From metabolic to epigenetic: Insight into trained macrophages in atherosclerosis (Review)

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Abstract. Atherosclerosis (AS) is a chronic inflammatory disease caused by the deposition of lipoproteins and sequent immune responses. Within the atherosclerotic plaque, macrophages are the most abundant immune cells and play a great part as protagonists and promoters of AS. In the past decade, the concept of 'trained immunity' has emerged, which highlights the memory characteristics of innate immunity, thus opening up a new avenue of research. Evidence suggests that trained immunity may regulate the onset and progression of AS with trained macrophages playing an important and dynamic role in atherogenesis. The present review provided a summary of concepts related to trained immunity and its relationship with AS. Furthermore, different phenotypes of macrophages responding to various stimuli within the atherosclerotic plaque were presented, along with the complex mechanisms of metabolic and epigenetic reprogramming in the cells. Finally, several promising therapeutic approaches for AS cardiovascular disease were discussed, which may shed light on new clinical strategies.

Contents

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1. Introduction

Atherosclerotic cardiovascular disease (ASCVD) is highly prevalent and accounts for the majority of mortalities worldwide (1). Characterized by a chronic non-resolving low-grade sterile inflammation of the arterial wall, atherosclerosis (AS) is mainly caused by low-density lipoprotein (LDL) (2). The main lesions in AS are characterized by LDL deposition in the intima, the innermost layer of the artery wall. Activated by such stimuli, endothelial cells express a leukocyte adhesion molecule, such as vascular cell adhesion molecule-1, interacting with its cognate ligands to promote the rolling and adherence of monocytes and lymphocytes, accompanied by smooth muscle cells and fibrous matrix proliferation, which gradually develop into the formation of an AS plaque (3). Lipoproteins sequestered in the arterial wall are susceptible to modifications, such as oxidation, which render these particles pro-inflammatory and immunogenic. Recruited monocytes mature into mononuclear phagocytes, take in normal or modified lipoproteins and transform into foam cells, a type of macrophage that promotes disease progression. Above various cell types in AS plaques, macrophages and foam cells are considered to be major contributors to atherogenesis: Not only do they respond inflammatorily through the secretion of pro-inflammatory mediators, matrix-degrading proteases and final death, but also release lipid contents and tissue factors, constituting a pro-thrombotic necrotic core after necrosis or apoptosis (4). Thus, the dynamics of macrophages should play a decisive part in atherogenesis.

In contrast to traditional immunology, researchers recently found the capacity of the innate immune system to build an inflammatory memory named trained immunity. Trained immunity possesses most prosperities from innate immunity and has the ability to give a rapid response in reinfection,

which is built on epigenetic and metabolic reprogramming. The inflammatory memory gifts the host both protection and susceptibility to inflammation-driven diseases such as AS. As monocytes and macrophages have a major role in atherogenesis and are widely investigated in the field of trained immunity, the present review will focus on them. After elaborating on trained immunity, phenotypes of macrophages under signals in AS plaques will be discussed. The mechanisms of this immune memory in trained AS macrophages in aspects of metabolism, transcription and epigenetics will then be presented, which may inspire future treatments of the disease.

2. Trained immunity: A novel mechanism of AS

Trained immunity. In the traditional cognation of immunology, in contrast of adaptive immunity, innate immunity is unable to build an immunological memory. However, prompted by evidence of protection against reinfection in plants and invertebrates, transplant rejection in invertebrates and cross-protection between infections in mammals with dysfunctional T and B lymphocytes, Netea *et al* (5) named the inflammatory memory in innate immune cells 'trained immunity', which represented hyper-responsiveness in the innate immune system facing a second stimulus.

Like innate immunity, trained immunity is built on pattern recognition receptors allowing recognition of different kinds of, but not individual, pathogens (6) and induced by stimuli, including pathogen-associated molecular patterns and damage-associated molecular patterns (7). Also, its properties have been described mainly in populations of innate immune cells, including monocytes, macrophages and natural killer (NK) cells. Studies have indicated the existence of trained hematopoietic stem cells and progenitor cells (8), along with an inflammatory memory in epidermal stem cells (9), showing a broader scale of trained immunity both in time and space. The innate immune memory lays its basis on epigenetic reprogramming in the first stimulation (10), which persists after the removal of the stimulus and leads to accessible chromatin and rapid recruitment of RNA polymerase II (11) in the reinfection.

