REVIEW

Immunology of chronic low-grade inflammation: relationship with metabolic function

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Abstract

Inflammation is part of the body’s innate immune response and is an essential process that not only defends against harmful bacteria and pathogens but also plays a key role in the maintenance and repair of tissues. Under pathological conditions, there is bilateral crosstalk between immune regulation and aberrant metabolism resulting in persistent inflammation in the absence of infection. This phenomenon is referred to as sterile metabolic inflammation (metainflammation) and occurs if the initiating stimulus is not removed or if the resolution process is disrupted. Disruption of this tightly regulated immune response and its failure to resolve as is evident in metabolic disorders is not only associated with disease progression but also leads to immune senescence and should not be neglected in the clinical management of patients. This review gives an overview of the mechanisms underlying chronic metabolic inflammation, the aberrant metabolic activation of innate immune cells (neutrophils, macrophages, mast cells, dendritic cells), and its role in disease progression using obesity–diabetes as a prime example. Addressing the underlying subclinical metabolic inflammation in addition to achieving glucose control may contribute significantly towards therapeutic interventions aimed at preventing the onset of co-morbidities in diabetic patients.

Introduction

Inflammation is part of the body’s innate immune response and is an essential process that not only defends against harmful bacteria and pathogens but also plays a key role in the maintenance and repair of tissues. It is mediated through the immune cell (leukocyte)-dependent mechanisms, the nutritional microenvironment, substrate availability as well as secreted factors (acute phase proteins, proteases, cytokines/chemokines, and complement factors) (Gasteiger et al. 2017, Kurita et al. 2021). Under pathological conditions, there is bilateral crosstalk between immune regulation and aberrant metabolism resulting in persistent inflammation in the absence of infection (Furman et al. 2019). This phenomenon is referred to as sterile metabolic inflammation (metainflammation) (for terminology see Box 1) and occurs if the initiating stimulus is not removed or if the resolution process is disrupted (Fig. 1 and 2). This low-grade chronic metabolic inflammation should not be neglected as it is significantly associated with all-cause mortality in the general population (Fest et al. 2019), negatively impacts insulin sensitivity (Blaszczak et al. 2020), and increases the risk for cancer development (Li et al. 2023).

The acute inflammatory process is well described in literature and consists of two sequential phases, the first involving neutrophil, monocyte/macrophage recruitment, and activation as illustrated in Fig. 3A. In the absence of infection, the primary aim of this initial response is phagocytosis of cellular/tissue debris to

Key Words
- neutrophils
- macrophages
- hyperglycaemia
- diabetes
- inflammation
prepare injured/apoptotic tissue for repair. Danger signals within the extracellular space released from the cytoplasm of lysing cells, damage-associated molecular patterns (DAMPs, also known as alarmins) are recognized by tissue-resident and infiltrating leukocytes through pattern recognizing receptors (PRRs). These PRRs include Toll-like receptors (TLR), Nod-like receptors, complement receptors, intracellular nucleic acid sensing receptors, and C-type lectin receptors, each of which initiates specific effector functions within leukocytes (Gasteiger et al. 2017, Chen et al. 2018, Furman et al. 2019). During this initial response there is a complex interaction between cells from the fast-acting innate immune system (neutrophils, monocytes/macrophages, invariant natural killer type cells (iNKT), myeloid-derived suppressor cells, innate lymphoid cells (ILC), basophils, eosinophils, and mast cells) and those from the adaptive immune system (lymphocytes including Th1/17, Th2, T-regulatory cells (Tregs), and B cells) (Gasteiger et al. 2017, SantaCruz-Calvo et al. 2022).

