in the lean AT contribute significantly to the maintenance of insulin sensitivity (26). In contrast, classically activated M1-polarised macrophages expressing CD11c and devoid of CD206 and MGL1 expression predominate in the obese AT (especially the visceral one). M1 cells express high levels of iNOS and the proinflammatory cytokines TNF and IL-6, which can directly inhibit insulin action in adipocytes, thereby promoting insulin resistance (19, 26-29). Additionally, a mixed M1/M2 phenotype has been observed in the obese AT; these cells are also thought to contribute to enhanced AT inflammation (19, 30). Selective depletion of CD11c+ macrophages by utilising mice expressing the diphtheria toxin receptor in CD11c+ cells resulted in reduction of the number of macrophages as well as of AT inflammation and led to improved insulin sensitivity in the diet-induced obesity-model (31).

Several mechanisms have been implicated in the shift of the M1/M2 balance in favour of M1 during diet-induced obesity. Free fatty acids promote inflammatory macrophage activation via ligation of the Toll like receptor (TLR)-4. For instance, lipid infusion in mice enhanced their AT inflammation and insulin resistance, in a manner dependent on TLR-4 (32). Consistently, haematopoietic deletion of TLR-4 (in irradiated mice that received TLR-4-/- bone marrow cells) prevented mice from AT inflammation and insulin resistance in the course of diet-induced obesity (33). In addition, a recent study demonstrated that fetuin-A, a carrier protein of free fatty acids, mediates the free fatty acid-induced TLR-4 activation. In particular, fetuin-A enhances free fatty acid-induced nuclear factor (NF)κb activation via TLR-4 in macrophages and adipocytes and reduces glucose uptake in adipocytes. Consistently, deficiency in fetuin-A or TLR-4 protected from insulin resistance and white AT inflammation in diet-induced obesity (34).

Furthermore, the macrophage expression of peroxisome proliferation-activated receptors γ and δ (PPARY and PPARD), which serve as fatty acid sensors and modulators of glucose and lipid metabolism, can regulate alternative activation to M2-like cells (35). Mice with myeloid-cell specific deficiency of PPARγ or PPARδ displayed reduced M2-polarised macrophages, increased AT inflammation and insulin resistance in diet-induced obesity (35). Similarly to PPARs, the transcription factor Krüppel-like factor 4 promotes alternative activation of macrophages; mice with myeloid-specific deficiency in Krüppel-like factor 4 developed insulin resistance in diet-induced obesity (36). Interestingly, a recent study demonstrated that exposure to cold temperature promoted alternative activation of macrophages in white AT and brown AT, which is required for metabolic adaptation to cold stress, in a manner dependent on IL-4 signalling (37).

Although AT macrophages derive from circulating monocytes that are recruited to the AT, only a few studies so far analysed the mechanisms that control trafficking of these cells into the obese AT. In this context, a role for monocyte chemoattractant protein-1 (MCP-1) and for its receptor C-C motif chemokine receptor 2 (CCR2), which orchestrate the infiltration of inflammatory monocytes and macrophages into inflamed tissues, has been suggested. The available data are, however, controversial. On one hand, mice with deficiency of MCP-1 or CCR2 were protected from insulin resistance due to reduced white AT inflammation (38, 39). Conversely, other studies found no difference or even more accumulated macrophages in the white AT and higher AT inflammation in MCP-1- or CCR2-deficient mice (25, 40, 41). Such conflicting results could be explained by differences in the genetic background, age of mice, composition of the experimental diets used or the presence of additional chemokines that compensate for the absence of MCP-1. In addition, lectin MGL1/CD301 is expressed in inflammatory monocytes and promotes the accumulation of M1 macrophages in the AT in the course of diet-induced obesity (28). Moreover, α4 integrin facilitates infiltration of monocytes into the AT (42). Mice with disturbed α4-integrin function due to a mutation (Y991A) showed decreased numbers of macrophages in the AT and were protected from insulin resistance in diet-induced obesity (42). On the other hand, the 3-phosphoinositide-dependent protein kinase 1 (Pdk1)/forkhead transcription factor (Foxo1) pathway modulates macrophage accumulation in white AT; whereas Foxo1 upregulates CCR2 expression, this effect is inhibited by Pdk1. Furthermore, Pdk1 in myeloid cells promotes M2 polarisation and obese mice lacking Pdk1 in myeloid cells displayed insulin resistance (43). In conclusion, macrophages and the polarisation thereof unequivocally contribute to the regulation of AT inflammation and insulin resistance. More studies are required to understand the underlying mechanisms of macrophage accumulation and activation in the AT.