While trained immunity guarantees the host a rapid response and better protection against restimulation, it brings the potential danger of inflammatory disorders and cancer. For instance, it was found that once infected, a pregnant individual with higher levels of interleukin-6 (IL-6) passed on the inflammatory memory to the fetal intestinal epithelium, which persisted to adulthood. As a result, inflammatory-primed intestinal stem cells showed both enhanced protection against *Salmonella* infection and worsened pathology in a model of colitis (12). Studies have been centered widely around the maladaptation of the innate immune memory and diseases, such as periodontitis (13) and Alzheimer's disease (14). AS, as a chronic inflammatory disease, is no exception.

Trained immunity in AS. Trained immunity can be induced by endogenous ligands, such as oxidized (ox)LDL (15), which has a major role in atherogenesis, indicating the contribution of this proinflammatory memory to AS. In fact, not only do traditional risk factors such as dyslipidemia and hypertension predispose individuals to ASCVD via trained immunity, but other factors, including lifestyle factors, inflammatory conditions and acute adverse cardiovascular events, were well classified in a review by Riksen *et al* (16).

Relevant research has also been conducted at the cellular level. For instance, isolated human monocytes were shown to mature into macrophages responding with enhanced production of tumor necrosis factor-α (TNF-α) and IL-6 after brief stimulation by oxLDL (15). Also, in a clinical trial, isolated monocytes from patients with ASCVD had a higher capacity of cytokine production than those from healthy subjects (17). The property was indicated to be maintained following conversion to macrophages. Furthermore, trained immunity was shown to be induced not only in circulating monocytes and tissue-resident macrophages, but also in hematopoietic stem and progenitor cells, termed central trained immunity, which guaranteed a long-term inflammatory memory (18). Other immune and non-immune cells related to AS, such as NK cells (19), neutrophils (20), dendritic cells (21) and endothelial cells (22), have also shown this potential, but as monocytes and monocyte-derived macrophages make a predominant contribution in all phases of AS (4), the present review will mainly focus on them.

3. Phenotypes of macrophages in AS

Aside from functional reprogramming of trained immunity, another form of immune cell adaption is differentiation (23). Monocytes infiltrating the sub-endothelium are exposed to multiple microenvironmental signals, such as pro- and anti-inflammatory cytokines, irons, calcium, lipids and their derivatives and heme from senescent erythrocytes. Initially, they differentiate into M0 macrophages, also known as resident-like macrophages, which can then become polarized into several categories of macrophages (24). Previously, these cells were classified by their activation states, such as classical, alternative and innate activation and deactivation (25); while, nowadays, they are mostly recognized by their secreta and the expression of various surface markers.

Above all of the subtypes, M1 and M2 were first described. M1 macrophages are classically activated by products of type-1 T-helper lymphocytes, such as interferon-γ. In AS plaques, observations suggested that cholesterol crystals (26), oxLDL (27,28) and pro-inflammatory cytokines drive, alone or in combination, M1 activation. Trained M1 macrophages secrete pro-inflammatory cytokines, such as IL-6, IL-1β, TNF, IL-23 and IL-12 (29), sustain inflammatory response and cause tissue damage. At the opposite end, M2 macrophages, mainly referred to as anti-inflammatory macrophages, are classically activated by IL-4 and IL-13 cytokines produced by type 2 T-helper cells (30,31). Activated by different signals, M2 macrophages have been classified into M2a, M2b and M2c, functioning separately. While M2a macrophages are classically regarded as 'wound healing macrophages', M2b and M2c macrophages are considered to be 'regulatory macrophages' (32). Studies on mice or humans have found other types of macrophages: Mox, M4, M(Hb) and Mhem macrophages. Mox macrophages found in mice are induced by oxLDL and act in a proatherogenic way. M4 macrophages are induced by platelets, while M(Hb) and Mhem macrophages are induced by the uptake of free hemoglobin (33). Of note, those polarized macrophages are able to depolarize and switch their phenotypes in different microenvironments (34) (Table I).

Table I. Phenotypes of macrophages in atherosclerosis.

