Immunometabolism orchestrates training of innate immunity in atherosclerosis

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Abstract

Atherosclerosis is characterized by a persistent, low-grade inflammation of the arterial wall. Monocytes and monocyte-derived macrophages play a pivotal role in the various stages of atherosclerosis. In the past few years, metabolic reprogramming has been identified as an important controller of myeloid cell activation status. In addition, metabolic and epigenetic reprogramming are key regulatory mechanisms of trained immunity, which denotes the non-specific innate immune memory that can develop after brief stimulation of monocytes with microbial or non-microbial stimuli. In this review, we build the case that metabolic reprogramming of monocytes and macrophages, and trained immunity in particular, contribute to the pathophysiology of atherosclerosis. We discuss the specific metabolic adaptations, including changes in glycolysis, oxidative phosphorylation, and cholesterol metabolism, that have been reported in atherogenic milieus in vitro and in vivo. In addition, we will focus on the role of these metabolic pathways in the development of trained immunity.

Keywords

Immunometabolism • Monocyte • Macrophage • Atherosclerosis • Trained immunity

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1. Background

Due to the growing and ageing world population, chronic diseases such as cardiovascular diseases (CVD) have become the most prevalent burden of disease. CVD, including myocardial infarction and stroke, are the leading cause of death worldwide.1 The vast majority of cardiovascular events is caused by rupture of atherosclerotic plaques in the arterial vessel wall and the subsequent formation of an occluding thrombus.

Monocytes and monocyte-derived macrophages play a pivotal role in the various stages of atherosclerosis.2,3 Atherosclerotic plaque formation is initiated by the recruitment of circulating monocytes, which are derived from myeloid progenitors in the bone marrow and a reservoir population in the spleen, into the intimal layer of the vessel wall. Here, monocytes differentiate into macrophages that can develop into lipid-laden foam cells, which eventually contribute to the growth of the necrotic core. In addition, damage-associated molecular patterns can further activate plaque macrophages by binding to membrane-bound pattern recognition receptors (PRR), such as toll-like receptors (TLR) and scavenger receptors.4 Therefore, the process of atherosclerosis can be augmented in situations of monocytosis or in the presence of circulating monocytes with a specific pro-inflammatory phenotype.4,5

Recently, two novel mechanisms have been revealed that control the inflammatory and atherogenic function of myeloid cells. First, various intracellular metabolic pathways of monocytes and macrophages impact on the functional state of these cells.6 In addition, we and others reported that brief stimulation of myeloid cells can result in a long-term pro-inflammatory and pro-atherogenic phenotype, which is denoted trained immunity.6 Metabolic and epigenetic rewiring have been identified as important mechanisms driving trained immunity.7 In this review, we will focus on how changes in the metabolism of myeloid cells can influence the pathophysiology of atherosclerosis and how metabolic reprogramming drives trained immunity.

2. A short introduction to innate immune memory in atherosclerosis

In contrast to the traditional immunological paradigm, cells of the innate immune system such as monocytes and NK cells can build an
imunological memory after previous encounters with micro-organisms.\textsuperscript{9–10} This is reflected by a persistent suppression (called immunotolerance) or a long-term augmentation of immune effector mechanisms, a phenomenon called ‘trained immunity’. Brief stimulation of monocytes with microbial products, such as the cell wall component of Candida albicans, β-glucan, or Bacillus Calmette Guérin (BCG), induces epigenetic reprogramming at the level of chromatin architecture, which is associated with an increased production of cytokines and chemokines in response to a (similar or unrelated) secondary stimulus. It is important to realize that trained immunity is not only triggered by infectious stimuli such as β-glucan and BCG, but also by endogenous non-microbial atherogenic stimuli, such as oxidized LDL (oxLDL) and lipoprotein(a) [Lp(a)].\textsuperscript{11,12} A complete overview of all triggers of trained immunity has recently been provided by van der Heijden et al.\textsuperscript{13} Brief stimulation of isolated human monocytes with oxLDL and Lp(a) induces a macrophase phenotype that, upon restimulation with TLR2 and TLR4 ligands, responds with an augmented production of pro-atherogenic factors, such as the cytokines tumour necrosis factor-α (TNF-α) and interleukin (IL)-6.\textsuperscript{11,12} Moreover, oxLDL trained cells show increased foam cell formation capacity by up-regulating scavenger receptors CD36 and SR-A, as well as reducing the expression of cholesterol efflux transporters adenosine triphosphate (ATP)-binding cassette transporter-A1 and G1 (ABCA1 and ABCG1), processes that have previously been shown to accelerate atherosclerosis.\textsuperscript{14–16} Finally, trained macrophages produce more collagenases, such as matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9), important in plaque destabilization.\textsuperscript{17} It is tempting to speculate that trained immunity could be a general underlying mechanism for other pro-atherogenic compounds, such as triglyceride-rich lipoproteins and IL-1β, which both stimulate macrophage foam cell formation and increase the production of pro-inflammatory cytokines.\textsuperscript{18} The effects of IL-6 and IL-1β are mediated by the activation of STAT3 and NFκB, which are critical transcription factors in the regulation of inflammation and atherosclerosis.\textsuperscript{19}