Granulocytes such as neutrophils are the primary cytotoxic effector cells that respond to inflammatory signals. Their activity precedes the recruitment of phagocytic monocytes/macrophages, dendritic cells, and several lymphocyte subtypes to sites of inflammation (Gasteiger et al. 2017, Furman et al. 2019). The recruitment of these immune cells by chemokines is mediated through cognate G-protein coupled chemokine receptors (termed according to their first cysteine residue into either C, C-C, CXC, CX3C categories) (Xue et al. 2019), whilst pro-inflammatory cytokines act through cell surface glycoprotein receptors to transmit paracrine signals through the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) and nuclear factor kappa B (NFκB) pathways to amplify inflammation (O’Shea & Murray 2008). The cytotoxic effector functions of phagocytic leukocytes cause nutrient depletion, increase oxygen consumption, and generate large quantities of reactive nitrogen and oxygen species (ROS) within tissue and have the potential to cause secondary tissue damage (Kominsky et al. 2010). The pro-inflammatory response is thus followed by a resolution phase during which neutrophils undergo programmed cell death (apoptosis), whilst phagocytic macrophages switch towards an anti-inflammatory phenotype to promote tissue repair and remodelling. Disruption of this tightly regulated immune response and its failure to resolve as is evident in metabolic disorders is not only associated with disease progression but also leads to immune senescence.
Inflammatory biomarkers such as the systemic immune-inflammatory index and the neutrophil-to-lymphocyte ratio (NLR) can be used as early indicators or predictors of clinical outcomes for a variety of diabetes-associated conditions including, nephropathy (microalbuminuria) (Chollangi et al. 2022, Qin et al. 2022), depression (Wang et al., 2021), macular oedema/retinopathy (Wan et al. 2020, Elbeyle et al. 2022), ketoacidosis (Cheng et al. 2021), foot ulcers (Vatankhah et al. 2017, Serban et al. 2021), cardiovascular disease-related adverse events (Saylik & Akbulut 2022), and pregnancy outcomes in gestational diabetes (GDM) (Wang et al. 2020, Pace & Vassallo 2021). Addressing the underlying subclinical metabolic inflammation in addition to achieving glucose control may thus contribute significantly towards therapeutic interventions aimed at preventing the onset of co-morbidities in diabetic patients.

Mechanisms underlying chronic metabolic inflammation

Adipose tissue expansion and subsequent insulin resistance-induced hyperglycaemia is considered the key driver of sterile metabolic inflammation in T2DM patients. The link between visceral adipose tissue (VAT) dysfunction and insulin resistance is well established (Calderón-DuPont et al. 2022, Kahn et al. 2022). Dysregulated expansion and hypertrophy of VAT during obesity induce tissue hypoxia; alter the secretory profile of adipokines, cytokines/chemokines, bioactive lipids, and modify the distribution and quantity of immune cells within the tissue (Fig. 2). Animal models have given significant insight into alterations that occur in the immune cell profile within VAT following high-fat...
diet-induced obesity. Using this model, several studies observed considerable infiltration of immune cells that promote inflammation (neutrophils, phagocytic macrophages, Th1 lymphocytes, dendritic cells, and mast cells) into the adipose tissue of obese mice compared to healthy controls, whereas regulatory immune cells that function to alleviate inflammation (Tregs, Th2, iNKT) become depleted (McLaughlin et al. 2017, Blaszczak et al. 2020). The rupture of hypertrophied adipocytes, excessive lipolysis, and release of pro-inflammatory mediators from dysfunctional adipose tissue is known to recruit phagocytic macrophages to form crown-like structures surrounding damaged/ruptured adipocytes (Murano et al. 2008, Gasteiger et al. 2017, Gugliucci 2022). The role of lymphocyte accumulation within dysfunctional adipose tissue is however less well defined but is thought to occur as a result of the altered VAT secretory profile.

Extensive analysis of the secretome of adipose tissue explants (subcutaneous (SAT) vs visceral (VAT)) derived from obese patients confirmed that dysfunctional visceral fat releases excessive amounts of pro-inflammatory cytokines, adipokines, and prostanoids as summarized in
Box 1  Terminology

**Definition:** Metabolic inflammation (also known as meta-inflammation) refers to chronic low-grade systemic inflammation fuelled by metabolic disturbances.

**Descriptive terms:** (associated with metabolic inflammation)

Subclinical or low-grade is indicative of inflammation that is not severe enough to present definite or readily observable symptoms.