Phenotype	Stimulus	Secreta
M1 macrophage	Cholesterol crystals, lipopolysaccharide, proinflammatory cytokines, oxidized LDL	IL-6, IL-1β, TNF-α, IL-23, IL-12
M2 macrophage		
M2a	IL-4, IL-13	Fibronectin, insulin-like growth factor, TGF-β
M2b	Immune complexes, IL-1β, lipopolysaccharide	IL-6, IL-1β, TNF-α, IL-10, TGF-β1
M2c	IL-10, glucocorticoids, TGF-β	IL-10, TGF-β, Pentraxin-3, MERTK[a]
Mox macrophage	Oxidized phospholipids	IL-1β, cyclooxygenase 2
M4 macrophage	C-X-C motif chemokine ligand 4	IL-6, TNF-α, MMP-7
M(Hb) macrophage	Haemoglobin/haptoglobin	IL-10
Mhem macrophage	Haem	IL-10 HMOX-1

[a]According to a previous article (100). LDL, low-density lipoprotein; TGF-β, transforming growth factor β; MERTK, myeloid-epithelial-reproductive tyrosine kinase; HMOX-1, heme oxygenase 1.

In the context of AS, M1 and M2 macrophages were shown to constitute 40 and 20% of total AS lesion macrophages, respectively, in mice (35). Furthermore, they possess distinct properties and separate tissue localizations: While M1 macrophages localize near the lipid core (36) and impair wound healing (37), M2 macrophages are more enriched in neo-angiogenic areas within the plaque (36) and more related to AS regression (38). Since M1 and M2 macrophages play a significant role in AS and have been widely studied, this review will mainly focus on them.

4. Metabolic changes in trained macrophages

Cellular metabolism not only follows and meets energy demands, but also creates intermediate metabolites serving important biological roles and responds to environmental cues by regulating the functional state of cells (39,40). The metabolism of macrophages in AS is no exception. Within AS plaques, through external stimuli and corresponding signaling pathways, intracellular metabolism reprograms and induces epigenetic reprogramming, which mediates trained immunity and macrophage phenotypes (16). Furthermore, metabolic reprogramming occurs not only in macrophages located in AS plaques, but also in circulating monocytes and their bone marrow progenitors exposed to pro-atherogenic stimuli such as lipoproteins (41), creating a long-lasting inflammatory memory.

Via pathway analysis, genome-wide transcriptome and histone modification profiling, Cheng *et al* (42) found that β-glucan-trained murine monocytes showed elevated aerobic glycolysis with a reduced basal respiration rate, as well as increased glucose consumption and lactate production; further investigation suggested the dectin-1/Akt/mTOR-hypoxia-inducible factor (HIF)-1α pathway responsible for the metabolic shift. The results indicated aerobic glycolysis (Warburg effect) as a metabolic basis of trained immunity. Further metabolic pathways, such as fatty acid oxidation (FAO) and fatty acid synthesis (FAS), also have important roles in metabolic reprogramming, as will be discussed below (Fig. 1).

Glucose metabolism. Monocytes or macrophages encountering inflammation-induced stimuli such as oxLDL increase the expression of HIF-1α through signaling pathways, including the nuclear factor κ light-chain-enhancer of activated B cells (NF-κB) pathway. In addition, hypoxia in regions rich in plaques is also an important activator of the HIF-1α transcription factor (TF). HIF-1α increases the expression of the glucose transporter 1 to enhance the uptake of glucose. Furthermore, the TF initiates glycolytic metabolism, as well as increases the expression of key glycolytic enzymes, such as hexokinase II (HK-II) and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB3), resulting in increased glycolytic flux (43). Research has shown that monocytes from patients with symptomatic AS expressed higher levels of glycolysis-related genes, including HK-II, PFKFB3 and pyruvate kinase M (PKM)1 (44).

Though less efficient, the enhancement of glucose uptake and glycolysis accelerate ATP production and become the major pathway for energy production (45). In addition, certain relevant enzymes contribute to the inflammatory states. For instance, PKM2, shown to be upregulated in pro-inflammatory macrophages, phosphorylates the TF STAT3, which consequently enhances the production of pro-inflammatory cytokines IL-6 and IL-1β (17). Apart from fueling the inflammation in atherogenesis, glycolysis also accelerates the pentose phosphate pathway (PPP) to synthesize amino acids needed for the increased protein, RNA and DNA synthesis burden of trained macrophages. An observation of only trained macrophages with an activated PPP (42) suggests that PPP is vital to the increased cellular dynamics of trained macrophages and takes part in the inflammatory process of atherogenesis (46). In addition, activated PPP produces NADPH. NADPH can further act as the electron donor in the production of oxygen radicals, which contribute to the oxidative stress within the AS plaque.