3. The mechanistic training tools in trained immunity

In order to promote trained immunity to boost resistance to infections in vulnerable patients or to dampen trained immunity in an attempt to prevent or reduce atherosclerosis, it is of crucial importance to thoroughly understand its underlying mechanism. In the past few years, we and others have tried to elucidate the intracellular mechanism that drives the memory function of trained immunity in mature circulating monocytes. To date, two key mechanisms have been identified: epigenetic and metabolic reprogramming. In this section, we will further delineate the involvement and interdependence of these mechanisms in trained immunity.

3.1 Epigenetic reprogramming

Epigenetic reprogramming at the level of the chromatin architecture, mainly occurring via histone modifications, is a central mechanism that underpins the enhanced functional state of trained innate immune cells (an in-depth review on epigenetics and trained immunity can be found elsewhere).\textsuperscript{20} Epigenetic regulation is defined by the regulation of gene expression without an alteration in the DNA sequence itself. This can be due to DNA methylation, histone modifications, or post-translational modulation by non-coding RNAs. These methylation and acetylation marks can regulate the accessibility of the DNA for the transcriptional machinery. DNA hypermethylation is typically associated with gene silencing. \textsuperscript{21} Modifications of histones, however, can either lead to activation or repression of gene transcription. Histone acetylation neutralizes the positive charge of the lysine residues, which will stimulate the binding of transcription factors and, as such, stimulates activation of gene transcription.\textsuperscript{22} The effect of histone methylation on gene transcription is dependent on the specific lysine residue that is involved and the amount of methyl groups added. For example, trimethylation of lysine 4 at histone 3 (H3K4me3) is associated with an open chromatin that allows gene transcription, whereas H3K27me3 compact the chromatin, leading to gene silencing.\textsuperscript{23} Interestingly, individual histone modifications can influence each other and can also interact with DNA methylation marks, through modulation of the activity of protein complexes that bind these histone/DNA modifications.\textsuperscript{24}

Analyses of several activating histone methylation and acetylation marks in the in vitro model for trained immunity showed distinct epigenetic characteristics for either naïve, tolerant, or β-glucan-trained macrophages.\textsuperscript{25} Notably, inhibition of epigenetic reprogramming with the non-specific histone methyltransferase inhibitor 5′-Methylthioadenosine completely prevented training.\textsuperscript{26} Regarding the role of DNA methylation in trained immunity, Novakovic et al.\textsuperscript{27} recently reported that DNA methylation was not significantly changed in β-glucan-trained macrophages.

In the context of atherosclerosis, in vitro training with oxLDL corresponds with enrichment of H3K4me3 at the promoters of at least TNFα, IL-6, MCP-1, IL-8, CD36, SR-A, MMP-2, and MMP-9.\textsuperscript{28} Moreover, in patients with established atherosclerosis, circulating monocytes demonstrate increased cytokine production capacity, which was associated with a lower presence of repressive histone modifications on the cytokine promoters.\textsuperscript{29} The differences in the specific histone modifications might be caused by the training stimulus, which is oxLDL in the in vitro experiments, but can also be yet another atherogenic stimulus in these patients in vivo. Additional studies using an unbiased whole genome approach to determine epigenetic reprogramming are clearly warranted in this context.