Sterile refers to inflammation that occurs in the absence of any infection.

Persistent or chronic refers to inflammation that does not resolve over time.

Systemic relates to the whole-body system as opposed to a particular area.

Fig. 2 (Kahn et al. 2022). This skewed secretome not only mediates local immune cell infiltration but is also released into circulation via the portal vein leading to systemic inflammation and has a subsequent functional impact on the insulin responsiveness of various other tissues including SAT, the liver, and skeletal muscle (Kahn et al. 2022, Ren et al. 2022). In the *ex vivo* study done by Kahn et al. (2022), the authors demonstrated that if the factors released by dysfunctional visceral fat explants are applied to either hepatocytes or skeletal muscle myotubes, their insulin sensitivity is negatively impacted and glucose handling is impaired. There is thus an accumulation of extracellular glucose (hyperglycaemia) in circulation, whereas intracellular glucose availability for glycerol and fatty acid synthesis is reduced due to insulin resistance. This leads to a further reduction in lipogenesis (esterification and synthesis of signalling bioactive lipids) within adipose tissue, whilst the impaired antilipolytic effects of insulin result in an increase in circulating free fatty acids (Calderón-DuPont et al. 2022). Together this leads to glucotoxicity and lipotoxicity inducing a myriad of complications related to multisystem cellular senescence and organ failure.

Prolonged hyperglycaemia causes glucose to form covalent bonds with various plasma proteins (albumin, fibrinogen, globulins, collagen), intracellular lipids, and nucleic acids to form different types of advanced glycation end products (AGEs) that interfere with the normal function of these molecules (Singh et al. 2014). Glycation of proteins disrupts their molecular conformation, alters enzyme activity, and interferes with receptor functioning. Extracellular AGEs bind to the receptor for advanced glycation end products (RAGE) on plasma membranes and alter intracellular signalling and gene expression to amplify inflammation through NFκB signalling (Tóbon-Velasco et al. 2014, Ramasamy et al. 2016). These receptors are known to be expressed on T-lymphocytes, monocytes, and macrophages and play a key role in priming the immune response (Yan et al. 2008).

A metabolic shift in numerous cell types and tissues furthermore causes the excess glucose to enter the polyol pathway in which glucose is reduced to the intermediate product, sorbitol, by aldose reductase in a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reaction. Sorbitol is subsequently oxidized to fructose by sorbitol dehydrogenase. Upon phosphorylation, fructose can re-enter the glycolysis pathway as fructose-6-phospate. An imbalance in diabetes and overload of the polyol pathway results in NADPH depletion and the accumulation of sorbitol which in turn cause widespread glycation of respiratory chain proteins and triggers intracellular damage by inducing oxidative and endoplasmic reticulum stress through elevated production of cytosolic ROS (Yan 2018). The depletion of NADPH leads to glutathione deficiency and insufficient antioxidant buffering capacity to counter this phenomenon causing mitochondrial dysfunction, DNA damage, and telomere shortening (Kumar et al. 2022, Sekhar 2022). Immune cells are especially sensitive to these pathological changes and become senescent whilst remaining metabolically active with a senescent-associated secretory phenotype. There is thus a continuous release of pro-inflammatory signals of diverse origins within the pathological microenvironment associated with obesity and diabetes that persistently amplifies the inflammatory response.

The exact mechanisms underlying the failure to resolve inflammation in obesity and diabetes are not yet fully understood but are thought to entail multilevel interaction between the immune system and metabolic processes that lead to aberrant leucocyte activation. This includes (i) the effect of pathological microenvironment on the immune-metabolic responses; (ii) the effect of leucocytes’ own metabolism on their effector functions; and (iii) the influence of leucocytes on metabolic processes within the tissue. The focus of this review will be on the dysregulation of innate immune cells under conditions of metabolic inflammation, the role of adaptive immune cells is reviewed elsewhere (SantaCruz-Calvo et al. 2022).