The pro-inflammatory macrophages with aerobic glycolysis also maintain a tricarboxylic acid cycle, despite two blockades after citrate and succinate leading to the accumulation of the two metabolites (17,47). Citrate is

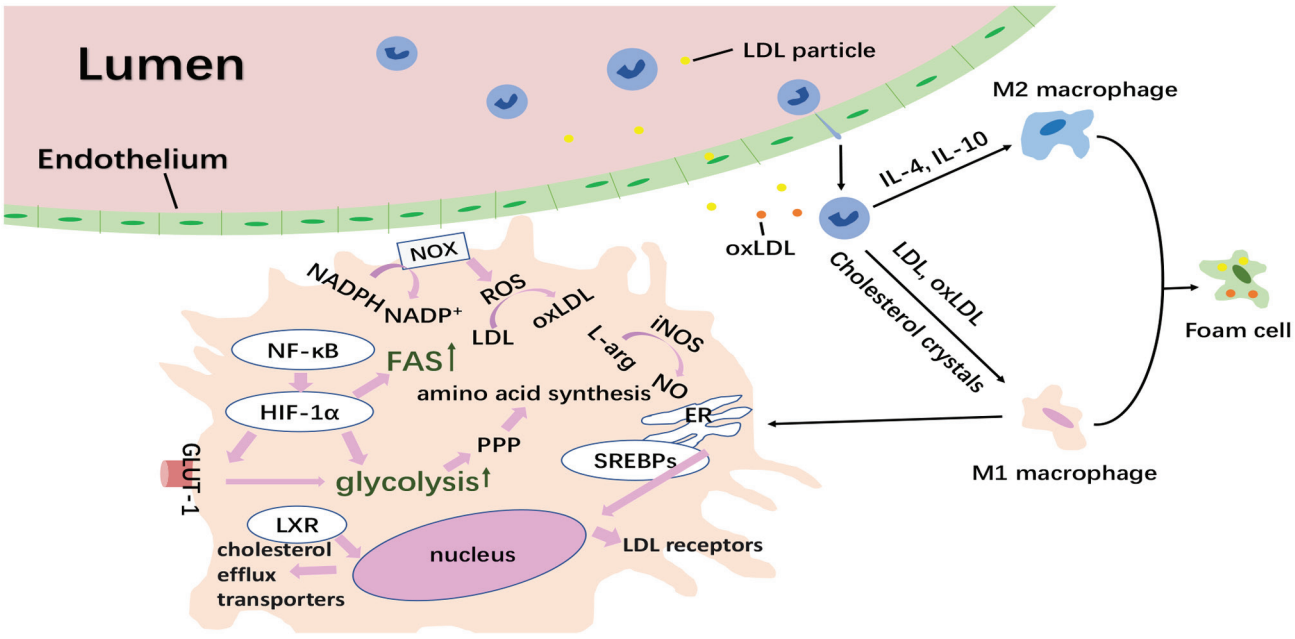

Figure 1. Overview of the formation of trained macrophages in atherosclerosis and their inner metabolic changes. LDL deposition in the intima activates endothelial cells to express a leukocyte adhesion molecule promoting the rolling and adherence of monocytes. Lipoproteins are modified occasionally and taken in by recruited monocytes, which then polarize into diverse phenotypes of macrophages according to their signals from the microenvironment. Monocytes or macrophages encountering oxLDL increase the expression of HIF-1α through the NF-κB pathway. The HIF-1α increases the expression of the GLUT-1, enhancing the uptake of glucose to support glycolysis, which then accelerates the PPP for amino acid synthesis. Activated PPP produces NADPH, which can further act as the electron donor in the production of oxygen radicals, contributing to oxidative stress. Also, the HIF1-α stimulates FAS. As for cholesterol metabolism, nuclear receptors LXR have the ability to reverse cholesterol transport and the SREBP family located within the ER can migrate into the nucleus and drive the expression of LDL receptors. The trained pro-inflammatory M1 macrophages synthesize NO from arginine via iNOS. oxLDL, oxidized low-density lipoprotein; arg, arginine; HIF-1α, hypoxia inducible factor-1α; SREBP, sterol-regulatory element binding protein; PPP, pentose phosphate pathway; GLUT1, glucose transporter 1; FAS, fatty acid synthesis; NF-κB, nuclear factor κ light-chain-enhancer of activated B cells; ROS, reactive oxygen species; ER, endoplasmic reticulum; iNOS, inducible nitric oxide synthase; LXR, liver X receptors; NOX, NADPH oxidase.