The importance of epigenetic regulation in atherosclerosis was also highlighted in a recent study by Fuster et al.,\textsuperscript{30} which investigated the role of clonal haemapoiesis induced by tet methylcytosine dioxygenase 2 (TET2) deficiency, a multifaceted transcriptional regulator, in LDLR\textsuperscript{-/-} mice. TET2 deficiency in macrophages promoted inflammation and aggravated atherosclerosis, which was mediated by increased production of IL-1β. Of note, Chromatin Immunoprecipitation (ChiP)-qPCR analysis revealed increased histone H3 acetylation at the Il1b gene promoter in TET2-deficient macrophages.\textsuperscript{31} With epigenetic reprogramming at the centre of trained immunity, it would be of great interest to explore whether clonal haemapoiesis could influence the induction of trained immunity.

3.2 Metabolic reprogramming

3.2.1 Differential routing of metabolic responses

Metabolic pathways play a major role in immune cell function. The glycolytic pathway, TCA cycle, pentose phosphate pathway (PPP), fatty acid
oxidation (FAO), fatty acid synthesis (FAS) and amino acid metabolism each have a unique purpose in the cell. However, at the same time, they are closely intertwined, as a consequence of shared fuel inputs and the reliance on the products of other pathways to serve as precursors. For a detailed description of the function of the various metabolic pathways in immune cells, we refer to some excellent recent reviews.3,34 Immune cells distinctly use these metabolic pathways to fine tune their function and to produce energy and building blocks for cell maintenance and proliferation, and modulation of cellular signalling.5 As such, innate immune cells can mount distinct metabolic responses to different inflammatory stimuli. The classical dichotomy for macrophage activation is an illustrative example of this phenomenon. Stimulation with lipopolysaccharide (LPS) and interferon-γ (IFNγ) induces a profound metabolic rewiring of macrophages which is characterized by increased aerobic glycolysis and impaired OXPHOS via the TCA cycle, leading to an accumulation of intermediates such as succinate.35 This switch from OXPHOS to glycolysis closely resembles the Warburg effect in tumour cells, in which pyruvate produced by the glycolytic pathway is metabolized to lactate, while TCA cycle intermediates accumulate, resulting in a decreased oxidative phosphorylation.36 Other up-regulated metabolic pathways in LPS/IFNγ macrophages are the PPP and FAS. Activation via these metabolic routes prepares the macrophage for host defence against microbial pathogens. On the other hand, when macrophages are activated via IL-4, they exhibit increased FAO and OXPHOS and become instrumental in immune-regulation and wound healing. Over the past few years, the classical dichotomy between M1 and M2 macrophages has been challenged and more metabolic blueprints for macrophage activation have been unravelled. Lachmandas et al. have recently studied the metabolic responses of human CD14+ monocytes to stimulation with several different ligands, including TLR ligands such as Pam3cys (TRL2), poly(I:C) (TLR3) and with whole pathogen lysates.8 These experiments illustrate that various intermediate metabolites can function as substrates or co-factors for epigenetic enzymes. Vinyl acetate produced by the glycolytic pathway is metabolized to lactate, while TCA cycle intermediates accumulate, resulting in a decreased oxidative phosphorylation.36 Other up-regulated metabolic pathways in LPS/IFNγ macrophages are the PPP and FAS. Activation via these metabolic routes prepares the macrophage for host defence against microbial pathogens. On the other hand, when macrophages are activated via IL-4, they exhibit increased FAO and OXPHOS and become instrumental in immune-regulation and wound healing. Over the past few years, the classical dichotomy between M1 and M2 macrophages has been challenged and more metabolic blueprints for macrophage activation have been unravelled. Lachmandas et al. have recently studied the metabolic responses of human CD14+ monocytes to stimulation with several different ligands, including TLR ligands such as Pam3cys (TRL2), poly(I:C) (TLR3) and with whole pathogen lysates from Escherichia coli, Staphylococcus aureus, or Mycobacterium tuberculosis. Upon these stimulations, monocytes exhibited both increased glycolysis and oxidative phosphorylation.8 Also, IL-1β production of these cells in response to both LPS and Pam3cys (P3C) was restricted when glycolysis was blocked with 2-deoxyglucose (2-DG). Blocking the OXPHOS with rotenone, however, restricted IL-1β production only in P3C-stimulated cells. This and other studies have highlighted the complexity of metabolic rewiring in human innate immune cells and demonstrate that there is no universal response to pathogenic stimuli, but that different stimuli can induce divergent macrophage responses through the differential activation of metabolic routes.