**Dysregulation of innate immune cells**

**Neutrophils**

Neutrophils (also known as polymorphonuclear leukocytes) are considered the predominant effector cells in sterile metabolic inflammation that respond to DAMPs. They are...
produced in the bone marrow through the process of granulopoiesis, mature and are mobilized into circulation through chemotactic signals and infiltrate various tissues through diapedesis where it exerts its effector functions and undergo apoptosis. The effector functions of these leukocytes include (i) oxidative bursts and the release of lytic enzymes such as myeloperoxidase and neutrophil elastase (NE) (degranulation), (ii) phagocytosis and production of pro-inflammatory cytokines, and (iii) the formation of neutrophil extracellular traps (NETs). These cells are functionally versatile with phenotypic heterogeneity and are important mediators of the inflammatory response (Rosales 2018). The rapid generation and release of reactive oxygen intermediates upon activation are mediated by the NADPH oxidase (Nox) complex following integrin-dependent adhesion to the extracellular matrix within the tissue (Graham et al. 2007). The functional activity of neutrophils is however dependent on the maintenance of intracellular energetics and disturbances in intracellular metabolism can alter their effector functions (Kumar & Dikshit 2019, Sadiku et al. 2021). Neutrophils have a very small number of mitochondria compared to other cells and therefore mainly rely on glycolysis for energy production (Maianski et al. 2004).

Neutrophils can however survive in injured tissues with limited availability of oxygen and metabolic substrates by relying on intracellular glycogen stores and the dynamic regulation of various other intracellular metabolic pathways depending on their immediate functional requirements (Kumar & Dikshit 2019). In a recent study, Sadiku et al. (2021) indicated that under conditions of physiological stress (in the absence of glucose and oxygen), neutrophils utilize gluconeogenesis to generate glycolytic intermediates from non-glucose substrates to increase their glycolytic capacity. The authors further indicated that even though neutrophils have a high level of fatty acid oxidation at baseline, upon stimulation with inflammatory mediators, there is a significant increase in intracellular glycolytic flux and redox buffering capacity (Sadiku et al. 2021). Defects in intracellular glucose cycling can therefore negatively impact key neutrophil functions, whereas a disproportionate increase in glycolysis due to the excessive availability of extracellular glucose fuels persistent neutrophil activity (Sadiku et al. 2021).

Thimmappa et al. (2022) elucidated specific neutrophil responses following various activating signals by assessing the differential phosphorylation of proteins using an unbiased quantitative phosphoproteomics approach. The authors indicated that the ex vivo exposure of neutrophils to hyperglycaemia for a period of 3 h induced a 6-fold increase in NETs formation compared to only a 3-fold increase upon stimulation with either lipopolysaccharide (LPS) (bacterial infection) or the metabolic intermediate homocysteine for the same period. The excessive NETs formation under hyperglycaemic conditions was preceded by constitutive activation of neutrophils (Fig. 3B). This observation is supported by various animal studies indicating that obesity predisposes neutrophils to spontaneously release weak NETs without stimulation (Cichon et al. 2021, Burczyk et al. 2022). This process is thought to involve C-Jun-N-terminal kinase (JNK) induced Rho GTPase kinase activity which is known to play an important role in NADPH oxidase activation as well as regulation of actin cytoskeletal rearrangement, both of which are required for NETs formation (Lacy & Eitzen 2008, Gavillet et al. 2018, Thimmappa et al. 2022). The externalized components of NETs which include chromatin, citrullinated histones, cell-free DNA, nucleosomes, and NE persist in vasculature and have been linked to the pathogenesis of disease progression (Kaplan & Radic 2012, Papayannopoulos 2018). In the context of diabetes, hyperglycaemia-induced constitutive formation of weak NETs is considered the main pathophysiological cause of diabetic kidney disease (DKD).