crucial for cholesterol and phospholipid synthesis, leading to the formation of new membranes. As for succinate, together with α-ketoglutarate, it is critical for the activity of two families of enzymes controlling epigenetic modifications: The JMJ family of lysine demethylases and the TET family of methyl-cytosine hydroxylases (48,49), mediating the epigenetic reprogramming in innate immune memory. Succinate is also able to activate HIF-1α and induces IL-1β production (46), contributing to the inflammatory states of pro-inflammatory M1 macrophages.

Cholesterol metabolism. A major risk factor for the development of AS is the high levels of oxLDL within AS plaques, which is taken up by macrophages, resulting in cholesterol-loaded macrophage foam cells. Foam cells secrete pro-inflammatory cytokines and chemokines, as well as produce matrix metalloproteinases to degrade the extracellular matrix of the plaque, leading to rupture (50,51). Furthermore, oxLDLs are highly pro-inflammatory and can induce a trained immune macrophage phenotype, making the control of cholesterol influx vs. efflux vital for atherogenesis.

This regulation extensively relies on nuclear receptors liver X receptors (LXRs) including LXR-α and -β. Activated by oxysterols, LXRs promote reverse cholesterol transport and have potent anti-inflammatory effects (52). Studies have indicated that LXRs are able to suppress the expression of inflammatory genes in macrophages, such as lipopolysaccharide (LPS)-stimulated cultured macrophages (52-55) and foam cells (56,57). It has also been reported that LXR agonists are able to reduce AS plaque formation (50). A recent study also showed that myeloid LXR deficiency led to a marked increase in AS with increased monocyte entry, foam-cell formation and plaque inflammation (58), marking the importance of LXRs in atherogenesis.

LXRs are also vital to the expression of sterol-regulatory element binding protein (SREBP)-1c, which is upregulated in the pro-inflammatory M1 macrophages and turns on the FAS (59). SREBP-1c belongs to the SREBP family of TFs, which also include SREBP-1a and SREBP-2 and contribute to the regulation of cholesterol and fatty acid biosynthetic gene expression (60). The family is located within the endoplasmic reticulum, where they are retained by cholesterols, oxysterols and desmosterols. When the intracellular concentration of these metabolites declines, the SREBPs are released, migrate into the nucleus and drive the expression of LDL receptors and genes involved in the synthesis pathway of both cholesterol and fatty acid (61). Studies have shown that cholesterol synthesis is upregulated in macrophages trained with β-glucan or Bacillus Calmette-Guérin, and β-glucan-induced trained immunity both *in vivo* and *in vitro* can be abrogated by statins inhibiting cholesterol synthesis (62); however, the role of cholesterol synthesis in oxLDL-induced trained macrophages remains to be elucidated.

Lipid metabolism. While glycolysis predominantly energizes the pro-inflammatory M1 macrophages, FAO is the major