3.2.2 Metabolic reprogramming in trained immunity Two observations point towards an important role for the intracellular metabolism in the induction of trained immunity. First, pathway analysis of the top 500 up-regulated genes in β-glucan-trained monocytes displayed a marked activation of central metabolic pathways.26 Cheng et al. showed that trained immunity is mediated by increased aerobic glycolysis and lactate production via the activation of mammalian target of rapamycin (mTOR) and hypoxia-inducible factor-1α (HIF-1α). A close interaction between metabolism and epigenetics was subsequently illustrated by the observation that there was an enrichment of the activating histone modifications H3K4me3 and H3K27ac on the promoters of several key glycolytic enzymes in β-glucan-trained monocytes, 1 week after stimulation.27 Furthermore, it is increasingly appreciated that various intermediate metabolites serve as co-factors or substrates for epigenetic enzymes, which is reviewed in more detail by Keating and El-Osta.39 Genetic evidence for the impact of cellular metabolism on epigenetic remodelling in the myeloid lineage has previously been described.40,41 For instance, mutation of isocitrate dehydrogenase 1/2 (IDH1/2) enables this enzyme to convert α-ketoglutarate to 2-hydroxyglutarate in the citric acid cycle, which competitively acts on epigenetic modifiers that normally depend on α-ketoglutarate as a substrate.38

In the context of trained immunity, the glycolytic switch itself was also shown to impact on the epigenetic remodelling through NAD+/NADH-dependent effects on the sirtuin-1 class of histone deacetylase enzymes in β-glucan-trained cells.7 Moreover, an additional series of experiments illustrated that monocytes trained with β-glucan exhibit enhanced glutaminolysis, with a marked accumulation of fumarate and succinate, which was crucial for the induction of a trained macrophage phenotype.42 Notably, stimulation of monocytes with an excess of fumarate resulted in enhanced TNFα and IL-6 production 6 days later, which correlated with increased H3K4me3 at the promoters of their genes. It was demonstrated that fumarate directly inhibits histone demethylase KDM5, which correlated with increased training. Providing the macrophages with α-ketoglutarate, a co-factor for KDM5 activity, increased KDM5 biological activity and suppressed fumarate-induced training. Collectively, these experiments illustrate that various intermediate metabolites can function as substrates or co-factors for epigenetic enzymes.

In the following sections, we will describe the various metabolic pathways that can drive myeloid cell activation in the context of atherosclerosis. In particular, we will highlight the role of these pathways and the interaction with epigenetic reprogramming in trained immunity.

4. Immunometabolism in atherosclerosis and trained immunity

In the context of atherosclerosis, monocytes are exposed to a great variety of signals. Not only within the microenvironment of the atherosclerotic plaque itself, but also in the bone marrow niche and in the circulation. Over the past few years, many studies have sought to identify the distinct metabolic pathways induced by atherogenic stimuli, such as lipoproteins, glucose, and hypoxia. Here, we will discuss the preclinical and clinical data on the key metabolic pathways in myeloid cells that are modulated in the context of atherosclerosis and are important for trained immunity, including glycolysis, the PPP, and cholesterol metabolism (Figure 1).

4.1 Glycolysis is up-regulated in atherosclerosis

In monocytes and macrophages, glucose transporter 1 (GLUT-1) on the cell’s outer cell membrane initiates the uptake of glucose. Within the cell, glucose is phosphorylated by hexokinase to glucose-6-phosphate which is subsequently used in either the glycolysis, the PPP, or during FAS. During glycolysis, processing of glucose in the cytosol yields two ATPs as well as pyruvate. The pyruvate generated during glycolysis is either converted to lactate by lactate dehydrogenase or enters the TCA cycle in the mitochondria. Below, we will discuss the involvement of glycolysis in the various stages of atherosclerotic disease.