Both animal and human studies have shown a correlation between continuous NET formation and the severity of DKD (Gupta et al. 2022). Under high glucose conditions, NETs promote nucleotide-binding domain (NOD)-like receptor protein 3 (NLRP3) inflammasome activation in phagocytic macrophages which in turn amplifies inflammation through interleukin (IL)-1β activity causing endothelial/glomerular filtration barrier dysfunction and fibrosis (Gupta et al. 2022). The collateral microvascular damage causes the release of DAMPs which include nucleic acids, adenosine triphosphate (ATP) and various metabolites from injured endothelial cells into the extracellular space which in turn is recognized by PRRs on leukocytes leading to a hyperinflammatory response (Garcia-Martinez et al. 2015). Animal studies have demonstrated that inhibition of NETs formation by using a peptidyl arginine deiminase 4 (PAD4) inhibitor to prevent the citrullination of histones and subsequent destabilization of chromatin within neutrophils can ameliorate endothelial dysfunction (Gupta et al. 2022) and that inhibition of NLRP3 inflammasome activity reduces DKD severity (Zhang et al. 2019a). Similarly, various studies have also implicated neutrophil activity in diabetic retinopathy (He et al. 2022), acute lung injury (Jiang et al. 2014), peripheral neuropathy (Chen et al. 2021), and foot ulcers (Lee et al. 2020).
these studies confirm the role of constitutive neutrophil activity in disease progression under conditions of sterile metabolic inflammation.

In the clinical setting, glucose control on its own is however insufficient to prevent secondary tissue damage caused by persistent metabolic inflammation. In a randomized control trial, Menegazzo et al. (2018) indicated that insulin or dapagliflozin (sodium-glucose cotransporter 2 (SGLT2)-inhibitor) treatment in T2DM patients could not inhibit NETs formation despite achieving glucose control, whereas metformin treatment could reduce the concentrations of NET components in the plasma of prediabetic patients independently from glucose control by preventing the membrane translocation of protein kinase C-βII and activation of NADPH oxidase. The anti-inflammatory properties of metformin that occur independently of glucose control have also been shown to cause a 9% reduction in the NLR in T2DM patients after prolonged (8–16 months) treatment, whereas this phenomenon was not evident in patients on sulfonylurea monotherapy despite achieving similar levels of glucose control (Cameron et al. 2016). In a subsequent study, Tikhonova et al. (2020) showed that hyperglycaemia in T2DM patients causes disturbances in the mechanisms of NADPH oxidase activation and intracellular Ca²⁺ signalling systems within neutrophils. The authors, therefore, supported the findings of Menegazzo et al. (2018) and suggested that NADPH oxidase within granulocytes could be a promising target for clinical intervention to manage diabetic complications related to metabolic inflammation. There are several small molecules currently being developed in the pharmaceutical industry aimed at inhibiting excessive NADPH oxidase activity, unfortunately, due to several ‘off-target’ effects and the detrimental consequences of completely blocking ROS production, very few of these molecules have progressed to phase I clinical trials (Altenhöfer et al. 2015, Sassetti et al. 2021).

It is furthermore unclear if blocking persistent NADPH oxidase activity will restore the normal functional responses of neutrophils. Despite the high glycolytic potential of neutrophils and their ability to oxidize fatty acids, their continuous activation under high glucose conditions limits the responsiveness of these leukocytes to subsequent stimulation with LPS and impairs chemotaxis (Joshi et al. 2020, Roy et al. 2022, Thimmappa et al. 2022). A delay in neutrophil trafficking and inability to mount a sufficient phagocytic response together with limited production of additional stronger NETs due to this ‘exhausted phenotype’ make diabetic patients vulnerable to infection.