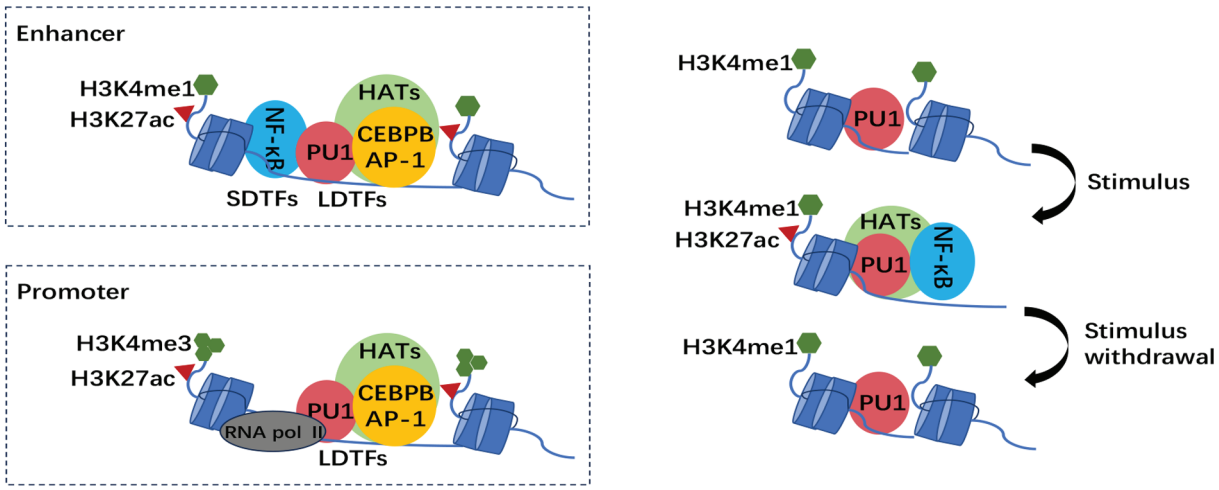

Figure 2. Epigenetic landscape and transcription factors binding in trained macrophages. Top left panel: Distal genetic elements are set up by LDTFs PU-1, CEBPB and AP-1 via deposition of H3K4me1 by HMEs. Signal-dependent TFs such as NF-κB recruit histone acetyltransferases, leading to histone acetylation, including H3K27ac and the formation of active chromatin. Bottom left panel: H3K4me3 accumulates on immune gene promoters, where RNA pol II, instead of SDTFs, binds and initiates gene transcription. Right-hand panel: During myeloid lineage differentiation, LDTF PU-1 and the accumulation of H3K4me1 prime the enhancers. Upon stimulation with factors such as oxLDL and cholesterol crystals, NF-κB binds pre-accessible chromatin and recruits HATs, leading to histone acetylation such as H3K27ac and the formation of active chromatin. The SDTF leaves and the H3K27ac marks are lost gradually after the removal of the initial stimulus, while H3K4me1 remains, keeping the enhancer in a primed state for subsequent activation. LDTFs, lineage-determining TFs; HATs, histone acetyltransferases; CEBPB, CCAAT/enhancer binding protein beta; AP-1, activator protein 1; H3K27ac, histone 3 lysine 27 acetylation; H3K4me3, histone 3 lysine 4 trimethylation; H3K4me1, histone 3 lysine 4 methylation; HME, histone-modifying enzyme; SDTF, signal-dependent TF; PU-1, Spi-1 proto-oncogene; NF-κB, nuclear factor κ light-chain-enhancer of activated B cells; TF, transcription factor; pol, polymerase.

source of energy production in M2 anti-inflammatory macrophages (63,64). Thanks to the overexpression of carnitine palmitoyl transferase 1 transporting long-chain fatty acids into the mitochondria, rates of FAO are predictably increased, accompanied with decreased production of inflammatory cytokines in these cells (65).

By contrast, FAS is generally associated with a pro-inflammatory macrophage phenotype (66). In this pathway, genes involved in the multi-complex enzyme, such as fatty acid synthase (FASN), are upregulated (59), and observation has shown that macrophage-targeted deletion of *FASN* reduced AS plaque formation and foam cell formation in *Apolipoprotein* $E^{-/-}$ mice (67). Hypoxia, together with NF-κB, activates the expression of HIF1-α, which stimulates stearoyl-coenzyme A desaturase (SCD), an important enzyme in FAS. That way, hypoxia enhances FAS, while suppressing FAO (68). SCD also drives the synthesis of monounsaturated fatty acids from palmitic acid. Increased intracellular levels of unsaturated fatty acids stimulate a pro-inflammatory phenotype by upregulating IL-1α production in foam cells (69).

Amino acid metabolism. Another classic example of metabolic reprogramming is amino acid metabolism, particularly that of arginine. While M2 macrophages catabolize arginine via arginase and finally break it down into molecules that support cell growth and division, as well as a building block for collagen production, the trained pro-inflammatory M1 macrophages synthesize nitric oxide (NO) from arginine via inducible-NO synthase (iNOS) (70). NO acts not only as a signal of important cues including vasodilation, insulin secretion and angiogenesis, but also as an important microbicidal agent, which is vital for the early stages of AS (71). Enhanced expression of the iNOS has been used to characterize activated M1 macrophages, and the expression of iNOS is demonstrated to be elevated compared to normal arterial tissue by *in situ* hybridization experiments (72,73).