Atherosclerotic plaques are characterized by local hypoxic regions, where oxygen availability is limited. Under hypoxic conditions, HIF-1α is stabilized and activates the glycolytic pathway, thereby reducing the cell’s dependency on OXPHOS. The increased glycolytic flux occurs through

References

[1] Cheng et al. [2] Lachmandas et al. [3] Keating and El-Osta [4] J. van Tuijl et al.
increased expression of key glycolytic proteins GLUT1, hexokinase II (HK2), and 6-phosphofructo-2kinase/fructose-2, 6-biphosphatase 3 (PFKFB3). Genetic deletion of HIF-1α in myeloid cells from Ldlr−/− mice reduced atherosclerotic plaque formation, which was likely mediated by a reduction in glycolytic rate. By using 18F radiolabelled glucose analogue fluorodeoxyglucose positron emission tomography CT (18F-FDG-PET/CT), several studies have assessed the involvement of glycolysis within human atherosclerotic plaques. Increased glucose uptake was found to co-localize with HIF-1α expression. Moreover, increased FDG uptake was associated with macrophage containing- and lipid-rich necrotic plaque areas. Tomas et al. performed metabolomics on human carotid atherosclerotic plaques, and revealed two distinct metabolic profiles associated with plaque vulnerability and inflammation. Vulnerable plaques were characterized by increased glycolysis, defective FAO, and increased anaplerosis of the TCA cycle. These plaques also had increased mRNA expression of glycolytic and PPP enzymes, next to increased protein levels of IL-1β, IL-6, and IL-18, but not TNFα, which resembles the cytokine production profile in macrophages of patients with atherosclerosis.

Directing our scope from the plaque to the bone marrow, support for a role of myeloid cell glycolysis in myelopoiesis and atherogenesis is found in the observation that in hypercholesterolaemic apoE−/− mice, leucocytes and HSPCs exhibit an increased GLUT1-dependent glucose uptake, which is associated with increased mitochondrial potential. This implies that the influx of glycolytic metabolites in these cells fuels the mitochondria for oxidative phosphorylation and ATP generation. Transplantation of bone marrow derived from GLUT1−/− mice into apoE−/− mice reversed HSPC proliferation and expansion, prevented myelopoiesis, and reduced atherosclerosis.

In contrast to the pro-atherogenic role of GLUT1 in HSPCs, GLUT1 can also facilitate anti-atherosclerotic actions in plaque macrophages. GLUT1 (also known as Slc2a1) expression is increased during the process of efferocytosis, which fuels increased glucose uptake and a shift from oxidative phosphorylation towards increased aerobic glycolysis, which is needed for effective clearance of apoptotic cells. Bone marrow transplantation from myeloid-targeted LysM-Cre Slc2a1fl/fl mice into Ldlr−/− mice on a Western type diet (WTD) increased the necrotic core in the aorta.

From a clinical perspective, circulating monocytes isolated from patients with atherosclerotic cardiovascular disease, showed an increased cytokine production capacity, which was associated with an up-regulation of glycolytic enzymes. This phenotype also persisted after in vitro differentiation into macrophages, showing a higher glycolytic flux accompanied by an increased oxygen consumption rate.

Thus, there is ample evidence that atherosclerosis is associated with an increased glycolysis in myeloid cells. A series of recent studies...
in vitro trained immunity has been validated by the observation that inflammatory gene expression. Furthermore, Parathath residing in the atherosclerotic plaque environment, exhibit an increased impact on the progression of atherosclerosis in humans remains to be linked to trained immunity as an underlying mechanism. Whether this that the up-regulated glycolysis observed in atherosclerosis, is intricately linked by the observation that primary human monocytes trained with the common pro-atherogenic stimulus oxLDL display increased lactate production, suggesting increased glycolysis and subsequent pyruvate fermentation in vitro. 

The collection of evidence discussed above mounts the hypothesis that the up-regulated glycosis observed in atherosclerosis, is intricately linked to trained immunity as an underlying mechanism. Whether this impacts on the progression of atherosclerosis in humans remains to be elucidated.