Monocytes/macrophages

Monocytes/macrophages play a critical role in the resolution of inflammation and their dysregulation has been implicated in various diabetes-related pathological conditions including adipose tissue dysfunction, atherosclerosis, non-healing wounds, and kidney disease. The recruitment of circulating monocytes and tissue-resident macrophages to sites of inflammation is initiated by the release of chemokines and apoptotic cell-derived extracellular vesicles from within tissue. Apoptotic vesicles (derived from apoptotic neutrophils and tissue) play an important role in intracellular communication and are thought to contain lipids, proteins, miRNAs, and immune-modulatory enzymes that together with DAMPs affect the activation status of macrophages (Torr et al. 2012, Grant et al. 2019, Ross et al. 2021). It is well established that macrophages have a continuum of different phenotypes that can either promote inflammation (M1 classically activated; regulated by hypoxia-inducible factor 1, NFκB, interferon-regulatory factor signalling) or facilitate tissue repair/remodelling (M2 alternatively activated; regulated by peroxisome proliferator-activated receptors (PPAR), nuclear factor erythroid 2-related factor, STAT6 signalling). The classically activated macrophages (M1) (surface markers CD14, CD80, CD86, CD38) mainly rely on glycolysis for energy production, whereas alternatively activated macrophages (M2) (surface markers CD36, CD206, CD163) have a higher rate of oxidative phosphorylation (Mantovani et al. 2004, Jha et al. 2015) (Fig. 3A). The phenotypic plasticity of macrophages is however very complex and their ability to switch phenotype is not only dependent on activating signals but also on intracellular bioenergetics and nutrient availability (Jha et al. 2015). These cells can thus have various intermediate roles on the spectrum between M1 and M2 activation statuses depending on the microenvironmental factors.

In the acute inflammatory response, the short-lived neutrophil activity is followed by that of M1 macrophages. The classically activated macrophages are phagocytic/cytotoxic and have a high glycolytic rate (Jha et al. 2015, Rasheed & Rayner 2021). These cells assist with the clearing of tissue debris (apoptotic bodies) and play a crucial role in recognizing apoptotic neutrophils and removing dead cells through the process of efferocytosis (Mahmoudi et al. 2022). This process is reviewed elsewhere but involves (i) recognition of dead/dying cells through ‘find me’ signals, which include soluble chemokines, nucleotides, and membrane lipids; (ii) phagocytic macrophages respond to ‘eat me’ signals such as a lack of phospholipid asymmetry...
on the plasma membrane, display of endoplasmic reticulum lumen proteins on the cell surface and the presence of lipid biomolecules (lysophosphatidylcholine, LPC) on apoptotic bodies; and iii) engulfs/destroys the apoptotic cell to recycle cellular components and restore homeostasis (Mahmoudi et al. 2022, Tajbakhsh et al. 2022).

During efferocytosis, there is activation of a molecular switch that drives phenotype and bioenergetics changes in macrophages from M1 (high glycolytic activity) towards M2 (oxidative phosphorylation) to resolve inflammation. The exact mechanism of this phenotype switch is still unclear but is thought to involve the activity of various specialized pro-resolving lipid mediators (SPMs) (such as eicosanoids, lipoxins, resolvins, protectins, maresins) (Ryan & Godson 2010, Börgeson & Godson 2012) and nuclear receptor signalling (PPARγ/α, liver X receptors, glucocorticoid receptors, orphan nuclear receptors (Nur)) (Stiefel et al. 2022). During this process, lysosomal degradation of apoptotic bodies through catabolism of cell corpses furthermore provides substrates for the metabolic switch to favour oxidative phosphorylation and triggers various nuclear transcription factors to induce the transcription of immunosuppressive cytokines such as IL10 and transforming growth factor-β (Zhang et al. 2019b). Disruption of efferocytosis as seen in metabolic disorders, such as diabetes, results in the accumulation of necrotic/pyroptotic neutrophil bodies which aggravate inflammation through increased activation of M1 macrophages, dendritic cells, and Th1/Th17 lymphocyte responses (Lee et al. 2022, Mahmoudi et al. 2022). In addition to continuous activation signals observed in obesity and diabetes, given the importance of intracellular metabolism in determining macrophage function, aberrant activation and reprogramming of macrophages also occur due to metabolic disturbances (An et al. 2019, Mahmoudi et al. 2022). There is however currently a lack of pharmaceutical agents to target the mitochondrial metabolism of macrophages to promote the resolution of inflammation. It is important to note that cell origin and tissue localization influence macrophage biology and that unique tissue-specific macrophage populations may respond differently towards metabolic dysregulation (Artyomov et al. 2016). Adipose tissue macrophages (ATMs) are at the centre of adipose tissue dysfunction and are sensitive to prolonged metabolic changes.