Experiments also found that glutamine had pro-AS effects on a murine macrophage-like cell line (74). Other research has shown the importance of glutamine in the induction of IL-1 by macrophages in response to LPS stimulation (75), highlighting the nonnegligible role of glutamine metabolism in trained immunity.

5. Transcriptional and epigenetic regulation in trained plaque macrophages

In the pathogenesis of AS, the cellular phenotype and function of macrophages, including differentiation and activation, are of great importance. The robust execution of this cascade of biological reactions is guaranteed by multilayered regulation, such as TF and co-factor binding, the epigenetic landscape, long-range interactions, DNA methylation, RNA editing and long non-coding (lnc)RNAs (76). Specifically, in trained macrophages in AS, studies have found that epigenetic processes, including histone modification, DNA methylation, modulation of microRNA and lncRNA expression (8), are at the basis of the innate immune memory (10,77) (Fig. 2).

Histone modification. Histone-modifying enzymes (HMEs) with histone-binding domains have the ability to recognize histone tails extended out of the octamers and catalyze the addition or removal of different histone modifications. Several modifications, such as methylation, acetylation, phosphorylation and ubiquitination, together with their role in supporting transcriptional processes, have been well-studied. Amid a vast repertoire of histone modifications, acetylation (lysine

residues) and methylation (arginine and lysine residues) are the most broadly studied and extensively characterized (78).

In the context of a trained immune response, epigenetic reprogramming takes place in different types of immune cells at a large number of immune genes and their distal genetic elements called enhancers. Upon primary stimulation, histone 3 lysine 4 trimethylation (H3K4me3) accumulates on immune gene promoters, with histone 3 lysine 4 methylation (H3K4me1) on enhancers and histone 3 lysine 27 acetylation (H3K27ac) marking active promoters and enhancers (79,80). H3K27ac marks are gradually lost after removal of the initial stimulus, while H3K4me1 and H3K4me3 modifications remain (49), suggesting that H3K27ac appears to function more as a mark of changes in promoter activity than H3K4me3, and H3K4me1 provides an epigenetic memory function in macrophages (81).

DNA methylation. CpG islands at promoters and enhancers can be recognized by DNA methyltransferases (DNMTs) and are generally related to transcriptional repression. DNA methylation has been shown to be more associated with monocyte-to-macrophage differentiation than subsequent activation (82,83). Epigenome-wide analyses have demonstrated a general loss of 5mc during *ex vivo* monocyte-to-macrophage differentiation (84). The role of DNA methylation in trained immunity is still in need of further investigation, and different combinations of histone and DNA modifications should determine the accessibility of DNA undoubtedly.

TF binding. TFs usually bind to specific DNA motifs, in requirement of chromatin accessibility, which is modulated by epigenetic modification. The macrophage epigenetic and chromatin accessibility landscape is established by the divergent binding of lineage-determining TFs (LDTFs) and signal-dependent TFs (SDTFs). LDTFs act as master regulators of the cell-specific epigenetic landscape by binding to closed chromatin, setting up a cell-specific regulatory landscape and driving cell-specific transcription programs (85). SDTFs can activate a stimulus-specific regulatory program (86,87) and can also activate epigenetically unmarked elements termed latent enhancers, which can remain marked after stimulus removal and ready for rapid response to a second activation, acting as cellular memory (81). In the context of AS, LDTFs such as PU-1 and CCAAT/enhancer binding protein β (C/EBPβ) instruct macrophage differentiation, while SDTFs such as NF-κB trigger macrophage activation regulated by cytokine stimulation (86).

NF-κB, for instance, is in the cytoplasm under basal conditions. Upon stimulation with substances such as oxLDL or cholesterol crystals, it translocates to the nucleus (88) and binds pre-accessible chromatin. Prior to this, classic enhancers are set up by LDTFs PU-1 and C/EBPβ (89) via deposition of H3K4me1 by HMEs. Upon binding, NF-κB recruits the histone acetyltransferase EP300, leading to histone acetylation such as H3K27ac and formation of active chromatin (90). Subsequently, the translation of genes such as HIF-1α is permitted and then causes changes in cellular metabolic and consequent biologic behavior. After removal of the stimulus, the SDTF leaves, and activating histone marks are removed under recruitment of histone deacetylases (HDACs), whereas H3K4me1 remains and keeps the enhancers in a primed state for subsequent activation.