### 4.2 Cholesterol metabolism as central player linking lipid metabolism and inflammation

The synthesis of cholesterol is not only important in maintaining the structural integrity of cell membranes, but the isoprenoid intermediates derived from the cholesterol synthesis pathway are also needed for the preylation of signalling and effector molecules involved in inflammatory responses. Therefore, the cholesterol homeostasis of a cell is tightly regulated by several transcription factors, including the Liver X receptor (LXR) and sterol regulatory element-binding protein (SREBP) family, which are both important in regulating inflammatory activity.

The importance of cholesterol homeostasis for immune function in the context of atherosclerosis is illustrated by various observations in murine models of atherosclerosis. Mice deficient in the cholesterol efflux transporters, ABCA1 and ABCG1, show accumulation of cholesterol in peritoneal macrophages and increased inflammatory responses to TLR ligands. In addition, Feig et al. showed that lipid-laden foam cells, residing in the atherosclerotic plaque environment, exhibit an increased inflammatory gene expression. Furthermore, Parathath et al. demonstrated that murine macrophages cultured under hypoxic conditions increase cellular sterol and triglycerides. This correlated with augmented activity and mRNA expression of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, thereby increasing the production of cholesterol. Moreover, hypoxia abrogated the ABCA1-mediated cholesterol efflux and these effects were reversed by reducing the expression of HIF-1α. Interestingly, Westerterp et al. demonstrated that Ldlr−/− mice transplanted with specific ABCA1 and ABCG1-deficient macrophages showed increased atherosclerotic lesions and a concomitant augmented expression of inflammatory chemokines within macrophage-containing areas.

While these studies imply that cholesterol accumulation mounts pro-inflammatory responses. Spann et al. on the contrary reported that foam cells derived from murine peritoneal macrophages have an anti-inflammatory phenotype due to the accumulation of desmosterol. This finding was recently confirmed in lipid-laden peritoneal macrophages from Ldlr−/− mice on a high-fat diet. In addition to LXR-activation by desmosterol, suppression of the PPP attenuated the inflammatory responses. This suggests that foam cell formation per se results in a predominantly anti-inflammatory macrophage phenotype, but that it requires extrinsic pro-inflammatory stimuli from the atherosclerotic milieu to adopt a pro-inflammatory phenotype.

Cholesterol metabolism not only impacts on disease progression in the atherosclerotic plaque itself, but could also play a role in earlier disease stages, i.e. at the level of the bone marrow. Transplantation of Abca1−/− and Abcg1−/− bone marrow cells into Ldr−/− mice resulted in increased haematopoietic stem and progenitor cells (HSPC) expansion and concurrent monocytyosis and accelerated atherosclerosis. The same effects occurred in apoE−/− mice, in which HSPCs also demonstrated a reduced ABCA1- and ABCG1-mediated cholesterol efflux.

Analysis of transcriptome data of β-glucan-trained macrophages revealed that the cholesterol synthesis pathway was also highly induced in trained cells. Bekkeren et al. have further unravelled the role of this pathway in trained immunity and revealed that not the isoprenylation pathway, nor the synthesis of cholesterol itself, were responsible for the trained phenotype, but that the accumulation of mevalonate induced epigenetic reprogramming and subsequent trained immunity. This occurred via the activation of the insulin-like-growth factor-1 receptor pathway and subsequent induction of mTOR signalling and glycolysis. Monocytes from patients with mevalonate accumulation in the context of the Hyper-IgD syndrome showed a trained immunity phenotype, which was characterized by an increased expression of cytokines and genes involved in the glycolytic pathway. Inhibition of the cholesterol synthesis pathway by statins inhibited the trained phenotype. Whether intracellular cholesterol metabolism also induces trained immunity in patients with cardiovascular disease remains a subject of further investigation.