In a study by Kratz et al. (2014), a proteomic approach demonstrated that macrophages derived from obese adipose tissue have neither an M1 nor M2 phenotype but rather represented a state of metabolic activation. The authors confirmed their findings by exposing macrophages to conditions characteristic of metabolic syndrome (high glucose, palmitate, insulin) and observed a metabolically activated status rather than the classical inflammatory phenotypes (Kratz et al. 2014). In agreement, Boutens et al. (2018) investigated the metabolic signatures of ATMs in lean and obese mice and demonstrated that obesity reprogrammes macrophages to have dual fuel bioenergetics to increase both glycolysis and oxidative phosphorylation. Using an ex vivo model in which macrophages were co-cultured with adipose tissue explants, the authors indicated that metabolic activation of macrophages was induced in a dose-dependent manner by factors derived from adipose tissue (Boutens et al. 2018). The constant exposure to excessive leptin under obesogenic conditions activates JAK2–STAT3 and phosphatidylinositol-3-kinase and the mammalian target of rapamycin (PI3K–Akt–mTOR) signalling cascades resulting in metabolic changes that increase glucose uptake, upregulate glycolytic enzymes, disrupt mitochondrial function, and subsequently increase phagocytic activity and the production of pro-inflammatory cytokines (Monteiro et al. 2019). In the study by Boutens et al. (2018), transcriptomic analysis furthermore confirmed that glycolysis was the main contributor to cytokine release in metabolically activated macrophages.

In disease states with high metabolic demand, glucose metabolism is the preferred fuel source for energy production, and glycolysis is thus favoured over oxidative phosphorylation in a phenomenon referred to as the Warburg effect (Rasheed & Rayner 2021). The rapid production of acetyl-CoA during glycolysis facilitates the expansion of the endoplasmic reticulum and Golgi apparatus for increased pro-inflammatory cytokine production. Increased glycolytic signalling that is mediated by both substrate availability and increased glucose transporter (GLUT)-1 membrane translocation is furthermore thought to imprint an M1-like phenotype on macrophages through epigenetic changes (Rasheed & Rayner 2021). These changes involve the interaction of glycolytic intermediates such as pyruvate kinase M2 and lactate with histone deacetylases causing chromatin remodelling and ultimately immune memory favouring pro-inflammatory macrophage identity (Guzik & Cosentino 2018, Rasheed & Rayner 2021). The epigenetic alterations in metabolic pathways in macrophages of T2DM patients furthermore cause dynamic changes in TLR4 expression and affect the ability of these cells to respond to subsequent activating signals (Davis et al. 2020).

These epigenetic changes and immune memory persist even after significant weight loss and achieving glucose
control. In an animal study, Blaszczyk et al. (2020) indicated that mice exposed to a high-fat diet for 3 months followed by a normal diet for 3 months maintained an obesogenic memory on the tissue level. Despite the significant weight loss, the immune cells did not normalize in these animals and adipose tissue inflammation persisted. Similarly, a study comparing the profile of SPMs in morbidly obese diabetic vs mildly obese non-diabetic patients before and after bariatric surgery, indicated that despite significant weight loss and remission of diabetes metabolic inflammation was sustained in the diabetic patients (Schulte et al. 2020).

The secretome of dysregulated ATMs is furthermore thought to influence the crosstalk between adipocytes and cancerous cells. In an ex vivo study, Vallega et al. (2022) investigated the effects of macrophages in the early stages of tumour development during obesity. The authors utilized a human co-culture system to study the paracrine interactions and demonstrated that pro-inflammatory macrophage-conditioned media intensified the crosstalk between breast cancer cells and adipocytes, causing the breast cancer cells to become more aggressive (Vallega et al. 2022). The exact molecular mechanisms underlying this interaction are still unclear but are likely multifactorial and related to the exchange of dysfunctional metabolites, disproportionate cytokine and growth factor production, and transfer of extracellular vesicle cargo (Vallega et al. 2022, Li et al. 2023). A better understanding of the properties and functions of dysregulated ATMs in obesity–diabetes is required before specific enzymes and/or pathways can be targeted for therapeutic intervention. Until novel therapies become available, early intervention in the prediabetic stage focussed on controlling inflammation (e.g. anti-inflammatory agents) and secondary cellular damage (e.g. antioxidants) is crucial to avoid the onset of epigenetic changes and long-term immune memory within macrophages.