Besides the above epigenetic and chromatin accessibility landscape, long-range interactions and ncRNAs are also found to be critical modulators of macrophage activation. For instance, an immune gene-priming lncRNA called upstream master lncRNA of the inflammatory chemokine locus is able to direct histone methyltransferase to immune-related genes topologically, leading to increased local H3K4me3, which is a ubiquitous epigenetic modification in trained immunity (91). The deposition of this mark and its persistence through the inhibition of histone demethylases provide specificity for trained immunity (16). Recent findings of epigenetic mechanisms and metabolic processes from the molecular basis of trained immunity were summarized in a previous review (92).

In an AS plaque environment, macrophages are exposed to a variety of signals and differentiate into complex phenotypes (93). Studies have shown that the identified pro-inflammatory and anti-inflammatory macrophage subsets in the plaque do not resemble the classical M1 and M2 macrophages and express both pro- and anti-inflammatory macrophage markers at the same time (94). Indeed, an epigenetic and transcriptional crosstalk between pro- and anti-inflammatory signaling in macrophages is suggested in several studies, highlighting the complexity of inflammatory signaling in an AS plaque *in vivo* (95,96).

6. Therapeutic approaches

AS is an inflammation-driven disease and macrophages have a central role in the modulation of inflammation. Due to the need to respond in a rapid and specific way, macrophages adopt a unique, permissive epigenetic landscape that is established and controlled by sequential binding of LDTFs and SDTFs, which shed light on therapy targeting epigenetic enzymes. Indeed, macrophage-specific genetic ablation or pharmacological inhibition of HMEs holds promise for the treatment of inflammatory diseases (97). For instance, HDAC3 is able to deactivate key factors needed for IL-4-dependent anti-inflammatory activation (98). Macrophage-specific knockout of *HDAC3* in mice promotes plaque stability and steers the cells into a more wound-healing, fibrotic and anti-inflammatory phenotype (99). Thus, the deletion of *HDAC3* may attenuate AS. However, owing to the broad action of epigenetic enzymes, strategies to increase specificity would be beneficial and possibly even required. There is a great demand to characterize the epigenetic landscape and pinpoint TFs, as well as upstream signaling pathways regulating cell type-specific gene expression programs.

Furthermore, due to the reprogramming of metabolism in trained macrophages in AS, new drugs interfering with key metabolic pathways such as glycolysis, which is involved in the activation and differentiation of monocytes, should be taken into consideration. Indeed, the observation that trained immunity is completely prevented by pharmacological blockers of glycolysis (42) not only validates the causal role of glycolysis in trained immunity but also enlightens future treatments of AS. Also, several animal studies have reported that LXR agonists can reduce AS plaque formation (50), making cholesterol metabolism a key pathway in AS treatment. For future employment, a targeted therapy would be required likewise.

Also, it may be worthwhile investigating to what extent the metabolic pathways determine the destiny of macrophages.

7. Conclusions

AS, a major threat to global public health, has its basis in chronic inflammation of the artery wall. Above all cells involved in atherogenesis, monocytes and macrophages have a major role in the pathogenesis, highlighting the importance of their biological activities. Trained immunity, the memory of innate immunity, has aroused the interest of investigators and may be a novel mechanism of atherogenesis. In the microenvironment of AS plaque, monocytes are activated and differentiate into various phenotypes of macrophages in response to a cascade of signals, mainly pro-inflammatory M1 and anti-inflammatory M2 cells. During this process, metabolic changes take place, such as enhanced glycolysis in trained pro-inflammatory macrophages. Epigenetic and transcriptional regulation acts as initiators in these metabolic changes.

Recent discoveries of trained immunity not only refresh the concept of immunology, but also inspire researchers to reconsider inflammatory processes in AS. In that way, researchers find heterogeneity of macrophages in AS plaque and further elucidate mechanisms that drive the differentiation. To meet the need for more experiments and findings, research on potential therapies for AS based on metabolism and gene expression is underway.

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Availability of data and materials

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Authors' contributions

TL and WF collected the references and were involved in the initial conception of this review. TL wrote most parts of the manuscript. TW and WY provided writing guidance and revisions. All authors have read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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