Dendritic cells

Dendritic cells (DC) are professional antigen presenting leukocytes of myeloid origin that play a role in innate as well as in adap-
Neutrophils

In acute inflammation, neutrophils are the leukocyte subpopulation arriving first in the inflamed tissue and promoting the subsequent recruitment of inflammatory monocytes by producing MCP-1 and other chemokines (51). It is likely that neutrophils operate in a similar fashion in the course of AT inflammation. Among other mediators, the neutrophil chemoattractant IL-8 is secreted by activated adipocytes (52). In men, elevated neutrophil markers, especially myeloperoxidase, are present in the plasma of obese subjects (53). Neutrophil infiltration into the visceral AT of mice was detected as early as three days after initiation of a high-fat diet (13). According to these data, neutrophil infiltration was transient and after seven days on high-fat diet, no neutrophils were detected in the AT. In a recent paper, Talukdar et al. found that the early infiltration of neutrophils was sustained over a longer period; the percentage of neutrophils, defined as the CD11c<sup>+</sup>Ly-6g<sup>+</sup>CD11c<sup>F4/80</sup> by flow cytometry, remained unaltered at around 1.5% of the total stromal vascular fraction cells over 90 days on high-fat diet (15). Corresponding to the amount of infiltrated neutrophils, the activity of neutrophil-specific elastase was also elevated in the AT and the liver of diet-induced obesity mice (15). Inhibition or genetic inactivation of neutrophil elastase in mice improved glucose tolerance (15) and improved AT and liver insulin sensitivity, accompanied by a marked reduction in neutrophil recruitment to the white AT (15).

Intriguingly, neutrophil elastase can degrade the insulin receptor substrate 1 (15, 54) and reduce insulin-induced Akt phosphorylation in adipocytes (15). This mechanism may be involved in the neutrophil- and elastase-dependent effect on insulin resistance in diet-induced obesity, as the levels of insulin receptor substrate 1 were higher in the AT and liver of elastase-deficient mice, as compared to elastase-sufficient mice (15).

Furthermore, elastase could contribute to AT inflammation via activation of signalling pathways downstream of TLR-4. Elastase can increase the expression of pro-inflammatory genes in peritoneal macrophages in a TLR-4-dependent manner (15). Consistently, elastase-deficient white AT displayed a decrease in expression of pro-inflammatory markers concomitantly with an increase of anti-inflammatory markers, while reduced numbers of M1-polarised macrophages were observed in the elastase-deficient obese AT (15). Thus, a dual mechanism of action of neutrophil elastase, involving interference with insulin signalling, as well as activation of the TLR-4-dependent AT inflammation, could account for its role in promoting development of insulin resistance.

The profound phenotype of elastase deficiency on AT inflammation and insulin resistance is intriguing. Further mechanisms of action of elastase could also account for its pro-inflammatory functions in the obese AT. Elastase can activate immune cells via protease activated receptors and by interacting with β2-integrins, thereby activating leukocyte adhesion (55, 56). Moreover, elastase can modify the chemokine and cytokine network by proteolytic activation or inactivation (57). Together, these mechanisms could also contribute to the phenotype of elastase deficiency in obesity-associated insulin resistance, which merits investigation in future studies.

Interestingly, not only neutrophils play a role in obesity and insulin resistance, but also obesity may affect neutrophil function. Hypercholesterolaemia induces elevation of blood neutrophils (58, 59). Despite the elevated CD11b expression on circulating neutrophils in obese patients (60), the number of blood neutrophils in both diet-induced obesity and db/db mice lacking leptin receptor is increased and mouse neutrophils display impaired chemotaxis towards CXCL1 chemokine (61). Along this line, neutrophil recruitment in acute lung injury was attenuated in models of diet-induced obesity and genetic obesity, i.e. in db/db mice (61). Lower neutrophil recruitment could be attributed to diminished expression of the receptor for CXCL1 and CXCL8 chemokines, CXCR2 (61). A negative feedback loop exists between IL-8 and CXCR1/2 receptors expression (62), which could link the two independent observations that neutrophils from obese mice express less CXCR2 (61), while IL-8 levels in the plasma of obese patients are elevated (63).