**Dendritic cells, mast cells, basophils, invariant natural killer cells**

Several other, less prominent innate immune cells such as dendritic cells, mast cells, and basophils are thought to have a pathological role in the development of obesity and insulin resistance (Wu & Van Kaer 2013, Park et al. 2018, Żelechowska et al. 2018, Zatterale et al. 2019). Animal studies have clearly demonstrated an accumulation of pro-inflammatory innate immune cells which include mast cells, dendritic cells, and basophils within adipose tissue during high-fat diet-induced obesity (Liu et al. 2009, Cho et al. 2016, McLaughlin et al. 2017). Basophils and mast cells are primarily responsible for host defence against parasitic infections and play a role in allergic reactions, whereas dendritic cells are specialized antigen-presenting cells that link the innate and adaptive immune systems by presenting antigens to lymphocytes (Gasteiger et al. 2017).

The accumulation of mast cells and dendritic cells within VAT is associated with the pro-inflammatory status of adipose tissue and indirectly impacts the attraction and activation of neutrophils, macrophages, and lymphocytes through cytokine/chemokine release (Żelechowska et al. 2018). The effect of hyperglycaemia on human mast cells has been illustrated in an in vitro study, which indicated that high glucose levels promoted the phosphorylation of extracellular signal-regulated kinase, JNK, and p38 mitogen-activated protein kinases to increase the intracellular production of inflammatory cytokines such as TNFα, IL1β, and IL6 (Nagai et al. 2012). The accumulation of mast cells and dendritic cells within adipose-associated connective tissue furthermore affects the healthy expansion of adipose tissue (preadipocyte differentiation), alters the release of key lipid mediators, enhances extracellular matrix protein expression/fibrosis, and contributes to the overall dysfunction of adipose tissue and subsequent insulin resistance (Żelechowska et al. 2018, Aldan et al. 2019, Zatterale et al. 2019). This has been confirmed in animal models showing that knockout of either dendritic cells or mast cells reduces the infiltration of macrophages into adipose tissue and alleviates insulin resistance under obesogenic conditions (Liu et al. 2009, Cho et al. 2016). In contrast, regulatory cells such as ILC2s and iNKTs are sensitive to lipid overload within adipose tissue and upon activation respond to alleviate inflammation (Park et al. 2018). These cells are however depleted in obese individuals and can therefore not exert their immunomodulatory function. Taken together, these studies support the notion that immune activation and upregulation of inflammatory cytokines begin during the prediabetic obesogenic state; further research is however required to understand how prolonged exposure to the pathological microenvironment associated with T2DM affects the function of these less prominent innate immune cells.

**Conclusion**

The clinical management of diabetic patients should be expanded to not only focus on weight loss and glucose control but should also include an independent anti-inflammatory strategy to improve the overall outcomes and delay disease progression. Although it is beyond the scope...
of this review to discuss potential therapeutic strategies and the efficacy of various pharmaceutical molecules, it highlights the necessity of addressing this underlying condition in T2DM patients. Given the complexity and multifactorial metabolic and immune dysregulation associated with low-grade metabolic inflammation, preclinical studies are only now beginning to shed light on potential therapeutic targets. There are thus numerous gaps in our knowledge related to the failure of inflammation to resolve and long-term innate immune memory. Based on current evidence, early intervention in the obesogenic prediabetic stage is recommended to prevent aberrant leukocyte activation, epigenetic alterations, and long-term immune memory.

**Declaration of Interest**
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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