### 5. Getting to the core of the training course: trained immunity in the bone marrow

Atherosclerotic lesions grow through a combination of continuous recruitment of circulating monocytes as well as local macrophage proliferation. We previously hypothesized that trained circulating monocytes could therefore aggravate atherosclerotic lesion development. Interestingly, in humans in vivo, a trained immune phenotype of circulating monocytes remains apparent even 3 months after vaccination with BCG. Given the short circulating half-life of monocytes of only 1–4 days, it is likely that training already occurs at the level of myeloid progenitor cells in the bone marrow. Indeed, a recent series of papers unequivocally demonstrate that training occurs in myeloid progenitor cells in the bone marrow, at least in mouse models.

Mitroulis et al. reported that in mice, administration of β-glucan induced a sustained increased proliferation and myeloid skewing of haematopoietic stem cells (HSCs) which was associated with mitigated LPS-induced DNA damage upon a subsequent LPS challenge. Moreover, the β-glucan-trained haematopoietic precursors were more efficiently protected against DNA damage and cell death induced by myeloablative chemotherapy. Interestingly, RNAseq analysis of long-term HSCs revealed profound metabolic reprogramming, especially involving genes in glycolysis, cholesterol biosynthesis, and the mevalonate pathway. Pharmacological inhibition with the IL-1 receptor antagonist Anakinra revealed that the up-regulation of glycolytic enzymes, via IL-1β signaling, was essential for HSC proliferation following β-glucan administration.
Similar to β-glucan-induced reprogramming of HSCs, BCG vaccination in mice led to sustained enhanced myelopoiesis in HSCs and multipotent progenitor cells. Macrophages derived from BCG-educated HSCs showed profound reprogramming of activating histone modifications, showing that these cells are epigenetically primed to initiate a more protective response against a subsequent infection with M. tuberculosis.

That trained immunity at the bone marrow level also plays a role in the context of atherosclerosis, was recently shown by Christ et al.66 In an apoE−/− mouse model of atherosclerosis, a 4-week WTD induced a persistent pro-inflammatory reprogramming of innate immune progenitor cells in the bone marrow. This pro-atherogenic and pro-inflammatory phenotype persisted even 4 weeks after reversing to a normal chow diet. Gene Ontology term enrichment network analysis of differentially expressed genes in granulocyte-monocyte progenitor (GMP) cells from these WTD-fed mice revealed up-regulation of various metabolic processes, including the cholesterol biosynthesis pathway. Moreover, they investigated the epigenetic profiling of GMPs from mice fed a WTD compared with chow diet by assay for transposase-accessible chromatin sequencing (ATAC-seq) and revealed that the WTD induced changes in the chromatin landscape. Also in this model, activation of the NLRP3 inflammasome and IL-1β signalling was fundamental for persistent GMP reprogramming.63 Pietras et al.66 showed that IL-1 exposure can drive myeloid differentiation in HSCs. Whereas IL-1 administration always enhances myeloid cell production, it can severely compromise HSC function and blood regeneration in conditions of chronic exposure. However, when mice were injected with IL-1β for 20 days and allowed to rest without further treatment for 8 weeks, most of the bone marrow changes had reverted to untreated levels, with normal numbers of HSCs and multipotent progenitors. The only persisting differences were increased GMP and decreased MMP4 numbers.64 As the effects on the HSCs were largely resolved after IL-1 withdrawal, it remains elusive whether the epigenetic modifications seen in GMPs also occur in the HSCs of WTD-fed mice. If these are restricted to GMPs, the longevity of the epigenetic changes and increased inflammatory response in myeloid cells might be determined by the turnover of these progenitors or the stimulus.

Since increased levels of IL-1β and other pro-inflammatory cytokines are a hallmark of various chronic inflammatory conditions, including rheumatoid arthritis, inflammatory bowel disease, psoriasis, obesity and metabolic syndrome, trained immunity at the level of bone marrow myeloid progenitors might provide a general mechanism driving the progression of atherosclerosis.67–69 It was recently reported that in rheumatoid arthritis, atherosclerosis is accelerated due to increased proliferation and myeloid skewing of HSCs in the bone marrow.70 Notably, these HSCs were characterized by an increased cholesterol content, due to defects in the cholesterol efflux pathways. Even after mobilization into the circulation, the mature myeloid cells retained these defects in their cholesterol metabolism. Moreover, the HSCs showed increased cell surface levels of the common β-subunit of the IL-3/γc-receptor stimulating factor (GM-CSF) receptor (IL-3Rβ),70 which phenocopies the HSC phenotype after β-glucan training.64

6. Clinical relevance and future directions

Evidence that central metabolic pathways, including glycolysis and cholesterol metabolism, are profoundly reprogrammed in myeloid cells in the different stages of atherosclerosis is rapidly accumulating. These processes also play major roles in the induction of trained immunity, with an intimate connection to epigenetic reprogramming.