Eosinophils

Eosinophils can modulate obesity-associated AT inflammation by regulating macrophage polarisation. In contrast to neutrophils that promote pro-inflammatory M1 polarisation, eosinophils were recently found to assist in glucose homeostasis by sustaining the levels of anti-inflammatory M2 macrophages in the white AT (64). The cytokines IL-4 and IL-13 contribute to the M2-polarisation of macrophages in white AT (35) and signalling through the IL-4/STAT6 axis attenuates white AT inflammation, while disruption of IL-4/STAT6 signalling decreased insulin sensitivity (65). Eos-
Eosinophils resident in the white AT were identified as the main source of IL-4 and IL-13, thereby contributing to the anti-inflammatory phenotype of lean AT (64). Eosinophils represent about 5% of the stromal vascular fraction cells in the lean white AT and their presence decreases with obesity. By engaging eosinophil-deficient and hypereosinophilic mice, Wu et al. showed that the number of M2 macrophages in AT is dependent on the eosinophil-derived IL-4 and IL-13 in the AT. In the absence of these two cytokines, the number of M2 macrophages was dramatically reduced, while total macrophage numbers remained unaltered. Consistently, hypereosinophilic mice displayed improved glucose tolerance, while eosinophil-deficient mice had higher total body fat, impaired glucose tolerance and insulin resistance (64).

Another source of IL-4 could be natural killer T (NKT) cells (66). Similarly to eosinophils, NKT cells are not present in chronically obese AT, promote M2 macrophage polarisation via IL-4/STAT6 signalling, and when activated, they improve glucose tolerance (66, 67). Thus, eosinophils and NKT cells promote and sustain M2 macrophage polarisation in the AT, and whether they do so independent of each other or acting in concert, is not known.

Eosinophil function may also be altered in obese individuals. Despite lower numbers of eosinophils in the obese AT, the production of eosinophils in the bone marrow and their infiltration into the lung in the course of allergic disease are increased in the obese state (68). Increased migration of eosinophils may result from the effect of leptin that facilitates eosinophil responsiveness to eotaxin (69). The prevalence of allergy is positively correlated with obesity (70) and the change in eosinophil function in obesity could be the common denominator between these two diseases (71).

Natural killer cells

Natural killer (NK) cells are of lymphoid origin, but unlike T or B cells, they do not express any antigen-specific receptors and are therefore considered as part of the innate immunity. NK cells participate in antigen-independent lysis of virus-infected cells or in tumour immunity. NK cell maturation and function require certain signals, e.g. IL-1, IL-15 and IL-18 (86, 87). NK cells secrete cytokines like IFN-γ or TNF and several chemokines, including CCL2 (MCP-1) and CXCL8 (IL-8). An intense crosstalk between NK cells and other leukocytes, including lymphocytes, macrophages and neutrophils exists (87, 88). The role of NK cells in obesity and AT inflammation is not entirely clear. While several groups reported unchanged number of circulating NK cells in obesity (89-91), others observed significantly lower numbers of circulating NK cells in obese subjects than in lean controls (92). In addition, a decrease in circulating NK cell counts was observed in obese women that were put on a caloric restriction diet (93). Metabolically obese healthy subjects had more circulating non-activated NK cells, while lower NK counts and higher NK cell activity was found in unhealthy obese patients (92). Higher numbers of NK cells positive for the activating receptor NKG2D were detected by experiments with the MC-stabilising drugs cromolyn and ketotifen, commonly used in allergy and asthma. These treatments improved metabolic dysregulation in diet-induced obese mice and reversed the weight gain of mice that were fed a high-fat diet for 12 weeks. Moreover, reconstitution of Kit$^{W-sh/W-sh}$ mice with bone marrow-derived MC from WT and Tnf$^{-/-}$, but not of If-6$^{-/-}$ or of Ifng$^{-/-}$ mice reversed the MC-deficiency phenotype (14). These data suggest an important role of MC-derived IL-6 and interferon (IFN)-γ in obesity.

In addition to IL-6 and IFN-γ, MC secrete a wide range of mediators, which could modulate the environment of the inflamed white AT. The MC mediator 15-deoxy-delta12,14-prostaglandin J2 was found to be a direct endogenous ligand and activator of PPARγ, thereby promoting adipocyte differentiation (82).

Although there is still little known about how specific MC products affect AT inflammation and obesity, MC can regulate activation of macrophages via several ways, including MC-derived IFN-γ and matrix metalloproteinase 9 (83), or MC-derived phospholipase A2 (84). MC proteases that are released from preformed granules upon MC activation, could significantly influence the AT microenvironment. For instance, chymase could promote angiogenesis (85) and, indeed, reduced angiogenesis was observed in the AT of MC-deficient mice and upon MC stabilisation (14).
were found (94). Interestingly, Rag2-/- mice with T and B lymphocyte deficiency displayed elevated numbers of NK cells in the obese white AT in diet-induced obesity, as compared to the white AT of lean Rag2-/- or obese wild-type mice (94). These data suggest that lymphocytes may prevent NK cells from entering the inflamed white AT.

The presence of NK cells in the AT depends on the expression of the cytokine IL-15. Overexpression of IL-15 in mice increased the numbers of NK cells in the visceral white AT, while IL-15-/− mice had almost no detectable NK cells in the white AT. The level of IL-15 expression negatively correlated with weight gain, and vice versa, obesity negatively correlated with circulating IL-15 levels (95, 96).

Another link between NK cell activation and obesity is leptin signalling (97). Leptin regulates NK cell development, as leptin receptor-deficient db/db mice had diminished number of NK cells in the liver, spleen, lung and blood (97). NK cell cytotoxicity was lower in db/db mice, and leptin treatment affected cytotoxicity in wild-type, but not in db/db mice (97). Short-term exposure to leptin-stimulated production of IFN-γ, expression of CD69 and increased cytotoxicity in NK cells, while long-term leptin signalling negatively affected NK cell function (98). This is in keeping with another study, whereby NK cells from lean mice displayed four times higher activation than NK cells from obese mice after in vivo leptin administration (99). An important implication of these studies is that attenuation of NK cell function due to obesity could be permissive for cancer development in obese patients, given the important role of NK cells in tumour surveillance (100).

The role of the inflammasome in obesity

The inflammasome is a multi-protein complex that functions as a danger-sensing intracellular sensor. It is activated by many stimuli, like viruses, bacteria or by certain types of endogenous metabolites, including those associated with obesity. Inflammasome com-
plex comprises an intracellular sensor, usually from the family of Nod-like receptors, e.g. NLRP1, NLRP3 or NLRC4, the cystein protease caspase-1 and finally the adaptor protein apoptosis-associated speck-like protein containing a caspase-recruitment domain (PYCARD). Activation of the inflammasome leads to activation of caspase-1 and subsequent release of the cytokines IL-1β and IL-18 that promote inflammation (101). The role of the NLRP3 inflammasome in obesity–associated insulin resistance has been recently established by using mice deficient in various NLRP3 components. Caspase-1 promotes insulin resistance in adipocytes in a manner dependent on IL-1β. Adipocytes deficient in caspase-1 and NLRP3 displayed higher metabolic activity. Moreover, mice deficient in caspase-1 and NLRP3 were significantly protected from insulin resistance (102). Saturated, but not unsaturated free fatty acids and their metabolic products like ceramide, can activate NLRP3, caspase-1 and IL-1β release from macrophages, leading to increased inflammation (103, 104). Furthermore, the deletion of NLRP3 prevents from liver and AT associated inflammasome activation and results in reduced production of IL-18, IFN-γ and attenuated T cell activation in the AT (104). Together, several studies implicate inflammasome activation in obesity, thereby resulting in the proinflammatory environment of the obese AT and the liver.

Conclusion and future outlook

Besides the well-established action of macrophages in AT inflammation and insulin resistance, the present review focused on the role of further innate immune cells, such as DC, neutrophils, eosinophils, MC and NK cells in the obese AT, which appear to be more relevant than previously appreciated (Figure 1). Neutrophils infiltrate white AT early after nutrient excess, and increased neutrophil recruitment to the obese AT is seemingly sustained over a longer period (15). Neutrophil-derived elastase in the obese AT may promote insulin resistance (15). It remains open, whether other neutrophil proteases, such as cathepsin G or proteinase 3 might also contribute to AT inflammation. In contrast, eosinophils help sustain the anti-inflammatory milieu of the lean AT by promoting M2 polarisation of macrophages (64). The role of eosinophils could reveal an interesting connection between obesity and allergy, two conditions that correlate with each other (70). MC also appear to be important players in obesity and the metabolic syndrome (14). MC possess a wide range of secretory effectors and their role in chronic white AT inflammation is likely very complex. Mechanistic studies on the role of main MC-derived mediators in AT inflammation and obesity are needed. NK cells are involved in several obesity-related complications, and their activation is somehow dependent on leptin signalling (97, 98). Their role in obesity-induced AT inflammation is not yet clear and requires further attention.

Taken together, innate immune cells, apart from macrophages, have been appreciated in obesity and metabolic research only in the last few years. Several recent publications have shed light on the role of these cells as regulators of obesity-related insulin resistance. Future studies are required to understand the mechanisms underlying the role of these innate immune cells and the crosstalk thereof in obese AT inflammation and obesity.

Conflicts of interest
None declared.

References