To determine the point at which the metabolic adaptations of monocytes and macrophages become disease promoting, it is required to study the metabolic changes in the multiple phases of the development of atherosclerosis. These stages range from adaptation of myeloid precursors in the bone marrow to foam cell formation in atherosclerotic plaques, and most importantly rupture-prone plaques.

A potential non-invasive tool to visualize metabolic changes in atherosclerosis in humans is in vivo visualization of macrophage glycolysis with either FDG-PET/CT or hyperpolarized MRL. Lewis et al.71 used hyperpolarized [1-13C]pyruvate MR spectroscopy as a novel method to detect glycolytic metabolism in a macrophage-like cell suspension. Upon macrophage activation with LPS, the hyperpolarized lactate label flux rates almost doubled. Inhibition of glycolysis in these activated cells by 2-deoxyglucose normalized the rates and concurrently inhibited the production of key pro-inflammatory cytokines.

Targeting the disease-promoting metabolic changes in myeloid cells during atherogenesis could be an exciting novel therapeutic strategy. Notably, metformin and statins, drugs that have already been used in clinical practice for years, interfere with key metabolic pathways that also drive innate immune activation in atherosclerosis.72,73 In a study by Duivenvoorden et al.,74 an injectable reconstituted high-density lipoprotein (rHDL) nanoparticle carrier vehicle was developed that is able to directly deliver statins to myeloid cells within atherosclerotic plaques. In vitro statin-rHDL showed an anti-inflammatory effect, which was mediated through the inhibition of the mevalonate pathway. When the statin-rHDL nanoparticles were subsequently applied in vivo in an apoE−/− mouse model, they accumulated in the atherosclerotic plaques, where they directly affected the plaque residing macrophages.74 A low-dose statin-rHDL treatment regimen during 3 months inhibited plaque progression, while in more advanced atherosclerotic plaques a 1-week high-dose regimen was able to markedly decrease inflammation.

In addition to drugs targeting intracellular metabolic pathways, epigenetic reprogramming is also amenable to pharmacological modulation and could serve as a potential tool to manipulate macrophage inflammatory responses in atherosclerosis (an extensive recent review can be found elsewhere75). Several drugs that inhibit epigenetic enzymes, such as histone deacetylase inhibitors, are already approved for clinical use in the fields of oncology and haematology and have been shown to also reduce inflammation in inflammatory diseases.76,77

Finally, since inflammatory cytokines have been identified as potent inducers of trained immunity, inhibition of these cytokines might also serve as a potential therapeutic target. For instance, the studies by Mitroulis et al.64 and Christ et al.63 have implied that inflammasome-mediated products such as IL-1β could be the central endogenous mediator in the induction of trained immunity. It is tempting to speculate that these mechanisms are part of the clinical benefit observed in the recent CANTOS trial, which studied IL-1 blockade in humans at cardiovascular risk.78 The CIRT study on the other hand, which was running parallel to the CANTOS trial, investigated whether inhibition of the CRP/IL-6/LI-1 axis with low-dose methotrexate (MTX) could reduce cardiovascular events. Among patients with stable atherosclerosis, low-dose MTX did not reduce levels of IL-1β, IL-6, or C-reactive protein and did not result in reduced cardiovascular events compared with placebo.79 Potentially, this could be explained by the fact that MTX affects S-adenosylmethionine levels, which is the main methyl donor for DNA and histone methylation, and as such may interfere with epigenetic remodelling and subsequent inflammatory responses.
In conclusion, further elucidation of the metabolic and epigenetic adaptations that control the development of atherosclerotic plaques will allow the future development of new classes of drugs to prevent or treat atherosclerosis.

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